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看板論文張貼時段

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P001

Nutrient deprivation induces the Warburg effect through ROS/AMPK-dependent activation of pyruvate dehydrogenase kinase.

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The Warburg effect is known to be crucial for cancer cells to acquire energy. Nutrient deficiencies are an important phenomenon in solid tumors, but the effect on cancer cell metabolism is not yet clear. In this study, we demonstrate that starvation of HeLa cells by incubation with Hank's buffered salt solution (HBSS) induced cell apoptosis, which was accompanied by the induction of reactive oxygen species (ROS) production and AMP-activated protein kinase (AMPK) phosphorylation. Notably, HBSS starvation increased lactate production, cytoplasmic pyruvate content and decreased oxygen consumption, but failed to change the lactate dehydrogenase (LDH) activity or the glucose uptake. We found that HBSS starvation rapidly induced pyruvate dehydrogenase kinase (PDK) activation and pyruvate dehydrogenase (PDH) phosphorylation, both of which were inhibited by compound C (an AMPK inhibitor), NAC (a ROS scavenger), and the dominant negative mutant of AMPK. Our data further revealed the involvement of ROS production in AMPK activation. Moreover, DCA (a PDK inhibitor), NAC, and compound C all significantly decreased HBSS starvation-induced lactate production accompanied by enhancement of HBSS starvation-induced cell apoptosis. Not only in HeLa cells, HBSS-induced lactate production and PDH phosphorylation were also observed in CL1.5, A431 and human umbilical vein endothelial cells. Taken together, we for the first time demonstrated that a low-nutrient condition drives cancer cells to utilize glycolysis to produce ATP, and this increases the Warburg effect through a novel mechanism involving ROS/AMPK-dependent activation of PDK. Such an event contributes to protecting cells from apoptosis upon nutrient deprivation.

P002

The antimicrobial peptide, epinecidin-1, mediates secretion of cytokines in the immune response to bacterial infection in mice

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Backgrounds:
To confirm whether epinecidin-1 is clinically valuable as a candidate for an antimicrobial drug, we evaluated the effects of synthetic epinecidin-1 on mice, by comparing the antibacterial neutralization efficiency, and measuring serum cytokine levels of immunoglobulin G (IgG), IgM, IgG1, IgG2a, interferon (IFN)-γ, interleukin (IL)-10, IL-12, and others.
Materials and Methods:
That the synthetic epinecidin-1 peptide induced significant secretion of immunoglobulin G1 (IgG1) in mice co-injected with P. aeruginosa. Moreover, after injection of 40, 100, 200, or 500 μg epinecidin-1/mouse, we detected IgM, IgG, IgG1, and IgG2a in mice treated for 1, 2, 3, 7, 14, 21, and 28 days.
Results:
Results showed that there were no significant differences in IgM, IgG, or IgG2a between mice injected with epinecidin-1 alone. IgG1 increased to a peak at 24 h, 7 days, and 28 days after an epinecidin-1 (40 μg/mouse) injection. Injection of 500 μg epinecidin-1/mouse increased IgG1 to peaks at 2 and 3 days; injection of 100 μg epinecidin-1/mouse increased IgG1 to a peak at 21 days.
Conclusion:
This supports epinecidin-1 being able to activate the Th2 cell response (enhance IgG1 production) against P. aeruginosa infection. Treatment with different concentrations of epinecidin-1 in mice elevated plasma interleukin (IL)-10 to initial peaks at 24 and 48 h, and it showed a second peak at 16 days. In RAW264.7 cells, treatment with epinecidin-1 alone did not produce significant changes in tumor necrosis factor (TNF)-α protein secretion at 1, 6, or 24h after treatment with 3.75, 7.5, or 15 μg/ml epinecidin-1 compared to the lipopolysaccharide group.

P003

HMG-CoA reductase inhibitors activate caspase-1 in human monocytes depending on ATP release and P2X7 activation

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Recent studies have demonstrated the stimulatory effects of HMG-CoA reductase inhibitors, statins, on IL-1β secretion in monocytes and suggest a crucial role for isoprenoids in the inhibition of caspase-1 activity. In this study, we further elucidated the molecular mechanisms underlying the stimulatory effects of statins on caspase-1. Three commonly recognized mechanistic models for NLRP3 inflammasome activation (i.e., ATP/P2X7/K^+ efflux, ROS production, and lysosomal rupture) were investigated in statin-stimulated human THP-1 monocytes. We found that fluvastatin and lovastatin can synergize with LPS to trigger inflammasome activation. Moreover, statin-induced caspase-1 activation and IL-1β production in LPS-primed THP-1 cells are dependent on GGPP deficiency and P2X7 activation. In particular, increased ATP release accounts for the action of statins in P2X7 activation. We also provide evidence that statin-induced moderate ROS elevation is involved in this event. Moreover, the cathepsin B inhibitor was shown to reduce statin-induced IL-1β secretion. Consistently statins can induce cathepsin B activation and lysosomal rupture, as evidenced by LysoTracker staining. Statins also increase intracellular ATP secretion and IL-1β release in primary human monocytes and murine macrophages. Notably, exogenous ATP-elicited P2X7 activation and consequent IL-1β release, an index of direct NLRP3 inflammasome activation, were not altered by statins. Taken together, statininduced enhancement of inflammasome activation in monocytes and macrophages covers multiple mechanisms, including increases in ATP release, ROS productionand lysosomal rupture. These data not only shed new insight into isoprenylation-dependent regulation of caspase-1 but also unmask mechanisms for statin-elicited inflammasome activation. *J. Leukoc. Biol.* 92:(2013).

P004

Antiplatelet Effect of a New Synthetic Compound HPW-RX40

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Backgrounds:
HPW-RX40 is a chemical derivative of 3,4-methylenedioxy-nitrostyrene (MNS). Although MNS has been reported to inhibit the activation of GPIIb/IIIa and platelet aggregation, the effect of HPW-RX40 on platelets, the pivotal players in atherothrombosis, has not been studied.
Materials and Methods:
The inhibitory effect of HPW-RX40 on human platelet aggregation and adhesion was assessed using aggregometry and flow chamber assay. Western blotting was used to evaluate the activation of Akt, protein kinase C (PKC), protein tyrosine kinases, and Rap1.
Results:
HPW-RX40 dose-dependently inhibited human platelet aggregation induced by thrombin, collagen and U46619 with IC50 values of 1.58, 1.23 and 1.31 μM. In addition, HPW-RX40 reduced platelet adhesion to a collagen-coated surface under whole blood flow conditions. It also markedly prevented thrombin- and collagen- induced Akt phosphorylation and Rap1 activation. Unlike MNS, it had no effect on protein tyrosine kinases. To further elucidate the mechanism of action of HPW-RX40, the protein kinase C activator 12-O-tetradecanoylphorbol-13-acetate (TPA) and the calcium ionophore A23187 were used to induce platelet aggregation and Rap1 activation. We found that HPW-RX40 could inhibit platelet aggregation induced by both agents; however, HPW-RX40 only suppressed Rap1 activation induced by A23187, but not TPA.
Conclusion:
Our results have demonstrated that HPW-RX40 potently inhibited platelet aggregation and reduced platelet adhesion to collagen in flowing whole blood. The mechanism of antiplatelet action of HPW-RX40 may be related to Rap1 inhibition; however, further studies were still needed.

P005
The Correlation Between Serum Atorvastatin Concentrations and Adverse Effects in Hyperlipidemic Patients

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Background:
Atorvastatin has been shown to efficiently reduce the levels of atherogenic lipoproteins. The adverse effects of myalgia and elevated ALT have been noticed in hyperlipidemic patients taking atorvastatin. However, the association between serum concentrations of atorvastatin and the occurrence of adverse effects has not been reported. Thus, serum concentrations of atorvastatin were determined to evaluate their significant difference in patients with and without adverse effects.

Patients and Methods:
Sixty-nine patients who received a daily dose of atorvastatin of 10 mg or 40 mg for at least 2 weeks were included in this study. Serum concentrations of atorvastatin were quantitated by HPLC analysis and β-naphthoflavone was used as the internal standard.

Results:
Serum concentrations (mean ± SD) of atorvastatin in non-myalgia (n = 62) and myalgia groups (n = 7) were 9.98 ± 22.77 and 6.38 ± 4.53 ng/ml, respectively. Serum concentrations of atorvastatin in groups with normal ALT level (< 40 U/L, n = 59) and abnormal ALT (> 40 U/L, n = 10) were 7.61 ± 18.94 and 21.44 ± 32.29 ng/ml, respectively. The serum atorvastatin concentrations in patients with elevated ALT were significantly higher than in patients with normal ALT. However, there was no significant difference between groups with and without the compliance of myalgia.

Conclusion:
Our findings reveal that patients with high ALT values had significantly higher serum atorvastatin concentrations. Factors affecting the serum atorvastatin concentration and the hepatotoxicity of atorvastatin can be important in the future study.

P006
The Regulatory Effect of Radix of *Hyptis Suavelens* on High-Fructose Induced Nonalcoholic Fatty Liver Disease in Murine Model.

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Backgrounds:
The hapatic manifestation of the metabolic syndrome in nonalcoholic fatty liver disease (NAFLD) is increase dramatically throughout the last two decade. It comprises a wide spectrum of stages of hepatosteatosis to liver cirrhosis and present a threat to public health issue. NAFLD has been proved that high-fructose intake in daily breveage and this is why NAFLD is more and more popular.

Materials and Methods:
In this research, we tried to use a traditional Chinese medicine – radix of *Hyptis suavelens* (RHS) and treat the C57BL/6 mouse which induced NAFLD with daily 30% fructose water. In this experiment, we divided into 4 groups, include naïve group, treatment groups including various doses (100 and 500 mg/kg) of RHS and control group offer the 30% fructose solution for 8 weeks administration. At the end of 8 weeks administration, triglyceride and cholesterol determination in serum or liver and oral glucose tolerance test (OGTT), 4-hydroxynonenal (4-HNE), Glucose transporter type 4 (GLUT4), and lipid accumulation in liver tissue sections by immuno- histochemical, Oil Red O staining were determinate.

Results:
After 8 weeks, RHS has the significant different in the gross of liver with control group. RHS reduced ALT and triglycerides levels in serum, and also decreased accumulation of triglycerides in liver. We provide information on the distribution of inflammatory factors in the liver and/or adipose organ, where their aberrant expression in NAFLD. GLUT4 play a central role in the regulatory glucose transport in progressions of NAFLD.

Conclusion:
These data suggest that RHS enhanced glucose sensitivity, reduced triglyceride level and prevent hepatic steatosis and is able to improve the NAFLD symptoms and defer the NAFLD occur, and has potential value to be healthy benefit.

P007
The Beneficial Effect of Celastrol on Lipopolysccharide-Induced Septic Shock in Rats

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Backgrounds:
The aim of this study is to evaluate the beneficial effects of celastrol on multiple organ failure during sepsis induced by LPS.

Materials and Methods:
Male Wistar-Kyoto rats (280-340 g) were anesthetized and assigned into five groups: (I) celastrol only: treated with celastrol (0.5 and 1 mg/kg in 5% DMSO, i.v.); (II) LPS only: 10 mg/kg, i.v.; (III) 0.5 mg/kg celastrol + LPS 10 mg/kg, i.v.; (IV) 1 mg/kg celastrol + LPS 10 mg/kg, i.v.; (V) Post-treatment: LPS 10 mg/kg + 1 mg/kg celastrol, i.v. Celastrol was treated 2 hours before LPS administration. The mean arterial pressure (MAP) and heart rate were monitored throughout the experiment. Plasma levels of ALT, BUN, creatinine, and LDH were detected to observe organ function. The superoxide anion production in thoracic aorta was measured by luminescence measurement system. Plasma levels of nitrite/nitrate were determinate by NO analyzer.

Results:
Pretreatment with celastrol (0.5 & 1.0 mg/kg) prevented LPS-induced circulatory failure. After LPS challenge, the plasma levels of LDH and ALT increased markedly, which can be significantly reduced by pretreatment with celastrol. The superoxide anion production in aorta was significantly decreased in celastrol-pretreated groups when compared with the LPS only group. The levels of plasma nitrate/nitrite and iNOS protein expression in aorta were markedly increased after LPS administration, which were attenuated by celastrol.

Conclusion:
Celastrol possesses protective effects on LPS-induced circulatory failure in rats, which is likely attributed to its antioxidant capacity and inhibitory effect on vascular iNOS induction.

P008
Activation of C-Met by Tabacco-Specific Carcinogen Benzo(a)pyrene Causes EGFR TKIs Resistance in Lung Cancer

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Backgrounds:
To investigate how cigarette smoking affects the therapeutic efficacy of EGFR tyrosine kinase inhibitors including gefitinib and erlotinib in NSCLC.

Materials and Methods:
NSCLC cell lines were cultured in medium containing cigarette smoke extract (CSE), tobacco-specific carcinogen benzo(a)pyrene (BaP), or nicotine-derived nitrosamine ketone (NNK) to establish stable clones. These stable clones and their parental cells were treated with EGFR tyrosine kinase inhibitors to determine cell viability in MTT, cell counting, and clonogenic assays. Pharmacological inhibitor or specific shRNA against EGFR and c-Met was employed to study the underlying mechanism of CSE-induced TKI resistance.

Results:
In comparison to their parental cells, CSE- and BaP-treated but not NNK-treated H292stable clones were more insensitive to EGFR TKIs. EGFR downstream survival signals, including Akt and ERK pathways, were not inhibited by EGFR TKIs in BaP-treated H292 cells. The compensatory Akt activity was resulted from the increase in c-Met tyrosine kinase activity.

Conclusion:
Our study indicates that tobacco-specific carcinogen benzo(a)pyene can lead cell resistance to EGFR tyrosine kinase inhibitors through activation of proto-oncogene c-Met, suggesting that combination with c-Met inhibitor may synergize the therapeutic efficacy of EGFR TKIs in NSCLC patients with cigarette smoke history.

P009
Exploring the beneficial effect of an enriched environment combined with imipramine for treating depressive-like behaviors induced by neuropathic pain

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Backgrounds:
Depression is one of the most common and most serious mental health problems facing people today. Despite years of research and efforts to develop effective treatments, currently available medications have significant limitations, most notably low response rate, time lag for treatment response and high toxicity. Therefore, developing a rapid onset of action and more effective treatment strategies are urgently needed.
Materials and methods:
We used a combinational therapy of an enriched environment (EE) and a tricyclic antidepressant imipramine for relieving depressive-like behaviors induced by neuropathic pain in a mouse model of spared nerve injury (SNI).
Results:
As compared with sham-operated animals, SNI mice exhibited mechanical allodynia and displayed an increased depressive-like behavior, as revealed by a loss of body weight, a decrease in sucrose preference, and an increase in immobility time during the tail suspension test. A subchronic (4 days) combination of an EE and imipramine treatment produced robust and rapid antidepressant-like responses compared with subchronic single treatment. These antidepressant effects are accompanied by a reversal of dendritic and synaptic reorganization brought about by SNI.
Conclusions:
Combination therapy is more effective in providing antidepressive effects than a single treatment. These findings highlight a novel strategy of combining multiple molecular targets to rescue depressive-like symptoms.

P010
Methamphetamine Inhibits Large Conductance Calcium-activated Potassium Channels in NG108-15 Neuroblastoma Cells

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Methamphetamine (MA) is a powerful synthetic drug that stimulates the central nervous system. The actions of MA on ion channels activity in neurons remain unclear. The present study investigated the effects of MA on large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) using whole-cell and cell-attached patch clamp techniques. The cell membrane potential was examined by current-clamp configuration in NG108-15 neuroblastoma cells. In whole-cell recordings, the whole-cell K^+ currents carried by BK_{Ca} channels were inhibited by MA with an EC_{50} of 146 μM, but not by dopamine (20μM). This implies that dopamine is not involved in the effects of MA on BK_{Ca} currents. In cell-attached patches, MA significantly decreased BKCa channels activity, but not modify single-channel conductance. Moreover, MA significantly decreased the BK_{Ca} channel opener NS1619-induced BK_{Ca} channel activity. Under current-clamp configuration, MA depolarized the cell from -37 ± 2 to -16 ± 4 mV and the membrane potential returns to resting potential after application of NS1619. These findings suggest that MA might act as a novel inhibitor of BK channels, and thereby increase the neuronal excitability.

P011
Adiponectin increases VEGF expression in human chondrosarcoma through PI3K and Akt signaling pathway.

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Background:
Vascular endothelial growth factor (VEGF) is an angiogenic mediator in tumors and has been implicated in the pathogenesis and progression of cancer. Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation. Recently, adiponectin was also shown to modulate angiogenesis. However, the effect of adiponectin on VEGF expression in human chondrosarcoma cells is mostly unknown.
Materials & Methods:
The qPCR was used to examine the mRNA expression of VEGF. The PI3K and Akt phosphorylation was examined by using Western blot method. A transient transfection protocol was used to examine HIF activity.
Results:
We found that adiponectin increased the VEGF expression in human chondrosarcoma cells. Adiponectin-mediated VEGF expression was attenuated by PI3K inhibitors (LY294002 and wortmannine), Akt inhibitor, and HIF-1α inhibitor. Activations of PI3K, Akt, and HIF-1α pathways after adiponectin treatment was demonstrated. Adiponectin-induced HIF-1α activation was inhibited by the specific inhibitor and mutant of PI3K and Akt cascade.
Conclusion:
This study showed for the first time that the adiponectin mediates VEGF expression of human chondrosarcoma cells. One of the mechanisms underlying adiponectin induced VEGF expression was activation of PI3K, Akt, and HIF-1α pathways.

P012
The influence of physical state on stress responsiveness and stress memory formation

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Backgrouds:
Stressful life events increase the risk of developing psychopathology such as depression or post-traumatic stress disorder. Every individual experiences stressful life events, but shows a high degree of heterogeneity of responses. For some individuals, exposure to acute or chronic stress leads to a pathologically excessive response, and ultimately results in psychopathology. Importantly, stress often occurs within the context of homeostatic challenge, requiring integration of physiological and psychological needs into appropriate hormonal, cardiovascular and behavioral responses. However, the neural mechanisms underlying stress integration within the context of homeostatic adversity remain poorly understood. The objective of this study is to examine how physical state affects responding to psychogenic stressors.
Materials and Methods:
Healthy adolescent male Sprague-Dawley rat (35-41 days old) were used in these experiments. Behavioral stress was evoked by forced swimming stress for 15 min in 25 ℃ water. Rast were injected subcutaneously with 1m of 2.0 M NaCl to induce a state of hypernatremia. One hour after injection, rats were subjected to behavioral stress and hippocampal slice (400 μm-thick) were prepared immediately after stress for extracellular recording. Blood sample were obtained from the tail before and at different time after stress to measure corticosterone levers.
Results:
We found that experienced forced swimming stress enhanced long-term potentiation(LTP) induction in the dentate gyrus of the rat hippocampus in vitro. The effect of stress on LTP was prevented when the animals were adrenalectomized or given mineralocorticoid receptor antagonist RU28318 before experiencing stress. Interestingly, the enhancing effect of stress on LTP was significantly ameliorated by treatment with MEK1/2 inhibitors, U0126 or PD98059, respectivity. In addition, we observed that hypernatremic challenge can effectively elevate plasma sodium concentration and attenuate stress-induced enhancement of LTP, which was prevented by adrenalectomy. Furthermore, a single psychogenic stress experience also elicited a long-lasting consequence for future behavioral response to same stress.
Conclusion:
We provide novel evidence for reduced responding to psychogenic stressors by an acute physical challenge.

P013
CEBPD-Regulated Thrombospondin 1 via Mir-135a in Astrocytes of Alzheimer's Disease

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Yu-Yi Chu,[1] Shao-Ming Wang[2]

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Backgrounds:

Astrocytes are essential in normal brain tissue and can secrete neurotrophic factors, including glial cell-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), to support normal neuronal function and survival. Alzheimer's disease (AD) is an irreversible neurodegenerative disease accompanied by cognitive/memory impairments. The *AppTg* mice showed impaired memory, but this effect was minor in *AppTg/Cebpd-/-* mice. However, the details remain unknown.

Materials and Methods:

The conditioned medium of astrocyte expressing CEBPD was used to assess the effects on neuron survival and number of synapses. By analyzing CEBPD-regulated genes and miRNA profilings in astrocytes, we identified that neurotrophic factor THBS-1 and miR135a were down-regulated and up-regulated, respectively, in astrocytes. The RT-PCR, reporter assay and chromatin immunoprecipitation (ChIP) assay were conducted to assess whether CEBPD can activate the promoter region of non-coding gene with miR135a. The reporters of THBS1 3'UTR with or without miR135a binding site and miR135a inhibitor were used to examine whether THBS-1 is a miR135a target.

Results:

In this study, as mentioned above, AppTg/Cebpd-/- mice showed a minor impaired memory. Comparing regular conditioned medium of astrocytes, the conditioned medium of astrocyte expressing CEBPD promoted neuron death. We demonstrated that CEBPD negatively regulates THBS1 transcription in astrocytes and miR135a is a CEBPD responsive miRNA. miR135a contributed to the repression of THBS1 transcription by directly targeting the THBS1 3'UTR. Moreover, a miR135a inhibitor can reverse CEBPD-repressed THBS1 transcript. Furthermore, conditioned medium of astrocytes expressing miR135a inhibited the growth of nurite length in neuron cells.

Conclusion:

Our results indicated that CEBPD contributes to the repression of THBS1 transcription by activating the expression of miR135a in astrocytes. We provided a new insight of astrocytic CEBPD in increasing cognitive decline of AD pathogenesis. The discovery strengthened the disadvantage of CEBPD activation in astrocytes and also implied the CEBPD/miR135a/THBS1 axis could be a therapeutic target of AD.

P014
Evaluation of in vivo anti-melanogenic activity of hyaluronic acid containing gold nanoparticles and sclareol.

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Backgrounds:

In this study, we would like to investigate whether hyaluronic acid containing gold nanoparticles (HA-AU NPs) and sclareol would inhibit the formation of melanin.

Materials and Methods:

MTT assay, Determine mushroom tyrosinase activity, Determine melanin content, Determine murine tyrosinase activity, Western blot.

Results:

In MTT assay, the IC20 dose of HA-AU NPs and sclareol in murine melanoma B16-F10 cells was 15 ppm and 25 μM after 48 hours treatment, respectively. Melanogenesis is significantly affected by tyrosinase, So we test the tyrosinase activity in HA-AU NPs and sclareol treatment. In cell free assay, the result was shown that IC20 dose (15 ppm and 25 μM) of HA-AU NPs and sclareol obviously attenuated mushroom tyrosinase activity. In cellular assay, the IC20 dose of HA-AU NPs and sclareol had potent inhibition of melanin production. And then the IC20 dose of sclareol significantly decreased murine tyrosinase activity, which indicated that it was more potent than positive control arbutin, but not shown in HA-AU NPs treatment.

Conclusion:

Our observations suggested that HA-AU NPs and sclareol have an inhibitory effect on melanogenesis. Sclareol showed efficient tyrosinase inhibitory effect in vitro. Our finding suggests that HA-AU NPs and sclareol can be an effective skin-whitening agent.

P015
Bullatacin Induces Apoptosis Through the Regulation of Fas, Caspase8/9 and Bax/Bcl-2 Protein Expression in Human Hepatoma Cell.

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Backgrounds:

Bullatacin, an anonaceous acetogenin(AA), contains adjacent bis-tetrahydrofuran and terminal beta-lactone ring. AAs have been demonstrated to possess pesticidal, anti-malarial, T-cell suppressant, anti-parasitic, antimicrobial, cytotoxic, and in vivo antitumor effects. In our previous reports, we have indicated that bullatacin cause a significant cytotoxic effect in hepatoma cell may be through the cell cycle and apoptosis induction.

Materials and Methods:

Bullatacin is purified from the fruit of Annona atemaya and dissolved in DMSO for several concentrations. Cells were treat bullatacin for 24, 48, 72h and estimated cell viability by XTT and methylene blue assay. Immunofluorescent stained for protein activation and protein location change. After treatment, harvest cells were stained with PI and AnnexinV dye, then pro-apoptotic cell were analyzed by flow cytometry. Moreover, whole cell lysates were be extracted and apoptotic protein were estimated by Western blotting analysis.

Results:

Our data have demonstrated that bullatacin increased apoptosis signals protein expression as a time and dose-dependent manner. Bullatacin induced apoptosis through DNA damage and activate check point pathways, which occurred in the apoptosis early stage, these effects may trigger the apoptotic signals Fas, caspase 8/9, Bax and Bad activation.

Conclusion:

Bullatacin decrease cell viability and induce apoptosis in Human Hepatoma cell through the increase of the DNA damage signal γ-H2Ax, chk2 expression and apoptotic signals expression (Fas, caspase8/9, Bax and Bad). By contrast, bullatacin reduced cell survival signal Ras, Raf and Bcl-2 level.

P016
Dlgap2 Mutant Mice Demonstrate Exacerbated Aggressive Behaviors and Orbitofrontal Cortex Deficits

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Backgrounds:

Many human clinical studies point out genetic mutations in synaptic proteins may lead to a constellation of psychiatry disorders. A specialized group of macromolecules within the synapse, postsynaptic scaffolding proteins complex, plays a role as the master organizer of synaptic proteins in postsynaptic density (PSD), it orchestrates receptors, protein kinases, and cytoskeletons in correct assembly for proper synaptic functions. Dlgap2/Sapap2 is one of the main components of postsynaptic scaffolding proteins complex. Recently, a large-scale copy number variation (CNV) analysis study clearly demonstrates its role in Autism Spectrum Disorder (ASD). We generated Dlgap2 mutant mice to investigate its role in psychiatry disorders.

Materials and Methods:

We used the three-chamber test and resident-intruder task to investigate the social behaviors of Dlgap2 mutant mice. With synaptosomal fraction analysis and Golgi staining, we analyzed the synaptic proteins and spine density.

Results:

Dlgap2 mutant mice displayed novelty-induced hyperactivity, enhanced social approach in the three-chamber test, and exacerbated aggression in the resident-intruder paradigm. With synatosomal fraction analysis, Dlgap2 mutant mice exhibited down-regulation of synaptic receptors and other scaffold proteins in the neocortex. With Golgi staining, Dlgap2 mutant mice demonstrated decreased spine density in orbitofrontal cortex.

Conclusion:

Dlgap2 plays a critical role in social behaviors and synaptic functions in a mouse model. Disruption of this gene causes synaptic dysfunction in the orbitofrontal cortex, which is a negative regulator of aggressive behaviors. Without proper cortical inhibition, Dlgap2 mutant mice demonstrate exacerbated aggression.

P017

The Protective Effect of Aldehyde Dehydrogenase Activator-1 (Alda-1) against 4-Hydroxynonenal (4-HNE) Induced Neurotoxicity in Neuronal Cells

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Backgrounds:

The lipid peroxidation products 4-hydroxynonenal (4-HNE) have been implicated in the etiology and pathogenesis of many disease that involve an increase in oxidative stress such as alcoholic liver disease, metabolic syndrome, as well as neurodegenerative diseases such as Alzheimer`s, Parkinson`s and multiple sclerosis. Aldehyde dehydrogenases (ALDHs) are involved in the oxidation of these aldehydes into their corresponding acids which are less toxic and easier to eliminate. It has been proposed that ALDH2 has an important role in the elimination of lipid peroxidation byproducts in neurodegenerative diseases.

Materials and Methods:

In this work, it was determined that aldehyde dehydrogenase activator-1 (Alda-1), an ALDH2 agonist, showed the highest protective effect from 4-HNE induced neurotoxicity in neuronal cells (SH-SY5Y).

Results:

In the result, 4-HNE exhibited dose-dependent neurotoxicity effects, such as increased oxidative stress (superoxide anion), apoptosis effect, mitochondrial dysfunction, decreased cell survival, and ALDH enzyme activity. Remarkably, pre-treatment of Alda-1 exerted highly protection against those neurotoxicity induced by 4-HNE. On the other hand, co-treatment of disulfiram, an ALDH inhibitor, with Alda-1, the protective effects of Alda-1 was reversed and additive the toxicity of 4-HNE.

Conclusion:

These results support the hypothesis that improved detoxification of aldehydes may be important in pathophysiology of neurodegenerative diseases. In the future, the signal pathways such as JNK and P38 pathways,Bcl2 family and NFκB pathway will be evaluated the influence of Alda-1 in the lipid peroxidation product of neurotoxicity.

P018

The inhibitory effect of Savia miltiorrhiza on thioacetamide-induced liver fibrosis and preneoplastic liver in rats

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Backgrounds:

To investigate the effects of Salvia miltiorrhiza (Danshen) on liver fibrosis and preneoplastic liver induced by thioacetamide (TAA) in rats.

Materials and Methods:

Hepatic fibrosis and preneoplastic liver were induced by TAA treatments three times a week for 10 weeks. The maleWistar rats with TAA treatments were separated into groups randomly and administrated with H2O and Danshen (100 and 300 mg/kg) via gastrogavage during the experiment period. After 10 weeks, macroscopic features of the liver and weight ratio of spleen to body were measured. Liver fibrosis of the rats was evaluated by Sirius Red staining. Activities of serum aminotransferase and γ-glutamyl transpeptidase (γ-GT) were measured using clinical jits and a spectrophotometric system. The protein expression of α-smooth muscle actin (α-SMA) was detected by Western blotting analysis. Liver sections were subjected to immunohistochemistry for glutathione transferase-P.

Results:

The rats that received the Danshen treatment had significantly reduced plasma aminotransferase andγ-GT activities, relative spleen weights, and hepatic hydroxyproline contents. A histological examination also confirmed that Danshen reduced the degree of fibrosis caused by TAA treatment. Western blot analysis showed that Danshen treatment reduced protein expression of α-SMA. An immunohistochemical examination also indicated Danshen reduced the biomarker for preneoplastic liver, such as glutathione transferase-P expression.

Conclusion:

Danshen could inhibit TAA-induced liver fibrosis and hepatoma in rats.

P019

Association of Ulcerative Colitis and Diabetes Mellitus: Analysis of 2005-2010 Nationwide Health Insurance Database

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Backgrounds:

Intracellular signaling pathways activated by inflammatory responses were associated insulin resistance. The correlation of diabetes mellitus (DM) with gastrointestinal diseases is not known. The aim of this study was to investigate the association of DM and ulcerative colitis (UC).

Materials and methods:

UC was identified by using a catastrophic illness certificate. Patients with DM were classified as having diabetes if they had at least one diabetes admission code or 3 outpatient codes within 365 calendar days. UC patients were divided into three categories: mild, moderate and severe. Prevalence of UC and health insurance burden were compared between the groups.

Results:

The prevalence of DM was 69/1,180 to 113/1,961 (5.85-5.76%) in those with UC, and 972,001/22,314,647 to 1,282,961/23,074,487 (4.36-5.56%) in controls in year 2005 to 2010 (OR 1.294, 1.100–1.522, p<0.01). Since moderate to severe symptoms usually require aggressive medication to control inflammation, it's it possible that mediation may also prevention of its associated with DM. The prevalence of DM in mild UC patients was 58/940 to 94/1,535 (5.81-5.776%), and 11/171 to 19/315 (6.04-5.72%) in moderate to severe UC patients in year 2005 to 2010 (OR 0.908, 0.643–1.285, p=0.606). Although there are no significant difference in DM prevalence rate between mild and moderate to severe UC patients, the medication costs were increased according to disease severity and DM comobility.

Conclusion:

DM is more common in UC than the general population although the mechanism is uncertain but may be related to inflammation. Medication costs were adversely affected by UC disease severity and DM comobility.

P020

Endothelin-1 promotes VEGF-dependent angiogenesis and MMP-13-dependent migration in human chondrosarcoma

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Background:

Chondrosarcoma is the second most common sarcoma in bone malignancy and characterized by a high metastatic potential. Endothelin-1 (ET-1) has been implicated in tumor angiogenesis and metastasis. However, the relationship of ET-1 on vascular endothelial growth factor (VEGF)-dependent angiogenesis and matrix metalloproteinase (MMP)-13-denpentent migration in human chondrosarcoma (JJ012 cells) is mostly unknown.

Materials and Methods:

We examined the effect of ET-1 in chondrosarcoma cell with multiple inhibitors and siRNA to confirm the result in vitro. On the other hand, in vivo result measured by lung metastasis, tumor xenograft study and Matrigel plug assay.

Result:

We found that the ET-1 and VEGF expression was correlated with tumor stage and significantly higher than that in normal cartilage. Exogenous ET-1 with JJ012 cells promoted VEGF expression and angiogenesis through ETAR, integrin-linked kinase (ILK), Akt, and hypoxia-inducible factor-1 alpha (HIF-1 α) signaling cascades. We also found the ET-1 increased MMP-13 expression and migration through focal adhesion kinase (FAK), phosphatidylinositol 3-kinase (PI3K), Akt, mammalian target of rapamycin (mTOR), and NF-κB signaling cascades. On the other hand, knockdown ET-1 decreased VEGF and MMP-13 expression and abolished angiogenesis and metastasis *in vitro* as well as in vivo. In addition, using xenograft tumor angiogenesis model, knockdown ET-1 significantly reduced tumor growth and tumor-associated angiogenesis.

Conclusion:

Taken together, these results indicate that ET-1 promotes VEGF and MMP-13 expression and contributing the angiogenesis, metastasis, and tumor growth of human chondrosarcoma cells.

P021

Naringenin Suppresses Neuroinflammatory Responses Through Inducing Suppressor of Cytokine Signaling 3 in Microglia

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Neuroinflammation is closely associated with the pathogenesis of neurological disorders. The hallmark of neuroinflammation is considered to be microglial activation. Suppressor of cytokine signaling (SOCS) proteins play a crucial role in regulating cytokine signaling and inflammatory gene expression. Naringenin, a flavonoid found in grapefruits, has been reported to have antioxidant, anti-inflammatory and antitumor activity. However, the effect of naringenin on neuroinflammatory responses in microglia is poorly understood. This study shows that naringenin significantly inhibits LPS-induced nitric oxide synthase (iNOS), cyclo-oxygenase-2 (COX-2) expression as well as nitric oxide (NO) production in microglial cells. On the other hand, naringenin up-regulates SOCS3 protein expression in a concentration-dependent manner. Transfection with siRNA against SOCS3 reversed the inhibitory effects of naringenin in microglia. Moreover, naringenin also induced PKCδ (Thr505) and AMPK (Thr172) phosphorylation in a time-dependent manner. Treatment with AMPK and PKCδ inhibitors reduced naringenin-enhanced SOCS3 expression. Moreover, the inhibitory effect of naringenin on LPS-induced NO production was also abolished by AMPK and PKCδ inhibitors. These results indicate that naringenin activates AMPK and PKCδ signaling pathways, resulting in enhanced SOCS3 expression, which is involved in anti-neuroinflammation.

P022

Antitumor agent ZYX-1, a Novel 4β-[(4"-benzamido)-amino]-4'-O-demethyl-epipodophyllotoxin Derivative, Induces Cell Apoptosis through Topoisomerase II Inhibition in Human Renal Carcinoma Cells

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Backgrounds:

ZYX-1 is a novel 4β-[(4"-benzamido)-amino]-4'-O-demethyl-epipodophyllotoxin derivative. Previous research has shown that ZYX-1 has anticancer activity in KB (nasopharyngeal) and KB-7d (the etoposide-resistant) cells.

Materials and Methods:

MTT assay, In situ labeling of apoptotic cells, Cell death detection assay, Western blot analysis, Flow cytometry analysis, Comet assay, Topoisomerase II relaxation assay, Band depletion assay, In vivo complex of enzyme (ICE) assay, Small interfering RNA transfection.

Results:

ZYX-1 exhibited potent and highly selective activity in the human renal carcinoma cells A498, with an IC50 value of 2.38 μM by MTT assay, more potent than VP-16. The molecular mechanism of ZYX-1-induced cell cycle G1 arrest was via DNA double-strand breaks in the A498 cells. ZYX-1 induced phosphorylation of ataxia telangiectasia mutated (ATM) protein kinase at Ser1981, which led to the activation of DNA damage signaling pathways including Chk2, Histone H2AX and p53/p21. To detect upstream signals, the data of *in vivo* complex of enzyme (ICE) assay showed that ZYX-1 acted on and stabilized the topoisomerase II-DNA complex, leading to the formation of ZYX-1-trapped cleavage complexes (TOP2cc). In addition, ZYX-1-induced DNA damage signaling and apoptotic death were reversed by knockdown of topo IIα or topo IIβ.

Conclusion:

ZYX-1 appears to be a novel DNA damaging agent that displays siginificant anticancer activity.

P023

WLV-1 Attenuates Nociception and Spinal Neuroinflammation in Streptozotocin-Induced Diabetic Rats

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Backgrounds:

Diabetes mellitus is a metabolic disease that can have long-term complications, including diabetic peripheral neuropathy (DPN). We had found, through preliminary screening, that WLV-1, a marine-derived compound, has potential *in vitro* anti-inflammatory effects.

Materials & Methods: Results

In the present study, we investigated the antinociceptive effects on intrathecal (i.t.) and oral administration of WLV-1 in streptozotocin (STZ)-induced diabetic neuropathic rats.

Results:

First, we observed that a single i.t. administration of WLV-1 results in dose-dependent antinociceptive effects in diabetic rats. Moreover, ziconotide, a positive control, caused serious side effects at analgesic doses in diabetic rats, whereas WLV-1 did not. Immunohistochemistry analyses showed that i.t. WLV-1 significantly attenuated STZ-induced activation of microglia and astrocytes, as well as the upregulation of inflammatory mediator interleukin-1β (IL-1β), on the lumbar superficial dorsal horn. In addition, we observed the upregulation of the anti-inflammatory cytokine transforming growth factor-β1 (TGF-β1) in the lumbar superficial dorsal horn of diabetic rats after i.t. administration of WLV-1. Next, we utilized oral administration of WLV-1 to further characterize its systemic effect on neuropathic pain in diabetic rats. We found that oral administration of WLV-1 and gabapentin had antinociceptive effects on STZ-induced pain behaviors, without changes in blood sugar levels. The activation of spinal microglia and astrocytes, as well as the upregulation of IL-1β, in STZ-rats were significantly suppressed by oral WLV-1.

Conclusion:

In conclusion, we have demonstrated the antinociceptive ability of central and systemic WLV-1, which is probably due to its anti-neuroinflammatory effects. Therefore, our findings indicate that WLV-1 is a potential candidate compound for drug development to treat neuropathic pain in patients with diabetes.

P024

Mechanism of antiarrhythmic effects by (-)-epicatechin-3-gallate in cultured cardiomyocytes

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(-)-Epicatechin-3-gallate (ECG), a green tea polyphenol, has been proposed to improve the cardiac contractility. The therapeutic potential of ECG on treatment of arrhythmia remains uncertain. We investigated the direct effects of ECG on the modulations of ion currents and cellular excitability in the primary culture of neonatal rat ventricular myocytes for analysis of hypertrophic myocardial arrhythmias. By using the patch-clamp configurations, we found ECG enhanced the slow component of voltage-gated sodium current (I_{Na}) inactivation in a concentration-dependent manner (0.1-100 μM) with an EC50 value of 3.8 μM. ECG not merely shifted the I-V relationship of peak I_{Na} to hyperpolarizing direction but also accelerated INa recovery kinetics. Working at a concentration level of I_{Na} enhancement, ECG has no notable effect on both voltage-gated K^+ current and L-type Ca^{2+} current. ECG increased the frequency of normal sAP about twofold without any proarrhythmic risk. Interestingly, the bradycardia-dependent EAD could be significantly restored by ECG in fast firing rate to normal sAP waveform. Our results reveal how ECG, the novel I_{Na} agonist, may act as a promising candidate in clinical applications on cardiac arrhythmias.

P025

MJ-66, a quinazolinone analog, induces malignant glioma cell death through DNA damage and mitotic catastrophe

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Backgrounds:

Malignant gliomas are the most common primary brain tumor in adults and are usually growing rapidly and life-threatening. Despite recent advances in treatment of malignant gliomas, the prognosis of patients remains very poor. In the previous study, our lab found that 2-(Naphthalene-1-yl)-6- pyrrolidinyl-4-quinazolinone (MJ-66) significantly caused glioma cell death through inducing G2/M arrest and mitotic catastrophe. The purpose of this study is to investigate how MJ-66 induced malignant glioma cell death through mitotic catastrophe.

Materials and Methods:

Human glioma U87 cells treated with MJ-66 were collected for indicated times. The mechanism of action was measured by western blotting and immunofluorescence staining. Inhibition of proliferation was evaluated by WST-1 assay and Trypan blue exclusion assay. Therapeutic effects of MJ-66 were measured in the intracranial glioma xenograft animal model after intraperitoneal or oral administration for 10 days.

Results:

We found that MJ-66 caused DNA damage through activating gH2AX in glioma cells. Subsequently, MJ-66 interfered with G2/M DNA damage checkpoint through increasing the level of p-Chk1 and p-Cdc25C. Moreover, we used a pharmacological combination to investigate the effects of MJ-66 and minocycline in gliomas. We demonstrated that the combination of MJ-66 and minocycline had additive effects to inhibit glioma cell growth and reduce cell viability. Finally, intracranial glioma xenograft animal model showed that MJ-66 inhibited tumor growth in vivo.

Conclusion:

These results suggest that MJ-66 induces malignant glioma cell death through DNA damage and mitotic catastrophe. These findings also provide the evidence that MJ-66 has antineoplastic effects in the animal model which may be developed as a new potent drug against malignant gliomas.

P026

The Effect of Amitriptyline on the Leptin-induced Cell Proliferaion in Rat Aortic Smooth Muscle Cell

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Backgrounds:

Atherosclerosis is one of the cardiovascular diseases. The control events of atherosclerosis include endothelial dysfunction, inflammation, vascular smooth muscle cells proliferation and matrix alteration. The proliferation of vascular smooth muscle cells (VSMCs) plays a key role in the development of atherosclerosis. Leptin exerts many potentially atherogenic effects such as induction of endothelial dysfunction, stimulation of inflammatory reaction, migration, hypertrophy and proliferation of vascular smooth muscle cells. According to literature, Amitriptyline is an antidepressant drug. There are studies, Amitriptyline inhibit platelet aggregation, the impact of the combination with endothelial cells and smooth muscle cells. Therefore, the aim of this study was to investigate whether Amitriptyline can inhibit the VSMC proliferation induced by leptin and the possible molecular mechanism of its action.

Materials and Methods:

Rat aortic smooth muscle cells (A10 cells) were serum-starved and subsequently treated with leptin at increasing concentrations 0.06, 0.6, or 6 nM (1, 10, and 100ng/ml) for 72h. In transwell migration assay, the A10 cells were seeded into the insert and move through the pores of the membrane at the bottom of the insert in a dose-dependent.

Results:

Leptin significantly induced A10 cells proliferation. Pretreatment with Amitriptyline (1 μM) significantly decreased the cell proliferation induced by leptin in A10 cells. It is well know that p44/42 MAPK activation plays an important role in cell proliferation. Thus, the contribution of p44/42 MAPK activation to VSMCs proliferation induced by leptin was investigated. The expression of phosphorylated p44/42 MAPK was significantly increased by leptin in A10 cell in a concentration-dependent manner, and it was inhibited by the pre-treatment of Amitriptyline (1 μM). The expression of cyclin D1, was a key cell-cycle signaling protein in cell-cycle progression, was significantly elevated by leptin in A10 cells. Meanwhile, the expression of p21, was a potent cyclin-dependent kinase inhibitor, was decrease by leptin in A10 cells. These effects of lepin were all reversed by the pretreatment of Amitriptyline.

Conclusion:

The beneficial effect of Amitriptyline on leptin-induced cell proliferation may result from its inhibition of cyclin D1 expression and p44/42 MAPK phosphorylation and the increased of $p21^{cip1}$ expression. Amitriptyline may thus prove a potential agent against atherosclerosis in obesity.

P027

The Interaction between c-Met and Notch1 Signal Pathways in Gastric Cancer SC-M1 Cells

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Backgrounds:

In Taiwan, gastric cancer ranked number fifth in total cancer mortality in 2010. Hepatocyte growth factor (HGF) and its receptor c-Met played an important role in gastric cancer progression and metastasis. In addition, we found a positive correlation between the expression of c-Met and Jagged1 in gastric cancer. Our recent study revealed that expression of Notch1 ligand Jagged1 was related to poor prognosis of gastric cancer patients. Interestingly, the recent studies showed that both HGF/c-Met and Notch1 signaling triggered the cyclooxygenase-2 (COX-2) promoter and elevated the expression of COX-2 protein. The objective of this study was to examine the possible interaction between HGF/c-Met and Jagged1/Notch1 signaling pathways in gastric cancer cells.

Materials and Methods:

The SC-M1 gastric cancer cells were transfected with pcDNA or HAN1IC (Notch1 intracellular domain). SC-M1/pcDNA and SC-M1/HAN1IC cells were treated with/without HGF and/or NS398 (COX-2 inhibitor) and their migration ability was examined using transwell assay. The protein expression of p-Met, c-Met, Jagged1, Notch1, COX-2, p-Src, Src, p-Erk, Erk and HES-1 were investigated by Western blotting after treatment of cells with or without HGF.

Results:

Results from transwell assay showed that the migration ability of SC-M1/HAN1IC cells was higher than SC-M1/pcDNA cells. Treatment of SC-M1/pcDNA cells with HGF at 24 hours increased the migration ability. However, no change in the migration ability of SC-M1/HAN1IC cells was found after treatment with HGF. In addition, the Western blotting showed that COX-2 expression of SC-M1/pcDNA cells and SC-M1/HAN1IC cells was increased after treatment with HGF for 24 hours. However, the migration ability of SC-M1/pcDNA cells and SC-M1/HAN1IC cells with/without HGF were inhibited by NS398 treatment. These results implied that COX-2 might play an important role on the migration ability of SC-M1/pcDNA cells and SC-M1/HAN1IC cells. Furthermore, the Western blotting showed that the p-Met expression was increased at 1 hour after treatment of SC-M1/pcDNA cells with HGF. The expression of c-Met in SC-M1/pcDNA cells was higher than that of SC-M1/HAN1IC cells. The HGF induced the downstream protein p-Erk expression was biphasic in the SC-M1/pcDNA and SC-M1/HAN1IC cells. On the other hand, the c-Met downstream protein p-Src was increased in a time-dependent manner after treatment of the SC-M1/pcDNA and SC-M1/HAN1IC cells with HGF. These results showed that HGF activated the c-Met signaling transduction and leads to COX-2 activation. The Notch1 pathway was indirectly activated by c-Met signaling pathway, because the Jagged1 and the Notch1 downstream protein Hes-1 expression were increased after HGF treatment of the SC-M1/pcDNA cells.

Conclusion:

We conclude that cross interaction between c-Met receptor and Jagged1/Notch1 signaling pathway are important in regulation of migration of gastric cancer cells.

P028

Mechanisms of Diastolic Dysfunction in Early-stage Chronic Kidney Disease

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Backgrounds:

Chronic kidney disease (CKD) is associated with increased cardiovascular morbidity and mortality. Although evidence suggests that cardiac remodeling occurs in early CKD, the underlying molecular mechanisms are poorly understood. Therefore, we examined structural and functional cardiac changes in a rat model of early-stage CKD induced by single nephrectomy (SNx).

Materials and Methods:

Adult male Sprague Dawley rats were randomly separated into the SNx or the sham group. All rats were anesthetized with 2% isoflurane and supported by a rodent ventilator. For single nephrectomy, the left renal artery and vein were ligated with 4/0 silk suture, and the left kidney was removed without bleeding. After eight weeks, both Doppler echocardiography and invasive catheter-mediated pressure-volume loop analysis were used to assay diastolic function of rat hearts. Electrophysiologic remodeling was examined by patch clamp technique and Fura-2 based calcium transient measurement. Immunoprecipitate and blotting of SERCA2a were used to determine the nitrosylation modification and cellular remodeling. Finally, low-density lipoprotein (LDL) isolated from animal plasma was analyzed and compared.

Results:

Doppler echocardiography and pressure-volume relationship analyses in SNx and sham rats revealed impaired diastolic function in SNx rats. Compared to isolated cardiomyocytes of sham rats, those of SNx rats had greater intracellular Ca^{2+} transients and longer decay times that were mediated by the combined effects of transient outward potassium currents, reduction-induced action potential prolongation, and SERCA2a nitrosylation. The mitochondria permeability transition pores of cardiomyocytes from SNx rats but not sham rats were oversensitive to Ca^{2+}, indicating mitochondrial dysfunction and adenosine triphosphate depletion. Furthermore, the apolipoprotein content of LDL was different between SNx and sham rats, and the LDL of SNx rats but not sham rats induced apoptosis through the lectin-like oxidized LDL receptor, increased inducible nitric oxide synthase protein levels, and increased calcium transient decay time in rat cardiomyocytes.

Conclusion:

In conclusion, we have shown that mild renal insufficiency in rats induced by SNx causes diastolic dysfunction of the heart. The operating mechanisms include both electrophysiologic and cellular remodeling, which may be driven by the altered LDL composition seen in early-stage CKD.

P029
Melanogenesis inhibitor(s) from Phyla nodiflora extract

李江文

Overexpression of tyrosinase can cause excessive production of melanin and lead to hyperpigmentation disorders, including melasma and freckles. Recently, agents obtained from plants are being used as alternative medicines to down-regulate tyrosinase synthesis and decrease melanin production. We used the extract of *Phyla nodiflora* Greene (Verbenaceae), a plant used in Taiwanese folk medicine for treating and preventing inflammatory diseases such as hepatitis and dermatitis. However, the antimelanogenesis activity of and molecular biological mechanism underlying the activity of the methanolic extract of P. *nodiflora* (PNM) has not been investigated to date. Our results showed that PNM treatment was not cytotoxic and significantly reduced the cellular melanin content and tyrosinase activity in a dose-dependent manner ($P < 0.05$). Further, PNM exhibited a significant antimelanogenesis effect ($P < 0.05$) by reducing the levels of phospho-cAMP response element-binding protein and microphthalmia-associated transcription factor (MITF); inhibiting the synthesis of tyrosinase, tyrosinase-related protein-1 (TRP-1), and TRP-2; and decreasing the cellular melanin content. Moreover, PNM significantly activated the phosphorylation of mitogen-activated protein kinases, including phospho-extracellular signal-regulated kinase, c-Jun N-terminal kinase, and phospho-p38, and inhibited the synthesis of MITF, thus decreasing melanogenesis. These properties suggest that PNM could be used as a clinical and cosmetic skin-whitening agent to cure and/or prevent hyperpigmentation.

P030
Allodynia Reduction Ability for a Single Compound ABL-006

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Background:

ABL-006 was purified from an traditional multi-purpose herb. It is reported anti-inflammatory and anti-oxidation. Astrocytic inflammatory reaction were reported to mediate pathologic pain. We have previously discovered that ABL-006 inhibit astrocytic inflammatory reactions.

Purpose:.

To test if ABL alleviate allodynia caused by pathologic pain.

Method:

Sciatic nerves of mice were transected on the right hindlimb to generate central pathological pain. The sensation of pain was assessed on 3rd, 7th and 14th day in terms of mechanical allodynia by von frey tests. The mice hind paw withdrawal threshold and withdraw rate are used for indication of allodynia. Experiment groups are as follows: 1. Normal: non-operated, not ABL-006 treated. 2. OP-saline: operated and treated with saline. 3. OP-006: operated and treated with ABL-006 (5 mg/kg animal weight) everyday following operation.

Results:

The withdrawl thresholds of Op-006 group were significantly increased compared to OP-saline group. Op-006 group showed lower withdrawl rate than Op-saline group in two different weight scales (0.02, 0.07g). Immunoreactivity for IL-1 in OP-006 group is significantly less than Op-saline group.

Conclusion:

The data suggested ABL-006 alleviate allodynia, partly by down-regulating inflammatory reactions in CNS.

P031
Effect of aciculatin on adipogenesis and lipolysis: study the mechanisms of action

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Background :

Obesity was mainly due to the excessive fat tissue in the body to increase food intake and energy expenditure are out of balance, and potentially also cause health hazards, which led to the occurrence of a number of diseases such as type II diabetes, dyslipidemia, hypertension and coronary heart diseases and metabolic abnormalities. The present study was performed to elucidate the mechanism of action of aciculatin, one of the main components of *Chrysopogon aciculatus*, with special attention to the adipocytes as the tissue primarily involved in the pathology of metabolic diseases.

Materials and Methods:

Mouse-derived adipocyte precursor 3T3-L1 cells were treated with differentiation inducers in the presence or absence of aciculatin, respectively. Adipogenesis was measured by oil red o staining and triacylglycerol accumulation. Immunoblot analysis was also performed to analyze the expression of differentiation markers (C/EBP β, C/EBP δ , C/EBP α and PPARγ).

Results:

Concurrent administration of aciculatin and differentiation inducers resulted in a significant inhibition of differentiation into mature adipocytes. Aciculatin also exhibited significant inhibitory action on the protein expression of PPARγ and C/EBP, and caused a reduction in the triacylglycerol accumulation. Further, aciculatin increased the basal lipolysis as indicated by the release of glycerol into the culture medium.

Conclusion:

This study revealed that aciculatin can prevent the differentiatioin of preadipocyte and stimulate basal lipolysis of mature adipocytes, avoiding the accumulation of lipid.

P032
The effect of minocycline on organ function and survival rate after heat stroke in rats

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Backgrounds:

Heat stroke (HS) is a life-threatening illness that is characterized clinically by central nervous system dysfunction, including delirium, seizures, or coma and severe hyperthermia. Minocycline is a tetracycline antibiotic with demonstrated properties against reperfusion injury including its anti-inflammatory, anti-apoptotic, and blood-brain barrier-protecting actions.

Materials and Methods:

Rats were divided into four groups: (1) Sham (2) Sham + Minocycline (10 mg/kg), (3) HS (rats in the heating-chamber of 42 ℃ for 58 min), (4) HS + Minocycline (10 mg/kg). The mean arterial pressure (MAP), heart rate, and rectal temperature (Tco) were measured. Whole blood samples were collected for the measurements of platelet count. Plasma levels of LDH, GOT, GPT, BUN, creatinine, creatine phosphate kinase, IL-1β, and TNF-α were also determined. At the end of experiment, hypothalamus, medulla, liver and kidney were collected for the measurement of inflammation-related protein expression levels. Survival rate were calculated 6 h after HS.

Results:

Our preliminary results showed that the MAP and heart rate in the HS group were significantly lower than those of the minocycline-treated group. The Tco and survival rate of HS rats pretreated with minocycline were significantly higher than those of HS group. The plasma levels of LDH, GOT, GPT, BUN, creatinine, creatine phosphate kinase also markedly reduced in the minocycline-tread group when compared with the HS group.

Conclusion:

Minocycline reduced the injuries and increased the survival rate of rats after HS. It showed promising to be a potential agent for treatment of HS.

P033

The H3K4 methyltransferase component Ash2l interacts with Oct4 and required for Oct4 downstream gene activation.

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Backgrounds:

Generation of induced pluripotent stem cell (iPSC) from somatic cells offers tremendous potential for therapeutics, the study of disease states, and elucidation of developmental processes. iPSC production study delivery the stemness genes that are capable to initiate the reprogramming, into a somatic cell to induce pluripotent cellular properties that closely resemble those of ES cells. Overexpression of stemness genes, such as Oct4, Sox2 and Nanog, were contributed to the promotion of pluripotency. Chromatin-state regulation was also reported as a fundamental mechanism contributed in stem cell pluripotency, differentiation, and the establishment of cell type-specific gene expression profiles. Transcriptional and epigenetic mechanism contribute to pluripotency and govern the biological behavior in stem cell. Both the expression of stemness gene and chromatin-state regulation are important in the pluripotency, however, the interaction between stemness associated transcription factors and chromatin-modifying protein are still unclear.

Materials and Methods:

Ash2l, a core subunit of the histone 3 lysine 4 (H3K4) methyltransferase (HMT) complex which is essential for H3K4 methylation and mouse embryonic development. H3K4-trimethylation by the HMT of proteins contributes to loose chromatin, whereby allows gene expression. Chromatin regulation is a fundamental mechanism underlying stem cell pluripotency, differentiation, and the establishment of cell type-specific gene expression profiles. Ash2l is required for ES cell pluripotency and the global, active chromatin modifications associated with ES cells. Therefore we sought to test the role of Ash2l about transcriptional and epigenetic changes during stem cell and cellular reprogramming by knockdown it.

Results:

We demonstrated that Ash2l, an ‘‘effector’’ of H3K4 methylation, interacted with the pluripotency transcription factor Oct4 by co-immunoprecipitation. Next, we found out this interaction may activated Oct4-target downstream genes expression by luciferase reporter assay. In the other hand, suppression of Ash2l in iPSC by shRNA, notably suppressed some of Oct4-target downstream genes expression.

Conclusion:

Overall we propose that the Ash2l-Oct4 partnership enhances Oct4 expression through transcriptional activation of its promoter and activated downstream genes expression. These results imply that Ash2l has essential functions as a stem cell co-activator for maintenance of ES cell pluripotency.

P034

Anticancer Activity of The Novel Histone Deacetylase Inhibitor, MPT0G028, in Human Colorectal Cancer Cells *in vitro* and *in vivo*

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Backgrounds:

In the past few decades, cancer has been the top of ten leading causes of death in economically developed countries and the second leading cause of death in developing countries. Moreover, colorectal cancer (CRC) has been the third leading cause of death in Taiwan. It has been reported that overexpression of histone deacetylases (HDACs) were observed in many types of cancers, including CRC. Therefore, small molecules targeting HDACs may have benefits in developing new anticancer drugs for CRC patients. In the present study, we investigated the anticancer activity of a novel synthetic HDAC inhibitor, MPT0G028, in human colorectal cancer cells *in vivo* and *in vitro*.

Materials and methods:

Anticancer activity was evaluated by SRB (Sulforhodamine B) and MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay in HCT-116 and HT-29 colorectal cancer cell lines. Cell cycle distribution was accessed by flow cytometry and protein expression was examined by Western blotting. HDAC activities were measured by fluorogenic HDAC assay kit. HCT-116 xenograft model was used to examine the antitumor activity *in vivo*.

Results:

First, MPT0G028 showed a better anti-proliferation effect than Vorinostat in human CRC cell lines. MPT0G028 exhibited significant inhibitory effects against class I HDACs by using *in vitro* biochemical assay and repressed total HDAC enzymatic activity in HCT-116 cells. MPT0G028 induced global acetylation on histone H3 and to a lesser extent on α-tubulin. Moreover, MPT0G028 can induce apoptotic cell death in a time- and concentration-dependent manner as evidenced by flow cytometry and Western blotting. The data also showed that the repression of Wnt/β-catenin pathway might involve in MPT0G028-induced apoptosis. Finally, MPT0G028 has a potent inhibitory effect on the tumor growth in the HCT-116 xenograft model.

Conclusion:

Based on the observed results, MPT0G028 is a novel HDAC inhibitor with a better potency than the FDA-approved HDAC inhibitor, Vorinostat, and induces dramatic apoptosis in CRC cells. We hope that this study will have contributions to the new drug development and clinical benefits for CRC patients.

P035

Perivascular Adipose Tissue Inhibits Thoracic Aorta Endothelial Function through cav-1-dependent Inhibition of Nitric Oxide Production

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Backgrounds:

Perivascular adipose tissue (PVAT) has been proposed as a cause of atherosclerosis. The location of PVAT is in association with the artery wall, which enables diffusion of adipokines (e.g. superoxide anion, angiotensin II (Ang II)). The adipokines are capable of inducing endothelial dysfunction in vessels. Caveolin-1 (Cav-1) is present in most of cells involved in the development of atherosclerosis. Cav-1 inhibits nitric oxide (NO) production by occupying the calcium/calmodulin binding site of endothelial NO synthase. The inhibition of NO production is a characteristic of endothelial dysfunction.

Materials and Methods:

Adult male Wistar rats weighing 265 to 305 g were euthanatized by intravenous injection of sodium pentobarbital. Thoracic aortas were isolated, dissected without the PVAT, divided into endothelium-intact (+E) and endothelium-denuded (-E) groups, and mounted in organ bath containing a Krebs’ solution bubbled with 95% O2 and 5% CO_2 at 37 ℃ . PVAT was incubated in Krebs’ solution for 30 minutes and transferred to organ bath before the isometric tension studies. The remaining aortas were frozen for Western blotting. The dose-response curve of phenylephrine, acetylcholine, and sodium nitroprusside were performed to examine the vascular reactivity and endothelial function.

Results:

The vasocontractility effect of PVAT was endothelial NO-dependent. Inhibition of NO level by PVAT does not via reduction of eNOS and influence of phosphorylate sites on eNOS, but via cav-1-dependent pathway.

Conclusion:

We found that the unknown factor(s) released from PVAT increased cav-1 protein expression and induced a vasocontractile effect via inhibition of endothelial NO production in rat thoracic aorta.

P036

Investigation on the Pharmacokinetics of Rivaroxaban in Rats by High-performance Liquid Chromatography with Ultraviolet Detection

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Backgrounds:

Rivaroxaban is an oral, direct, specific inhibitor of factor Xa, which is indicated for the prophylaxis of deep vein thrombosis after major orthopaedic surgery of the lower limbs. Being newly launched, the pharmacokinetic information of rivaroxaban is limited. For pharmacokinetic studies, a suitable sensitive method that allows an accurate measurement of rivaroxaban in biological matrices is needed.

Materials and Methods:

The UV spectra of rivaroxaban and the effects of pH on its UV absorbance were measured to identify the optimal detection wavelength for developing HPLC assay. The selection of stationary phase and the composition of mobile phase were also optimized to achieve adequate retention and resolution for rivaroxaban and the internal standard. Plasma samples (50 μL) were deproteined by acetonitrile. The supernatant was evaporated to dryness and the resulting residue reconstituted with 150 μL of acetonitrile-50 mM phosphate buffer solution. An aliquot of the solution was injected onto the column for HPLC analysis.

Results:

Chromatographic separation was achieved on a C_{18} column by isocratic elution with the mobile phase of acetonitrile-water containing 50 mM phosphate buffer, at a flow rate of 1 mL/min. The eluants were measured by UV detection at 300 nm. The retention time was about 12 min for rivaroxaban. No endogenous substances were found to interfere. Calibration curves were linear from 10 to 10000 ng/mL. The lower limit of quantification was 10 ng/mL. The accuracy, precision, specificity, recovery and stability of this assay have also been validated.

Conclusion:

A simple and sensitive HPLC method was successfully developed to analyze rivaroxaban. The method is suitable in pharmacokinetic investigation and monitoring rivaroxaban concentration.

P037

IL-18-induced interaction of IMP3 and HuR contributes to COX-2 expression through a post-transcriptional regulation in leukemia

汪含穎

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Backgrounds:

COX-2 has been suggested to participate in antiapoptosis and anticancer drug resistance of leukemia. A recent study showed that IL-18 can induce COX-2 expression in leukemia. However, the regulation and consequence of IL-18-induced COX-2 transcription remains uninvestigated.

Material and methods:

Two acute myeloid leukemia cell lines U937 and THP-1 were used to assess the IL-18-induced COX-2 transcription. The mRNA stability assay, COX-2 and COX-2 3'UTR reporters were used to determine whether IL-18 can induce COX-2 transcription through a posttranscriptional regulation. The immunoprecipitation assay, in vivo RNA binding assay and COX-2 3'UTR reporters were used to assess the effects of IL-18 and IL-18-regualted interaction of RNA binding proteins HuR and IMP-3. Signaling inhibitors including SP600126 (a JNK inhibitor), SB203580 (a p38 inhibitor) and PD98059 (a MEK1/ERK1/2 inhibitor) were used to determine the IL-18-induced nucleocytoplasmic shuttle of HuR and IMP-3 and consequent effect on IL-18-induced activity of COX-2 3'UTR reporters.

Results:

IL-18 contributed to antiapoptosis, but not proliferation, of U937 and THP-1 cells. IL-18 can induce COX-2 expression through a posttranscriptional regulation in U937 cells, especially in PMA and Vitamin D3-differentiated U937 cells. IL-18 induced the shuttle of nuclear HuR and IMP-3 to cytoplasm that further contributed to mediate IL-18-stabilized COX-2 mRNA by a direct binding to the 3'UTR of COX-2 mRNA. Moreover, JNK and ERK1/2 signaling pathways were involved in IL-18-induced nucleocytoplasmic shuttle of HuR and COX-3 3'UTR reporter activity.

Conclusion:

We revealed a JNK- and/or ERK1/2-regualted HuR nucleocytoplasmic shuttle in response to IL-18 and contribute the stabilization of COX-2 mRNA that could be a therapeutic target for the therapy of leukemia. In addition, this novel discovery provides a speculation that IL-18-induced COX-2 mRNA stability may also contribute to the differentiation-related anticancer drug resistance in leukemia

P038

Effects of Oil Extracted from Antrodia Cinnamonea Fruiting Bodies on 3T3-L1 Preadipocyte Differentiation and Adipogenesis

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Backgrounds:

Adipogenesis is the process of cell differentiation by which preadipocytes become adipocytes. This study investigated the effects of oil extracted from Antrodia cinnamonea fruiting bodies (OE) on 3T3-L1 preadipocyte during insulin-induced adipogenesis and whereby the mechanism.

Materials and Methods:

The 3T3-L1 preadipocytes were induced to differentiate with 10% FBS/DMEM and a hormonal cocktail for 48 hr at two days post-confluence (designed as Day 0), and then next allowed to maturate for 14 days in the 10% FBS/DMEM with 10 ug/ml insulin in the absence or presence of supplemented OE. Lipid droplets formation was assessed by oil-red O staining. Adipogenesis-related genes, including peroxisome proliferator-activated receptor gamma (PPARγ), adipocyte protein 2 (aP2), glucose transporter 4 (GLUT4), fatty acid synthase (FAS), CD36 and lipoprotein lipase (LPL) as well as transcription factors CCAAT-enhancer-binding proteins (C/EBP)-α, β and δ expression was determined by RT-PCR. The activation of PPARγ and phosphorylation of ERK and Akt was detected by western blot. The lipolysis was measured by fatty acid release.

Results:

OE dose-dependently inhibits the proliferation of undifferentiated 3T3-L1 cells. OE also dose-dependently decreases the insulin-stimulated lipid droplet accumulation, and increases fatty acids release during the preadipocyte maturation. OE decreases the mRNA expression of PPARγ, aP2, GLUT4, FAS, CD36 and LPL during insulin-induced maturation in 3T3-L1 cell. OE treatment also decreases nuclear translocation of C/EBP-α, β and δ during 3T3-L1 cell differentiation. ERK and Akt are pivotal signal mediators during insulin-induced adipogenic differentiation. We found that OE inhibits PPARγ activation, ERK and Akt phosphorylation in 3T3-L1 cells after insulin stimulation.

Conclusion:

OE inhibits adipogenic progression in 3T3-L1 cell by regulating C/EBPs, ERK and PPARγ expression.

P039

Involvement of Arc protein in transfer of learnt motor skill in the primary motor cortex

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Although repeated practice is required for better performance of motor skill, the improvement of motor skill is also benefit from previous easy task learning. Neuroimage studies of human subjects reveal that primary motor cortex plays an important role in this transfer of previously acquired motor skill. However, the neuronal substrate for the transfer of previously acquired experience is still uncertain. This study aimed to investigate, through measurement of immediate-early genes in the primary motor cortex, the neuronal substrates associated with the transfer process of two task-specific trainings: easy acrobatic and difficult acrobatic motor skill learning. Sprague-Dawley rats were trained on a runway apparatus to acquire the motor skill needed to complete the acrobatic motor skill learning. Based on the difficulties to complete the task, the training protocols were divided into easy acrobatic and difficult acrobatic tasks. Animals in 4 groups: control sedentary (C), easy (E), difficult (D), and easy + difficult training (E+D), were trained to traverse the runway for seven days. At the last day of training, animals were sacrificed and their primary motor cortices were isolated for subsequent analysis of western blot and immunohistochemistry. The result demonstrated that previously learnt easy task may provide behavioral benefit for the performance of difficult task. In addition, an effect of training protocol on the expression of activity-related cytoskeleton associated protein (Arc) in the primary motor cortex was observed. Expression of Arc was significantly increased in E group compared to C group, whereas no significant changes in Arc level in D and E+D groups. The immunohistochemical observation also showed an increase in Arc immuno-positive signals in the primary motor cortex from E group. Because of the involvement of Arc in modulating the consolidation of memory, the increased expression of Arc in the primary motor cortex after easy acrobatic motor learning reveals the possible engagement of Arc in the easy task.

P040

Mitogen-activated protein kinases p38-beta contributes to TNF-alpha resistance in oral cancer mediated BAD ser112 phosphorylation

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Backgrounds:

When oral cancer is diagnosed, the three-year survival rate prediction of this patient is only 58% and can only be increased to 74% after surgery in Taiwan. Before the squamous cell carcinoma (SCC) formed, hyperlosia is an initial stage symptom induced by EGFR over expression in a long term inflammation. Thus, tumor necrosis factor-alpha (TNF-α) releasing in inflammation lose it original anti-tumor function and a tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance might happen in mane cases. In our pervious study indicated p38β MAPK over expression in oral cancer might be associated with TRAIL resistance through serine 122 of BAD phosphorylation, and which is a gatekeeper of BAD-mediated apoptosis.

Materials and Methods:

In this research, a cell line T28 from 4-nitroquinoline-N-oxode (4-NQO) induced oral cancer in C57B mouse and human tongue squamous cell carcinoma cell line SCC4 were screened in this TNF-α resistance issue. All proteins from cell were analysis by immune blot assay.

Results:

TNF-α releasing is through p38 mitogen-activated protein kinases and TNF-α resistance exists in both T28 and SCC4 cell line. Further, the serine 136 of BAD phosphorylation was promoted by p38α MAPK isoform and the serine 122 and 155 of BAD phosphorylation were promoted by p38β MAPK and also block the apoptosis cause by TNF-α. A p38β MAPK inhibitor SB202190 (10μM) was used, the cell cycle arrested at G2 phase from 9.5% to 17.36% within 24h treatment in SCC4 cells.

Conclusion:

Over expression of p38β MAPK in oral cancer indeed caused TNF-α induced apoptosis resistance by BAD phosphorylation. And serine 122 of BAD is control by p38β MAPK. This suggests that p38β MAPK is a possible anticancer target in oral cancer therapy.

P041

The Intervention of Nitric Oxide in Oxidative Toxicity : Re-injure or Rescue Neuroglia ?

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Backgrounds:

Oxidative stress is an important factor leading to the occurrence of human neurological diseases. Besides neurons, glia itself may be also damaged by oxidative toxicity, being due to its overactivation in response to neuronal injury or inflammatory stimulation, and subsequently abundant self-secretion of superoxide anion (·O2-), hydrogen peroxide (H_2O_2), and nitric oxide (NO). The reaction of NO and ·O2- to form peroxynitrite (ONOO-) is toxic to cells. Conversely, NO has been demonstrated to be protective against reactive oxygen species (ROS) toxicity. Nevertheless, the interactions between H2O2 and NO under oxidative stress, which may promote or attenuate death of neuroglia, remains unclear.

Materials and Methods:

Primary cell cultures, including neuroglia, microglia and astrocytes, were used as model system to examine the effect of NO on oxidative toxicity. The cells, treated with different concentrations of H_2O_2, NO donor S-nitroso-N-acetyl-D, L-penicillamine (SNAP) or both, were subjected to a series of assays and then analyzed.

Results:

In neuroglial cultures H_2O_2 and SNAP respectively elicited cell death in a concentration-dependent manner. Sublytic concentrations of H_2O_2 (30 uM) plus SNAP (300 uM) were sufficient to damage mixed glia. Noteworthily, toxic H_2O_2 insult (300 uM) could be reduced by sublytic SNAP (30 or 100 uM), detected by LDH activity, MTT reduction and fluorescence staining of apoptotic nuclei. Furthermore, in rat microglia- or astrocytes-enriched cultures the additive toxicity of combinations of lower H_2O_2 and higher SNAP was observed. Dissimilarly, the inhibition of lower SNAP (30 or 100 uM) against H_2O_2 toxicity (300 uM) was occurred only in microglia-enriched, but not astrocytes-enriched cultures.

Conclusion:

NO has a concentration-dependent dual effects revealing either enhanced or slack H_2O_2 toxicity on the viability of microglia.

P042

Estrogen affects L-type Ca2+ channel expression and function in H9c2 cardiomyoblasts differentiation

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Background:

Endogenous cardiac progenitor cells are activated and recruited by cytokines which released from infarcted heart. Differentiation into mature cardiomyocytes compensates cardiomyocytes death in infarcted heart. However, not only cytokines but endocrine such as estrogen (E2) might involve in. E2 can induce myoblasts differentiation into skeletal muscle through estrogen receptor alpha-dependent pathway, but for cardiomyoblasts remain unknown. Also, E2 enhances cardiac L-type Ca^{2+} current in mature cardiomyocytes in short-term exposure through ERα; however, in cardiomyoblast after long-term exposure of E2 still unknown. The purpose of our study is whether E2 induces cardiomyoblasts differentiation into cardiomyocytes by enhancing cardiac L-type Ca^{2+} channels.

Materials and Methods:

Experimental model was H9c2 undifferentiated cardiomyoblasts in 1%FBS culture condition.

Results:

DMSO at 10nM was a negative control. RA at 10nM was a positive control. A 3-days treatment with DMSO enhanced MyoG expression, decrease L-type Ca^{2+} channel protein and current expression, after 7-days it fused into multinucleated skeletal muscle with spindle shape. Compare with DMSO at the same incubation period, RA did not affect on MyoG expression, but enhance L-type calcium protein and current expression, and maintain morphology in round shape; E2 did not affect on MyoG expression, but enhance L-type Ca^{2+} channel protein and current expression, and maintain morphology in round shape. DPN (ERβ agonist) decrease MyoG expressions, but enhance

L-type Ca^{2+} channel protein and current expression, and maintain morphology in round shape.

Conclusion:

Our study suggests that H9c2 differentiation into cardiomyocytes by enhancing cardiac L-type Ca^{2+} channel via ERβ in low serum condition.

P043

Prior Manipulation Therapy induced Primary Osteosarcoma Metastasis – From Clinical to Basic Research

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Backgrounds:

Our previous clinical study demonstrated that manipulation therapy (MT) on osteosarcoma (OS) patients prior to diagnosis resulted in poor prognosis after surgical treatment. This study was aimed to provide the evidence from clinical to basic for MT-induced metastasis in primary osterosarcoma.

Materials and Methods:

Eight-week-old male GFP-labeled human OS cells-transferred nude mice were randomly allocated into 2 groups, namely, MT (+) and MT (-) groups. MT was conducted with repeated massage on tumor site twice a week for 7 or 15 weeks. The parameters evaluated were x-ray diagnosis, micro-PET/CT scan, histopathology and serum metalloproteinase 9 (MMP9) level.

Results:

The results showed that MT (+) mice showed a decreased body weight (30.5±0.65g) and an increased tumor volume (8.3±1.18 mm3) compared to MT (-) group with body weight (35·8±0·40g, p<0·0001) and tumor volume (3.9±1.34mm3, p=0.038), respectively. There was an increased signal intensity over lymph node region of hind limb by micro-PET/CT and the GFP-labeled human OS cells were detected in the lung and bilateral lymph nodes in MT (+) group, while there were no such findings in MT (-) group. The serum MMP9 level was higher in MT (+) group (27.1±1.29 ng/ml) than in MT (-) group (17.8±1.97 ng/ml, p=0.048).

Conclusion:

Taken previous clinical observation and the present in vivo evidence together, we conclude that physicians should pay more attention on those patients who seek MT before diagnosis or during treatment for osteosarcoma.

P044

Teroxirone Eradicates Proliferation of Human Non-Small Cell Lung Cancer Cells by Modulating Tumor Suppressor p53

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In this work, we described that the proliferation of human non-small-cell-lung-cancer cells H460 and A549 cells can be inhibited by low concentrations of teroxirone *in vitro* and *in vivo*.

Teroxirone-mediated apoptosis is dependent on the status of *p53*. Transient expression of p53 activates downstream p21, cytochrome c and caspase-3. The presence of caspase-3 inhibitor reverted apoptotic phenotype. The experimental *in vivo* evidence of growth suppression was also demonstrated in xenograft tumors as established in nude mice. As a potential therapeutic agent capable of restraining cell growth by apoptotic death at low concentrations, teroxirone provided a new perspective as an alternative approach in reversing tumorigenic phenotype of human lung cancer.

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$GABA_B$ Receptor-Mediated Tonic Inhibition Is Involved in Regulation of Spontaneous Firing of Locus Coeruleus Neurons in Developing and SSRI treated Rats

王瀚穎 **Han-Ying Wang**

Purpose:

To study the regulation of $GABA_B$ receptor-mediated tonic inhibition in spontaneous firing rate (SFR) of LC neurons in developing and SSRI exposed rats.

Materials and methods:

1.Sagittal brain slices (300 μm) containing LC were prepared from postnatal day (PN) 2-4, PN8-10, and PN 14-20 Sprague-Dawley (SD) rats.

2.LC neurons were identified by IR-DIC microscopy video, and those neurons were then recorded by whole-cell patch clamp using K-gluconate, Cs-gluconate, or Cs-Cl (for locally evoking IPSC or $GABA_A$R-mediated tonic current) based internal solution. All recordings were routinely made with addition of 5 mM kynurenic acid (KA), 1 μM strychnine (STR), and 100 μM picrotoxin (PTX) in aCSF for blocking synaptic effects.

3. In all these experiments, biocytin was included in the pipette solution and filled the recorded neurons, which then could be double-stained with tyrosine-hydroxylase (TH) to verify the identity of the neurons.

Results:

Application of 20μM CGP52466, a selective $GABA_B$ receptor ($GABA_A$-R) antagonist, resulted in inward currents of PN2-4, PN8-10, and PN 14-20 rats; each averaged from 8-12 LC neurons are 4.6±0.8p_A, 9.3±0.6p_A, and 9.5±0.4pA respectively which was not induced when GIRK channels was blocked by addition of 1mM Ba^{2+} into the bath medium. In PN2-4 rats, application of 100 μM GABA or 100μM baclofen induced outward currents ($I_{GABAB-R}$) 33±2pA and 35±3pA, respectively, which is significant smaller than currents induced in PN8-10 rats ($p<0.001$). Nevertheless, there is no significant difference in currents induced in PN8-10 rats and in PN14-20 rats. Furthermore, tonic inhibition had similar developmental increase pattern as currents induced by 100μM GABA or 100μM baclofen application and SFR; it was 55±6% at PN2-4, significantly increased to 117±12% at PN8-10 ($p<0.01$), and again remained unchanged at PN14-20. Bath application of 10μM (s)-SNAP5114 plus 50μM NNC711 dramatically and significantly decreased LC neuron SFR from 0.3±0.1 Hz to 0.1±0.07 Hz ($p < 0.01$); subsequent application of CGP52466 not only reversed the effect of GABA transporter (GAT) 1 and GAT2 blockers, but also further increased discharge rate to 104±6%, 217±8% and 223±16% for PN2-4, PN8-10, and PN14-20 rats respectively. The SFRs of LC neurons recorded in slices from CTM treated male rats was 0.45±0.07 Hz, which was significant higher than control male animals ($p<0.01$). Finally, $GABA_B$-R mediated standing current and $I_{GABAB-R}$ induced by 100μM GABA or 100μM baclofen were both significantly reduced in CTM treated male but not in female rats.

Conclusion:

In our studies, $GABA_B$R-mediated tonic inhibition could modulate and maintain spontaneous firing at stable level in the LC nucleus of developing rats. Moreover, we determined that ambient GABA concentrations in LC nucleus were much similar among three developmental periods, suggesting $GABA_B$R could sense subtle change of ambient GABA concentration to modulate SFRs. Furthermore, we found GABABR-mediated tonic inhibition was retarded and caused an increased SFR in perinatal CTM treated male but not in female rats. Taking together, $GABA_B$R-mediated tonic inhibition in LC nucleus was gradually changing in development and could be attenuated by exposure of SSRI in a sexually dimorphism.

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MicroRNA-X Suppresses Expression of N-Cadherin in Non-Small Cell Lung Cancer Cell

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Backgrounds:

Metastases are the main cause of human cancer deaths. Brain metastasis appears in 25% of patients with non-small cell lung cancer (NSCLC). Despite its clinical importance, little is known about the genetic determinants of metastasis. Previously, we used microarrays to screen both genes and miRNAs involved in brain metastasis from NSCLC. Among these differentially expressed genes and miRNAs, we identified that N-cadherin (CDH2) may be regulated by some miRNA candidates. Therefore, the purpose of this study is to investigate whether CDH2 is regulated by miR-X. (Note: the full name of miR-X is not allowed to be disclosed due to confidential agreement.)

Materials and Methods:

The cancer cells from metastatic brain, Bm7, and its parental lung cancer cells, CL1-5, were used in this study. Quantitative real-time PCR were conducted to measure expression levels of mRNA and miRNA. A bioinformatics tool TargetScan was used to search the candidate miRNA binding sites within the 3' untranslated region (UTR) of CDH2. Luciferase assays were used to investigate the interaction between CDH2 and candidate miRNA.

Results:

Quantitative real-time PCR showed that CDH2 was significantly up-regulated and miR-X was significantly down-regulated in Bm7 cells. The binding sites of miR-X could be found in 3'UTR of CDH2 by in silico analysis and the binding activity was confirmed by luciferase assays. Furthermore, overexpression of miR-X in Bm7 markedly reduced the protein level of CDH2.

Conclusion:

Our result suggested that CDH2 was subject to miR-X regulation, which may provide useful information to prevent brain metastasis of l

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Green tea (−)-epigallocatechin gallate inhibits IGF-I stimulation of glucose uptake in 3T3-L1 adipocytes

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Purpose:

This study investigated the pathways involved in EGCG modulation of IGF-I-stimulated glucose uptake in 3T3-L1 adipocytes.

Materials and Methods:

Glucose uptake was assayed to measure the uptake of ^{3}H-2-deoxyglucose. Western blot analysis was performed to measure levels of Glucose transporters (GLUTs) and IGF-I signaling molecules.

Results:

EGCG inhibited IGF-I stimulation of adipocyte glucose uptake in dose- and time-dependent manners. Treatment with 20 μM EGCG for 2 h significantly decreased IGF-I-stimulated glucose uptake by 59%. The EGCG receptor [also known as the 67-kDa laminin receptor (67LR)] was discovered in fat cells. Pretreatment of adipocytes with a 67LR antibody, but not normal rabbit immunoglobulin, prevented the effects of EGCG on IGF-I-increased glucose uptake. Moreover, pretreatment with an AMP-activated protein kinase (AMPK) inhibitor, such as compound C, but not with a glutathione activator, such as *N*-acetyl-L-cysteine, blocked the anti-IGF-I effect of EGCG on adipocyte glucose uptake. Further analysis for subcellular fractions indicated that EGCG decreased the IGF-I-induced increases in the GLUT4 translocation from the cytosol to the plasma membrane and that EGCG had no effects on the level of GLUT1 translocation and total amounts of GLUT-1 and GLUT-4 proteins. In addition, EGCG suppressed the IGF-I-stimulated phosphorylation of PKCζ/λ, but not Akt or ERK, proteins.

Conclusion:

These results suggest that EGCG inhibits IGF-I stimulation of 3T3-L1 adipocyte glucose uptake through downregulated GLUT-4 translocation and IGF-I signaling and through 67LR- and AMPK-dependent pathways.

P048

Activation of Estrogen Receptor β (ERβ)-induced Breast Cancer Cells Migration through Transient Receptor Potential Vanilloid Type 1 Ion Channel (TRPV1)

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Backgrounds:

Activation of estrogen receptor β (ERβ) by 17β-estradiol (E2) plays an important role in breast cancer development. E2 induces migration in breast cancer cells by activation of ERβ. Activation of transient Receptor Potential Vanilloid type 1 Channel (TRPV1), a voltage-independent calcium channel, enhances cells migration. However, the role of TRPV1 in ERβ-induced migration of breast cancer cells remains unknown. Thus, we hypothesized that activated TRPV1 is involved in E2-induced migration in breast cancer cells.

Materials & Methods:

4T1 cells, ERα-/ERβ+ mouse breast cancer cell line, were used in this study. Western blot analysis was to measure the relative abundance of TRPV1. Migration assay was used to determine the number of migrated cells in Boyden chambers.

Results:

Western blot analysis showed the presence of TRPV1 protein in 4T1. Migration assay showed that E2 and DPN (ERβ agonist) increased the number of migrated cells. PHTPP (ERβ antagonist) inhibited the increased number of migrated cells by E2 and DPN. In addition, capsaicin (TRPV1 agonist) increased the number of migrated cells. Capsazepine (TRPV1 antagonist) inhibited the increased number of migrated cells by capsaicin. Moreover, Capsazepine inhibited the increased number of migrated cells by E2 and DPN.

P049

Slowly Infusion Rate of Doxorubicin Induced Higher Pro-inflammatory Cytokines Production in Early Phase of Treatment

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Backgrounds:

The different infusion rates of Doxorubicin have been used for treating human malignancies. However, no final conclusion about optimal infusion rate has yet been determined. In this study, we examine the effects of different infusion rate of Doxorubicin treatment in early phase.

Materials and Methods:

Thirty male Wistar–Kyoto rats were randomly divided into 3 groups, the 5 minutes, 15 minutes and 30 minutes infusion rate groups. A single dose of Doxorubicin (8.3mg/kg i.v.) was given. Blood samples were taken from femoral artery at 0, 1, 3, 6, 9, 12, 18, 24, 36 and 48 hours for blood cells counting and blood biochemistry analysis after Doxorubicin administration.

Results:

Our findings show that the liver (GOT and GPT) and renal (BUN and creatinine) injury markers had marked elevation in 30minutes group and higher than other groups, CK-MB and LDH level also significant elevated after Doxorubicin administration. The 5 minutes group had lower GOT, GPT, CK and LDH levels than other groups. Otherwise, 30 minutes group had higher TNF-α level and IL-6 level than 5 and 15 minutes groups. However, the hematologic toxicity of Doxorubicin didn't significant change between three groups.

Conclusions:

These results indicated that 30 minutes infusion rate may produce higher pro-inflammatory

cytokines than 5and 15 minutes infusion in acute phase of Doxorubicin treatment.

P050

The Role of Membrane Cholesterol in Adipokine Secretion in 3T3-L1 Adipocytes

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Backgrounds:

Obesity is caused by the disorders of body energy balance. Excess energy accumulates in the form of triglycerides in adipocytes when energy absorption is more than energy depletion, not only resulting in increased adipocyte volume, but also the aberrant secretion of adipokines, the cytokines secreted by adipocytes. Lipid raft is a membrane structure which is enriched in cholesterol. It plays an important role in cell endocytosis, secretion and signal pathway; however its role in adipokine secretion remains unknown. Moreover, hypertrophied adipocytes were characterized by decreased cholesterol level on the plasma membrane in obesity. Talking together, we hypothesize that the reduction of cholesterol in plasma membranes of adipocytes, which leads to aberrant function of lipid raft, may cause abnormal function of adipokine secretion in obesity.

Materials and Methods:

We applied MβCD (Methyl-β-cyclodextrin), a drug with high affinity for cholesterol, on differentiated 3T3-L1 adipocytes to reduce the cholesterol content of plasma membrane and examined the secretion of adipokines by ELISA.

Results:

We found that MβCD treatments reduced basal and insulin-stimulated leptin secretion. MβCD treatments also reduced insulin-stimulated Akt phosphorylation. On the other hand, MβCD treatment increased the secretion of a chemokine monocyte chemoattractant protein 1 (MCP-1), and also increased ERK/MAPK phosphorylation.

Conclusion:

Therefore, reducing membrane cholesterol by MβCD treatment may influence the function of secretion in adipocytes. Further experiments will be required to elucidate the mechanism. This study will increase our knowledge of adipokine secretion in adipocyte, and provide new directions for treating metabolic diseases in the future.

P051

Role of Soluble Epoxide Hydrolase in Experimental Obstructive Nephropathy

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Purpose:

Obstructive nephropathy is a common cause of end-stage renal disease. However, the detailed mechanism of this disease is still elusive. Soluble epoxide hydrolase (sEH), the key enzyme for the conversion of epoxyecicosatrienoic acid to dihydroxyeicosatrienoic acid, is abundantly expressed in kidney. In this study, we investigated the role of sEH in pathogenesis of obstructive nephropathy by use of unilateral ureteral obstruction (UUO) mouse model.

Materials and Methods:

In current study, 8 week-old wild-type (WT) and sEH deficient ($sEH^{-/-}$) mice were subjected to the UUO surgery. Histological changed was examined by hematoxylin & eosin staining, Masson's trichrome staining and Periodic Acid-Schiff staining. Protein expression was analyzed by western blotting. Cytokines were measured by ELISA kits.

Results:

The protein level of sEH in kidney was increased in UUO group compared to that observed in sham group. Additionally, UUO induced an increase in renal tubular injury, glomerulosclerosis, inflammation and fibrosis in WT mice. Genetic deletion of sEH ameliorated the UUO-induced renal tubular injury, inflammation and fibrosis but did not affect glomerulosclerosis. Moreover, $sEH^{-/-}$ with UUO showed lower levels of fibrosis-related or inflammation-related protein such as collagen1A1, α-actin, transforming growth factor-β, interlukin-1β (IL-1β), IL-6, monocyte chemoattractant protein-1, macrophage inflammatory proein-2 and inducible nitric oxide synthase than UUO-WT mice.

Conclusion:

Collectively, our findings suggest that sEH may play an important role in the pathogenesis of experimental obstructive nephropathy and inhibition of sEH may be a therapeutic strategy for the treatment of obstructive nephropathy-related diseases.

P052

Examinations of corticosterone and adrenal gland weights in lithium chloride- and morphine-induced conditioned suppression in rats

吳季文 , 黃智偉 *

Chi-Wen Wu and Andrew Chih Wei Huang*
Department of Psychology Fo Guang University Abstract

Backgrounds:

Whether an abused drug, morphine, as well as an emetic drug, lithium chloride (LiCl), induces taste aversion learning and reflecting in the changes of the brain stress system, releasing corticosterone and changing adrenal gland weights, should be investigated.

Materials and Methods:

The study used conditioned taste aversion models to test this issue, and moreover, measured the taste aversion behavior, releasing corticosterone levels, and the alternations of the adrenal gland weights on consecutive 3, 6, 12 days. During the test phase, all rats were given a 0.1% saccharin solution and then intraperitoneally injected 4ml/kg of 0.15M LiCl and 10mg/kg of morphine. The taste aversion learning, corticosterone releasing, and adrenal gland weights were tested.

Results:

The present results showed that LiCl as well as morphine induced conditioned taste aversion in behavior on 3, 6, and 12 days, although morphine may induce an overtraining effect in taste aversion learning, causing it did not induce a taste aversive learning in behavior. Only morphine on 3 days increased the corticosterone releasing. However, LiCl did not affect the corticosterone releasing compared to the control. LiCl as well as morphine decreased the adrenal gland weights than the control, particularly on 3 days.

Conclusion:

The present findings related to the involvements of the brain stress system in LiCl- and morphine-induced taste aversion learning should be discussed.

P053

Attentional set shifting test in methamphetamine-induced schizophrenia rats: A pilot study

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Backgrounds:

For such a lone time, methamphetamine was demonstrated to utilize to intimate the positive symptoms of schizophrenia in animal model. However, whether methamphetamine (MAMPH) can induce an attentional deficit needs to be scrutinized.

Materials and Methods:

The present study utilizes MAMPH to induce the schizophrenic symptoms, and then tests the attentional set sifting task. All rats were given 1mg/kg MAMPH or its vehicle saline by intraperitoneal injection before behavioral testing. The attentional set shifting task respectively includes a series of discrimination sessions: simple 1 (SD1), simple 2 (SD2), reversal 1 (R1), intradimentional shift (IDS), reversal 2 (R2), extradimentional shift (EDS), and reversal 3 (R3).

Results:

The present results indicated that there was a non-significant difference in the group factor. Sessions was a significant difference. The post hoc test indicated that non-significant differences occurred in SD1, R1, IDS, R2, and EDS between Saline and MAMPH groups. However, the correct trial numbers of the MAMPH group may be decreased than those of the Saline group in SD2 and R3 sessions, although the pilot data showed both were non-significant differences.

Conclusion:

Taken together, MAMPH may cause the attention deficit, and it is a great pharmacological way to intimate an animal model of schizophrenia.

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Bowel Obstruction Triggers Commensal Bacterial Endocytosis by Enterocytes: Role of IFNγ-Induced Terminal Web Myosin Phosphorylation and Brush Border Fanning

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Bacterial dissemination is commonly seen in intestinal obstruction (IO). Paracellular influx was mediated by ROCK signaling for phosphorylated myosin light chain (pMLC) and tight junctional disruption. Myosin phosphorylation in terminal web (TW) is associated with brush border (BB) fanning. The mechanism of bacterial endocytosis in enterocytes remains unclear.

Aim:

To investigate the role of IFNγ in TW pMLC for bacterial endocytosis.

Methods:

Mouse small intestines were obstructed by a loop ligation in which ML-7 (a MLCK inhibitor), Y27632 (a ROCK inhibitor) or vehicle was luminally administered. Next, mice were injected i.p. with anti-IFNγ. Bacterial diversity was examined using denaturing gradient gel electrophoresis and gene sequencing. Bacterial endocytosis in isolated enterocytes was measured by gentamycin resistance assay and fluorescence *in situ* hybridization. Epithelial Ca^{2+}/ATP-dependent TW contraction and BB fanning were visualized using transmission electron microscopy. Caco-2 cells were exposed to IFNr for 48 hours, and transepithelial resistance (TER), dextran permeability, phosphorylation MLC in TW region, *E. coli-GFP* endocytosis and translocation were measured.

Results:

Small intestinal bacterial overgrowth was noted with increased amount of *Escherichia, Staphylococcus, and Lactobacillus* after IO. Bacterial endocytosis was observed without paracellular permeability change at 3-6 h of IO. Enterocytic pMLC localized to TW was associated with BB fanning, which may be inhibited by ML-7 but not Y27632. IO-induced bacterial endocytosis through intermicrovillus space was reduced by ML-7 or anti-IFNγ. Caco-2 cells treated with IFNγ showed MLCK-dependent TW contraction and *E. coli* endocytosis and translocation.

Conclusions:

IFNγ-induced epithelial MLCK-dependent TW contraction and BB fanning is involved in bacterial endocytosis.

P055

Dysfunction of sodium current is responsible for the occurrence of early after-depolarization in cultured cardiomyocytes

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Voltage-gated sodium current (I_{Na}) contributes to cardiac action potential (AP) upstroke, influences AP duration (APD), and may be responsible for early after-depolarization (EAD). Understanding such arrhythmogenic mechanism would facilitate the development of novel therapeutic strategies for arrhythmias. However, the critical underlying mechanism remains uncertain. By using the whole-cell patch-clamp configurations, we performed the electrophysiological characterizations of spontaneous action potential (sAP) in long-term cultured neonatal rat ventricular myocytes. Two stages of sAP alterations were significantly observed, including the suppression of both amplitude and frequency, whereas APD prolongation. Gradual changes of sAP were strongly correlated with the decay of INa but not IK. The expression of dominant cardiac sodium channel subunit, Nav 1.5 was not significantly changed during the culture periods. sAP could be modulated by tetradotoxin in early but not in late culture stage. The bradycardia-dependent EAD was driven by Ca^{2+} inward current. The novel I_{Na} agonist, (-)-epicatechin-3-gallate, could restore the EAD to normal sAP with fast firing rate. INa suppression leads to retardation of automaticity, APD prolongation, and EAD. Our study demonstrated that I_{Na} is a rate-determinant to maintain automaticity.

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Functional Brain Imaging Study of Central Post-Stroke Pain in Rats

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Stroke is the major cause of disability worldwide and more than 8% of the stroke patients will develop central post-stroke pain (CPSP) 6 months later. Several studies have proposed that the spinothalamic tract and the thalamus are involved in the development of CPSP. However, which brain areas are involved in the functional changes remains uncertain. In this study, we utilize an autoradiographic approach, [^{14}C]-iodoantipyrine radioreactivity, to investigate the brain area associated with CPSP in the rat model. The concentration of 0.125U/0.5μl type 4 collagenase was injected into ventral basal thalamus (VB), and behavior tests were performed for once trial each week for 5 weeks to show the development the pain symptom of CPSP. After 4 weeks, the right external jugular vein was cannulated with a PE20 tube, and animals were allowed to recover for 1 week. On the last week, [^{14}C]-iodoantipyrine (125μCi/kg in 300μl of 0.9% saline) was intravenously infused after following immediate anesthesia. Local tissue radioactivity was analyzed by autoradiographic brain slice images. The results indicated that von Frey (Mechanical pain) test and the Plantar (Thermal pain) test both revealed the increasing of somatosensory sensitivity in the hindpaw contralateral to the side of the hemorrhagic lesion. The results of the radioactivity ratio showed that IL, GI-1, AID, AIV, Hippocampus, and PAG of the left brain of the CPSP group were significantly different from those in sham group. No significant difference was found in the other brain areas between the CPSP and the control groups. In contrast, the right brain showed significant differences at IL, PrL, Cg1, and VB. Furthermore, the connectivity between different brain regions such as the cortex, striatum, hippocampus, thalamus, hypothalamus, amygdala, and PAG were assessed by the radioactivity correlation between CPSP and control rats. The results indicated that the cortex was strongly correlated with the nucleus of the thalamus. The thalamus are strongly associated with the nucleus of the hypothalamus. The [^{14}C]-iodoantipyrine radioactivity approach might be an appropriate way to test which specific brain areas are involved in the symptoms of CPSP. How the cortex, thalamus, hypothalamus, and amygdala interact with each other during the development of CPSP symptoms should be discussed in the further studies.

Key Words:

Chronic Post-Stroke Pain, Isotope, Functional Brain Imaging.

P057

The effect of Acetaminophen on physiological status of acute fracture stage in a rat model

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Backgrounds:

Fracture caused inflammation and pain trouble orthopedic doctors and patients usually. Acetaminophen is a popular and widely used analgesic for the treatment of the pain-related symptoms. But few studies focus on its effect on acute stage of trauma. This study examined the influence of acetaminophen on physiological status of acute fracture stage in a rat model.

Materials and Methods:

Thirty-two 30-week-old male Wistar rats were divided into four groups: sham group, fracture group, acetaminophen-fracture group and acetaminophen group. Fracture rats were prepared by breaking the unilateral tibia and fibula. The inflammation condition was evaluated by serum cytokine level. The physiological status was evaluated by estimating central temperature, heart rate and mean blood pressure. The hepatic and renal adverse effect was also assessed by measuring the serum levels of aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), and creatinine (Cr.). Muscle related trauma condition was evaluated by measuring the serum levels of creatine phosphokinase (CPK) and lactate dehydrogenase(LDH).

Results:

Higher central temperature was noted at the fracture group fed with acetaminophen. Accumulated hepatic injury presented as climbing-up curve of sGOT and sGPT. All the four groups of rats were kept in steady status and revealed high consistency.

Conclusion:

We concluded that acetaminophen may have accumulated and exacerbated side effect for acute stage of injury as an analgesic agent. Alternative therapy or combined protection method could be considered at this stage.

P058

Medication Behavior and Its Influencing Factors of The Elderly in Remote Districts: Study on The Jiasian and Liouguei Districts, Kaohsiung City

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Backgrounds:

Aging is a normal, natural phenomenon, often accompanied by degradation of physiological functions and suffering from a variety of diseases. The elderly are the main users of prescription drugs and non-prescription drugs in Taiwan. In addition, since special geographic environments in some of Taiwan's outlying islands and in remote areas, the elderly difficult to get good medical care. Therefore, understanding of the elderly medication behavior and related influencing factors in remote districts is an important public health issues. The aim of this study was to examine the medication behavior and its influencing factors of older adults in remote districts.

Materials and Methods:

The study mainly focused on elderly people over the age of 65 in the Jiasian and Liouguei districts, Kaohsiung city. A cross-sectional study using random samplings by way of a face-to-face interview survey and data further analyzed using SPSS computer software.

Results:

The 100 elderly peoples surveyed in this study, and the results revealed information on the following categories: Health Condition and Medication Characteristic section, Medication Behavior and Medication Compliance section, and Influencing Factors section. In the Health Condition and Medication Characteristic section, the most common type of medical treatments is Western medicine (81%), and elderlies with chronic disease accounted for up to 82%. Our also found that most elderly had displayed better medication behavior and medication compliance (76.8%). In addition, their medication behavior was mainly affected by healthcare professionals such as doctors and pharmacists (86%), relatives and family (36%), and advertisements, such as found on TV and radio stations (24%). Our further found that the elderly themselves taking or purchase of non-prescription medications accounted for 18% and frequently used non-prescription medication was drugs advertised on TV or radio stations (17%).

Conclusion:

Our analysis indicated that most elderly in remote district had improper medication knowledge and their medication behaviors susceptible to external influencing factors such as non-healthcare professionals. Therefore, strengthen education of both elderly and their relatives can further reduce the risk of medication errors by the elderly in remote district.

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P059

Palmitic Acid-Induced Lipotoxicity and Its Protection by Catechin in Rat Cortical Astrocytes

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Backgrounds:

Astrocytes, the most abundant cells in the central nervous system (CNS), do not only metabolize neurotransmitters but also play an active role in modulating synaptic transmission by releasing gliotransmitters. Palmitic acid (PA) is a saturated fatty acid which, when being excessive, is a significant risk factor for development of metabolic syndromes or stroke. Activation of astrocytes by PA has been shown to cause neuronal inflammation and demyelination. However, direct damage by PA to astrocytes is relatively unexplored. The aim of this study was to identify the mechanism(s) of PA-induced cytotoxicity in rat cortical astrocytes.

Results:

Exposure of astrocytes to PA (100 μM) for 24 h resulted in 50 % cell death. Cell death was apoptotic (as revealed by TUNEL assay) and unrelated to endoplasmic reticulum (ER) stress and cytosolic Ca^{2+} elevation. Exposure of astrocytes to PA for 30 min to 5 h was associated with significant mitochondria membrane potential (MMP) collapse and reactive oxygen species (ROS) production. Co-treatment of astrocytes with catechin (300 μM), an anti-oxidant found abundantly in green tea, significantly prevented PA-induced MMP collapse, ROS production and cell death.

Conclusion:

Our results suggest that PA-induced cytotoxicity in astrocytes may involve MMP collapse and ROS, which can be protected by catechin.

P060

Association of *Cyclooxygenase 2* Single Nucleotide Polymorphisms and Hepatocellular Carcinoma Susceptibility

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Background:

Hepatocellular carcinoma (HCC) is a worldwide neoplasm for which early diagnosis is difficult and the prognosis is usually poor. Overexpression of *cyclooxygenase 2 (COX-2)* has been suggested to be associated with hepatocarcinogenesis. Although several C*OX-2* inhibitors have been used in hepatoma therapy, the genetic background between *COX-2* and HCC remains largely unknown.

Materials and Methods:

In this study, the association of genotypic polymorphisms in *COX-2* with HCC was investigated. 298 patients with HCC and 298 healthy controls recruited from the China Medical Hospital in Taiwan were genotyped by a PCR-RFLP method. We have investigated six polymorphic variants of *COX-2*, including A-1195G, G- 765C, T+8473C, and variants in introns 1, 5 and 6, and analyzed the association of specific genotype(s) with susceptibility to HCC.

Results:

The results showed that, for each of the six genotypes, no differences in distribution between the HCC and control groups were found. There was neither obvious joint effect of *COX-2* G-765C/intron 6 haplotype nor its genotypes with smoking or alcohol consumption on HCC risk. Environmental factors, other than smoking and alcohol drinking, may affect the post-natal expression of *COX-2* in the etiology of HCC, which is an outcome of complex genetic and environmental interactions. Moreover , our immunohistochemistrical results indicated that the *COX-2* protein was significantly over-expressed in well-differentiated HCC, but not significantly increased in expression in poorly-differentiated HCC.

Conclusion:

We suggest that *COX-2* may be a determinant of the differentiation grade of HCC.

P061
ATP Stimulates PGE2/Cyclin D1-Dependent VSMCs Proliferation via STAT3 Activation: Role of PKCs-Dependent NADPH Oxidase/ROS Generation

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Backgrounds:

Vascular smooth muscle cells (VSMCs) that function as synthetic units play important roles in cardiovascular diseases. Extracellular nucleotides, such as ATP, have been shown to act via activation of P_2 purinoceptors implicated in various inflammatory diseases, we hypothesized that extracellular nucleotides contribute to vascular diseases via up-regulation of inflammatory proteins, including cyclooxygenase-2 (COX-2) and cytosolic phospholipase A_2 ($cPLA_2$) in VSMCs. However, the mechanisms of ATP-induced $cPLA_2$ and COX-2 expression and PGE_2 synthesis remain largely unclear.

Materials and Methods:

VSMCs were used in the study. The mechanisms of ATPγS-induced $cPLA_2$/COX-2 expression were investigated by Western blot, RT-PCR, real time-PCR, and promoter assay. Finally, PGE_2 production was detected by an ELISA kit.

Results:

We showed that pretreatment with the inhibitors of STAT3 (CBE), NADPH oxidase [diphenyleneiodonium chloride (DPI) or apocynin (APO)], ROS [N-acetyl-L-cysteine (NAC)], and PKC (Ro-318220, Gö6983, or Rottlerin) or transfection with siRNAs of STAT3 and $p47^{phox}$ markedly inhibited ATPγS-induced $cPLA_2$ and COX-2 mRNA/protein expression and promoter activity and PGE_2 secretion. ATPγS further stimulated PKC, $p47^{phox}$, and STAT3 translocation. Moreover, ATPγS-induced STAT3 phosphorylation and translocation was inhibited by pretreatment with the inhibitors of PKC, NADPH oxidase, and ROS. ATPγS enhanced NADPH oxidase activity and ROS generation in VSMCs, which were reduced by pretreatment with Ro-318220, Gö6983, or Rottlerin. Finally, we found that ATPγS significantly induced cyclin D1 expression and VSMCs proliferation, which were inhibited by pretreatment with NAC, APO, DPI, Ro-318220, Gö6983, Rottlerin, or CBE or transfection with siRNAs of COX-2 and cyclin D1. We also demonstrated that ATPγS induced cyclin D1 expression via a PGE_2-dependent pathway.

Conclusion:

These results suggested that ATPγS-induced $cPLA_2$/COX-2 expression and PGE_2 secretion is mediated through a PKC/NADPH oxidase/ROS/STAT3-dependent pathway in VSMCs.

P062
The Regulatory Effect of Lactoferrin on High-Fructose Induced Nonalcoholic Fatty Liver Disease in Murine Model.

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Backgrounds:

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide and is characterized by aberrant lipid storage in hepatocyte and inflammatory progression to nonalcoholic steatohepatitis. Dietary fructose is the most important risk factors for the development of NAFLD. In this study, we want to evaluate lactoferrin protects against the onset of non-alcoholic fatty liver disease in a mouse model treated by high-fructose diet.

Materials and Methods:

C57BL/6 mice administered with lactoferrin (50, 100 and 200 mg/kg/day) while drinking with 30% fructose solution for 8 weeks. At the end of 8 weeks administration, serum alanine aminotransferase (ALT), triglyceride and cholesterol determination in serum or liver and oral glucose tolerance test (OGTT), Interleukin-6 (IL-6), 4-hydroxynonenal (4-HNE), thymic stromal lymphopoietin (TSLP), Toll-like receptor-4 (TLR-4) and lipid accumulation in liver tissue sections by immunohistochemical, Oil Red O staining were determinate.

Results:

Lactoferrin reduced ALT and triglycerides levels in serum, and also decreased accumulation of triglycerides in liver. IL-6 expression was markedly increased in the livers of mice with nonalcoholic steatohepatitis and insulin resistance. Lactoferrin might regulate activation of kupffer cell via inhibition of IL-6 expression. We provide information on the distribution of inflammatory factors in the liver and/or adipose organ, where their aberrant expression in NAFLD. TSLP play a central role in the progressions of NAFLD. Our results indicated that the treatment with lactoferrin can reduce NAFLD and insulin resistance by high-fructose induced hepatic steatosis.

Conclusion:

These data suggest that lactoferrin enhanced glucose sensitivity, reduced triglyceride level and prevent hepatic steatosis. Lactoferrin prevent high-fructose induced hepatic steatohepatitis in mice through involvement in down-regulated inflammation, including IL-6, TSLP and TLR-4, and further hepatoprevention to release ALT.

P063
Study of the Association of *Cyclin D1* Genetic Polymorphisms with Nasopharyngeal Carcinoma Risk.

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Background:

The cell cycle regulator *cyclin D1 (CCND1)* is a critical regulator of the G1/S phase transition and plays an important part in several tumor types. This study aimed at investigating the association of *CCND1* with and examining the interaction among *CCND1* genotype and individual smoking habit in nasopharyngeal carcinoma susceptibility.

Materials and Methods:

A total of 352 native Taiwanese consisting of 176 cases and 176 controls were enrolled in this hospital-based study, and *CCND1* A870G (rs9344) and C1722G (rs678653) genotyping were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and partially verified by direct sequencing.

Results:

The results showed that there were significant differences between nasopharyngeal carcinoma and control groups in the distribution of the genotypic (p=0.0222) and allelic (p=0.0322) frequencies in the *CCND1* A870G genotype. Individuals who carried at least one G allele (GG or AG) had a 0.71-fold lower risk of developing nasopharyngeal carcinoma compared to those who had the AA genotype (95% confidence interval=0.53-0.96). In addition, there is an obvious joint effect of *CCND1* A870G genotype with smoking habit on nasopharyngeal carcinoma susceptibility.

Conclusion:

These findings support the conclusion that the cell cycle regulation may play a role in nasopharyngeal carcinoma development and that *CCND1* A870G polymorphism maybe a useful biomarker for nasopharyngeal carcinoma progression.

P064
Role of Glycine N-methyltransferase in Experimental Ulcerative Colitis

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Inflammatory bowel diseases (IBDs) are chronic inflammatory disorders with unclear etiology and mechanism(s). Glycine N-methyltransferase (GNMT) plays a central role in inflammatory diseases such as hepatitis and atherosclerosis. However, little is known about the impact of GNMT and the involved mechanism in the pathogenesis of IBD. In the current study, we investigated the role of GNMT in colitis with the mouse model of dextran sulfate sodium (DSS)-induced colitis. GNMT was expressed in the epithelium of the colon under normal conditions, and with DSS treatment, its expression was predominant in infiltrated leukocytes of lesions. Mice with genetic deletion of *GNMT* (*GNMT*$^{-/-}$) showed increased susceptibility to DSS induction of colitis, as revealed by the progression of colitis. Additionally, severe colonic inflammation, including increased crypt loss, leukocyte infiltration and hemorrhage, was greater with DSS treatment in *GNMT*$^{-/-}$ than wild-type mice. Furthermore, the expression of adhesion molecule and inflammatory mediators in the colon was significantly higher with DSS treatment in *GNMT*$^{-/-}$ than wild-type mice. Moreover, loss of *GNMT* decreased cell apoptosis in colitis lesions with DSS treatment. Collectively, our findings suggest that GNMT may be a crucial molecule in the pathogenesis of DSS-induced colitis. This finding may provide new information for a potential therapeutic target in treating IBD.

P065
Induction of Autophagy and Apoptosis in Rat Liver following Silver Nanoparticle Administration

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Backgrounds:

Exposures to silver nanoparticles (Ag-nps) have been shown to distribute and accumulate in the liver and induce hepatotoxicity in animal studies. It was considered to be related to reactive oxygen species-induced oxidative stress and abnormalities in energy metabolism leading to cellular damage. For energy homeostasis and maintains organ function at critical situations, autophagy has been known to be a self-degradative process to recycle cytoplasmic components. In this study, we investigated dynamically the role of autophagy in Ag-nps induced hepatotoxicity, and its relationship with apoptosis was discussed.

Materials and Methods:

Role of autophagy in association with apoptosis were studied in Sprague Dawley rat liver following an intraperitoneally administration of Ag-nps. Animals were sacrificed at different time points (1-30 days) and blood and liver were collected for analysis. Liver function was determined based on enzymatic analysis of aspartate aminotransferase (AST) and alanine aminotrasferase (ALT) activities. Inflammatory reaction was observed by histological characteristics of liver tissues. Autophagy was evaluated based on evidence of LC3-II (autophagy biomarker) protein expression and morphological evaluation. Cellular energy state was determined by ATP content. To further assess the extent of cell death by Ag-nps, apoptosis was evaluated by caspase-3 protein expression and TUNEL-positive cells staining.

Results:

Autophagy increased and peaked in early stage (1 day post-exposure) followed by a rapid decrease during mid stage (4-10 days post-exposure) and remained decreased during late stage (11-30 days post-exposure). Inflammatory reaction was induced during mid stage and proceeded to an advanced degree during late stage. Apoptosis began to rise sigmoidally during mid stage and peaked during mid and late stages. Cellular energy state decreased rapidly in early stage (-64% in day 1) and remained decreased through mid and late stages. Liver function was impaired in late stage.

Conclusion:

These results indicate that following Ag-nps administration, autophagy was induced; however, failure to preserve autophagy compounded with energy reduction led to apoptosis and impaired liver function. The study provides an *in-vivo* evidence of hepatotoxicity by insidiously continuous exposure of Ag-nps in rats.

P066
Retrograde Synaptic Plasticity at Dentate Granule Cells

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Backgrounds:

Activity-induced modification of synaptic transmission is considered as a cellular substrate for learning and memory. Because induction of long-term potentiation (LTP) and long-term depression (LTD) usually requires correlated pre- and post-synaptic activation, it is generally thought that these modifications are synapse-specific. However, accumulating studies demonstrate that LTP/LTD may spread to adjacent synapses, so called heterosynaptic plasticity. More interestingly, Poo and his colleagues showed that LTP/LTD can retrogradely spread to dendritic inputs of presynaptic neurons in cultured hippocampal neurons and in a developing tadpole retinotectal system. However, whether retrograde synaptic plasticity occurs in the mammalian brain remains largely unknown.

Materials and Methods:

Using acute hippocampal slices from 18- to 25-d-old Sprague Dawley rats, we performed paired recordings from synaptically coupled granule cells (GCs) and basket cells (BCs) in the dentate gyrus. Synaptic transmission at perforant path (PP)-GC synapses was evoked by extracellular stimulation of the PP input.

Results:

We found that LTP induced at GC-BC synapse led to downregulation (84% of baseline) of synaptic efficacy at perforant path (PP)-GC synapse, whereas there was no significant change in synaptic efficacy at PP-BC synapse. We also observed that high-frquency stimulation (HFS) applied to GCs alone had no effect on synaptic efficacy at PP-GC synapse.

Conclusion:

Our data show that synaptic strengthening at GC-BC synapse has a depressive effect on PP-GC transmission suggesting homeostatic regulation along PP-GC-BC connections.

P067
Effect of Noni Juice on Male Reproductive Function of Streptozotocin-Nicotinamide Induced Diabetic Rats

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Backgrounds:

Diabetes mellitus (DM) continues to be a major healthcare issue, it is also multifaceted, potentially leading to significant disturbance of numerous physiologic processes. Male reproductive dysfunction is a consequence of DM, but the underlying mechanisms are poorly understood. *Morinda citrifolia* (noni) has been used in folk remedies by Polynesians for over 2000 years, and is reported to have a broad range of therapeutic effects, including anti-inflammatory and anti-diabetic activity. This study aims to investigate the possible ameliorating effect of Noni juice dietary supplement on streptozotocin (STZ) and nicotinamide (NA) induced male reproductive alterations in Sprague-Dawley (SD) rats.

Materials and Methods:

Sixty adult male SD rats were randomly divided into six groups: group I, control, normal rats; group II, STZ-NA induced, untreated DM rats; group III, positive control, STZ-NA induced and treated rosiglitazone in DM rats; group IV, STZ-NA induced DM rats supplemented with 1.24 g/kg bw of noni juice; group V, STZ-NA induced DM rats supplemented with 2.48g/kg bw of noni juice; group VI, STZ-NA induced DM rats supplemented with 6.2g/kg bw of noni juice. In this study, mean organ weight, epididymal sperm counts, sperm motility, percentage sperm abnormality, as well as, testicular malondialdehyde (MDA) level, the activities of the enzymes superoxide dismutase (SOD) changes were determined. Oral glucose tolerance test (OGTT), MDA, SOD and testosterone levels were also estimated in blood samples. Data were expressed as mean ± standard deviation.

Results:

Noni juice supplementation were not caused a significant change in the mean organ weight of the SD rats, opposed to the diabetes groups. OGTT data show that noni juice can not improve the STZ-NA induced hyperglycemia. There was a statistically significant increase in the sperm count and motility in the diabetes rats supplemented with Noni juice. In rat testicular tissues, MDA were significantly elevated while SOD activities as well as testosterone level were significantly decreased in diabetic rats as compared with control group. Noni juice supplementation to diabetic rats restored the testicular SOD activity to almost control levels, in addition, MDA level decrease while testosterone increase significantly as compared with untreated diabetes group.

Conclusion:

Oral supplemented with noni juice restored the control level of sperm quantity and quality, plasma testosterone level in the STZ-NA induced diabetic rats. An important role of testicular oxidative damage in STZ-NA induced infertility could be suggested, supplement with noni juice has a protective protective effect by restoring antioxidant enzymatic activity and decrease lipid peroxidation level in testes tissue. Our study indicated that Noni juice have anti-oxidative properties to prevent diabetic induced male reproductive damage from oxidative stress.

P068
Nucleus accumbens lesions influence methamphetamine-induced the rewarding and aversive conditioned learning

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Department of Psychology Fo Guang University Abstract

Backgrounds:

Grigson and her co-workers suggest that abused drug-induced tastant suppression intake is not due to its aversion. However, they suggest the conditioned suppression results from that the fist tastant reward is outweighed of the second rewarding effects of abused drugs, in termed of reward comparison hypothesis.

Materials and Methods:

All rats were injected neuronal excitatory neurotoxin NMDA or its vehicle in the nucleus accumbens. After that, rats will be respectively given the rewarding conditioned place preference (CPP) and aversively conditioned taste aversion tests (CTA). The CPP was conducted a conditioned stimulus (CS) context exposure to associate with methamphetamine (unconditioned stimulus, US), whereas the CTS was the saccharin solution CS to be paired with US. Finally, rats will be tested in the CTA and thereby the CPP.

Results:

The present results indicated that NMDA microinjection into the nucleus accumbens could decrease the reward threshold and impaired the methamphetamine-induced conditioned taste aversion. Moreover, NMDA lesion facilitated the conditioned place preference.

Conclusion:

Altogether, the present data suggest that methamphetamine is not a rewarding effect but not an aversive effect in the conditioned taste aversion; resulting in the reduction of conditioned suppression to saccharin intake. However, methamphetamine might show a positive rewarding effect in conditioned place preference task. Therefore, the reward comparison hypothesis should be re-elucidated in the future study.

P069

The Involvement of Protein Kinase C Delta-mediated Cytoskeleton Remodeling in Aloe-emodin-induced Photo-killing of Human Lung Cancer Cells.

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Background:
Photodynamic therapy is becoming a widely accepted form of cancer treatment using a photosensitizing agent and light. Our previous study has demonstrated that photoactivated aloe-emodin induced anoikis and changes in cell morphology, which were in part mediated through its effect on cytoskeleton in lung carcinoma H460 cells. However, the molecular mechanisms of these photoactivated aloe-emodin-induced changes remain unknown.
Materials and Methods:
The present study demonstrated that the expression of protein kinase Cδ (PKCδ) was triggered by aloe-emodin and irradiation in H460 cells.
Results:
Furthermore, the photoactivated aloe-emodin-induced cell death and translocation of PKCδ from the cytosol to the nucleus was found to be significantly inhibited by rottlerin, a PKCδ-selective inhibitor. Western blot analysis demonstrated that rottlerin also reversed the decrease in protein expression of cytoskeleton-related proteins, such as rat sarcoma (RAS), ras homolog gene family member A (RHO), p38, heat shock protein 27 (HSP27), focal adhesion kinase (FAK), α-actinin and tubulin, induced by photoactivated aloe-emodin.
Conclusion:
Our findings suggest that the regulation of cytoskeleton-related proteins mediated by PKCδ may be the mechanisms for the protective effects of rottlerin against the photoactivated aloe-emodin induced H460 cell death.

P070

Voluntary and Involuntary Running in the Rat Show Different Patterns of Theta Rhythm, Physical Activity and Heart Rate

李嘉宜

Jia-Yi Li

Purpose:
Involuntary exercising rats undergo more physical and mental stress than voluntary exercising rats; however these findings still lack electrophysiological evidence. Many studies have reported that theta rhythm appears when there is mental stress and that it is affected by emotional status. Thus, we hypothesized that the differences between voluntary and involuntary movement should also exist in the hippocampal theta rhythm.
Materials and Methods:
Using the wheel and treadmill exercise models as voluntary and involuntary exercise models, respectively, this study wirelessly recorded the hippocampal electroencephalogram, electrocardiogram, and three dimensional accelerations of male young rats.
Results:
Treadmill and wheel exercise produce different theta patterns in the rats before and during running. Even though the waking baselines for the two exercise models were recorded in different environments, there is no significant difference between any of the recorded waking baseline values. When the same movement related parameters are considered, the treadmill running group showed more changes in their theta frequency (4-12 Hz), in their theta power between 9.5-12 Hz, and in their heart rate than the wheel running group. A positive correlation between the changes in high frequency (9.5-12 Hz) theta power and heart rate was identified.
Conclusion:
Our results imply that changes in high frequency theta activity may represent mental stress during involuntary running by rats.

P071

The effect of $1,25D_3$ on testosterone induced aromatase expression and action

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Purpose:
Abnormal ovulation is commonly caused by polycystic ovary syndrome (PCOS), which is concerned with excessive testosterone of ovarian follicles mediating developmental collapse of dominant follicle. Testosterone is converted into estradiol (E_2) by aromatase in ovarian granulosa cells and E_2 contributes to follicle maturation. However, excessive testosterone is able to inhibit aromatase activity in granulosa cells, in turn reducing E_2 production. Generally, $1,25D_3$ supplement help to improve symptoms of PCOS patients who have low level of $1,25D_3$ in blood. Therefore, our study investigate the the effects of $1,25D_3$ and testosterone on aromatase and estradiol secretion in rat granulosa cell.
Materials and Methods:
Rat granulosa cells were cultured in steroids/phenol free media and treated testosterone or $1,25D_3$ combined with testosterone for 24 hr. Cell lysates were subjected to western blot analysis of protein and phosphorylation level, and estradiol secretion in collected media was assessed using the RIA.
Results:
Results indicated that testosterone treated groups increased aromatase level, whereas testosterone-induced aromatase level was declined with $1,25D_3$ treated groups. However, $1,25D_3$ combined with testosterone exhibited increase in estradiol synthesis relative to without $1,25D_3$. Testosterone induced aromatase phosphotyrosine at 10 min, 30min and 1hr, whereas $1,25D_3$ improved the effect of testosterone lasted to 6hr and 24hr. The granulosa cells pretreated L-type calcium channel blocker or intracellular calcium chelater did not influence $1,25D_3$ regulated aromatae expression, aromatse phosphotyrosine and estradiol secretion.
Conclusion:
Thus, we demonstrate that $1,25D_3$ potential improve testosterone induced estradiol though regulating aromatase phosphotyrosine, but it's not involved in calcium increase in granulosa cells.

P072

Deficiency of C-C Chemokine Receptor Type 5 Ameliorate High-Fat Diet-Induced Obesity, Adipose Inflammation, and Insulin Resistance

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Backgrounds:
Recent investigations suggest that obesity gives rise to a state of chronic, low-grade inflammation that contributes to insulin resistance and diabetes. Although the tissues and cell types involved in this inflammatory response are not fully understood, there is significant interest in the role of adipose tissue macrophages and T lymphocytes in the inflammatory changes characteristic of obesity. CC-chemokine ligand 5 (RANTES) is chemotactic for T cells, eosinophils, and basophils, and plays an active role in recruiting leukocytes into inflammatory sites. The C-C chemokine receptor type 5 (CCR5) interactions with RANTES that is importance for T cell migration. Thus, the present study was aimed to investigate whether high-fat diet-induced obesity, adipose inflammation, and insulin resistant syndrome could be attenuated in CCR5 knockout mice.
Materials and Methods:
Female C57Bl/6 mice were fed with high-fat diet (HFD) for 24 weeks to induction of obesity and insulin resistance.
Results:
The HFD mice developed insulin resistant syndrome characterized by elevated body weight, hyperglycemia, hyperinsulinemia, and hyperlipidemia. In addition, HFD mice exhibited impairment of oral glucose tolerance test (OGTT), increasing of glucose-stimulated insulin secretion, and decreasing of insulin sensitivity during insulin tolerance test (ITT). In contrast, diet-induced obesity and insulin resistant syndrome were significantly ameliorated in CCR5 knockout mice. Deficiency of CCR5 also attenuated obesity-induced fatty liver. Histology revealed that the size of adipocytes was smaller in CCR5-/- mice than those in wild type mice by HFD treatment.
Conclusion:
These results indicate that CCR5 mediated adipose inflammation may play an important role in high-fat diet-induced obesity and insulin resistance.

P073
Altered Acoustic Reflexes of Young Rats after Early Sound Exposure at Moderate Levels

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Background:

Our earlier studies showed that after presenting sounds of moderate levels to young rats, neurons in the auditory cortex and midbrain expanded in size at high frequency region distant to the exposing tone, suggesting the possible involvement of tinnitus. If so, the auditory behavior which reflects perceptual changes at low sound levels related to tinnitus would be altered. Here we determined if the acoustic reflexes do change in these animals.

Method:

A continuous tone (4 kHz, 65 dB SPL) was delivered to young rats (S.D.) during night time from postnatal day 21 to 27. Four acoustic reflexes (head orienting, pinna reflex, vibrissa freezing and head freezing) in response to a noise burst presented free-field (0 to 65 dB SPL) were characterized using digital image processing techniques.

Result:

In the monotonic intensity-response functions (head and pinna reflexes), no difference was found between the exposed and the control groups. The only difference ($p < 0.05$) was found in the non-monotonic function of freezing responses (in particular the head freezing). The sound-exposure affected reflexes occurred at low sound levels (0 to 35 dB SPL), and not at high sound levels (35 to 65 dB SPL).

Conclusion:

Only the low-intensity acoustic reflex was affected by early sound exposure. Findings are consistent with a perceptual change at low sound levels overlapping with the intensity range where tinnitus is typically reported. Further work is needed to confirm if similar change occurs in the more established tinnitus models (e.g., loud sound exposure or salicylate over-dose).

P074
Inhibitory Effects of Microwave Radiation on LPS-Induced NFκB Cytokine Gene Expression in the THP-1 Monocytes

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Purpose:

Microwave radiations can be encountered regularly in daily lives. When WHO announced that microwave radiations were a kind of environmental energy which interfere with the physiological functions of the human body, great concerns have been raised over the damages microwave frequencies can do to human physiology. The immunological performance and the activities of the cellular inflammatory factor NFκB have been closely related in monocyte.

Materials and Methods:

Due to the effect of phorbol 12-myristate 13-acetate (PMA) on THP-1 monocytes, THP-1 monocytes would differentiate into macrophages and would then react with lipopolysacch- arides (LPS), and the amounts of NFκBp65 protein and mRNA increase in the THP-1 monocytes. Gene expression of NFκBp65 is affected when THP-1 monocytes are exposed to a frequency of 2450 MHz and at 900 W. We used the real-time PCR method to analyze the NFκBp65 gene expression of THP-1 monocytes both under microwave radiation and normal state.

Results:

THP-1 monocytes were stimulated with PMA and LPS to differentiate into macrophage, the amount of NFκBp65 protein and mRNA in cells increased exponentially, and the levels of NFκBp65 protein and mRNA expression were decreased by the exposure of microwave radiation by the real-time PCR analysis method.

Conclusion:

Microwave radiations were found to inhibit the activity functions of THP-1 monocytes stimulated with PMA and LPS.

P075
The Effect of the Blood-Activating and Stasis-Resolving Chinese Medicine in Zebrafish Embryo Development

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Purpose:

The purpose of this study is to demonstrate the effects of blood-activating and stasis-resolving Chinese medicine in different embryo developing stages of zebrafish (*Danio rerio*). Several kinds of Chinese medicine, such as Hirudo, Sanleng, and Ezhu, were applied in this study.

Materials and Methods:

The drugs were added at one of the follow time courses that 0 hour, 12 hour, or 24 hour after fertilization. The drug dosages (1000, 750, & 250 ppm) were had tested in this study.

Results:

It is found that (1) Adding drugs at oh after fertilization, Hirudo, Sanleng, and Ezhu could lead to 10 ~ 30% embryos dead and delay the incubation period. (2) However, giving the drugs at 12h, Hirudo, Sanleng, and Ezhu could shorten the incubation period, and it is faster than the control group. (3) Giving the drugs at 12h or 24h, Taoren would shorten the body length of lava and significantly decrease the body pigments. (4) It is shown that the developing heart or vessels of zebrafish embryo or lava would be affected by those blood-activating and stasis-resolving Chinese medicines.

Conclusion:

It is suggested that the effect of blood-activating and stasis-resolving Chinese medicines, such as the changes of incubation period or lava lethal ratio may be by the abnormal developing heart or vessels at the early stage.

P076
The Effect of the Tocolysis Chinese Medicine in the behavior of Zebrafish lava

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Purpose:

The purpose of this study is to show the effects of tocolysis Chinese medicine in the behavior of zebrafish lava.

Materials and Methods:

Three kinds of Chinese medicine were applied in this study, such as Xiong Gui Jiao Ai Tang(XGJAT), Dang Gui Shao Yao San(DGSYS) and Bao Chan Wu You Fang(BCWYF). The drug dosages (1000, 750, & 250 ppm) were conversion lower/equal to the human dosage. Those drugs were added at one of the follow time courses that 0 hour, 12 hour, or 24 hour after the eggs of Zebrafish (*Danio rerio*) had fertilized. The behavior of lava were recorded and analysis by the animal video behavior system (Diagnostic & Research Instruments Co., Ltd.).

Results:

4 days after the eggs hatched, it is showed that low dose groups of XGJAT and DGSYS could increase the move more distance than other dose groups. In the same time, the groups of adding BCWYF at 0h did not show exciting movements, but The groups of adding BCWYF at 24h had displayed significantly swimming behavior,. Although DGSYS increase the ATPase activity, it is had not shown enough directive effective evidence on the behavior.

Conclusion:

It is suggested that low dose XGJAT could use at the embryo early stage. The application of BCWYF should be competently at the late stage, but very cautiously at the early stage.

P077
Vitamin D Supplements may Exacerbate the Acute Liver Injury

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Backgrounds:

Vitamin D is a secosteroid hormone whose principal biological action is to regulate mineral and skeletal homeostasis. Vitamin D metabolism may also be locally regulated by pro-inflammatory signals acting on monocytes/ macrophages. However, there was little study to explore the effect of vitamin D supplements on the acute live injury animal model. In this study, we used vitamin D to study the effects on the acute liver damage with Thioacetamide (TAA) in conscious rats.

Materials and Methods:

Acute liver damage was induced by intravenous injection of 280 mg /kg in conscious rats. 30 minutes later, the rats received an intravenous injection of vitamin D (10 ng/kg in 0.5 mL/h normal saline) or normal saline (0.5 mL/h). Biochemical parameters measured during the 60-h period following vitamin D administration, included white blood cells (WBC), haemoglobulin (Hb), platelet, aspartate transferase (GOT), alanine transferase (GPT), creatine phosphokinase (CPK), glucose and lactate dehydrogenase (LDH) were measured at 0 hour, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 48 hours and 60 hours. Rats were sacrificed by decapitation 60 h after TAA administration and livers were removed immediately for pathology.

Results:

Vitamin D significantly increased blood GOT, GPT, CPK, LDH and glucose levels but decreased the blood platelet level. The levels of histopathological damage in the liver after with or without intravenous vitamin D administration were not different after TAA administration.

Conclusion:

We proposed that the Vitamin D supplements may not improved the acute liver injury with TAA.

Key words:

Vitamin D, acute liver injury, Thioacetamide (TAA)

P078
Age-Dependent Effects of Calorie Restriction on Metabolic Parameters and Exercise Performance of Rats

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Purpose:

To examine the influence of calorie restriction on exercise performance and metabolic parameters in animals at different ages.

Materials & Methods:

Young (4 month), middle-aged (9 month) and old (16 month) Sprague-Dawley rats were randomized into calorie restriction (CR) or ad libitum (control) groups. The CR group received 10% calorie restriction in the first week of intervention and the restriction increased to 25% in the second week and maintained at 40% restriction afterward. The diet intervention lasted for 14 weeks. Body weight (BW), systolic blood pressure (SBP) and intraperitoneal glucose tolerance test (IPGTT) were determined before, during and after intervention. Exercise performance was evaluated by grip test, rotarod test and treadmill test, which represented muscle strength, exercise capacity and coordination, respectively. All values were analyzed with independent t-tests and the significant level was set at $p<0.05$.

Result:

Rats with CR, no matter the ages of animals, had lower BW and SBP, and had better glucose tolerance than rat with ad libitum. Regarding exercise performance, we found young rats with CR had better grip strength and exercise capacity compared with the control group, though these differences were notobserved in middle-aged and old animals. In addition, CR did not affect coordinationestof rats at all age levels.

Conclusion:

CR is beneficial not only for old animals but also for young and middle-aged animals. Notably, CR at young age had positive effects on exercise performance.

P079
The Role of Syndecan-4 and Underlying Mechanisms in Dorsal Root Ganglion Mechanotransduction Using Controlled Polydimethylsiloxane Substrate Stiffness

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Backgrounds:

Mechanotransduction, the mechanical stimuli are transformed into a biological response, organize the physiological processes, such as senses of touch, hearing, makes a essential contribution to homeostasis. The syndecans are one of the adhesion receptor families that modulate the adhesion and as organizers of the extracellular matrix (ECM), there are four types of the syndecan family in mammals, and the syndecan-4 (S4) can signal to cause focal-adhesion formation and migration by increasing protein kinase Cα (PKCα) activation. In previous study, PKCα and focal adhesion kinase (FAK) are regulated by syndecan-4, but the mechanism and role of S4 in DRG neurons remain obscure.

Materials and Methods:

We harvested the DRG neurons from mice, and culturing DRGs on controlled polydimethylsiloxane (PDMS) substrates (PDMS ratio of base to curing agent of 35:1) coating with ECM, poly-lysine and fibronectin to investigate the mechanotransduction of DRG neurons. Use the Immunofluorescent microscopy, image analysis and western blotting to compared with poly-lysine glass group, fibronectin glass groups and each PDMS groups.

Results:

The neuron density, neurite length and neurite branch among different groups showed significant increase in fibronectin glass group. And the western blotting data showed that PKCα significant increase in the fibronectin groups, and the FAK-397 also expression in the fn groups.

Conclusion:

We suggested that fibronectin may participate in DRG neuron density, neurite branch and length through fibronectin SDC-4 interaction. And fibronectin SDC-4 interaction then to activate PKCα in DRG neurons.

P080
Sex Differences in Dopamine System and Stereotyped Behavior on Repeated Methamphetamine Administration in Adolescent Rats with Neonatal Status Epilepsy

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Backgrounds:

Seizures in immature stage can cause abnormal behavioral sensitization and neuroadaptations. The present study was designed to investigate whether juvenile Methamphetamine(MA) administration following early-life postnatal epilepticus alters stereotyped behavior and dopaminergic function in male and female rats.

Materials and Methods:

Male and female Sprague-Dawley (SD) rats in postnatal day 9 (P9) were randomly assigned to the following four groups with treatments of (1)saline (P9) / saline (6th week); (2)LiPC (P9~P10) / saline; (3)saline (P9) / MA (6th week); (4)LiPC (P9~P10) / MA (6th week). Epileptic seizure was induced at P10 by pilocarpine in rat pups pretreated 18h earlier with lithium chloride. After pilocarpine injection, pups behavioral manifestations of epileptic seizure activity were observed in isolation from mothers until the end of the experiment. With seizure activity lasting 3 to 4 hours, only pups displaying seizure behavior for more than one hour were used for the study. In the adolescenthood (6th week), the group 3 and 4 were received MA for 5 days and the control groups were received saline.

Behavioral locomotion and stereotypy induced by MA were rated according to Dougherty/Ellinwood rating scales, the locomotion-stereotypy score was evaluated for 140 min by a single observer blinded to the treatment conditions. The cumulative time spent in stereotype behavior such as sniffing or head movement was independently evaluated as described by Nakamura et al. during the 140 min after MA administration.

The animals were killed by decapitation. Three brain regions including prefrontal cortex and striatum were quickly dissected out. Tissue levels of dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxy-phenylacetic acid (HVA) in each of the tissue samples were determined by high performance liquid chromatography coupled with electrochemical detection.

Results:

1.LiPC-induced Neonatal Status Epilepsy (NeoSE) exhibited enhanced behavioral sensitization (MA-induced locomotion/stereotypy rate) to MA in adolescent male rats, not in the female. But, the augmented behavioral sensitization after MA challenge (5 mg/kg) on P55 was observed in female rats.
2.Altered tissue levels of DA and its metabolites in the prefrontal cortex and striatum in adolescence.
3. Rats with neonatal epilepsy followed by adolescent MA treatment increased mortality.

Conclusion:

These results suggest that LiPC-induced NeoSE exhibited enhanced behavioral sensitization to MA in adolescent male rats, not in the female. The female rats showed a prolonged timing effect of behavioral alteration in administration of MA with or without NeoSE. The different behavioral sensitization and DA turnover in MA administration in adolescent rats with NeoSE were attributed to sexual effects.

P081

The role of histone deacetylases (HDACs) in modulating TZD suppression of TNF-α-induced lipolysis

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Background:

In obesity, abnormal secretion of adipokines changes the energy balance. Among these adipokines, the inflammatory factors such as TNFα may increase lipolysis of adipocytes, resulting in elevated free fatty acids in the blood, leading to insulin resistance and diabetes. Thiazolidinediones (TZDs), through peroxisome proliferator-activated receptor γ (PPARγ), suppress inflammation and reduce the free fatty acids in the blood, thereby alleviating the symptoms of diabetes. However, TZDs have many known side effects. Therefore is important to understand the mechanism of TZD actions.

Methods:

We examined TNFα-induced lipolysis in differentiated 3T3-L1 adipocytes. Glycerol released from hydrolysis of triglycerides was measured for the level of lipolysis.

Results and conclusion:

PPARγ is a transcription factor. Its function can be regulated by corepressor and HDACs. We tested if HDAC activity is required for TZD suppression of TNFα-induced lipolysis using HDAC inhibitors. Our results showed that TZD inhibited TNFα-induced lipolysis. Treatment with Trichostatin A (TSA), a pan HDAC inhibitor, attenuated TZD action on suppressing TNFα-induced lipolysis. However, treatments with more specific HDAC inhibitors did not have similar effects, suggesting that HDACs may not be involved in this regulation. Then we wanted to know how TSA suppressed TZD inhibition of TNFα-induced lipolysis. TSA treatment reduced the amount of PPARγ. Moreover TZD has been shown to suppress TNFα-induced ERK phosphorylation. We found that ERK inhibition attenuated TZD action as TSA treatment, suggesting that ERK may play a role in TSA treatment. Further studies will be required to elucidate the mechanism mediating TZD suppression of TNFα-induced lipolysis.

P082

The Nuclear Chaperone Nucleophosmin Escorts an Epstein-Barr Virus Nuclear Antigen to Establish Transcriptional Cascades for Latent Infection in Human B Cells

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Epstein-Barr Virus (EBV) is an oncogenic c-herpesvirus that capably establishes both latent and lytic modes of infection in host cells and causes malignant diseases in humans. Nuclear antigen 2 (EBNA2)-mediated transcription of both cellular and viral genes is essential for the establishment and maintenance of the EBV latency program in B lymphocytes. Here, we employed a protein affinity pull-down and LC-MS/MS analysis to identify nucleophosmin (NPM1) as one of the cellular proteins bound to EBNA2. Additionally, the specific domains that are responsible for protein-protein interactions were characterized as EBNA2 residues 300 to 360 and the oligomerization domain (OD) of NPM1. As in c-MYC, dramatic NPM1 expression was induced in EBV positively infected B cells after three days of viral infection, and both EBNA2 and EBNALP were implicated in the transactivation of the NPM1 promoter. Depletion of NPM1 with the lentivirus-expressed short-hairpin RNAs (shRNAs) effectively abrogated EBNA2-dependent transcription and transformation outgrowth of lymphoblastoid cells. Notably, the ATP-bound state of NPM1 was required to induce assembly of a protein complex containing EBNA2, RBPJk, and NPM1 by stabilizing the interaction of EBNA2 with RBP-Jk. In a NPM1-knockdown cell line, we demonstrated that an EBNA2-mediated transcription defect was fully restored by the ectopic expression of NPM1. Our findings highlight the essential role of NPM1 in chaperoning EBNA2 onto the latency-associated membrane protein 1 (LMP1) promoters, which is coordinated with the subsequent activation of transcriptional cascades through RBP-Jk during EBV infection. These data advance our understanding of EBV pathology and further imply that NPM1 can be exploited as a therapeutic target for EBVassociated diseases.

P083

Reactive oxygen species mediated upregulation cofilin and c-myc induced cellular senescence phenomenon

Presented by Chia-chien Lo

Advisor : Yi-jang Lee

2011.11.14

Cellular senescence prevents unlimited proliferation and may lead to death. This phenomenon is believed to be associated with up-regulation of p53 and $p16^{INK4}$ tumor suppressor. Actin depolymerizing factor (ADF)/cofilin family protein is important for accelerating the pool of G-actin in cells. Recent study also demonstrates that oncogenes (c-myc and Ras) may be a potential characteristic of replicative senescence. And we investigated whether forced expression of cofilin-1 can influence the ratio of senescence.

we choose the lung primary cell, WI-38 and MRC5, and lung cancer cell line, A549 and H1299 (lacking p53 and $p16^{INK4}$ gene), were transfected c-myc plasmid. Redistribution of the cofilin and c-myc assay using Aleax Flour 594 and Aleax Flour 488 fluorescence to confocal fluorescence microscopic examination. Concomitantly, cellular senescence was demonstrated by senescence-associated β-galactosidase (SA-β-gal) assay. Then the protein or mRNA level expression analysis are by western blot or qRT-PCR. And use luciferase assay to detect the gene expresstion.

Our results provide evidence that ROS may induce cofilin-1, c-myc and replicative senescence via an alternative pathway. And also c-myc can induce cofilin-1 by confocal fluorescence microscopic examination. In addition, SA-β-gal and western blot data also show that c-myc can induce senescence.

That cofilin-1 may a senescence biomarker to detect the cellular senescence . It may interact with c-myc that provide an important implication for cancer treatment by promoting cell senescence through cofilin regulation.

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P084

PROGESTATIVE AND ANTI-PROGESTATIVE ACTIVITIES IN CHINESE MEDICINAL PLANTS

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Background:

Estrogen and progestins in current use have adverse effects and many of these adverse effects cause to progestins. Under the circumstances, many women have chosen to use botanical alternatives for hormone replacement therapy without knowing the mechanism of action and steroidogenic properties. Therefore, it is necessary to screening the herbs on the basis of progestative and anti-progestative properties of their extracts.

Materials and methods:

Ethanol extract of 12 Chinese medicinal plants were assayed for progestative and anti-progestative activities by using progesterone receptor driven luciferase reporter gene bioassay. Cytotoxicity of herbal extracts was investigated in procaine aortic endothelial cells using MTT assay.

Results:

Ethanol extracts of three species such as *Dipsacus asperoides, Cortex eucommiae and Perilla frutecens* exhibited progestogenic and seven species such as *Folium artemisiae, Scutellaria baicalensis, Radix glycyrrhizae, Euscaphis japonica, Ailanthus altissima, Angelica sinensis* and *Atractyodes macrocephala koidz* recognized to be anti-progestogenic like activities. Most potent progestative activity was in Dipsacus asperoides which 100 µg ethanol extracts displayed progestogenic activity was 8 folds higher than vehicle and it is comparable to 100 nM of progesterone. The extract of *Cortex eucommiae and Perilla frutecens* were exhibited maximum 1.5 fold higher progestative activity compared to vehicle. Herbs extracts which have the anti-progesterone like activity could significantly inhibit the 100 nM of progesterone activity in bioassay system. All herbs extracts did not show detectable cytotoxicity over the tested concentration up to 80 µg.

Conclusion:

This discovery will aid to select suitable herbs for hormone replacement therapy.

Key words:
Progestins, Anti-progesterone, Bioassay, Medicinal plants,

P085
Studies on the Effect and Mechanism of GRP78 Down-regulation after Areca Nut Extract Treatment in Oral Cancer Cell

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Backgrounds:

Oral squamous cell carcinoma (OSCC) is an aggressive tumor with high mortality and often occurs in betel quid chewers in Taiwan. We identified some ER stress related proteins that were differently expressed in two OSCC cell lines (OC2 and OCSL). One of these proteins, 78-kDa glucose-regulated protein (GRP78), was further investigated.

Materials and Methods:

Two domestic cell lines (OC2 and OCSL) derived from different grades of OSCC were established. We used the quantitative real-time PCR and Western blot to evaluate the gene expression in OSCC cell lines after areca nut extracts (ANE) treatment.

Results:

In our previous data, we found that GRP78 was up-regulated in low grade OC2, and down-regulated in high grade OCSL cell line. Overexpression of GRP78 in OC2 may decrease cell colony formation. Moreover, the expression of GRP78 decreased after ANE treatment in a dosage-dependent manner in OC2. Consistently, the transcription factor Yin Yang 1 (YY1), which was a GRP78 promoter binding protein for regulating GRP78 expression positively, was also down-regulated after ANE treatment. This YY1 regulated expression might be correlated with the histone modification and miRNA, but not with DNA methylation. On the other hand, the epithelial mesenchymal transition (EMT) markers, E-cadherin was down-regulated and vimentin was up-regulated after ANE treatment in OC2, respectively. We are currently investigating the correlation of GRP78 and YY1 with these EMT markers.

Conclusion:

Based on preliminary results, we conclude that GRP78 may be down-regulated after ANE treatment mediated through YY1, following by the regulations of E-cadherin and vimentin expression.

P086
Activation of Estrogen receptors (ERs) Down regulates Peroxisome Proliferator activated Receptor- Gamma in Hepato Cellular Carcinoma

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Background:

Hepato cellular carcinoma (HCC) occurs more often in men than in women; however a little is known about its underlying molecular mechanisms. Therefore, our present study is focused to investigate the effect of ERα, ERβ on PPARγ expression in Hep3B cells.

Materials and Methods:

We investigated PPARγ, ERα and ERβ mRNA and protein expression by RT PCR and Western blotting.

Results:

To determine whether Peroxisome Proliferator-activated Receptorγ (PPARγ) plays a central role in HCC, at first we screened for PPARγ expression in HCC tissues and in malignant HA22T, Huh-7, Hep3B, HepG2, and normal chang liver cells. PPARγ expression was found to highly express in in HCC tissues and in Hep3B cells. Next we aimed to compare the effects of estrogen receptors in ERα, ERβ overexpressing cells on PPARγ expression. E2 treatment decreased PPARγ expression without increasing ERα in ERα overexpressing cells, whereas in ERβ overexpressing cells; E2 treatment increased ERβ expression and decreased PPARγ expression in an E2 independent manner.

Conclusion:

In summary, the present study demonstrates that overexpression of ERα and ERβ plus E2 are primarily related to down regulation of PPARγ and thus provide a valuable therapeutic option for treating HCC patients.

P087
從缺

P088
Phosphorylation Modification of Vacuolar H^+-pyrophosphatase

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The plant vacuole occupies more than 90% of cell volume. There are two kinds of proton pumps in plant vacuoles, vacuolar-type H^+ transporting ATPase (V-ATPase) and vacuolar-type H^+ transporting pyrophosphatase (V-PPase). These proton pumps are used to generate proton gradient for storage of many ions and metabolites. Several studies directly indicated that V-ATPase is regulated by phosphorylation and 14-3-3 protein interaction. In addition, current report referred that a putative 14-3-3 consensus binding motif (RRQFNTIP) is localized in the loop 13 of V-PPase. However, limit information was presented in regulation and phosphorylation of V-PPase.

In this study, phosphorylation of V-PPase was validated utilizing immunoblot analysis with anti-phosphotheonine antibody. Phosphorylation site was then demonstrated to be Thr residue from plant cell extra and purified V-PPase heterologously expressed in yeast. These findings are in agreement with previous prediction that Thr phosphorylation might trig the 14-3-3 binding and subsequently regulate the V-PPase.

P089

Phosphorylation of STAT3 on Serine 727 promotes AR-mediated cell proliferation in prostate cancer cells.

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Prostate cancer is a common cancer in men. Signal transducer and activator of transcription 3 (STAT3) is an important transcription factor for cell proliferation in various cancer types. It has been shown that the transcriptional activity of STAT3 is correlated to Tyr-705 phosphorylation and followed by STAT3 dimerization and nuclear translocation for DNA binding. However, phosphorylation of STAT3 on Serine-727 which is necessary for its activation in response to a variety of stimuli contributes to the maximal transactivation through increasing the recruitment of transcriptional cofactor, such as AR. On the other hand, the correlation between AR activation and STAT3 Ser-727 phosphorylation under interleukin-6 treatment has been found. Our previous results demonstrated that Cdk5 positively regulates Ser-727 phosphorylation of STAT3 and promotes LNCaP cell proliferation. Moreover, Cdk5 also phosphorylates AR at Ser-81 through direct biochemical interaction and results in protein stabilization of AR in the presence of low androgen (0.1 nM R1881). Here we found that STAT Ser-727 phosphorylation might promote AR Ser-81 phosphorylation under Cdk5 control and contribute to AR activation and prostate cancer cell proliferation. These findings may elucidate the relationship between AR and STAT3 and the future investigation of the common upstream regulators becomes important.

P090

Combination Treatment with PCNA Peptide and 5-FU Results in Cooperative cell Growth Inhibition and Induces Apoptosis in Colorectal Cancer Cells

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Backgrounds:

The proliferating cell nuclear antigen (PCNA) is a most important protein to DNA synthesis and repair. Previous study showed that the PCNA agonist PCNA peptide (inhibition of phosphorylated PCNA) has a strong activity in prostate and breast cancers. In the current therapy of colorectal cancer (CRC), the anti-metabolite 5-fluorouracil (5-FU) is a cornerstone. However, whether co-treatment with low doses 5-FU and PCNA peptide could improve a therapeutic benefit is urgently needed.

Materials and Methods:

Four human CRC cell lines (RKO, HCT116 , SW480 and HT-29) we used in this study. We studied the growth inhibition, cytoxicity effects and the additional benefit of synergism of 5-FU and PCNA peptide on cultured CRC cell lines by using WST-1 assay. For the detection of apoptosis we showed both cleavaged caspase-3 and PARP protein expression on Western blot analysis.

Results:

Here, we found that PCNA Y211 phosphorylation was detected in four CRC cell lines via Western blot analysis. 5-FU and PCNA peptide have more inhibited effect and induced apoptosis to activate caspase-3 and PARP on the RKO and HCT116 cells which expressing wild type p53 than the SW480 and HT29 cells which expressing mutant p53. In addition, the combination treatment with PCNA peptide and 5-FU results in cooperative cell growth inhibition and induces apoptosis in CRC cells.

Conclusion:

This PCNA peptide has potential to be developed as a therapeutic agent in combination with traditional chemotherapy, 5-FU in CRC cells.

P091

Targeted Depletion of TDP-43 Expression in the Spinal Cord Motor Neurons Leads to the Development of ALS-like Phenotypes in Mice

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TDP-43 is a ubiquitously expressed nuclear protein highly conserved among *Caenorhabditis elegans, Drosophila,* mouse and human. TDP-43 has been implicated in the regulation of transcription, alternative splicing, translation, and neuronal plasticity. It has also been shown to be a disease signature protein associated with several neurodegenerative diseases including the frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). ALS is a progressive and fatal motor neuron disease with no effective medicine. Importantly, diseased neurons in the majority of the ALS cases are loss of nuclear TDP-43 with cytosolic TDP-43 proteinopathies characterized with TDP-43- positive, ubiquitin-positive inclusions (UBIs). However, the role of the mismetabolism of TDP-43 in the pathogenesis of ALS is unclear. In the present study, we have used gene targeting approach to disrupt the expression of TDP-43 in mouse. Loss of the TDP-43 expression results in peri-implantation lethality of mice between embryonic day (E) 3.5 to 6.5. Blastocysts of the homozygous *Tardbp* null mutants are morphologically normal but exhibit defective outgrowth of the inner cell mass (ICM) cells *in vitro*. To further address the tissue-specific role of TDP-43, we show that mice with inactivation of the *Tardbp* gene in the spinal cord motor neurons (HB9:Cre-*Tardbp*$^{tx/-}$) exhibit progressive and male-dominant development of ALS-related phenotypes including kyphosis, motor dysfunctions, muscle weakness/ atrophy, motor neuron loss, and astrocytosis in the spinal cord. Significantly, ubiquitinated proteins accumulate in the TDP-43-depleted motor neurons of the spinal cords of HB9:Cre–Tardbp$^{tx/-}$ mice with the ALS phenotypes. This study not only establishes an important role of TDP-43 in the long term survival and functioning of the mammalian spinal cord motor neurons, but it also establishes that loss-of-TDP-43 function could be one major cause for neurodegeneration in ALS with TDP-43 proteinopathies.

P092

Gene Expression of Multidrug Resistance-Associated Transporters Related to Arsenic Exclusion in Porcine Aortic Endothelial Cells

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Backgrounds:

Arsenic is a ubiquitous metal in the environment and the accumulation of arsenic in ground water and plants poses a health risk to both humans and animals. Arsenic-induced toxicity is associated with the cellular uptake and the exclusion of arsenic compounds. Multidrug resistance transporters are involved in the arsenic detoxification. The vascular endothelial cells are considered to be one of the primary targets for arsenic toxicity and are used extensively as the experimental model to examine cellular responses induced by arsenic. The objectives of this study were to identify the specific multidrug resistance transporters in the primary culture of porcine aortic endothelial cells (PECs), and to investigate the gene expression of these transporters associated with rapid arsenic excretion in PECs.

Materials and Methods:

The porcine aortic endothelial cells (PECs) were isolated, identified and used in this study. PECs were maintained routinely and passed in sterile basal medium (M199) supplemented with 10% fetal bovine serum (FBS) under a humidified atmosphere of 5% CO_2 and 95% air at 37℃ . All experiments were performed when the cells reach 80-90% confluent. To investigate the differential expression of specific multidrug resistance transporters during rapid arsenic excretion, PECs were preloaded with 200 μM arsenic trioxide (As_2O_3) for 2 hr, and the gene expression of the specific transporters were monitored after arsenic removal. Reverse-transcription polymerase chain reaction (RT-PCR) was used for detecting gene expression.

Results:

In this study, several multidrug resistance transporters, MDR1, MRP1, MRP2, MRP3, MRP5 and MRP9, were found to express in PECs by RT-PCR analysis. The RT-PCR results also indicated that the MRP2 mRNA was increased in PECs at 1-2 hr after As_2O_3 preloading, and MRP3 mRNA was decreased in PECs after As_2O_3 preloading.

Conclusion:

The results from this study indicate that MRP2 and MRP3 may be the major multidrug resistance transporters associated with rapid arsenic excretion during As-induced toxicity in PECs.

P093
A synthetic glycolipid induces autophagy in macrophages.

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Backgrounds:

Activation of Toll-like receptor 4 (TLR4) triggers innate immunity and subsequently leads to induction of adaptive immunity. Recent studies show that induction of TLR4 signaling in macrophages can induce autophagy which is involved in the elimination of intracellular pathogens and the enhancement of antigen presentation.

Our laboratory has previously developed a synthetic glycolipid, CCL-34, which can activate macrophages through TLR4 signaling pathway (Biochemical Pharmacology, 2007, 73:1957-1970) and induce TLR4-dependent anticancer immunity in vivo (Journal of Biological Chemistry, 2011, 286: 43782-43792). In this study, we used macrophages as a cell model to examine whether CCL-34 can induce autophagy and study its underlying molecular mechanism. Furthermore, whether CCL-34 can function as immune adjuvant was also investigated.

Materials and Methods:

A murine macrophage cell line, RAW264.7, was used as the cell model to study autophagy. The CCL-34 compound used in our study was synthesized by Dr. Chun-Cheng Lin in the Department of Chemistry of National Tsing Hua University. Lipopolysaccharide (LPS), a TLR4 ligand known to induce autophagy, serves as the positive control.

To detect incidence of autophagy, the expression and cellular location of LC3-II protein (the marker of autophagy) was measured. The protein level of LC3-II was measured by Western blot and a stable RAW264.7 cell clone expressing GFP-LC3 was established for monitoring cellular distribution of LC3-II under fluorescent microscope. The signaling molecules involved in TLR4-mediated autophagy were analyzed by Western Blot and co-immunoprecipitation. To test the adjuvant activity of CCL-34 *in vivo*, C3H/HeN mice were immunized by OVA, treated with vehicle, CCL-34, or alum (a positive control), then the serum level of OVA-specific IgG in all treated mice was detected using ELISA assays.

Results:

The stable cell clone expressing GFP-LC3 was established and the numbers of cells showing fluorescent punctuation patterns were increased after treatment with CCL-34. The expression level of LC3-II protein was induced after CCL-34 treatment (30μM, 24h). In CCL-34-treated RAW264.7 cells, p38 signaling pathway was activated and inhibition of p38 pathway in these cells further promoted autophagy. Moreover, the serum level of OVA-specific IgG was significantly elevated in CCL-34-treated OVA-immunized mice than in OVA-only treatment group.

Conclusion:

Our data demonstrate that CCL-34 can induce autophagy in macrophages and enhance immune response *in vivo*. Furthermore, the established stable cell line expressing GFP-LC3 can be further applied to screen for autophagy- inducing compounds.

P094
HB-EGF Inhibits Proliferation of Thyrocytes in Graves' Disease

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Backgrounds:

Graves' disease (GD) is an autoimmune thyrotoxic disorder usually with hypervascular diffuse goiter. Heparin-binding epidermal growth factor-like growth factor (HB-EGF), a member of the EGF family, is a potent paracrine and/or autocrine mitogen for fibroblasts, smooth muscle cells and keratinocytes. In previous experiments we found that HB-EGF was excellently expressed in primary thyrocytes from GD thyroid tissues. However, the action of HB-EGF on mitogenesis in GD remains still unknown. Therefore, here we investigated the mitogenic effect of HB-EGF on thyrocytes.

Materials and Methods:

The expression of ErbB family on thyrocytes was analyzed by Western blotting. For overexpression of HBEGF, the human thyroid carcinoma cells (CG3) was transfected with the HB-EGF construct. In other experiment, the cells treated with various concentrations of HB-EGF in the culture. Silencing of HBEGF expression was performed with siRNA. Transcript of HB-EGF was analyzed by RT-PCR, and HB-EGF expression was determined by ELISA and Western blotting. The cell proliferation was performed by MTT and BrdU assay.

Results:

The CG3 cells expressed EGFR, ErbB2 and ErbB4. Transfect of HB-EGF induced overexpression of HB-EGF in CG3. The specific siRNA inhibited intrinsic expression of HB-EGF in CG3 and primary thyrocytes. The overexpression of HB-EGF and treatment of HB-EGF inhibited the cell proliferation of CG3. By contrast, silencing of HB-EGF could slightly increase cell proliferation.

Conclusion:

These results suggest that HB-EGF inhibited proliferation of thyrocytes. Thus, HB-EGF might play a role for inhibiting of thyroid hyperplasia in GD.

P095
Role of Plzf plays in limb patterning and male germ cell renewal

靖永皓

Yung-Hao Ching

The zinc finger and BTB domain containing 16 *Zbtb16* (also called Plzf, Zfp145 or Green's luxoid) belongs to the POZ/zinc-finger family of transcription factors. It contains a BTB/POZ domain that mediates epigenetic transcriptional repression. ZBTB16 is essential for proper skeleton patterning and male germ cell renewal. Two alleles have been reported that display similar phenotypes: a targeted knock-out, and the spontaneous nonsense mutation luxoid. We describe a new ENU induced mis-sense allele of Zbtb16 called seven toes (Zbtb16 ^{7t}). Homozygous animals exhibit hindlimb and axial skeleton abnormalities. Whereas the skeletal abnormalities are similar to those of the other alleles, Zbtb16 ^{7t} differs in that it does not cause spermatogonial depletion and male infertility. Positional cloning revealed a point mutation changing the evolutionarily conserved amino acid Glu44 to Gly, possibly altering the BTB domain's activity. Therefore, Zbtb16 ^{7t} is a separation-of-function allele that reveals differential requirements for domains of ZBTB16 in different developmental milieus.

P096
The Inhibition of Fluoro-Rutaecarpine Derivatives to Epithelial-Mesenchymal Transition

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Backgrounds:

It had been proved that the microenvironment formed by the cells which under the state of inflammation was contribute to induce epithelial-mesenchymal transition (EMT) of cancer cell,and causes invasion and metastasis. We investigate the anti-inflammation mechanism of rutaecarpine derivatives, and by linkage the effect of rutaecarpine derivatives between inflammation and the EMT in order to figure out a novel candidate to suppress cancer progression and metastasis.

Materials and Methods:

Raw264.7 macrophage cell treated with Lipopolysaccharide (LPS,50ng /ml) and different concentration of fluoro-rutaecarpine derivatives for 24hr to test cell viability by MTT and the release of nitric oxide (NO) by Griess assay.Also use the same condition to treat Raw264.7 cell and extract the total protein lysate for western blotting. Ovarian cell line A2780 was treated with fluoro- rutaecarpine derivatives 24hr for migration assay, invasion assay and protein extraction .

Results:

In the results of MTT and Griess assay, fluoro-rutaecarpine derivatives were not toxic to Raw264.7 macrophage cell, and can inhibit the release of NO. In western blotting, data indicated that fluoro-rutaecarpine derivatives inhibited the protein expression of iNOS,COX-2 and IκB in Raw264.7 cell under the circumstances of LPS stimulation.Also, the protein expression of COX-2 and Slug also been inhibited in A2780 cell by fluoro-rutaecarpine derivatives. Besides, in migration and invasion assay fluoro-rutaecarpine derivatives showed inhibition to A2780 cell.

Conclusion:

Our results suggest that fluoro-rutaecarpine derivatives not only have anti-inflammation but anti-EMT effect. In the future, we will address the modulatory role in both the inflammatory and EMT cells to reveal the mechanism of fluoro-rutaecarpine derivatives suppressing cancer metastasis.

P097

Established optical fusion protein (mPlum-IFP1.4) for in vivo imaging based on near-infrared property

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Backgrounds:
Nowadays, utilizing the visibly fluorescent protein became an optical reporter monitoring in the cells level condition was not difficult already. But monitoring in the deep tissue and tumorgenesis of mice was been handicapped by poor penetration of emission light. In the previous study, infrared fluorescent proteins (IFPs) with the excitation/emission peaks maxima of 684/708nm and showed well fluorescent expression in the cell and mice which more increased fluorescence intensity by biliverdin. However, IFPs still had some limitations should be overcomed such as monitoring more deeper tissue that penetrated emission fluorescent light or before in vivo imaging needed intravenous biliverdin that not very convenient for each times.
Materials and methods:
Here we cloned the mPlum (Far red) and IFP1.4 fluorescent proteins to become an optical fusion protein (mPlum-IFP1.4) based on foster resonance energy transfer (FRET). Olympus confocal microscopy was used to characterize mPlum-IFP1.4 based on FRET and defined by acceptor photobleach technique. Both of IFP1.4 and mPlum-IFP1.4 fusion protein fluorescence were quantitative by In vivo imaging system (IVIS).
Results:
We expected that mPlum-IFP1.4 fusion protein not only increased the IFP1.4 fluorescent intensity but also more convenient operated during in vivo imaging that meaning unnecessary biliverdin. Each of cells level *in vitro* and mice level in vivo imaging, all the results showed mPlum-IFP1.4 fusion protein had stably expression fluorescence intensity than native IFP1.4 that based on FRET property and brightness intensity unaffected by biliverdin.
Conclusion:
Taken together, the mPlum-IFP1.4 fusion protein exhibits improved brightness compared to native IFP1.4, and it would be useful for in vivo optical imaging of deep tissue, cancer or stem cell viability via gene expression.

P098

The Effects of Diosgenin in Fibrosis Regulation of Renal Proximal Tubular Epithelial cells

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BACKGROUND:
Fibrosis is the important pathway of many diseases such as end-stage renal failure. Recent study has demonstrated that high concentration of glucose is the most important fibrogenesis-inducing and propagating cytokine. Diosgenin has been shown to be cancer-chemopreventive and and anti-inflammatory. In this study, renal proximal tubular epithelial cells (designated as HK-2) were treated with high concentration of glucose (HG, 27.5 mM) to determine whether the Diosgenin reduces fibrosis in vitro.
METHODS:
Cells were cultured in high concentration of glucose (HG, 27.5 mM) for 48 hours. Different concentrations of Diosgenin (0.1, 1, 10 μM) were added to cells for the last 24 hours. Cells were trypsinized and subjected to the following assays. ELISA was used to evaluate the secreted protein, such as fibronectin and Signal transducer EMT initiator (e.q Snail). Immunofluorescence staining was used to assay the in situ expression of proteins (e.q. fibronectin and Snail).
RESULTS:
We found that the 10 μM of Diosgenin exert optimal inhibitory effects on high glucose-induced fibronectin in HK-2 cells. Diosgenin markedly inhibited HG-induced increase in α-smooth muscle actin and snail. Whereas HG-induced decrease in E-cadherin expression was reversed as well.
CONCLUSION:
Diosgenin has the potential to inhibit high glucose-induced renal tubular fibrosis possibly through EMT pathway.

P099

Prior Manipulation Therapy induced Primary Osteosarcoma Metastasis – From Clinical to Basic Research

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Backgrounds:
Our previous clinical study demonstrated that manipulation therapy (MT) on osteosarcoma (OS) patients prior to diagnosis resulted in poor prognosis after surgical treatment. This study was aimed to provide the evidence from clinical to basic for MT-induced metastasis in primary osterosarcoma.
Materials and Methods:
Eight-week-old male GFP-labeled human OS cells-transferred nude mice were randomly allocated into 2 groups, namely, MT (+) and MT (-) groups. MT was conducted with repeated massage on tumor site twice a week for 7 or 15 weeks. The parameters evaluated were x-ray diagnosis, micro-PET/CT scan, histopathology and serum metalloproteinase 9 (MMP9) level.
Results:
The results showed that MT (+) mice showed a decreased body weight (30.5±0.65g) and an increased tumor volume (8.3±1.18 mm3) compared to MT (-) group with body weight (35·8±0·40g, p<0·0001) and tumor volume (3.9±1.34mm3, p=0.038), respectively. There was an increased signal intensity over lymph node region of hind limb by micro-PET/CT and the GFP-labeled human OS cells were detected in the lung and bilateral lymph nodes in MT (+) group, while there were no such findings in MT (-) group. The serum MMP9 level was higher in MT (+) group (27.1±1.29 ng/ml) than in MT (-) group (17.8±1.97 ng/ml, p=0.048).
Conclusion:
Taken previous clinical observation and the present in vivo evidence together, we conclude that physicians should pay more attention on those patients who seek MT before diagnosis or during treatment for osteosarcoma.

P100

GTP-binding Protein Involves in the Regulation of Axin Degradation

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Backgrounds:
Wnt are secreted lipoglycoproteins that function as signaling molecules to regulate embryonic development at different stages and tissue homeostasis. Without Wnt stimulation, Axin serves as a platform to form a protein complex with MACF1, GSK3β, CK1, and β-catenin in the cytoplasm. GSK3β and CK1 will phosphorylate β-catenin and target β-catenin for proteasome degradation. Upon Wnt stimulation, Wnt binds to its receptor Frizzled and co-receptor LRP5/6, and MACF1 helps Axin complex translocation from the cytoplasm to the cell membrane, then Axin is degraded and in some way β-catenin is accumulated in the cytoplasm for transducing Wnt signaling. So far, how Axin stability is regulated by Wnt stimulation remains elusive and is the focus of current study.
Materials and Methods:
Human embryonic kidney 293T cells and African green monkey kidney fibroblast-like COS7 cells were treated with several inhibitors to G protein-mediated signaling in control-conditioned medium or Wnt3a-conditioned medium, and Western blotting, immunofluorescence staining and TCF-mediated luciferase activity assay were performed to examine the involvement of G protein in the proteolysis of Axin.
Results:
By Western blotting, compare to control treatment, Axin was degraded and beta-catenin was accumulated in Wnt stimulated cells. When cells were treated with different concentrations of inhibitors to G protein-mediated signaling, Axin degradation was inhibited and beta-catenin accumulation was reduced. Immunofluorescence staining data showed that, as compare to control treatment, beta-catenin was accumulated in the cytoplasm and translocated into the nucleus in the Wnt-stimulated cells. This phenomenon was completely blocked by treatment with these inhibitors. We then performed the beta-catenin/TCF-mediated luciferase activity to examine the effects of these inhibitors on Wnt signaling. As compare to control treatment, the luciferase activity increased after Wnt stimulation. Treatment of cells with different concentrations of inhibitors decreased the luciferase activity.
Conclusion:
We conclude that the involvement of G proteins and the underlying signaling pathway in regulating Axin degradation.

P101
Study the Effects of Prostaglandin E2 Mediated Interferon-beta Production in Macrophages

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Backgrounds:

Accumulated evidence indicates that via induction of COX-2, the production of prostaglandin E2 (PGE2) is increased in activated macrophages. As one of the prostaglandin family member, PGE2 promotes inflammation, secretion of cytokines and angiogenesis.

Materials and Methods:

Previously, our laboratory observed enhanced expression and activity of Src was required for generation of interferon beta (IFN-beta) in double-stranded RNA (dsRNA)-stimulated macrophages. In this study, we reported that by virtue of elevation of the expression and activity of Src, PGE2 can cause the increment of IFN-beta in macrophages.

Results:

This could be evidenced by attenuation of Src decreased nuclear accumulation of IRF3 and IFR7 and IFN-beta production, while re-introduction of Src reversed all these defects. Intriguingly, the expression of Lyn, one of the myeloid Src relatives did not alter in the presence or absence of PGE2.

Conclusion:

Our findings indicated that PGE2-elicited Src expression and activation resulted in nuclear translocation of IRF3 and IRF7 that ultimately led to INF-beta production.

P102
The mechanism of Aβ N-terminal mutations in Alzheimer's disease.

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Backgrounds:

Alzheimer's disease (AD) is an aged-related neurodegenerative disease. Amyloid –β (Aβ) peptide produced from amyloid precursor protein (APP) is one of pathological factors in AD. Familial APP mutations near the secretase cleavage sites change Aβ levels or Aβ42/Aβ40 ratio. However, the pathological mechanisms of mutations located on N-terminal region of Aβ are still unclear. In this study, we want to investigate whether Aβ N-terminal familial mutations interfering APP processing, Aβ production and cellular toxicity induction.

Materials and Methods:

APP 770 wild type and with H677R, D678H, D678N mutants were transfected to HEK293 cell. The Aβ level in the medium from four different transfected cells was detected by ELISA. Western blotting was used to exam the levels of total APP and APP C-terminal fragments. The localization of APP mutants in cells was observed by confocal microscope. The cells transfected with different APPs were treated with H_2O_2 to test the cell survival by MTT assay.

Results:

After treating cycloheximide to inhibit protein biosynthesis, APP_{D678H} had the highest and APP_{H677R} had the lowest degradation rate among these APPs. APP_{D678H} and APP_{D678N} produced more C99 which cleaved by β–secretase than APP wild type (APP_{WT}), but APP_{D678H} produced more Aβ than other mutations. APP_{D678H} and APP_{D678N} accumulated in early-endosome when treated NH_4Cl to inhibit lysosome function. The cells expressing APP_{D678H} exhibited more sensitive to H_2O_2 and $ZnCl_2$.

Conclusion:

Our results showed that APP_{D678H} has higher β-secretase cleavage efficiency and produces more Aβ. APP_{D678H} expressing cell is more sensitive to oxidative stress. APP_{D678H} might have higher tendency to be processing via endosome-lysosome pathway. In conclusion, the Aβ N-terminal mutations might regulate APP processing and Aβ toxicity. Our study might discover the molecular pathway of APP mutations to contribute our knowledge toward AD.

P103
Overexpression of CTHRC1 in Hepatocellular Carcinoma Promotes Tumor Invasion and Predicts Poor Prognosis

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Backgrounds:

Collagen triple helix repeat containing-1 (CTHRC1) is a 25-kDa secreted glycoprotein. It may contribute to tissue repair in vascular remodeling and promoting cell migration. CTHRC1 was found to be expressed in 16 out of 19 types of human solid tumors including invasive melanoma but not in benign nevus and non-invasive melanoma. However, the functional role of CTHRC1 in HCC and other cancers is still uncharacterized.

Materials and Methods:

We use real time RT-PCR, western blot, adhesion and migration assay, immunofluorescence

staining and tumorigenicity assay to figure out the mechanism of CTHRC1 in HCC.

Results:

We found that the *CTHRC1* gene is overexpressed in hepatocellular carcinoma (HCC). Overexpression of CTHRC1 in HCC was associated with large tumor size and advanced tumor stage. Furthermore, expression of CTHRC1 was identified as an independent prognostic factor in the multivariate analysis. Suppression of CTHRC1 expression inhibited tumor migration and invasion whereas overexpression of CTHRC1 promoted tumor invasion. Activation of RhoA, but not Rac1 or Cdc42, was found to play a crucial role in CTHRC1 induced cell migration. CTHRC1 promoted adhesion of cancer cells to extracellular matrix through induction of integrin β1 expression and activation of focal adhesion kinase.

Conclusion

These results suggest CTHRC1 promotes tumor invasion and metastasis by enhancing the adhesive and migratory abilities of tumor cells. It is also a promising biomarker for predicting the prognosis of patients with HCC.

P104
Distant Metastasis of Breast Cancer to Endometrium-A Case Report

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Background:

The origin of adenocarcinoma on Pap smear may come from the cervix, endometrium, and extrauterine organ. Pathologists must identify the source clearly according to cytological characteristics and find out the real lesions which can provide important information for appropriate treatment.

Information (Materials and Methods):

The patient is a 42 years old, married female who had undergone breast mastectomy for breast cancer in 2004. In 2007, she had a cervical conization owing to the abnormal Pap smear (CINII). The follow up was normal after treatment until June 2012. Unusual adenocarcinoma cells were observed on Pap smear.

Results:

We review the Pap smear, cervical biopsy and breast specimen. (1) Cytologic findings: The cervical smear was showed clean background and several 3-dimensional malignant glandular clusters which were identified as extrauterine origin. (2) Endocervical curettage: the specimen showed malignant cells with pleomorphic nuclei arranged in glandular pattern. Because the morphology was unusual for tumor of the uterus, the immunohistological staining of GCDFP-15 was performed and showed positive result. These cancer cells were suggested as extrauterine neoplasm. (3) After reviewed the breast biopsy (2004), the morphology was similar to cervical biopsy .According to above data, a metastatic adenocarcinoma origin from breast is concluded.

Discussion and Conclusion:

Mostly, the extrauterine adenocarcinoma found in cervical smear may metastasize from peripheral organ. The cytologic features include a clean background or with morphology unusual for tumors of the uterus or cervix. In addition, the detailed history can offer critical information for proper diagnosis. Furthermore, immunohistochemical staining is also a useful tool to identify tumor source. In conclusion, cytologic interpretation can offer a rapid and accurate diagnosis for better treatment. In addition, the patient history and immunohistochemical staining can also provide useful information for the identification of cancer types and sources.

P105

Microenvironment of the mature ovarian follicle attracts chemotaxis of the transformed fimbriae cells, which requires a downregulation of CNN1 expression.

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Background:

High-grade serous carcinoma (HGSC) of the ovary is the most popular and lethal gynecologic malignancy. The early carcinoma lesion of HGSC was never found in the ovary but was found in fimbriae epithelium of the fallopian tube. The natural history of this early fimbriae lesion and its relationship to ovarian and microenvironments are unknown. We proposed a homing of fimbriae carcinoma cell toward ovulated ovarian follicle for subsequent development of HGSC.

Materials and methods:

We immortalized and transformed fimbriae epithelial cells to generate cell lines named FE25 and FE-RAS, respectively, and compared them with a HGSC cell SKOV3. Conditioned medium (CM) of matured ovarian follicular cells (FC), ovarian stromal cells (OVSC) and fallopian tube stromal cells (FTSC) were collected to treat fimbriae epithelial cells. Cell proliferation, migration, anchorage independent growth (AIG) and xenograft tumorigenesis were analyzed. Besides, Q-RT-PCR was performed to detect differentially expressed genes in transformed fimbriae cells. CNN1 was overexpressed in the FE-RAS and SKOV3 by transient transfection.

Results:

CM of follicular cells enhanced but that of FTSC suppressed the proliferation of immortalized fimbriae cells. The same CM also enhanced proliferation of transformed FE-RAS cells and ovarian cancer cell line SKOV3. In transwell assay, stromal cells of the ovary are highly chemo-attractive for the transformed fimbriae cells. CM of both stromal cells (OVSC and FTSC) suppressed AIG of transformed fimbriae cells. Coinjection of FTSC markedly suppressed tumorigenesis of SKOV3 and FE-RAS cells. OVSC also suppressed tumorigenesis of FE-RAS but not SKOV3. Calponin-1 (CNN1) was down-regulated in the transformed fimbriae epithelial cells. Overexpression of CNN1, a thin filament associated protein, conferred a reduced motility and a more “attached” morphology of both transformed fimbriae cells and SKOV3 cells.

P106

Teroxirone Eradicates Proliferation of Human Non-Small Cell Lung Cancer Cells by Modulating Tumor Suppressor p53

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In this work, we described that the proliferation of human non-small-cell-lung-cancer cells H460 and A549 cells can be inhibited by low concentrations of teroxirone *in vitro* and *in vivo.* Teroxirone-mediated apoptosis is dependent on the status of p53. Transient expression of *p53* activates downstream p21, cytochrome c and caspase-3. The presence of caspase-3 inhibitor reverted apoptotic phenotype. The experimental *in vivo* evidence of growth suppression was also demonstrated in xenograft tumors as established in nude mice. As a potential therapeutic agent capable of restraining cell growth by apoptotic death at low concentrations, teroxirone provided a new perspective as an alternative approach in reversing tumorigenic phenotype of human lung cancer.

P107

Interaction of CD49f and IGF-1R Signaling in Pluripotent Mouse Germline Stem Cells

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Background:

Stem cell niche is known to regulate the self-renew and differentiation in stem cells.This study aims to examine the possible role of CD49f and IGF-1R signaling in Oct-4 expression of mouse GSCs through HIF-2α.

Methods:

CD49f positive and alkaline phosphatase (AP) positive mGSCs were purified by magnetic-activated cell sorting (MACS) from 0~2 days postpartum (dpp) newborn ICR and culture in a laminin-coated culture plates. RNA interference and molecular inhibitors targeting on the IGF-I receptor (IGF-IR)-related signaling were used to examine the role of IGF-IR and its down-stream signaling in Oct4 expression of mGSCs. The protein expressions of the Oct-4 and HIF-2α were examined using western blotting.

Results:

The $CD49f^{+}AP^{+}$GSC cells showed the strong AP activity and expressed pluripotent-related genes including Oct-4, Sox-2, Blimp1, Nanog. IGF-1 dose-dependently increased the expression of HIF-2α as well as the Oct4 in CD49f+AP+GSC cells. Meanwhile, experiments using signal inhibitors such as picropodophyllin (PPP, a specific inhibitor of IGF-IR phosphoylation), LY2940002 (PI3K inhibitor), Rapamycin (mTOR inhibitor) effectively suppressed the IGF-I-induced HIF-2 and Oct-4 expression in GSCs. Furthermore, RNA interference targeting on IGF-IR (shIGF-IR) effectively suppressed both the IGF-I and/or hypoxia-induced HIF-2/Oct4 expression in the $CD49f^{+}AP^{+}$GSCs. Importantly, laminin synergistically cooperated with IGF-IR to stimulate the expression of Oct4 in mouse germline stem cells, highlighting the crosstalk of IGF-I and CD49f in germ cell stemness.

Conclusions:

In summary, our results demonstrated that IGF-1R signaling pathway may cooperate with CD49f to promote Oct-4 expression in pluripotent mouse CD49f+ germline stem cells.

P108

The Protective Roles of Carbonic Anhydrase Related Protein VIII in Human Cells Harboring A8344G Mitochondrial DNA Mutation

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Backgrounds:

Myoclonus epilepsy associated with ragged-red fibers (MERRF) is a mitochondrial maternally inherited disease with pathological features of myoclonus, generalized epilepsy and ataxia. The most common mutation in MERRF syndrome, A8344G mutation in mtDNA, is associated with defects in protein synthesis that affect assembly of complexes in electron transport chain and disrupt mitochondrial oxidative phosphorylation. In the present study, we showed a significant decrease of the carbonic anhydrase-related protein VIII (CA8) in mRNA and protein levels in cybrid cells harboring MERRF A8344G mutation. The biochemical functions of CA8 and it’s role in MERRF syndrome were also examined.

Materials and Methods:

Protein and mRNA expression levels were analyzed by Western blot and RT-PCR. Cells treated with MG132 or 3MA to block proteasome or autophagic activation were used for detection of CA8 degradation pathway. Additionally messenger RNA was blocked by 5,6-dichlororibofuranos ylbenzimidazole (DRB) treatment and mRNA stability was examined. Cells with overexpression or down regulation of CA8 were treated by staurosporine (STS) to assess cell apoptotic ratio. Finally, calcium release assay was performed and the signals were detected by Fura-4AM.

Results:

Even through CA8 was down-regulated in MERRF cybrids harboring A8344G mutations, inhibition of mitochondrial complex I does not influence CA8 expression. Overexpression of CA8 in mutant MERRF cybrids significantly decreased cell death, whereas CA8 knockdown in wild type cybrids significantly increased cell death under staurosporine treatment. To investigate the CA8 degradation pathway, we found that CA8 may be degraded by the autophagic pathway. Furthermore, our results indicated that overexpression of CA8 may up-regulate phospho-Akt and down-regulate Bad protein expression. Interestingly, an increase of LC3-II formation was observed in wild-type cybrids with CA8 knockdown and a decrease of LC3-II was found in mutant cybrids with CA8 overexpression. Moreover, in response to ATP treatment, a significantly increase in cytosolic Ca^{2+} level was observed in cybrids and in CA8 down-regulated cells, as compared to the wild type control.

Conclusion:

In this study, we proposed a specific role of CA8 to protect cells from apoptotic stress through regulation of phospho-Akt and pro-apoptotic signaling pathway involving Bad expression. Furthermore, we demonstrated that CA8 may be involved in regulating the autophagic pathway. Our results also indicated that CA8 may play an important role in regulating Ca^{2+} release to maintain autophagic homeostasis and cell bioenergetics. Regulating CA8 and the autophagic pathway might help develop therapeutic solutions for treatment of MERRF syndrome in the future.

P109

Study of The Therapeutic Efficacy and Mechanism of Action of A Novel HDAC Inhibitor, MPT0B390, On Hormonal Refractory Prostate Cancer

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Backgrounds:

Prostate cancer is a common male malignancy and a frequent cause of mortality worldwide, especially in western countries. Progression of primary prostate cancer to castration-resistant prostate cancer (CRPC) is associated with numerous genetic and epigenetic alterations that are thought to promote survival at metastatic sites. Among, pathogenesis of prostate cancer had been indicated to parallel by aberrant transcriptional regulation which involves gene silencing by histone deacetylase (HDAC). Thus, HDAC inhibitor is the promising anticancer agents for treating prostate cancer.

Materials and Methods:

We recently designed and synthesized a series of novel nitrogen-containing [6,5]-fused heterocyclic as HDAC-targeted agents. Among them, MPT0B390 was identified as a potential lead based on cytotoxic and HDAC inhibitory properties. We proposed that MPT0B390 might exert the potential therapeutic efficacy against prostate cancer growth; especially in hormonal refractory prostate cancer. In order to mimic the hormonal refractory prostate cancer cell, androgen-dependent and androgen independent cells, LNCap 104-S, LNCap 104-R1, and PC3, was used. Cell viability was determined by MTT assay. Western blot analysis was used to determine the effects of MPT0B390 on androgen-related signaling pathways on these cell lines. Cell cycle analysis after treating with MPT0B390 treated by flow cytometry. The MPT0B390-inducede apoptosis was determined by TUNEL assay. The effect of MPT0B390 on xenografted animal model was also performed.

Results:

MPT0B390 was identified as a class I /II HDAC inhibitor by *in vitro* enzymatic activity assay. Comparing the anti-proliferative activity against a panel of prostate cancer cells with SAHA, MPT0B390 is much more potent than that in SAHA (IC_{50} ranging from 0.2 to 0.5 μM *vs* 2 to 4 μM). Treatment of MPT0B390 in PCs arrests cell in Go/G1 phase and increases p21/waf1expression in a concentration-dependent manner. The results of TUNEL assay showed that MPT0B390 induce cell apoptosis in both androgen-dependent and –independent cells in a concentration-dependent manner. In addition, western blot analysis revealed an activation of caspase-8,-9 and -3, and the cleavage of poly(ADP-ribose) polymerase (PARP). Furthermore, MPT0B390 also induced autophagy, as evidenced by increasing LC3-B expression in a concentration-dependent manner. It was well-known that androgen receptor (AR) plays an important role in the prostate cancer progression. Our data showed that the expression levels of both AR protein and mRNA were down-regulated in both androgen-dependent and –independent prostate cancer cells. Importantly, MPT0B390's treatment resulted in shrinkage of PC3 cells tumor growth in xenografted animal model and was superior to the therapeutic effect of SAHA.

Conclusions:

MTP0B390, a new novel class I &II HDAC inhibitor, is active against both androgen-dependent and –independent prostate cancer cells both *in vitro* and *in vivo*. It is worthwhile for further development of this compound.

P110

Role of IGF-1R Signaling in Oct-4 Maintenance of Alkaline Phosphatase Positive Germline Stem Cells

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Backgrounds:

Recent studies using a transgenic mice model have demonstrated that knockout of hypoxia inducible factor 2α (HIF-2α) decreases Oct-4 and the number of migrated primordial germ cell (PGC) in genital ridge. However, the hypoxia-induced endocrinal signals which mediating PGC self-renewal and migration are still unknown.

Materials and Methods:

We have previously established a serum-free culture system to generate pluripotent CD49f/alkaline phosphatase positive germline stem cells (CD49f$^+$ AP$^+$ GSCs) *in vitro* from neonatal mouse testis. The purification of CD49f$^+$ AP$^+$ GSCs was performed in MACS magnetic beads system.

Results:

We found IGF-1 increased HIF-2α and Oct-4 protein expression level in dose-dependent manner. The blockage of PPP (IGF-1R phosphorylation inhibitor), LY294002 (PI3K inhibiter), and Rapamycin (mTOR inhibitor) decreased HIF-2α and Oct-4 protein expression level in both hypoxia and IGF-1 treatment condition. In addition, Knockdown IGF-1R also reduced HIF-2α and Oct-4 protein expression level in both hypoxia and IGF-1 treatment condition. Therefore, we suggested that IGF-1 promoted HIF-2α and Oct-4 protein expression level through IGF-1R/PI3K/Akt/mTOR signal pathway. Interestingly, Knockdown HIF-2α not only decreased Oct-4 protein expression level but also IGF-1R protein expression level. Nevertheless, we found that the treatment of MG132 (proteasome inhibitor) and IGF-1 maintained HIF-2α and Oct-4 protein expression level.

Conclusion:

The current work demonstrates that IGF-1/IGF-1R signaling promoted HIF-2α and Oct-4 protein expression via PI3K/Akt/mTOR signal pathway in CD49f$^+$ AP$^+$ GSCs model.

P111

Detect EGFR Gene Mutations by Combination of Fragment Length Difference and SNaPshot Assays

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The epidermal growth factor receptor (EGFR) belongs to the receptor tyrosine kinase (RTK) family. A mutant EGFR could remain constant phosphorylation which elicits downstream activation and signaling for cell proliferation and even tumor formation. Consequently, mutations of EGFR have been identified in several types of cancer, and it is the target of new anticancer therapies. Anticancer therapeutics directed against EGFR, including gefitinib and erlotinib for lung cancer, and cetuximab for colon cancer have shown better response rate than conventional chemotherapy. In general, EGFR mutations are most commonly observed with deletions of exon19 at frequency of 53.4% and L858R point mutation in exon 21 at frequency of 43.8%. Our study aim is to develop a novel detection method with combination of fragment length difference and SNaPshot assays for these two major EGFR gene mutations. In the present study, the fragment length assay can successfully demonstrate deletions of EGFR exon 19. The SnaPshot assay also showed good capability for detecting the L858R mutation. We have successfully combined two technologies and applied on eight clinical specimens. The preliminary results were fully consistent with the conventional Sanger sequencing result. The efficacy and limit of detection (LOD) of this combined assay will be further defined before applying on clinical specimens.

P112

The Characterizing and Assessments of The Modified fluorinated Cisplatin Analogues on Antitumor Effects

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Backgrounds:

Cisplatin is a common therapeutic anti-cancer drug for patients with several forms of cancers including testicular cancer, colorectal cancer, and lung cancer. Currently, however, it has been revealed that tumors can develop the resistance to cisplatin in clinical. Therefore, to develop new therapeutic replacement of cisplatin is critical and important.

Materials and Methods:

To improve the antitumor effects of cisplatin, the newly synthesized modified cisplatin analogues A and B with fluorinated bipyridine were examined. For comparing to the anti-tumor effects, the commercial cisplatin was used as a control. Four cancer cell lines including HepG2 (Liver hepatocellular carcinoma cells), COLO 205(Human colon adenocarcinoma cells), HT29 (Human colon adenocarcinoma cells) and MCF7 (human breast adenocarcinoma cell line) were investigated. The cell survival rate was determined by manual cell count and the cell death program will be evaluated by apoptotic assay, LDH assay, and DNA fragmentation. The improvement of cisplatin analogues on the anti-cancer effects has been under studying. In the end, the in vivo therapeutic assessment will be performed in the xenograft murine (nude mice) model coupling with IVIS imaging system.

Results:

These two cisplatin analogues A and B display are promising candidates, displaying more powerful cytotoxicity toward cancer cells, comparing to commercial cisplatin. In particular, analogues B shows the most profound cytotoxic effects on the COLO 205 cells with LD50 at 10μM and less than 5% of COLO205 were survived under the concentration of 40μM. Moreover, no resistance was observed.

Conclusion:

The analogues B displays powerful cytotoxicity in colorectal cancers and potentially can replace cisplatin to turn as a good therapeutic candidate for patients with colorectal cancers. We hope this study can generate a new chemotherapeutic drug for cancer patients with an improved outcome and prognosis.

P113

Characterization of Colorectal Cancer Migration-related MicroRNA MiR-338-5p and its Target Gene PIK3C3

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Background:

Colorectal cancer (CRC) has higher metastasis rate. MicroRNA is an epigenetic factor to regulate cell proliferation, tumor cell growth, cancer formation and metastasis by regulating tumor suppresser genes or oncogenes. The objective of this study is to identify miRNAs and target genes related to colorectal cancer migration and metastasis.

Materials and Methods:

Fifty six paired colorectal cancer specimens (tumor and adjacent non-tumor tissue) were collected at National Cheng Kung University hospital. The colon cancer cell lines SW480, SW620, HCT116 and HT29 were used for validation. The migration of colon cancer cell was evaluated by electric cell-substrate impedance-sensing (ECIS) wound healing assay.

Results:

Previously, we used microarray to demonstrate that miR-139-3p and miR-550* were down-regulated and miR-338-5p was up-regulated in recurrent CRC patients. In this study, the expression level of miR-338-5p in tumor tissues of metastatic patients is higher than non metastatic patients (P=0.0203). MiR-338-5p expression level is positively correlated with CRC cells with higher migration activity. High miR-338-5p expression induces the migration of colon cancer cell HCT 116 (P=0.008). Furthermore, miR-338-5p expression level is negatively correlated with its target gene phosphatidylinositol 3-kinase class 3(PIK3C3) in CRC cells. Over expression or down-regulation of miR-338-5p inhibits or increases PIK3C3 mRNA and protein expression in CRC cells HCT116. MiR-338-5p may further block autophagy through inhibition of PIK3C3. Furthermore, autophagy inhibits colon cancer cell HCT-116 migration after rapamycin treatment (P=0.0439).

Conclusion:

Collectively, MiR-338-5p is a potential microRNA related to migration of CRC. MiR-338-5p induces CRC cell migration through suppressing PIK3C3 expression and autophagy.

P114

Evaluation of Antioxidant Capacity with Aqueous Extract of Antrodia camphorate, Acxemonium implicatun and Rhodiola crenulata

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Backgrounds:

Previous study found that cancer, diabetes, Alzheimer's disease and cardiovascular disease had a close relationship with free radicals. In addition to the person's normal oxidative metabolism, there are many causes of the radicals, as well as some external factors, such as environmental pollution, sunlight, radiation, cigarettes, drugs, alcohol, viruses, parasites and fat food. Therefore, more intake of free radical scavenging antioxidants had a great help in slow the effects of aging, prevent cancer and protect the heart. In this study, a comparison with three aqueous extracts of Antrodia camphorate (ACAE), Acxemonium implicatun (AIAE) and Rhodiola crenulata (RCAE) is to investigate in the antioxidant capacity analysis including removal of H_2O_2 and O_3, and anti-oxidation for oleic acid.

Materials and Methods:

10 g of Antrodia camphorate, Acxemonium implicatun and Rhodiola crenulata powders was extracted with 200 ml d.d. H_2O in a 100 heated water bath for 6 hours. This cold extract was centrifuged and lyophilized. A crude brownish sample was weighted and resolved in PBS. Its recovery was about 63.12~70.58%. After centrifugation at 10,000xg for 30 min, the resolute was filtrated through a Millipore filter (0.2μm) and stored at 4 for the assay listed below. The aim of this study:(1) Analysis of the clearance rate of H_2O_2 with ACAE, AIAE and RCAE. (2) Analysis of the clearance rate of O_3 with ACAE, AIAE and RCAE. (3) Measure of the antioxidant capacity of ACAE, AIAE and RCAE to oleic acid.

Results:

Results showed that 5 μg /ml of ACAE, AIAE and RCAE induced increase16-48% in the clearance rate of H_2O_2, and RCAE expressed a best performance. 8 μg / ml of ACAE, AIAE and RCAE enhanced 14-40% in the clearance rate of O_3, and RCAE was also the most efficient. In 40 μg / ml of those extracts, revealed more than 40% increase in the anti-oxidation for oleic acid, and RCAE was still effectiveness.

Conclusion:

Data suggested that the aqueous extract of those three Chinese drugs had an antioxidant capacity, especially of *Rhodiola crenulata*. Furthermore, *Rhodiola crenulata* acts a most potential role as a free radical scavenging effect and enhance the antioxidant function for development of medical drugs and health food, and can be used in future research.

P115

Expression of Acid-sensing Ion Channels in the Amygdala

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Background:

Despite the high prevalence of fear and anxiety disorders, the pathogenic mechanisms and the underlying fear circuitry are incompletely understood. Accumulating evidence indicates that ASICs in the amygdala are essential for fear learning and memory. However, how exactly ASIC1a within the intricate amygdala circuitry contributes to conditioned fear memory is unclear. There are diverse neuronal populations in the fear circuitry. To better understand the role of ASICs in the fear learning and memory, it is essential to know the functional expression of ASICs in different cell types of amygdala.

Material and Method:

Acute amygdala slices of 300 μm thickness from the brains of 1 to 3-month-old C57BL/6 wild-type or GAD67-GFP mice were used to investigate the ASIC expression in fear circuitry. Following identification of cell types based on electrophysiological properties, ASIC currents were evoked by fast application of proton and measured in nucleated patch recording configuration. To further correlate the ASIC currents with gene expression profile, expression of ASIC1a, ASIC2a and ASIC2b subunit mRNAs of each neuron type was analyzed by single-cell reverse transcription (RT)-PCR.

Result:

Previous reports (Ziemann et al., 2008; Weng et al., 2010) showed that ASICs are differentially expressed in distinct types of neurons in the hippocampus. In contrast, ASIC expression in the amygdala is relatively homogeneous regardless of cell types. Furthermore, properties of ASIC currents in different types of amygdala neurons are also very similar, suggesting the same subunit composition of ASICs in different types of the amygdala neurons.

Conclusion:

Overall, ASIC density and subunit composition appear to be homogenous across entire populations in the amygdala, suggesting the role of ASIC in fear circuitry is different from other circuitries.

P116

Different effects of Nervous necrosis virus quasispecies coat proteins on grouper cells.

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We found different quasispecies of nodavirus derived from chronical nodavirus-infected grouper. To investigate the role of these variant CPs in pathogenesis, six variant CP expression plasmids were constructed and transiently transfected into grouper GF-1 cells. At different times, the cell cycle and cell proliferation were assayed using flow cytometry and methyl thiazolyl tetrazolium (MTT) assays, respectively. The proportion of G_2/M-phase GF-1 cells transfected with CP expression plasmids was higher than that of cells transfected with the blank plasmid, especially in regards to quasispecies 2 (QS2). The proliferation ratio of cells transfected with the CP expression plasmids was significantly higher than that of cells transfected with the blank plasmid, with the exception of QS6. We also found that the different quasispecies CPs downregulated the promoter activity of the tumor necrosis factor (TNF) gene to different degrees. Overall, this study represents the first comprehensive analysis of variant CPs from grouper with persistent nodavirus infections and their effects on different aspects of pathogenesis.

Keyword:

quasispecies, nodavirus

P117

The Study of Efficacy and Safety of Recombinant Toxin Subunit Vaccines Against Swine Progressive Atrophic Rhinitis

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Backgrounds:

Progressive atrophic rhinitis (PAR) is an important upper respiratory disease in swine. The clinical symptoms of PAR are characterized by degeneration nasal turbinate atrophy, twisting of the snout and growth retardation, which cause economic loss. Previous studies have shown that PAR is caused by toxigenic Pasteurella multocida (P. multocida) and virulent Bordetella bronchiseptica strains. P. multocida toxin (PMT) produced from P. multocida is the major virulence factor of the PAR in swine. In 1991, Petersen et al. have reported that recombinant PMT derivatives induce effective protection against P. multocida infection. Therefore, PMT has been considered as a good candidate for subunit vaccine development. The efficacy of a subunit vaccine is determined by immunogenic epitopes.

Materials and Methods:

Several PMT fragments designed by computer software were fused with molecular adjuvant as novel plasmids. Novel antigens were highly expressed in E. Coli expression system. The protective efficacy of novel vaccines upon toxin challenge was examined in a mouse animal model. Enzyme-linked immunosorbent (ELISA) and neutralizing antibody assay were carried out for the measurement of anti-PMT antibody and neutralizing antibody titer. In addition, mice and guinea pigs were used for safety assay.

Results:

The results revealed that this novel subunit toxin vaccine protects mice from toxin challenge and the survival rate increased significantly when compared to the control group. Moreover, high anti-PMT antibody and neutralizing antibody titers are detected post-vaccination, which indicates that the novel vaccine induces humoral immune responses. This vaccine is also harmless to mice and guinea pigs in safety assay.

Conclusion:

This novel recombinant toxin subunit vaccine shows highly potential to against progressive atrophic rhinitis in swine.

P118

Irisflorentin Attenuates The Maturation and Function of Mouse Bone Marrow-Derived Dendritic Cells through Blocking of IKK/NF-κB and MAPK Activities

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Backgrounds:

Dendritic cells (DCs) are key antigen presenting cells in the immune system. One active field of study is the handling of DCs as pharmacological targets to descovery novel biological modifiers for the treatment of inflammatory and autoimmune diseases.

Materials and Methods:

Irisflorentin (IFT), an isoflavone component derived from the roots of *Belamcanda chinensis* (L.) herbs which have been used for the treatment of inflammatory diseases in traditional Chinese medicine. In this study, we tested the potential of IFT to modulate lipopolysaccharide (LPS)-stimulated activation of mouse bone marrow-derived DCs.

Results:

Our results revealed that treatment with up to 10 μM IFT does not cause cytotoxicity in cells. IFT inhibited the production of TNF-α, IL-6, and IL-12p70 by LPS-stimulated DCs. The expression of LPS-induced MHC class II, CD40, and CD86 on DCs was also decreased by IFT, and the endocytic capacity of LPS-stimulated DCs was restored by IFT. In addition, LPS-stimulated DC-elicited allogeneic T-cell proliferation was blocked by IFT, and the migratory ability of LPS-stimulated DCs was reduced by IFT. Moreover, our results confirmed that IFT impairs the responses of LPS-stimulated activation of DCs through suppression of IκB kinase and mitogen-activated protein kinase activities. Coadministration of IFT with 2,4-dinitro-1-fluorobenzene prevented 2,4-dinitro-1-fluorobenzene-induced delayed-type hypersensitivity.

Conclusion:

IFT may be a new potent immunosuppressive agent and could be used in the prevention and treatment of inflammation, and autoimmunity through the blockage of DC maturation and function.

P119

Investigating the roles of Influenza A virus NEP protein and its functional interaction with the F1α/β subunits of mitochondria ATP synthase for viral egression

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Background:

Influenza A virus (IAV) is an enveloped virus with a genome made up of 8 segmented RNAs, which encode 11 viral proteins. The IAV Nuclear Exporting Protein (NEP) has been reported to involve in virus ribonucleoprotein (vRNP) nuclear export through Nuclear Export Signal (NES)-independent interaction with hCRM1, and can negatively regulate viral genome transcription/ replication. However, the functional relevance of NEP in virus life cycle remains largely unknown. Our previous results demonstrated that NEP interacts with mitochondria ATP synthase *in vivo* and may enter into mitochondria. Interestingly, while NEP affected the cellular ATP level and type-I IFN response, it did not induce apoptosis, cause cellular Reactive Oxygen Species (ROS), and disrupt mitochondria membrane potential. Because IAV, unlike many RNA viruses, lacks self-equipped motors similar to the α subunit of F1 to pack its genomes, we surmise that NEP is not only a multifunctional protein but also has a role in viral egression by interacting with the F1α/β ATP synthase. In this study, we report the *in vivo* and *in vitro* interaction relationship between NEP and the subunits of ATP synthase F1α and F1β, and aim to identify their interaction domain(s) critical for their functional association and viral egression.

Materials and Methods:

E.coli expression plasmids expressing truncated ATP synthase F1α or β domain in 3 different lengths with 6xHis tagged at the N-terminus were constructed to map the NEP-interacting domain(s) of ATP synthase. These plasmids were transformed into BL21(DE3) and the respective recombinant proteins were purified to study their interaction site(s) with a recombinant GST-tagged NEP by a GST pull-down assay.

Results:

We have confirmed our previous result that NEP affected the cellular ATP level and type-I IFN response, but did not induce apoptosis, cause cellular Reactive Oxygen Species (ROS), and disrupt mitochondria membrane potential. We also found that the *in vivo* interaction of ATP synthase with NEP requires RNA in the association, because the treatment of RNAse disrupts the interaction. Functional characterization of their association in IAV infectivity may be important to understand the role of ATPsynthase in viral egression.

Conclusion:

This study provides a significant insight on the roles of IAV NEP protein and its functional interaction with mitochondria ATP synthase α or β in IAV egression. Identification of the interactive domain(s) of the ATP synthase α or β subunits to NEP-RNA complex may help to design competitive peptides for therapeutic application.

P120

Cytoprotective Effect of Fisetin on the Hypoxia-induced Cell Death in PC12 Cells

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Backgrounds:

Hypoxia plays a major role on the promotion of neuronal cell damage in stroke, heart failure and neurodegenerative diseases. Therefore, cell adaptation to hypoxia is an effective way for neuroprotection. Fisetin (3,7,3,4-tetrahydroxyflavone), a flavonoid widely distributed in fruits and vegetables, is known to exhibit several biological activity including neuroprotection. In this study, we aim to investigate the neuroprotective effect and mechanism of fisetin against hypoxia-induced cell death in PC12 cells.

Materials and Methods:

PC12 cells were treated with cobalt chloride ($CoCl_2$), which serves as a hypoxia-induced agent, and cell survival was examined by MTT assay. The effect of fisetin on the ROS scavenging was performed in the flow cytometry. The quantitative real-time PCR and Western blot analysis were used to determine the expression of HIF-1α and the signaling molecules involved in the fisetin-mediated cytoprotection in PC12 cells.

Results:

We found that fisetin could significantly rescue $CoCl_2$-induced cell death in PC12 cells. It has been reported that $CoCl_2$ induces reactive oxygen species (ROS) and leads to cell death. However, our flow cytometric result showed that ROS scavenging was not involved in the fisetin-mediated cytoprotection. Hypoxia induced factor 1 alpha (HIF-1α) is a known protein against hypoxia; we next investigated the effect of fisetin on HIF-1α expression. The quantitative real-time PCR and Western blot analysis showed that fisetin promoted the HIF-1α gene expression. This result implied that HIF-1α might contribute to fisetin-mediated cytoprotection of PC12 cells. Furthermore, to investigate which signaling pathways are involved in the fisetin-mediated neuroprotection, the selectively inhibitors for specific kinase including MAPK/ERK 1/2 (MEK1/2), JNK, p38 MAPK and PI3/Akt were used. Our data showed that the protective effect of fisetin was abolished by inhibition of MAPK/ERK-, p38-, and PI3/Akt-dependent pathways in PC12 cells. Further investigation on the activation of these pathways by fisetin and their roles on the fisetin-mediated cytoprotection of PC12 cells are in progress.

Conclusion:

In conclusion, HIF-1α may contribute to fisetin-mediated neuroprotection in PC12 cells. Furthermore, modulation of signaling pathways such as MAPK are involved in channeling the fisetin stimulus for cell survival against hypoxia insults.

P121

Purification and Characterization of Cellulases from *Lentinus edodes*

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Backgrounds:

Tremendous amounts of various lignocellulosic substrates and other wastes produced from agricultural, forest, and food-processing industries have been used for the cultivation of edible mushrooms. In this study, we investigate the protein components in the waste substrate of cultivated mushrooms (*Lentinus edodes*).

Materials and Methods:

The major protein constituents in aqueous extract of sawdust waste are purified by DEAE-Sepharose and phenyl-Sepharose chromatographies. Homogeneity of individual purified protein is examined by electrophoresis analysis and further identified by MALDI-TOF and MS/MS analysis. Cellulase activity of purified proteins is prescreened on a carboxymethyl-cellulose (CMC) agar plate followed by staining with Congo red solution. The properties of cellulase activity are investigated by changing enzymatic conditions. Specificity of cellulase activity is further characterized by subjecting enzymatic reaction products to MALDI-TOF analysis.

Results:

Four major functionally unknown proteins revealed by electrophoresis analysis in aqueous extract are purified to apparent homogeneity using DEAE-Sepharose and phenyl-Sepharose chromatographies. Based upon the sequential order of elution from DEAE-Sepharose column, these proteins are termed Led-A, -B, -C, and -D, respectively. MALDI-TOF and MS/MS analysis identifies Led-A as an exo-β-1,3-glucanase with molecular mass of 80 kDa, and the other three Led proteins to be putative fungal cellulases. The putative cellulase activity is subsequently detected in both Led-C and -D proteins spotted on a CMC plate, but not observed in either Led-A or -B protein spots. MALDI-TOF analysis depicts that both Led-C and -D proteins act as endo-cellulases. They can hydrolyze CMC to release oligosaccharides composed of 3-6 glucose residues.

Conclusion:

We have purified four major enzymes from the sawdust waste of *Lentinus edodes* cultivation substrate. Led-C and -D proteins, with molecular mass of 29.6 and 26.3 kDa respectively, are confirmed to have endo-cellulase activity, cleaving CMC at every 3-6 glucose residues.

P122

Inhibition of CD24, CD44, CD133 expression and sphere formation by HOXA9 silence in human gastric cancer cells

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Background:

Epidemiological studies report that gastric cancer is one of the most common cancers worldwide, and is also the second leading cause of cancer-related mortality. The poor prognosis of gastric cancer may be partly attributed to the complicated molecular networks operating the aggressiveness of gastric cancer. Although a large body of studies has revealed the deregulation of certain genes in gastric carcinogenesis, the molecular mechanisms behind gastric tumor development are not yet fully understood.

Method and Material:

We measured the homeobox A9 (HOXA9) protein level in human gastric cell lines: normal cell lines (CSN) and cancer cell lines (AGS, CS12, NCI-N87 and MKN45). We applied the HOXA9 short haipinRNA (shRNA) to successfully knockdown the expression of HOXA9 protein and subsequently explore the role of HOXA9 in human gastric cancer cells. We measured the cell growth, the capacity of sphere formation, and the expression of CD24, CD44 and CD133.

Result:

In the present study, we found that HOXA9 is over-expressed in human gastric cancer cells CS12, N87 and MKN45. We further found that HOXA9 silencing reduces cell growth in human CS12 gastric cancer cells. The capacity of sphere formation of in CS12 and MKN45 cells are inhibited when expressing low HOXA9 protein level. We observed that HOXA9 knockdown inhibits the expression of cancer stem cell candidate makers CD24, CD44 and CD133 in CS12 (HOXA9 shRNA) and MKN45 (HOXA9 shRNA) cells.

Conclusion:

HOXA9 protein is over-expressed in malignant gastric cancer cells including CS12, N87 and MKN45. HOXA9 may play an important role in regulating the expression of cancer stem cell markers (CD24, CD44 and CD133), the cellular growth and the capacity of sphere formation in human gastric cancer cells.

P123

Impaired Antiviral Response due to Defective Redox Signaling upon HCoV 229E Infection in G6PD-knockdown A549 Cells

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Backgrounds:

Glucose-6-phosphate dehydrogenase (G6PD) provides NADPH as reducing power to meet the cellular needs for reductive biosynthesis and maintenance of the cellular redox homeostasis. Previously, G6PD-deficient cells have been reported to be more susceptible to viral infection comparing to normal counterparts. However, the mechanism of enhanced susceptibility to viral infection in G6PD-knockdown cells remains elusive.

Materials and methods:

The expression levels of mRNA and protein were measured by RT-PCR and Western blot, respectively. TNF-α secretion was detected by ELISA. NF-kB activity was analyzed by EMSA.

Results:

Toward this end, we analyzed the antiviral response upon human coronavirus 229E (HCoV 229E) infection in A549 and MRC-5 cells and found that the expression of TNF-α (*Tumor necrosis factor- alpha*), *OAS* (2'-5'-oligoadenylate synthetase) and *MX-1 (myxovirus resistance 1*) genes were transiently up-regulated at the early stage of viral infection. Further investigation found that the expression of *OAS* and *MX-1* upon HCoV 229E infection in G6PD-knockdown A549 cells was moderately but significantly decreased in G6PD-knockdown cells. In addition, the expression of antiviral cytokine, TNF-α, which was transiently up-regulated at 2 hour post-infection (p.i.), was lower in G6PD-knockdown than in control cells. Moreover, the reduced antiviral responses in G6PD-knockdown cells could be restored by complementation of G6PD activity. Knockdown of TNF-α expression by siRNA in A549 cells increased HCoV 229E replication. Still more, reduced IκB degradation and decreased NF-κB activation were observed in G6PD-knockdown A549 cells when compared with that of control. Furthermore, HSCARG protein, a NADPH sensor and NF-κB activation modulator, was up-regulated in G6PD-knockdown A549 cells leading to impaired antiviral gene expression.

Conclusions:

All in all, these data support the notion that enhanced susceptibility to viral infection in G6PD-knockdown cells is due to impaired NF-κB signaling as a consequence of redox imbalance-induced up-regulation of HSCARG expression and thus adversely affects the antiviral response.

P124

Immunization of Dengue DNA Vaccines Expressing Envelope Protein Domain III Elicits Neutralizing Antibodies and Specific T-cell Responses

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Background:

Dengue is a mosquito-borne viral disease which spreads widely in the world and there is no vaccine is available. The envelope (E) glycoprotein of dengue virus (DV) is the major antigen for protective immunity and its domainIII (EDIII) is the main target for the induction of neutralizing antibodies. We developed a DNA vaccine containing EDIII of four serotypes and investigated its immunogenicity against dengue virus.

Materials and Methods:

We constructed two DNA plasmids-pD13 and pD24, which contains the two EDIII of four dengue serotypes and fused with a signal peptide for secretory pathway. Grouped BALB/c mice received three times with 10μg or 100μg of equal amount of pD13 and pD24 or 100μg of vector control by intramuscularly injection. Sera samples were tested for the presence of specific antibodies by ELISA. To reveal T cell immune responses, production of IFN-γ and IL-4 were measured by ELISPOT assay. Neutralizing antibody titers of the sera were also detected by plaque reduction assay.

Results:

After immunization, the EDIII-specific antibody response was induced in dengue DNA vaccine immunized mice but not vector control. The neutralizing antibody was also detected in DNA vaccine immunized mice and demonstrated protective titer against all DV except DV-4. For T cell response, that only IFN-γ but not IL-4 detected in dengue DNA vaccine immunized mice suggested a Th1 response was elicited. The strength of IFN-γ production was dose-dependent on the amount of DNA plasmids. Interestingly, a serotype-specific IFN-γ response correlated to the neutralizing activity was noticed that DV-4 specific T-cell response was the lowest.

Conclusion:

The EDIII based DNA vaccine could enhance both T and B cell responses including a protective neutralizing antibody response. These results indicate that this DNA vaccine has the potential to develop as a new generation of vaccines against DV.

P125

The Autophagic Effect of Justicidin A, A Purified Compound from *Justicia procumbens*, and Its Role on Apoptosis in Human Hepatocellular Carcinoma Cells.

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Backgrounds:

Our previous published report (FEBS Letter 580:3185-3191, 2006) indicated that Justicidin A (JA) induced apoptosis of human hepatocellular carcinoma (HCC) cells *in vitro* and *in vivo* via caspase 8 and mitochondria-related pathway. The present study was proposed to investigate the autophagic effect of JA in HCC and to find how the autophagic effect influences the process of JA-induced apoptosis.

Materials and Methods:

HCC Hep 3B cells were treated with JA. The markers of autophagy, LC3-II and p62, and autophagy-related signaling molecules were determined using Western blot. As autophagy was induced, the acidic vesicular organelles (AVOs) were produced in cells which can be stained with acridine orange (AO) and analyzed by flow cytometry. To further clarify the autophagy effect on process of apoptosis, Hep 3B cells were pretreated with the autophagy inhibitor Bafilomycin A1 (BAF), and then stained with propidium iodide (PI) followed by flow cytometry to determined the percentages of cells at the sub-G1 phase, representing the proportion of apoptotic cells.

Results:

By Western blot analysis, the expressions of LC3-II and p62 increased but that of Akt decreased as the incubation time increased with JA, suggesting that JA induced autophagy of HCC Hep 3B cells. JA also increased the percentages of AO-positive cells in a time- and dose-related manner, confirming JA induced autophagy. In BAF-pretreated Hep 3B cells, JA elevated the percentages of cells at sub-G1 phase and raised the expression of cleaved caspase 3, indicating that the use of autophagy inhibitor promoted JA-induced apoptosis.

Conclusion:

JA induced autophagy of HCC Hep 3B cells characterized by increase of AVOs-positive cells and induction of LC3-II and p62 expression. Administration of autophagy inhibitor further increased the process of apoptosis, suggesting that JA-induced autophagy decreased JA-induced apoptosis. Combination of autophagy inhibitor with JA is suggested to optimize the anticancer effect of JA in HCC.

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P126

The function of HLTF and SHPRH in the drug resistant phenotype of nasopharyngeal carcinoma

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Backgrounds:

Post-replication repair (PRR) plays an important role in bypassing DNA lesions during DNA replication. One pathway of PRR involves the K63-linked polyubiquitination of PCNA mediated by the E3 ubiquitin ligases HLTF and SHPRH that allow bypass of DNA lesions by the template-switching mechanism. We recently discovered that HLTF and SHPRH are highly expressed in some lung cancer tissues and cisplatin resistant nasopharyngeal carcinoma (NPC) cell lines, indicating the importance of HLTF and SHPRH in the lung cancer development and drug resistant phenotype.

Materials and Methods:

We used lung cancer samples including six lung adenocarcinomas ranging from T1 to T4 stages and two lung squamous cell carcinomas ranging from T1 to T2. These lung cancer and control tissues from same patients were collected from Thoracic Oncology Team at NCKU Hospital. The mRNA was isolated from these samples and the expression level of genes was determined by qRT-PCR. In addition, the cisplatin sensitive and resistant NPC cell lines were also used in this study.

Results:

Our results demonstrate that HLTF and SHPRH are highly expressed in lung cancer tissues and cisplatin resistant NPC cells. The cisplatin treatment can induce DNA damage response both in cisplatin sensitive and resistant NPC cells. However, cisplatin resistant NPC can progress through cell cycle, while the cisplatin sensitive NPC cells are arrest in S-phase after the cisplatin treatment.

Conclusion:

Our results suggest that cisplatin resistant NPC cells enhance the PRR pathway, thus conferring the NPC cells resistant to cisplatin. Since HLTF and SHPRH are also highly expressed in some lung cancer cells, our results indicate the importance of HLTF and SHPRH during the cancer development.

P127

UVA-Activated Photosensitizer 2-(4-aminophenyl)-7-methoxybenzothiazole Suppresses Proliferation and Induces Apoptosis in Keloid Fibroblasts: a Potential Therapy for Keloids

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Background:

Photodynamic therapy (PDT), employing a photosensitizer and a specific wavelength of light, is an emerging therapeutic method for treating neoplastic and non-neoplastic diseases. Keloids are fibroproliferative dermal lesions characterized by the proliferation of fibroblasts. Recently, PDT has been proven to be a potential modality in treating keloids. The aim of this study was to investigate the effects of our newly synthesized photosensitizer namely 2-(4-aminophenyl)-7-methoxybenzothiazole (6d), plus UVA irradiation (UVA-activated 6d) on proliferation and apoptosis in keloid fibroblasts.

Methods:

Fibroblasts cultured from healthy adult human foreskins and keloids were treated with UVA-activated 6d. The influence on cell viability and proliferation were assessed by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay and BrdU incorporation assay, respectively. The uptake of 6d in keloid fibroblasts was evaluated by immunofluorescence assay. Flow cytometry was performed to determine cell cycle distribution, intracellular ROS production, mitochondrial membrane potential (ΔΨm) change, and active caspase-3 expression.

Results:

Our results demonstrated that the combinations of 6d 2.0 μM and 5.0 μM plus UVA 0.5 J/cm2 irradiation treatments, which did not significantly influence the viability of normal fibroblasts, significantly decreased the viability and proliferation in keloid fibroblasts. Cell cycle analysis showed significant G0/G1 arrest and increased sub-G1 proportion in keloid fibroblasts treated with UVA-activated 6d. UVA-activated 6d treatment significantly induced intracellular ROS generation. The loss of mitochondrial membrane potential (ΔΨm) and increased active caspase-3 expression were noticed in keloid fibroblasts after UVA-activated 6d treatment. Moreover, the increased active caspase-3 expressions in keloid fibroblasts treated with UVA-activated 5.0 μM 6d was inhibited by N-Acetyl-L-cysteine (ROS scavenger) pretreatment, implying the involvement of ROS on the induction of apoptosis.

Conclusion:

The present data indicated that UVA-activated 6d treatment exerted anti-proliferative

P128

The pregnancy outcomes for human embryos cultured in single step medium and sequential medium

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Backgrounds:

The objective of this study was to examine the effects of different culture systems on the development of early human embryos in vitro. The percentage of cleavage and the morphological appearance of embryos were recorded daily for 72 hours in each system using an inverted phase-contrast microscope.

Materials and Methods:

A total of 210 patients were included and was divided into two groups in this prospective randomized study during the time period from May 2012 to October 2012. All of the selected embryos had a good outcome as determined by postinsemination parameters (at least one two-pronuclei [2PN] embryos were available). In this study, two culture systems were compared: one was single step medium (Global medium) and the other was sequential medium (Q1 medium), each embryo was culture from day 1 to 3. We compared the effects of these two culture systems on embryo quality, and pregnancy rate.

Results:

Results of single step medium (Global medium) and sequential medium (Q1 medium), respectively were fertilization (75.42% vs. 77.7%) and Day 2 cleavage (98.74% vs. 99.1%) rates were not different, Day 3 cleavage (99.84% vs. 99.85%) rates were not different too. On Day 3, a significantly better result of single step medium than sequential medium embryos had ≧ 6 cells (77.64% vs. 70.39%, P<0.001) was noted. But pregnancy rate (43.93% vs. 47.57%) were not different.

Conclusion:

Assessing the degree of compaction can be a valuable addition to traditional morphologic

assessment in identifying optimal embryos for transfer on day 3. Routine morphologic assessment on day 3 includes cell number, extent of fragmentation, and degree of asymmetry , all of which have been shown to be correlated with pregnancy rates after day 3 transfer.

In conclusion, in combination with traditional morphologic assessment of cell number and percent fragmentation, the present results indicate that early compaction can be a valuable tool in selecting which ≧ 6-cell embryos to transfer on day 3. In embryos with ≧ 6 cells and with minimal fragmentation, early compaction portends a favorable prognosis, whereas in embryos with ≧ 6 cells and with a higher degree of fragmentation, compaction is negatively associated with implantation potential.

P129
Targeted Drug Delivery Systems Mediated by A Novel Peptide in Breast Cancer Therapy and Imaging

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Backgrounds:

Targeted delivery of drugs to tumors represents a significant advance in cancer diagnosis and therapy. Therefore, development of novel tumor-specific ligands or pharmaceutical nanocarriers is highly desirable.

Materials and Methods:

In this study, we utilized phage display to identify a new targeting peptide, SP90, which specifically binds to breast cancer cells, and recognizes tumor tissues from breast cancer patients.

Results:

We used confocal and electron microscopy to reveal that conjugation of SP90 with liposomes enables efficient delivery of drugs into cancer cells through endocytosis. Furthermore, in vivo fluorescent imaging demonstrated that SP90-conjugated quantum dots possess tumor-targeting properties. In tumor xenograft and orthotopic models, SP90-conjugated liposomal doxorubicin was found to improve the therapeutic index of the chemotherapeutic drug by selectively increasing its accumulation in tumors.

Conclusion:

We conclude that the targeting peptide SP90 has significant potential in improving the clinical benefits of chemotherapy in the treatment and the diagnosis of breast cancer.

P130
An Methylarginine-containing RNA Binding Protein SERBP1 is Related with Stress Granules Formation

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Backgrounds:

Stress granules are cytoplasmic ribonucleoprotein granules formed following various stresses that inhibit translation. They contain messenger RNA, small ribosomal subunits, eukaryotic initiation factors, and a large variety of RNA binding proteins (RBPs). Some of these RBPs are methylarginine-containing proteins such as FMRP. Arginine methylation by protein arginine N-methyltransferases (PRMTs) has been shown to be involved in modulating the subcellular localization or protein-protein/ protein-RNA interactions. SERBP1 (SERPINE1 mRNA binding protein 1) is an RNA binding protein that can be methylated by PRMT1 and the modification affects protein interaction and intracellular localization. It was also found in stress granules.

Materials & Methods:

In the present study we determined the localization of SERBP1 in stress granules under oxidative stress by immunofluorescent analyses. We also examined the interaction of SERBP1 with stress granule-associated proteins by co-immunoprecipitation.

Results:

Immunofluorescent analyses showed that full-length and truncated SERBP1 proteins co-localized with TIA-1, a typical stress granule marker, in the cytoplasic granule after arsenite treatment. Treatment of a methylation inhibitor adenosine periodate (AdOx) delayed the disassembly of SERBP1-associated stress granules. Co-immunoprecipitation analyses indicated that SERBP1 interacts with some stress granule-associated proteins, such as FMRP and hnRNPA1. The interaction is RNA-dependent.

Conclusion:

SERBP1 is a stress granule-associated protein and arginine methylation can affect the disassembly of SERBP1-associated stress granules It interacts with some arginine methylated and stress granule-associated proteins in an RNA-dependent manner. Whether arginine methylation of SERBP1 might be related to the function of stress granules requires further studies.

P131
Study the Effects of Berberine on Lipopolyssacharide (LPS)-Mediated in Interleukin-1 beta Production in Macrophages

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Backgrounds:

The Toll-like receptors (TLRs) recognize the hightly conserved, pathogen-expressed molecules and play a critical role in innate immunity. As the key players in innate immune system, macrophages can recognize lipopolysaccharide (LPS) via TLR4. It is well established that LPS is one of the pathogen-associated molecular patterns (PAMPs) that can trigger TLR4 activation and the generaton of proinflammatory cytokines such as interleukin-1 beta (IL-1β). As an isoquinoline alkaloid present in numerous plants of the generation Berberis and Coptis, berberine has a wide range of pharmacologic actions including anti-inflammation, anti-cardiovascular diseases and anti-diarrhea. Previously, we have demonstrated that berberine impaired LPS-elicited macrophage locomotion by suppression of the expression of Src as well as the activity of Src and FAK.

Materials and Methods:

The murine macrophage cell line, Raw264.7 (American Type Culture Collection), the cells lysates were resolved on 8% SDS-polyacrylamide gels, transferred to nitrocellulose membranes and probed with respective antibodies followed by horseradish peroxidase (HRP)-conjugated protein A or HRP-conjugated rabbit anti-mouse IgG and detected by Enhanced Chemiluminescence method (Amersham) (Rockford, IL, USA).

Results:

Berberine treatment reduced the level of total tyrosyl phosphorylated proteins in LPS-stimulated macrophage. And LPS-elicited Src induction was berberine sensitive, then I find when berberine treatment reduced the level of caspase-1 in LPS-stimulated macrophage.

Conclusion:

Here, I would interested to study the effect of berberine on LPS-mediated IL-1β production and its underlying mechanisms.

P132
Activation of PI3K Pathway by P-Rex2 is Inhibited by GNMT

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Glycine N-methyltransferase (GNMT) is a tumor suppressor for hepatocellular carcinoma (HCC). Previously, we reported that GNMT regulates mTOR signaling pathway through interacting with an mTOR binding protein-DEPTOR. Sequence blasting and phylogenetic analysis demonstrated that the first DEP domain of DEPTOR clustered with P-Rex2, a regulator of the small guanosine triphosphatase Rac. Since P-Rex2 has been showed to be able to antagonize PTEN in PI3K pathway, the aims of this study was to analyze the interaction between GNMT and P-Rex2 and their association in PI3K-Akt signaling pathway. Gel filtration assay demonstrated that GNMT and P-Rex2 were eluted in the same fractions. Reciprocal co-immunoprecipitation experiments demonstrated that GNMT interacts with different domains of P-Rex2 including DH, PH, PDZ and InsPx4-Phosphatase. Overexpression of P-Rex2 enhanced Akt activation in Huh7 cells and such effect was blocked by over-expression of GNMT. Pulse-chase experiments showed that overexpression of GNMT caused significant reduction of the half-life of P-Rex2 from 15 hr to 9.5 hr. In addition, overexpression of GNMT resulted in a proteasome-dependent degradation and ubiquitination of P-Rex2. By screening a panel of E3 ubiquitin ligases, we found an E3 ligase that can enhance the ubiquitination of P-Rex2. Therefore, these results suggested that GNMT could affect PI3K-Akt pathway via binding with P-REX2 and enhance its degradation.

P133
The Role of KDEL Motif of GRP78 in JEV Life Cycle

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Background:

Recent evidences showed the co-migration of glucose regulation protein 78 (GRP78) with Japanese Encephalitis Virus (JEV) virion and it co-opted the assembly of viral particles during the viral life cycle. It has also been known that the C-terminal tetra-peptide, KDEL, was able to prevent GRP78 secretion and maintain it within the ER lumen. Since the components of KDEL in GRP78 is a key factor for its subcellular localization, in this study, we investigate the role of KDEL motif of GRP78 in JEV infected cells.

Materials and Methods:

Using Immunoblotting to compare the KDEL motif of GRP78 deleted in JEV and ER stress secretion medium, and the protein-protein interactions between GRP78 and JEV viral proteins will be examined by co-immunoprecipitaion. Finally, overexpression of KDEL-truncated GRP78 in the GRP78-silenced cells to verify the role of KDEL motif in GRP78 involved in JEV life cycle.

Results:

Immunoblotting result showed the JEV-induced extracellular GRP78 does not contain the KDEL motif using anti-KDEL specific antibody, indicating that deletion of KDEL motif assist the release of GRP78 upon JEV infection. The detection was also been observed when the cell was treated with tunicamycin (TM), an ER stress inducer, revealing that deletion of KDEL motif is required for its transportation from ER to cell surface or even release outside of cell. Overexpression of KDEL-truncated GRP78 in the GRP78-silenced cells resulting in no effect on the viral RNA replication, and Immunoblotting result showed the condition is similar to the production of viral NS5 protein.

Conclusion:

Altogether, these results show that deletion of KDEL motif for GRP78 trafficking from ER to outside of cell is required upon JE viral infection. And overexpression of KDEL-truncated GRP78 in the GRP78-silenced cells increasing the viral replication and the production of viral NS5 protein, similar to endogeneity GRP78, implying this motif is not involved in not only viral replication but also the production of viral NS5 protein.

P134
The application of Antrodia camphorata extracts used for maintaining stem cell pluripotency

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Backgrounds:

Embryonic stem (ES) cells are pluripotent stem cells that mean they have the ability to differentiate into all types of cells. For this reason, ES cells have the potential to clinical application. However, culture of the ES cells need leukemia inhibitory factor (LIF) which resulted in increasing the gene expression of Sox2 and Oct4 and maintain ES cells self-renewal. Unfortunately, LIF is an expensive reagent. In this study, we wanted to find out a traditional Chinese medicine which can replace LIF and maintain the pluripotency of ES cells. In our previous study, we found out that Antrodia camphorate (AC) could increase the gene expression leves of Sox2 and Oct4. For this reason, AC has the potential to replace LIF, in stem cell cultivation.

Materials and Methods:

First, we identified the cytotoxicity of Ethanol Extracts Antrodia Camphorata (EEAC) in mouse embryonic fibroblasts (MEF) cell by MTT assay. Q-PCR was used to determine the gene expression of Sox2 and Oct4 in MEF cells. Alkaline phosphatease (AP) and immunofluorescent staining were used to identify ES cell pluripotency. Finally, we wanted to know why EEAC could maintain the stem cell pluripotency and find the major pathway by western blot.

Results:

By the data of MTT assay, we choose the different concentrations of EEAC which can not inhibit the cells growth. And we observed that EEAC can increase the gene expression of Oct-4 and Sox-2 in MEF cells.The data of AP and immunofluorescent staining suggest that EEAC can maintain ES cell pluripotency.

Conclusion:

Our data suggest that EEAC not only can increase the gene expression of Oct-4 and Sox-2, but also can maintain ES cell self-renewl.

P135
Zinc-Dependent Interactions Between JAB1 and Pre-S2 Mutant Large Surface Antigen of Hepatitis B Virus

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Background:

Chronic hepatitis B virus (HBV) infection is a major cause of hepatocellular carcinoma (HCC) worldwide. The pre-S2 mutant large HBV surface (Δ2 LHBS) protein, which contains an in-frame deletion in LHBS, is highly associated with HBV-induced HCC and is a biomarker for prognosis of HCC patients after hepatetomy surgery. We previously found that Δ2 LHBS interacted with the Jun activation domain-binding protein 1 (JAB1), a zinc metalloprotease, which causes degradation of the cell cycle regulator $p27^{Kip1}$. This is believed to be a major mechanism for Δ2 LHBS-induced HCC.

Materials and Methods:

To test binding affinities between Δ2 LHBS and JAB1, we performed *in vitro* GST pull-down assays and co-immunoprecipitation by using the *E. coli* recombinant protein in the presence of various ions. To test the Δ2 LHBS-JAB1 in the context of whole HBV genome in human hepatocytes, we also constructed Δ2 HBS mutant expressed in the whole HBV genome plasmid then performed co-immunoprecipitation analysis to test interactions Δ2 LHBS and JAB1 in the liver cells.

Results:

By using *E. coli* recombinant protein, GST pull-down assays revealed that interaction betweenΔ2 LHBS and JAB1 was a zinc-dependent process. Zinc ions in 20μM were essential for the interaction between Δ2 LHBS and JAB1. The JAB1-Δ2 LHBS interaction in the context of whole HBV genome in the human hepatoma HuH7 cell lysates was also dependent on zinc, similarly to the E. coli recombinant proteins.

Conclusion:

Zinc plays an important role in mediating protein binding between JAB1 and Δ2 LHBS viral oncoprotein. Chelating zinc therefore is a potential promising approach to disrupt the Δ2 LHBS-JAB1 complex and ameliorate the oncogenic effect of Δ2 LHBS.

P136
Potential Roles of Genes (*NFIX and ZFP36L1*) Associated with Megakaryocytic Differentiation

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Backgrounds:

Megakaryopoiesis is a multi-stage process regulated by many specific genes, but the underlying mechanisms are still unknown. HEL (human erythroleukemia) cell line can go through either erythrocytic or megakaryocytic lineage differentiation upon treatments with different inducers. Our previous studies demonstrated that *Bacillus anthracis* lethal toxin (LT) could suppress TPA (12-O-tetradecanoyl-phorbol 13-acetate) induced megakaryocytic differentiation in HEL cells. To identify the novel genes that may regulate megakaryocytic differentiation, microarray analysis was performed. Our data revealed that NFIX (nuclear factor I/X) and ZFP36L1 (zinc finger protein 36, C3H type-like 1) were up-regulated after TPA treatments and down-regulated upon LT pre-treatments. We hypothesized that there two genes may play major roles in megakaryocytic differentiation.

Materials and Methods:

The quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to verify the expression of these two genes upon TPA treatment. In order to see whether these two genes is associated with megakaryocytic differentiation, the expressions of these two genes were knockdown by short hairpin RNAs (shRNAs) in HEL cells and then the hallmarks of megakaryocytic differentiation (the expression of megakaryocytic specific surface marker-CD41b and DNA contents -polyploidization) were examined by flow cytometry. The dynamic expressions of these two genes during megakaryocytic differentiation were also verified by qRT-PCR in human umbilical cord blood derived $CD34^+$ hematopoietic stem cells (HSCs) model.

Results:

Our data indicated that the expressions of these two genes were up-regulated upon TPA treatments. The hallmarks of megakaryocytic differentiation were suppressed when each of these two genes were down-regulated by shRNAs knockdown. The expressions of these two genes were elevated as differentiation process proceeds in human umbilical cord blood derived $CD34^+$ hematopoietic stem cells (HSCs) model.

Conclusion:

We demonstrated that NFIX and ZFP36L1 play major roles in megakaryocytic differentiation.

P137
Effects of G6PD Deficiency and Oxidative Stress on the Expression of MicroRNAs

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Background:

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy. G6PD-deficient cells suffer increased oxidative stress due to redox imbalance. This study aimed to determine whether microRNAs (miRNAs) are affected by G6PD status and responsive to oxidative stress.

Materials and Methods:

G6PD-deficient A549 cells were used as cell models to investigate the effects of G6PD status on miRNA expression. The expression levels of messenger RNA (mRNA) and miRNA were measured by quantitative real-time polymerase chain reaction (qRT-PCR). Protein expression was determined by Western blot.

Results:

By microRNA profiling, the expression level of miR-200b was found to be three-fold decrease in G6PD-knockdown A549 cells compared to scramble ones. G6PD knockdown also reduced expression levels of miR-200b family, including miR-200a, miR-429 and miR-141. Furthermore, the reduced miR-200b expression in G6PD-knockdown cells could be restored by complementation of G6PD activity. However, there was no significant difference in cell growth rate and miR-200b targeted gene expression, like cyclin A2, cyclin E2, cyclin dependent kinase 2 (CDK2), c-Myc, bcl-2 and p38, between scramble and G6PD-knockdown A549 cells. Further investigation showed that G6PD knockdown reduced H2O2-induced miR-200b expression but enhanced the magnitude and duration of H2O2-induced phosphorylation of p38 and JNK in A549 cell.

Conclusion:

Taken together, these data suggest that miR-200b could be a redox sensitive miRNA involving in the regulation of cell death upon oxidant challenge.

P138
The Therapeutic Approach for Stroke Mice Model by Using Novel iPS Cells.

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Backgrounds:

Stroke is the third leading disease cause of death in Taiwan. Until now, stroke has no effective therapeutic methods. iPS cell are the novel stem cell type that have highly potential for cell therapy. So we want to evaluate the therapeutic effective of the iPS cells derived neural stem cells to stroke mice model.

Materials and Methods:

Previously, Our laboratory was generated iPS by non-viral infections and without oncogenes called iPS-OSH cells, and then we differentiated the iPS-OSH cells into neural stem cells (NSC) to transplant to MCAO stroke mice brain. Immunofluorescence staining was used for the differentiated confirm. Flow was used to detecte population of NSC after transplantation. Locomotor activity measured by quantitated mice spontaneous activity in the open field. Beam walking was used to assess active balance. Motor coordination and balance are measured by performance on the rotarod. Immunohistochemistry used for recovery assessment.

Results:

The data of immunofluorescence showed that differentiated iPS expressed high level of Tuj1 and Nestin (neural cell markers), and flow data indicated that 63.2% of P75 (neural stem cell marker) positive cell in all population. All of the behavioral tests improved after cell transplanted than the control group. Immunohistochemistry data pointed out that neural differentiated markers (NeuN, GFAP and Map2) expressed in therapy group and co-localized with transplanted cells.

Conclusion:

Our study indicated that iPS-OSH derive NSC can survival in stroke mice brain and can differentiate into neuron cells to recover the brain function.

P139
Probucol Alleviates High Glucose-induced Renal Tubular Hypertrophy via Suppressed the Src/PI3K/ERK Signaling

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Backgrounds:

High glucose (HG) act to increase oxidative stress, promote extracellular matrix protein synthesis, and tubulointerstitial fibrosis. However, the mechanisms of antioxidants on the HG-induced renal tubular hypertrophy in diabetic nephropathy remain unclear. Probucol is a diphenolic compound with lipid-lowering effect and antioxidant property. Thus, the effects of probucol on HG-induced renal tubular hypertrophy and the Src/PI3K/ERK signaling were examined.

Materials and Methods:

Cultured human renal proximal tubular cells, exposed to HG (500 mg/dl) in the presence of antioxidant probucol, the Src family kinase inhibitor PP2, the PI3K inhibitor LY294002, and the ERK inhibitor PD98059 were assessed for cellular growth by MTT assay and LDH assay. Cell lysates were processed for a glutathione S-transferase activity ELISA, and Western blotting to measure key cellular hypertrophic markers.

Results:

HG significantly enhanced Src/PI3K/ERK activation and reduced cyclin D1, DMP1, and Ets-1 protein synthesis. These effects were not observed when cells were treated with the osmotic control high mannitol. In addition, we found that probucol, the Src family kinase inhibitor PP2, the PI3K inhibitor LY294002, and the ERK inhibitor PD98059 treatments significantly attenuated HG-inhibited cellular growth and HG-induced the Src/PI3K/ERK activation and cellular hypertrophy. Moreover, probucol and N-acetylcystein treatments reversed HG-reduced the antioxidizing enzyme glutathione S-transferase activity in these cells. The ability of probucol, PP2, LY294002, or PD98059 to ameliorate tubular hypertrophy was also verified by the observation that it significantly blocked HG-increased the protein levels of collagen IV, plasminogen activator inhibitor-1, α-smooth muscle actin, $p27^{Kip1}$, and $p21^{Waf1/Cip1}$.

Conclusion:

These results suggested that probucol has potent inhibitory effect against HG-induced renal tubular hypertrophy, and the Src/PI3K/ERK signaling pathway may be important target of probucol.

P140
The Development of Chicken Monoclonal Antibody Against New Human Breast Cancer Marker Protein

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Backgrounds:

To screen and identify single chain Fv (scFv) antibodies against new breast cancer maker protein from the constructed chicken phage display library.

Materials and Methods:

Recombinant phages which express short linker and long linker scFv specific for recombinant breast cancer protein marker were enriched after four-round screening (panning). The enriched recombinant phages were used to infect E.coli Top10'F to express soluble scFv antibody that can be purified by Ni+ sepharose affinity chromatography. The binding activity of the soluble antibodies was further identified specific to recombinant antigen protein and human breast cancer cells by ELISA, Western blotting and flow cytometry

Results:

The specific scFv against recombinant breast cancer were enriched after four rounds panning. We selected and expressed 13 colonies short linker scFv and 3 colonies long linker scFv with recombinant antigen in ELISA and Western blotting. After sequence alignment, we isolated 6 different colonies short linker scFv and 1 colony long linker scFv. Western blotting and flow cytometry analysis revealed that one colony short linker scFv had the affinity to breast cancer.

Conclusion:

We selected and expressed successfully scFv specific to human breast cancer. The scFv may be applied to diagnosis and therapy of breast cancer.

P141
Adiponectin/PPARβ signaling pathway—PPARβ associated protein(s)

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Backgounds:
PPARβ, an ligand-activated transcription factor, regulate pleiotropic biological functions including regulatory roles in anti-inflammation, lipid, and glucose homeostasis. It was shown that PPARβ is a downstream signaling molecule of adiponectin, an anti-diabetic and anti-atherogenic adipokine. Plasma levels of adiponectin have been reported to be significantly reduced in obese/diabetic mice and human. Although upstream signaling of adiponectin/AdipoR/APPL1/AMPK pathway has been established recently, the more downstream especially related to PPARβ is largely unknown. We proposed to investigate the possible PPARβ associated protein(s) involved adiponectin/PPARβ signaling pathway.

Materials and Methods:
Western blot analysis was used to examine AMPK phosphorylation after globular adipionectin treatment on C2C12 myoblasts and HEK 293T cells. To characterize the abundance of PPARβ protein for further investigate, the endogenous PPARβ protein amounts in C2C12 myoblasts, myotubes, and HEK 293T cells were compared simultaneously. We also constructed GFP-PPARβ plasmids to be able to obtain GFP tagged protein and perform the IP experiment. Furthermore, FLAG-PPARβ, pGL3 mCPT-1 luciferase reporter gene, and renilla internal control were cotransfected into HEK293T cells to perform dual luciferase reporter assay. This is to access its physiological function such as fatty acid β-oxidation in advance.

Results:
Adiponectin increased AMPK phosphorylation in both C2C12 myoblasts and HEK 293T cells. These experiments established proper conditions for adiponectin treatments in C2C12 myoblasts and HEK 293T cells to continue following studies. In addition, the expression of PPARβ in C2C12 myotubes and HEK 293T cells is more than the one in C2C12 myoblasts. Furthermore, The GFP-PPARβ expressed only in nucleus and can be immunoprecipitated by PPARβ antibody. Finally, the FLAG-PPARβ plasmid expressed and functioned in a dose-dependent manner by measurements of dual luciferase reporter assay.

Conclusions:
The data confirmed that adiponectin, recombinant PPARβ plasmids, and PPARβ antibody are functional. Hopefully, the protein(s) involved in adiponectin/PPARβ pathway and its role in glucose uptake and/or fatty acid β-oxidation will be revealed eventually.

P142
Small Ubiquitin-Modifier (SUMO) interacted with NNV-coat protein in Orange-spotted grouper

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Small ubiquitin-like modifiers (SUMOs), structurally similar to ubiquitin, ligated to lysine residues within sumoylation target proteins. Sumoylation and ubiquitination exhibit similar biological processes for post-translational modification which was participated in a number of cellular processes such as nuclear transport, transcriptional regulation, apoptosis and protein stability. In the present study, the cDNA of orange-spotted grouper *SUMO*, terms *osgSUMO*, was cloned by the combination of homology cloning and rapid amplification of cDNA ends polymerase chain reaction (RACE-PCR) approaches. The SUMO1 and SUMO2-yellow fluorescent protein (YFP) were distributed in the cytoplasm. Our results show that SUMO1 was capable of decreasing coat protein (CP) of nucleus localization ratio, not SUMO2. Thus, we demonstrate here that SUMO1 was able to interact with CP by using analysis of fluorescence resonance energy transfer (FRET). Taken together, grouper SUMO can partially influence on localization of CP.

Key words:
SUMO, orange-spotted grouper, RACE-PCR, FRET

P143
A novel adenoviruses vector armed with human osteonectin promoter driven PML inhibits human renal cell carcinoma growth

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Backgrounds:
Advanced renal cell carcinoma (RCC) is highly resistant to conventional therapies. Patients with advanced RCC have an extremely poor outcome with an estimated median survival of less than 1 year. It is medically urgent to develop new therapies to overcome this disease. The promyelocytic leukaemia gene *PML* is a pleiotropic tumor suppressor, which opposes mTOR-HIF1a-VEGF signaling in hypoxia. We hypothesize that *PML* may be a promising therapeutic agent for RCC targeted gene therapy as mTOR is critical for RCC tumor progression. Osteonectin is a matricellular protein and is implicated in the angiogenesis, invasion, and metastasis of human malignancies. Human osteonectin (hON) promoter has been used for gene delivery to certain types of cancer, but it has not been investigated in RCC. Herein, we tested the feasibility of hON promoter for RCC transcriptional targeting, and examined the anti-tumor effect of adenoviral vectors carrying either wild type or degradation-resistant mutant (S518A) *PML* driven by hON promoter toward RCC.

Materials and Methods:
The expression of hON and PML in normal (HRE) and malignant renal cell lines (RCC42, RCC52) was determined using western blot, and further validated in clinical specimen by IHC. A previously constructed 522-bp hON promoter was tested in cancer cell lines for transcription activity using luciferase-reporter assay. The different *PML* gene constructs (Cancer cell 20, 214-228, August 16, 2011) were kindly provided by Dr. Ruey-Hwa Chen at Institute of Biological Chemistry, Academia Sinica, and then subcloned into adenoviral vector with hON promoter. *In vitro* cell killing assay was performed to compare the cytotoxicity of cancer cell lines by PML-based adenoviral vectors.

Results:
Western blotting demonstrated a significant downregulation of PML in RCC42 and RCC52 compared to normal HRE cells. IHC of hON in normal kidney and malignant kidney tissues revealed a higher level of hON expression in both RCC cells and cancer adjacent stroma. In consistent with the endogenous hON expression, the 522 bp-hON promoter exhibited a strong transcriptional activity in RCC42 and RCC52 cells with an average of 14- and 44-fold higher luciferase reporter expression than control pGL3 vector, respectively. Adenovirus vectors carrying hON promoter-driven PML-1 and PML-4, either wild type or S518A mutant were constructed and confirmed their expression in RCC cells under both normoxia and hypoxia conditions. We found that Ad-hON-PML, regardless of wild type or mutant efficiently kill RCC42 and RCC52 cells at a moi rang of 30-100 in a dose-dependent manner. No significant different in cytotoxicity of RCC cells by Ad-hON-PML between normoxia and hypoxia culture condition.

Conclusion:
Expression of tumor suppressor PML gene under the control of 522 bp- hON promoter led to the growth inhibition of RCC *in vitro*. We are testing the anticancer effect of Ad-hON-PML in an orthotopic mouse model of human RCC.

P144
Screening Chinese Herbal Extracts That Can Reduce Thioacetamide-induced Acute Hepatitis in Rats

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Backgrounds:
Macrophages play an important role in the host defense system against foreign pathogens, it is associated with occurrences of many diseases and their progressions, including a variety of infections and inflammation. When macrophages are stimulated by the foreign matter such as lipopolysaccharide (LPS), it will start the defense mechanisms by activation and release of inflammatory mediators, including nitric oxide (NO).

Materials and Methods:
Ethanol extracts of 78 kinds of Chinese herbals and natural plants were used in the LPS-activated murine macrophage RAW264.7 cells to preform anti-inflammatory tests. These tests utilized the inhibitions of NO productions as a preliminary screen of anti-inflammatory effect. Extracts with high NO inhibition rates were further subjected to test their effects of cell viabilities by MTT assay.

Results:
Results show that seven extracts from *Artemisia indica* Willd,*Passiflora edulis* leaf,*Toona sinensis root,Toona sinensis* leaf, *Colocasia esculenta* leaf, *Cortex Phellodendri* and *Solanum tuberosum* leaf have significant anti-inflammatory effects with more than 90% NO inhibition rates in concentration of 100μg/mL. Results of MTT assays show that the increases in extracts' concentrations (25-200μg/mL) gradually decrease the cell viabilities. In the concentration of 100μg/mL, all of these seven extracts show above 75% of cell viabilities.

Conclusion:
These preliminary results indicate that seven herbal or plant extracts may have anti-inflammatory effects. After detailed cell tests to select the most effective anti-inflammatory extract(s), we will perform the *in vivo* anti-inflammatory tests in rats by intraperitoneal thioacetamide injection which can induce acute liver injury.

P145

Study on the biological activity and antibacterial efficacy of Ocotea, Blue Cypress, Copaiba, Lemon, Lavender, Hinoki, Peppermint, and Thieves essential oils

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Backgrounds:

In this research, nine commercial oils essential oils (EO) including, Ocotea (*Ocotea quixos*), Copaiba (*Copaifera reticulata*), Blue Cypress (Callitris intratropica), Lemon, Lavender, Hinoki, Peppermint, and Thieve essential oils were evaluated for its antibacterial effect against four pathogens, *Eschericia coli, Salmonella typhimurium, Staphylococcus aureus and Pseudomonas aeruginosa*. The preliminary screening showed Thieves essential oil as a strong inhibitor towards indicator pathogens. In our further study, Thieves essential oil was further investigated for additional efficacy when combined with antibiotics. Fourteen antibiotics including ciproflox 5ug, ofloxacin 5 ug, erythromycin 15ug, cephalothin 30ug, cefoxitin 30 ug, tetracyclin 30ug, chloramphenicol 30ug, bacitr 10 iu/ie/ui, penicillin 10 iu/ie/ui, nalidixic 30 ug, gentamicin 10UG, streptomycin 10 ug, kanamycin 30 ug, and ampicillin 10ug, was also investigated to evaluate its synergic, antagonist, indifference or antagonism influence.

Materials and Methods:

The antibacterial effects of EOs were initially screened against *Eschericia coli, Salmonella typhimurium, Staphylococcus aureus* and *Pseudomonas aeruginosa* using the disk diffusion method. The influence of varying concentrations of EO combinations on efficacy was also monitored in 100%, 75%, and 50% concentration, respectively. To evaluate the combination effect of potent EOs and antibiotics against indicator pathogens, filtered disk paper containing antibiotic was used and placed on agar growth medium that have been inoculated with indicator pathogens.

Results:

This preliminary study showed promising results for Thieves essential oils as pathogen inhibitors against *Eschericia coli, Salmonella typhimurium, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Mixture of Thieves essential oils with tetracyclin 30 ug, kanamycin 30 ug, and gentamicin 10UG showed additive efficacy against indicator pathogens. On the contrary, Thieves essential oil showed more effective antibacterial effect when it was applied alone without combination with the remainder selected antibiotics.

Conclusion:

This study showed that Thieves essential oil might be more effective against *Eschericia coli, Salmonella typhimurium, Staphylococcus aureus* and *Pseudomonas aeruginosa* when applied in combination with selected antibiotics.

P146

Luteolin Attenuates Mucin Production Via Inhibition Of $GABA_A$ Receptor Activation

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Backgrounds:

Airway mucus overproduction is one of the most important symptoms of asthma and causes severe clinical outcomes in patients. Despite of the effectiveness of general therapies to asthma, specific treatment to prevent mucus overproduction in asthma are still largly lacking. Recent studies have found that activation of the A type γ-aminobutyric acid receptors ($GABA_AR$) is important for promoting micus oversecretion in airway epithelia of the lung in asthma.Here, we report that luteolin, a natural flavonoids compound found in *peanut hull, Perilla frutescens*, and other herbs, suppresses mucus overproduction by functionally inhibiting the GABAergic system. This hypothesis was investigated by testing the effects of luteolin on pulmonary inflsmmstion, goblet cell hyperplasia, excessive mucus secretion, and GABAergic transmission with histological ans electrophysiological approaches.

Materials and Methods:

The *in vivo* asthma model was established by sensitizing 6 to 8-week male BAL/c mice with i.p. injection of 50μg of ovalbumin (OVA, adsorbed in 2mg aluminium hydyoxide in 200μl PBS) on day 0, 7, and 14, followed by challenging the mice with i.t. instillation of OVA (100μg in 40μl of saline) on day 21, 22, and 23. Survived mice were divided into four treatment groups, including the vehicle, 0.1, 1, and 10 mg/kg/day of luteolin. Electrophysiology study was performed in A549 cells, a human alveolar basal epitheial cell line, by examining $GABA_AR$-mediated currents with whole-cell patch clamp recordings.

Result:

10 mg/kg luteolin significantly decreased accumulation of leucocytes in the bronchoalveolar lavage fluid (BALF) and lung tissue. Inhibition of muscus overproduction by luteolin was demonstrated by the periodic acid-Schiff (PAS) stain method. In addition, electrophysiology results showed that luteolin inhibits GABAAR-mediated whole-cell currents in A549 cells.

Conclusion:

Our observation demonstrates that luteolin effectively attenuates mucus overproduction in asthma, at least partially via a mechanism of inhibiting $GABA_ARs$. These data suggest a possible therapeutic application of luteolin in treating mucus overoduction in asthma.

P147

BT284 Affect Telomerase Activity and Apoptosis in K562 Cell

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Backgrounds:

BT284 is a carboxamide analog which can inhibit the proliferation in human promyelocytic leukemia HL-60cells. To understand the molecular mechanisms of BT284 in human erythromyeloblastoid leukemia K562 cells, we investigate the telomerase activity, apoptosis and differentiation in BT284treated K562 cells.

Materials and Methods:

K562 cells were incubated with different concentrations of BT284 to explore the toxicity of BT284 by using MTT assay. After 24 h or 48 h treatment of BT284, cell morphology changes were observed with May-Grünwald/Giemsa stain. Meanwhile, the effects of BT284 on telomerase activity and differentiation were evaluated by telomere repeat amplification protocol and alkaline phosphatase assay, respectively. BT284-induced apoptosis was examined by western blotting.

Results:

IC_{50} of BT284 is around 10 nM in K562 for 24 h treatment. K562 cells undergo into differentiation with all trans retinoic acid and slightly morphology changes with different concentrations (0, 2, 4 and 6 nM) of BT284 by May-Grünwald/Giemsa stain. In addition, the inhibitory effect of BT284 on telomerase activity was concentration dependent.

Conclusion:

When the concentration of BT284 was under than IC_{50}, cell morphology changed and differentiated. Once differentiated, cells are triggering apoptosis. Afterwards, BT284 can be candidate treatment in combination with anti-cancer drug.

P148

The Role Of Thrombomodulin In Osteoclastogenesis

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Backgrounds:

Thrombomodulin (TM), a glycoprotein on the cell surface, is highly expressed in endothelial cells and other numerous cell types such as keratinocyes, osteoblasts and myeloid mononuclear cells. It is first discovered as an anticoagulant factor, and recent studies demonstrate that it has multi-functions including angiogenesis, cell proliferation and complement activation. TM in monocyte/macrophage is associated with inflammation and the expression level is altered in physiological and pathophysiological conditions. In osteoclastogenesis, monocyte /macrophages obtained from bone marrow or vascular-blood system can differentiate to osteoclasts by cell-cell fusion to form multinucleated cells, which are involved in bone resorption. Until now, the role of TM in macrophage in osteoclastogenesis has not been investigated.

Materials and Methods:

Osteoclast precusor was isolated from human peripheral blood mononuclear cells (PBMC) and bone marrow haematopoietic cells of the myeloid-specific TM-deficent mice ($LysMcre/TM^{flox/flox}$). The differentiation was induced by macrophage-stimulating factor and receptor activator of nuclear factor kappa-light-chain-enhancer of activated B cells ligand, and tartrate-resistant acid phosphatase staining as well as wesern blot of protein level were performed to distinguish osteoclasts and undifferentiated macrophages.

Results:

The expression level of TM was decreased in primary hematopoietic cells from murine bone marrow and PBMC during osteoclasts differentiation. In addition, the osteoclasts differentiation was enhanced in macrophages derived from $LysMcre/TM^{flox/flox}$ mice in comparison with those from wild type mice.

Conclusion:

Taken together, we showed that TM expression was inversely correlated with the differentiation of osteoclasts. The results suggested that TM may participate in osteoclastogenesis, and which may provide a novel application on osteometabolic diseases.

P149
Shikonin Inhibits the Production of Macrophage-derived IL-1beta via Decreasing the Inflammasome Activation

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Backgrounds:

Shikonin, a naphthoquinone compound extracted from Lithospermum erythrorhizo, has been shown to exert a wide range of pharmacological properties, including anti-inflammatory activities. Recently, it has been shown that the inflammasome activation plays a crucial role in the production of macrophage-derived IL-1beta, which mediated the chronic inflammation. Previous studies showed that shikonin decreased the productions of the proinflammatory cytokines including TNF-alpha and interleukin 1β both in monocytes and macrophages via interfering with different signaling molecules.

In the present study, we analyzed the role of shikonin in activation of NLRP3 inflammasome in human macrophage-like cell line, THP-1.

Materials and Methods:

After being differentiated by phorbol myristate acetate (PMA), macrophage-like THP-1 cells were treated with LPS or LPS plus shikonin for different time periods. The supernatants and cell pellets were collected separately for investigating the effect of shikonin on the expression of IL-1β by ELISA and the activation of NLRP3 inflammasome molecules by western blot analysis. The viability of macrophage-like THP-1 cells treated with shiokonin was analyzed by the cell counting kit 8 (CCK8) assay for 1-3 days.

Results:

In macrophage-like THP-1 cells, shikonin doses at less than 0.5 uM had no detectable toxic effects. Minor toxicity was first noted at >0.5 uM, and the toxic IC50 for 24 hours was approximately 0.67 uM in THP-1 cells. We found that, after treatment with the low concentration of shikonin, the production of IL-1β is was inhibited. The decreasing levels of NLRP3 and caspase-1 were also detected.

Conclusion:

Low concentration of Shikonin can effectively suppressed IL-1β expression in THP-1 macrophages via downregulation of inflammasome activation but not affect NF-kB activity. Suggesting that low concentrations of shikonin in less affect the state of the immune cells, and as a drug to treatment IL-1β-associated autoinflammatory diseases.

P150
The Effect of Gonadotropins on the Expression of Pituitary Adenylate Cyclase-Activating Polypeptide and Its Receptor and Steroidogenesis in the Gonads of Tilapia (*Oreochromis mossambicus*)

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Backgrounds:

The gonad development, gametogenesis and steroidogenesis in vertebrates are under the regulation of gonadotropins (GTHs) – follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted by pituitary gland. Pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide, has diverse functions including the regulation of proliferation, differentiation, metabolism, endocrine and immune systems in animals. Our previous studies showed that PACAP and its receptor expressed in the gonads of tilapia (*O. mossambicus*) and were involved in the cAMP-protein kinase A signaling pathway, and GTHs could affect their expression and steroidogenesis. Aim of this study was to identify the effect of gonadotropins on the expressions of $PACAP_{38}$ and its type I receptor (PAC_1-R), and steroidogenesis in tilapia gonads.

Materials and Methods:

Tilapia testes and ovarian follicles were cultured in DMEM with different concentrations of human chorionic gonadotropin (hCG; 5, 15, 50 IU) or pregnant mare's serum gonadotropin (PMSG; 5, 15, 50 and 100 IU) for 2 hours, respectively. The expression of $PACAP_{38}$ and PAC_1-R mRNA was investigated by semi-quantitative RT-PCR. Concentration of gonadal steroids (estradiol, testosterone, 11-keto testosterone, and progesterone) was analyzed by enzyme immunoassay (ELISA Kit, Cayman Chemical Company). Results from three independent experiments were expressed as the mean ± standard error of the mean. In all experiments, significance values ($P < 0.05$) were compared by a Duncan multiple range test after one-way analysis of variance to determine differences among the means of the groups and genders.

Results:

The expression levels of $PACAP_{38}$ mRNA and concentrations of gonadal steroids (estradiol, testosterone, 11-keto testosterone, and progesterone) increased in a dose-dependent manner, and the inductive function of gonadotropins could be suppressed by the addition of protein kinase A inhibitor H89 (10 μM). However, higher concentrations of GTHs (hCG 50 IU and PMSG 100 IU) had negative effect on the expression of $PACAP_{38}$ and PAC1-R and steroidogenesis. The expression levels of PAC_1-R mRNA were not significantly affected by hCG or PMSG in either sex.

Conclusion:

The expression of $PACAP_{38}$ and PAC_1-R in tilapia gonads suggested that PACAP may regulate gonadotropins action and enhance steroidogenesis via paracrine/autocrine mechanisms in this bony fish.

P151
Analysis of the Efficacy of Rigid Gas-Permeable Contact Lens for Prevention Against UVB-induced Vision Degeneration

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Backgrounds:

UV irradiation may cause a variety of ocular diseases. Particularly, the short wave UV at 312 nm wavelength inflicts most on corneal health after long term exposure. Previous animal studies have been focused on the protective effects by soft contact lenses and relatively few evaluations on rigid gas-permeable (RGP) lenses were reported.

Materials and Methods:

This study investigated the persistence and reliability of RGP under the influence of UVB. ICR mice were randomly divided into 4 groups: (1) blank control without UVB exposure, (2) with UVB exposure, (3) with UVB exposure, and with TMVC RGP, and (4) with UVB exposure, and with Sinsaki RGP. Following anesthesia, the mice of groups (3) and (4) were covered with RGP lenses and exposed to daily UVB for a 5-day period.

Results:

UVB exposure lead to corneal surface damages, including reduced smoothness and transparency, impaired cornea sensitivity and visual acuity. Histological analysis revealed that corneal thickness was decreased and fewer p63+ basal cells were observed. Immunohistochemistry assay showed alterations of Cox-2, NF-κB, Fas, MMP-9 and Cytokeratin-5 expression. With TMVC or Sinsaki RGP lenses to shield UVB irradiation, the tissues damages and the alterations of cellular and inflammatory markers were all ameliorated.

Conclusion:

This study demonstrated the bio-protective effects of RGP by using mouse as a study model for the assessment of UV shield efficacy.

P152
Transcription Factor HBP1 Regulates Cellular Migration and Invasion in Oral Cancer Cells

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Background:

Transcription factor HMG box-containing protein 1 (HBP1) functions as a potential tumor suppressor in various types of cancer. Previously, we demonstrated that HBP1 modulates cell growth in oral cancer. Analysis of human oral cancer specimens revealed that the mean HBP1 mRNA level in invasive tumors was significantly lower than that of the normal tissues suggesting a potential role of HBP1 in cell invasion. Therefore, the objective of the current study is to examine whether HBP1 regulates cellular motility in invasive oral cancer and to elucidate the potential mechanism.

Materials and Methods:

Human oral cancer cell lines, HSC-3 and FaDu cells, were employed in the current study. A 3-D Matrigel culture was used to test the effect of HBP1 on anchorage-independent colony formation. To examine the effect of HBP1 on cell invasiveness, HBP1-specific siRNA or HBP1 cDNA construct was used to either suppress or over-express HBP1. Wound-healing and Matrigel invasion assays were performed to examine cell motility. Effects of HBP1 on the expression and activity of matrix metalloproteinases (MMPs) were detected by reverse transcription-polymerase chain reaction (RT-PCR), Western blotting, and gelatin zymographic assays.

Results:

HBP1 overexpression inhibited anchorage-independent growth in both HSC-3 and FaDu cells. In addition, HBP1 knockdown in both HSC-3 and FaDu cells enhanced cell migration, whereas ectopic expression of HBP1 resulted in a delayed healing time and invasion. Furthermore, HBP1 inhibited the expression and activity of the MMP family members.

Conclusion:

Our study demonstrated that HBP1 expression is negatively correlated with cell migration, invasion, and anchorage-independent growth in oral cancer cells. These data suggest HBP1 as a potential predictor of the aggressiveness in the development of oral cancer.

P153
N-methyl-D-aspartate receptor plays a role during zebrafish myogenesis

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Backgrounds:
N-methyl-D-aspartate receptor (NMDAR, NR) is most often composed of two NR1 and two NR2 (A, B, C, and D) subunits, and plays a key role for controlling synaptic plasticity and memory. It was reported that NMDAR is involved in the Ca^{2+} influx of C2C12 myoblasts during muscle differentiation, however, it's biological function during myogenesis is still controversial. In this present study, we use zebrafish as a model, follow by genetic knockdown approach to further clarify the role of NMDAR during myogenesis.

Materials and Methods:
In our study, we designed the zebrafish NR2D polyclonal antibody to detect the protein level of NR2D in zebrafsih embryos, and colocalized with muscle cell membrane marker of Dystrophin polyclonal antibody. Also, we designed a specific NR2D morpholino, follow by genetic knockdown approach to further clarify the role of NMDAR during myogenesis. We stained with the monoclonal antibodiy F59 and phalloidin to detect muscle fiber alignment. And used the MyoD and Myogenin RNA probes as a myoblast differentiation marker, to detect the mRNA expression in zebrafish embryos. Then, we used the Pax7 monoclonal antibody as a marker to detect the myosatellite cells in the lateral somite at the early stage of myogenesis.

Results:
First of all, endogenous NR2D expressions were detected by a peptide antibody against zebrafish NR2D, and results revealed that NR2D signals were detected in notochord, somites and somite boundaries. These expression domains corresponds well with that of dystrophin (a muscle-specific membrane protein), suggesting that NR2D not only express in the neuron precursors but also locate in the membrane of muscle cells. Then, we injected a specific morpholino (NR2D-MO) and found that NR2D-MO-injected embryos displayed phenotypic abnormalities, such as trunk flexion, shorten body length and malformed somite. Interestingly, the percentages of embryos with phenotypic abnormalities increased as the injection doses of NR2D-MO increased. Staining with specific monoclonal antibodies F59 and phalloidin, it revealed that NR2D-MO treatment disturbs actin and muscle fiber alignment. Whole mount in situ hybridization showed that myoD and myogenin expression domains are largely reduced in NR2D-MO-injected embryos compared to the corresponding no-injection control group. Furthermore, we found that numbers of Pax7-positive muscle stem cells were largely increased in lateral somites of tail regions of NR2D morphants.

Conclusion:
Our results suggest that NMDAR plays a role during myogenesis, especially in the process of muscle stem cells differentiate to myoblasts. It may provide novel insight in studying muscle development in vertebrates.

P154
Hypermethylation of FHIT, a putative tumor suppressor plays a role of radiation resistance in Oral Carcinoma

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Oral cancer comprises of the major cancer incidence and mortality in Southern Taiwan. Although radiotherapy is a major treatment modality in oral cancer patients after surgical operation, recurrent cancer with history of radiotherapy demonstrated a high radio-resistance phenotype and more aggressive cancer behavior. However, the molecular mechanism of radiation resistance is not fully understood. We aim to investigate the epigenetic changes in selected radio-resistant oral cancer cell line. Through step-wise fractionated ionizing radiation up to 50Gy, we established a radio-resistant cells in OML1 oral cancer cell line, namely OML1-A50. Global methylation analysis using methylation microarray showed that there are 129 and 147 genes showing significant hypo-methylation or hyper-methylation respectively in OML1-A50 as compared to the control cells ($p<0.001$). Most of hyper-methylated genes involved in metabolic process. We focused on fragile histidine triad protein, FHIT a diadenosine triphosphate hydrolase that reported to have tumor suppressive function. Firstly, we confirmed that methylation increment of FHIT promoter and loss of mRNA expression in OML1-A50 cells. Moreover, restoration of FHIT in OML1-A50 cells resensitized the cell to single fractional of high Gray radiation. Lastly, we observed that promoter hyper-methylation of FHIT correlated with higher risk of recurrence in oral cancer patients. In conclusion, ionizing radiation may induce epigenetic silencing of FHIT in oral cancer cells. The role of FHIT in radio-resistance of oral cancer deserves further investigation.

P155
Screening of CUG repeat RNA toxicity genetic modifiers in *C.elegans*

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Backgrounds:
Myotonic Dystrophy type 1 (DM1) is a dominant neuromuscular disease caused by expanded CUG repeat RNA. Our laboratory has established a *C. elegans* model of DM1 and further demonstrated that the DM1 phenotypes could be alleviated by decreasing the expression of CUG repeat RNA. Recent reports indicate that the transcription of expanded trinucleotide repeats requires the participation of certain transcription factors. These findings suggest a potential strategy for DM1 treatment by reducing mutant gene expression through down-regulating the expression of specific genes.

Materials and Methods:
In this study, I have first tried to identify the genes which are required for or may facilitate the expression of expanded CUG repeats 83 in transgenic *C. elegans* using RNAi library. Then, I investigated if knocking down the expression of candidate genes may specifically reduce the expression of expanded CTG repeats-containing genes expression but does not affect normal gene expression by quantitative RT-PCR and western blotting. Finally, the effect of candidate genes shRNA treatment on the lifespan of DM1 worms was examined.

Results:
5000 genes have been screened and 8 candidate genes which may reduce the expression of CUG83 RNA expression were picked up. Among these genes, Y57G11C.23, Y73F8A.g, Y73F8A.l and *ccg-1*, were shown to be able to reverse the shortened life span of DM1 worms. Currently, the effect of knocking down the expression of these genes on other phenotypes, including brood size and muscle structure and function, of DM1 worms are under investigation.

Conclusion:
Our results suggest the existence of CUG repeat RNA toxicity modifiers. However, further experiments are required to confirm the efficacy and underlying mechanism in rescuing the DM1 phenotypes.

P156
Evaluation the Extraction Methods of Human Peripheral Blood Mononuclear Cells (PBMCs)

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Backgrounds:
Human peripheral blood mononuclear cells (PBMCs) are the blood mononuclear cells collectively, including lymphocytes, monocytes, and macrophages. When human body is under infection, the PBMCs will attack the pathogens and induce inflammation responses. Therefore, the PBMCs are widely used to investigate immune-relative researches. In general, the BD vacutainer CPTTM Cell Preparation tubes with Sodium Citrate and Sigma-Aldrich HISTOPAQUE-1077 are methods that are widely used to isolate the PBMCs. To observe the differences between these two kinds of methods, we compared the category and purity of PBMCs separated from them.

Materials and Methods:
We adopted two methods, including the BD vacutainer CPTTM Cell Preparation tubes with Sodium Citrate and the Sigma-Aldrich HISTOPAQUE-1077, to isolate the PBMCs and evaluated their purity and category by flow cytometry.

Results:
To compare the purity of lymphocytes, PBMCs isolated from the methods mentioned-above were analyzed. The results showed that lymphocytes isolated from the BD vacutainer CPTTM Cell Preparation tubes with Sodium Citrate were more pure than those from the Sigma-Aldrich HISTOPAQUE-1077. In addition, monocytes can be effectively isolated only from BD vacutainer CPTTM Cell Preparation tubes with Sodium Citrate, but not from the Sigma-Aldrich HISTOPAQUE-1077.

Conclusion:
In summary, BD vacutainer CPTTM Cell Preparation tubes with Sodium Citrate is better than the Sigma-Aldrich HISTOPAQUE-1077 for the isolation of lymphocytes; furthermore, only the BD vacutainer CPTTM tube can effectively isolate monocytes from whole blood. We suggest that BD vacutainer CPTTM Cell Preparation tubes with Sodium Citrate is an appropriate method for the isolation of PBMCs in the future research.

P157
Plasmid-Mediated Quinolone Resistance in *Salmonella enterica* Isolates from Taiwan

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Backgrounds:

Resistance to quinolones in Enterobacteriaceae often derived from the accumulation of mutations in DNA gyrase (mainly GyrA) and topoisomerase IV (mainly ParC). In addition, quinolone resistance can be associated with decreased membrane permeability or overexpression of efflux pump systems. The principle mechanisms of resistance to quinolones are generally chromosome-encoded. However, since 1998, the emergence of plasmid-mediated quinolone resistance (PMQR) has been reported. Although these PMQR determinants confer low-level resistance to quinolones, they represent additional transferable quinolone resistance mechanisms. To date, three major PMQR mechanisms have been identified: (i) genes qnrA, *qnrB, qnrC, qnrD*, and *qnrS* encoded the synthesis of Qnr proteins which protect target enzymes (GyrA and ParC) from quinolone inhibition. (ii) *aac(6')-Ib-cr* encoded the synthesis of aminoglycoside acetyltransferase which acetylates several fluoroquinolones. (iii) QepA-type or OqxAB-type efflux pumps extrude fluoroquinolones from the bacterial cell.

Materials and Methods

In this study, a total of ten *Salmonella enterica* isolates collected from Chang Gung Memorial Hospital in 2011 was screened for PMQR determinants by PCR and Southern blotting.

Results:

The results indicated *oqxAB* was present in seven isolates, while *aac(6')-Ib-cr* was present in six isolates (three of these isolates were also *oqxAB* positive). However, the MIC values (0.125-0.25μg/ml) showed these isolates were susceptible to CIP (ciprofloxacin). The correlation between *oqxAB /aac(6')-Ib-cr* and drug susceptibility remained to be investigated further.

Conclusion:

In addition, our data seem to imply that there might be a high prevalence of PMQR determinants in *S. enterica* isolates from Taiwan which should be closely monitored.

P158
Acetaminophen Inhibitions of Ethanol Metabolism with Human Alcohol and Aldehyde Dehydrogenases

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Backgrounds:

Acetaminophen, i.e. *p*-acetamidophenol, is widely used over-the-counter analgesic, antipyretic medication. The drug's hepatotoxicity can be significantly enhanced by chronic excessive alcohol consumption. ADH and ALDH are the principal enzymes responsible for metabolism of ethanol in humans. The goal of this study was to investigate the acetaminophen inhibitions of ethanol oxidation with recombinant human Alcohol and Aldehyde Dehydrogenases.

Materials and Methods:

Recombinant human alcohol dehydrogenases, ADH1A, ADH1B1, ADH1B2, ADH1B3, ADH1C1, ADH1C2, ADH2, and ADH4, and recombinant human acetaldehyde dehydrogenases, ALDH1A1 and ALDH2 were assessed the inhibitions of oxidation with acetaminophen at near physiological pH 7.5 and a cellular coenzyme concentration, 0.5 mM NAD. For comparison, *S*-hydroxymethylglutathione and benzaldehyde were used as substrates for human ADH3 and ALDH3A1, respectively, which are virtually inactive in ethanol metabolism due to their very high K_m for ethanol or for acetaldehyde.

Results:

Acetaminophen acted as a noncompetitive inhibitor (I) for all of the ADH family members studied with the slope inhibition constants (K_{is}) ranging from 0.90 to 20 mM, and the intercept inhibition constants (K_{ii}), 1.4 to 19 mM, suggesting that two abortive ternary-complex intermediates, E-NAD-I and E-NADH-I, can be formed during catalytic reaction. This is consistent with that dissociation of E-NADH being rate-limiting step in catalysis for ADH family. Acetaminophen was a noncompetitive inhibitor for ALDH2 (Kis = 3.0 mM and Kii = 2.2 mM), but a competitive inhibitor for ALDH1A1 (Kis = 0.96 mM).

Conclusion:

Kinetic simulations using the experimentally determined numerical steady-state rate equations of human ADH and ALDH families show that the ethanol-oxidizing activities of ADH1C1, ADH1C2, ADH2 and ADH4, and the acetaldehyde-oxidizing activity of ALDH1A1 can be significantly inhibited in ethanol metabolism at therapeutically attainable doses of acetaminophen, thus potentially reducing hepatic and gastric first-pass metabolism of ethanol.

P159
The Effect of β2-Glycoprotein I on Vascular Endothelial Growth Factor-Induced Angiogenesis and Tumor Progression

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Backgrounds:

β2-glycoprotein I (β2-GPI) is a plasma glycoprotein with diverse functions, but the molecular effect of β2-GPI on vascular biology are still unclear. In our previous works, we found that β2-GPI could inhibit vascular endothelial growth factor (VEGF)-induced human aortic endothelial cells growth and migration. VEGF plays a key role in angiogenesis. Tumor progression depends on vascularization and angiogenesis in malignant tissue. Thus, anti-angiogenesis targeting VEGF has been considered as an important strategy for cancer therapy. The aims of this study were to investigate the role of β2-GPI in the regulation of angiogenesis and tumor progression in vivo and in vitro.

Materials and Methods:

β2-GPI was purified from normal human plasma. We evaluated β2-GPI anti-angiogenesis activity by matrigel plug assay and angioreactor angiogenesis assay in vivo. The anti-tumor activity of β2-GPI was evaluated in C57BL/6 mice bearing B16-F10 melanoma. The growth inhibitory effects of β2-GPI on B16-F10 melanoma cells were assessed using the MTT assay. Boyden chamber assay were performed to evaluate the migration and invasion ability of B16-F10 melanoma cell

Results:

We found that β2-GPI suppressed VEGF-induced neovascularization in C57BL/6 mice. Xenografts tumor assay and MTT assay revealed that β2-GPI inhibited B16-F10 melanoma cell growth. Furthermore, we found that β2-GPI inhibited B16-F10 melanoma cell migration and invasion in vitro.

Conclusion:

Our results indicate that β2-GPI inhibits tumor progression through suppressing angiogenesis and inhibiting the proliferation and migration of tumor cells.

P160
Proofreading exonuclease of DNA polymerase I is a bona fide exonuclease in endonuclease V-mediated repair pathway

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Backgrounds:

Deamination of adenine can occur spontaneously under physiological conditions, generating highly mutagenic lesion, hypoxanthine. The process is enhanced by exposure of DNA to ionizing radiation, UV light, nitrous acid, or heat. Hypoxanthine tends to pair with cytosine during DNA replication, and would result in A:T to G:C transition mutations. In *Escherichia coli*, deoxyinosine (Hypoxanthine deoxyribonucleotide) is primarily removed through an alternative excision repair pathway initiated by endonuclease V.

Materials and Methods:

We constructed deoxyinosine-containing heteroduplex DNA substrates to analyze the repair mechanism in vitro. We designed three mutagenic deoxyinosine lesions of A-I, G-I, and T-I in the recognition sequences or at the cleavage site of a specific restriction endonuclease. In the presence of deoxyinosine, each of the substrates was refractory to a specific restriction endonuclease cleavage. After the deoxyinosine was repaired, the DNA became sensitive to the restriction endonuclease so that we could evaluate the repair level by cleavage assay.

Results:

Using cell-free extracts from different genetic backgrounds, we found that gene products of *nfi* (endonuclease V) and *polA* (DNA polymerase I) were the most important components for processing all of the three deoxyinosine lesions. We also showed that the 3'-5' exonuclease activity of DNA polymerase I is the *bona fide* exonuclease for removing 3' penultimate deoxyinosine of endonuclease V nicking products.

Conclusion:

Our results could complete a multistep working model for endonuclease V-mediate repair pathway.

P161

The Proofreading Activity of DNA Polymerase I on 3' Penultimate Mismatches within a DNA Primer and the Implications in DNA Repair

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Backgrounds:

Proofreading activity of DNA polymerase is an important factor in maintaining the high fidelity of genetic information during DNA replication. Proofreading by DNA polymerase I is accomplished in a 3'-5' exonuclease site that will excise the mis-incorporated base. We want to investigate whether proofreading 3'-5' exonuclease activity of polymerase I contributes to DNA repair.

Materials and Methods:

We designed heteroduplexes containing a mismatch at the 3' penultimate base of a single-stranded DNA nick, a configuration that represents an intermediate in the repair of deoxyinosine-containing DNA through incision by *Eschreichia coli* endonuclease V. The mismatched bases were designed within the same sequence environment of several overlapping restriction endonuclease sites so that the proofreading activity can be scored by restriction endonuclease assay and repair efficiencies of different mis-incorporation can be systemically compared. All 12 possible mismatches were constructed and proofreading activity was analyzed along with endonuclease V treated deoxyinosine-containing DNA.

Results:

All mismatches were successfully corrected by pol I. Modified bases, such as deoxyinosine, at 3' penultimate site were also corrected with similar efficiency.

Conclusion:

The results of this study improve our knowledge of the capacity of DNA polymerases to recognize and correct mis-incorporation errors during DNA replication. This study also provides further support for the idea that 3'-5' exonuclease activity of *E. coli* Polymerase I is the bona fide exonuclease activity required for endonuclease V-mediated alternative excision repair for deoxyinosine.

P162

Effect of Arecoline on the Fetal Development of Zebrafish Embryo

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Arecoline, one of the main ingredients in betel nuts, has been known to elicit a great impact on the growth of embryo. Previous studies have also found that the concentration of arecoline in the water is directly associated with the survival rate of zebrafish embryos. (If incubated in a concentration of 0.04%, death of the embryo will be incurred in an average of 48 hours; if incubated in a concentration of 0.02%, it will then be lengthened to 72 hours). The present study is focused on how a zebrafish embryo will be deformed in arecoline incubation, given that the concentration is low enough for its survival. Ten zebrafish embryos from different stages were incubated in the medium containing 0.001、0.01、0.02%、0.04% of arecoline for 24h, 48h, 72h, 96h and 120h and the morphological changes of the phenotype were examined. Immuno-histochemistry (IHC) is applied to compare the phenotypical changes in great detail. By this study, we also observed that the concentration of the arecoline and the retarding development of the segmentation, muscular system of the zebrafish are normally correlated.

P163

The Role of Microglia in Retinal Degeneration of Mice with Defective NR2E3 Gene

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Backgrounds:

To investigate the role of microglia in *rd7/rd7* mice, which are a mouse model of enhanced S-cone syndrome (ESCS)

Materials and Methods:

rd7/rd7 mice were outcrossed to Mafia (Tg/Tg) mice. The two-generation outcross–intercross series was used to generate rd7/rd7;Tg/Tg double homozygous for Nr2e3 rd7/rd7 mutation and Tg/Tg. Offspring from an incross of *rd7/rd7;Tg/Tg* mice were used *in* this study. AP20187 was used to systemic ablation of bone marrow-derived microglia in rd7/rd7;Tg/Tg mice. Immunohistochemical analysis, flow cytometry, electron microscopy, and western blot were used in this study.

Results:

Sequential analyses of retinal rosettes and AF spots revealed that retinal rosettes appeared earlier than AF spots, suggesting that microglia are recruited and appear after the formation of the outer nuclear layer folding. We demonstrated that the majority of microglial cells present in the retina of rd7/rd7;Tg/Tg mice expressed green fluorescence protein. After systemic depletion of circulating bone marrow (BM)-derived microglia, the cilioretinal flatmount in rd7/rd7;Tg/Tg mice showed characteristics that mimic those of later stages of retinal degeneration in rd7 mice. Further analyses of the outer nuclear layer confirmed a decrease in the number of nuclei in rd7/rd7;Tg/Tg mice injected with AP20187. Photoreceptor loss after systemic depletion of circulating BM-derived cells may be associated with the upregulation of cytotoxic molecules, such as TNF-a and IL-1B.

Conclusion:

Future studies dedicated to the search for therapeutic agents to intervene in the inflammatory processes involved in retinal degeneration should investigate the molecular signals that act between microglial activation and photoreceptor loss.

P164

Proinflammatory Cytokines May Induce Skeletal Myoblast C2C12 Cells Atrophy and Fibrosis via Caveolin-3 Mediated Signaling Pathways

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Backgrounds:

Chronic diseases, such as chronic obstructive pulmonary disease (COPD) and cancer, may induce cachexia and consequently cause muscle dystrophy involving skeletal muscle atrophy and fibrosis. Previous studies found that the action of proinflammatory cytokines including TNF-α, IL-1β, IL-6 may trigger this chronic diseases-related myopathy, and caveolin-3, myostatin and connective tissue growth factor (CTGF) are also involved, however, the signaling mechanism(s) is still unclear.

Materials and Methods:

In order to understand the possible roles of caveolin-3, myostatin and CTGF and underlying signaling pathways, we apply western blotting, immunocytochemistry to detect the morphological changes, the expresssion of these proteins and phosphorylation state of related signaling molecules in proinflammatory cytokines-treated skeletal myoblast C2C12 cells. Moreover, by using of caveolin-3 overexpression model, we further investigate the relationship between these signaling molecules.

Results:

Our studies found that proinflammatory cytokines down-regulate the expression of caveolin-3 but up-regulate the expression of myostatin and CTGF in atrophic C2C12 cells as well as the phosphorylation of upstream signaling molecules Akt, NF-κB and CREB. Therefore, chronic diseases may cause skeletal muscle atrophy and fibrosis via a variety of signaling pathways to effect the expressions of myostatin and CTGF in which caveolin-3 may play an important regulatory role.

Conclusion:

Proinflammatory cytokines may induce skeletal myoblast C2C12 cells atrophy and fibrosis via caveolin-3 mediated signaling pathways. The possibility of crosstalk between upstream signaling pathways and strategies in clinical application to decrease cachexia-related muscle atrophy and fibrosis will be further to explored.

P165

Comprehensive Analysis of Neurobehavior Related to Histomorphology Alteration in Chronic Constrictive Nerve Injury Model by CatWalk XT System

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Background:

Neuropathic pain is a debilitating disease significantly affecting physical, psychological and social interaction in patients with chronic pain. The neurobehavior of animal model of chronic constriction injury appeared analogous to human neuropathic pain. However, there were no data depicting the severity of histomorphology alteration of nerve system related to grading of neurobehavior. In this study, we conducted the different intensity of chronic constrictive injury by applying various number of nerve ligature to determine the severity of histomorphology alteration related to neurobehavior detected by CatWalk XT system.

Material and methods:

One hundred twenty Sprague–Dawley rats weighing 250–300 g were used in this study. These animals were randomizedly allocated into five groups including sham, one, two, three and four 3-0 chromic gut ligatures loosely ligated around left sciatic nerve. These neurobehavior were assessed by CatWalk XT, thermal hyperalgesia and mechanic allodynia before injury and then periodically after injury. The nerve tissue from skin to dorsal spinal cord was obtained for histomorphology analysis one week after injury and the evoked potential of brain were analyzed 4 weeks after injury.

Results:

Significant different expression of NGF existed in skin related to intensity of nerve injury. High expression of CD 68 and TNF-α, and decreased S-100 expression in the distal end of nerve showed the same trends. Increased expression of synaptophysin in dorsal root ganglion and dorsal spinal cord were in line with intensity of injury. The increased amplitude of sensory evoked potential paralleled with increased severity of nerve damage. Either mechanic allodynia or thermal hyperalgesia were remarkably detected in all groups but not related to severity of nerve damage. In CatWalk XT gait analysis, significant difference of print area, maximum contact maximum intensity, stand phase, swing phase, single stance and regular index existed either compared to sham or in intra-group comparison.

Conclusion:

The histomorphology and electrophysiology alteration were related to severity of nerve damage. The subtle neurobehavior difference was only detected by CatWalk XT system but not in mechanical allodynia or therapy hyperalgesia. Thus, CatWalk should be a useful tool to monitor the neuropathic pain especially mandatory for detecting subtle alteration.

P166

Elevated Telomerase Bioactivity in Peripheral Blood Mononuclear Cells Is Associated with Human Coronary Stenosis

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Backgrounds:

The initiation, progression and/or development of coronary atherosclerosis are suggested attributable to widespread inflammation process through systemic activation of immune cells. Emerging evidence has demonstrated the replicative senescence in circulating blood leukocytes and human arterial tissues in cardiovascular disease, as shown by shorter telomeres, unique structures at the extreme end of the chromosomes and essential in protection of chromosome ends. However, no attempt has been paid to explore the telomerase bioactivity in peripheral mononuclear cells in patients with angiographic coronary artery disease. We aimed to fill the gap between the telomerase bioactivity in peripheral blood mononuclear cells (PBMC) and age-associated coronary artery disease.

Materials & Methods:

Whole blood from a total of 322 subjects undergoing coronary angiography in National Taiwan University Hospital was collected transcatheterly and purified for PBMC. Functional activity of telomerase and mean terminal restriction fragments (mTRF) were assayed by telomeric repeat amplification protocol (TRAP) and Southern blot hybridization, respectively.

Results:

Our results showed the elevated telomerase activity was positively associated with the presence, maximal luminal stenosis of the culprit lesion, number-of-diseased-vessels and the Duke prognostic score, after adjustment of traditional risk factors, clinical features, medical history and mTRF (p=0.004, 0.001, 0.004 and 0.001, respectively). Receiver-operating characteristic (ROC) curve analysis represented a cut-off value of 160.8 ymol of telomerase-elongation products predictive of angiographic stenosis. A subgroup of patients with 8-month follow-up angiography was analyzed and revealed a close relationship of telomerase regulation with the short-term changes in the stenotic extent within the same subject (p=0.043, N=38). Moreover, comparing telomerase alone, adding mTRF elevated the risk-prediction of coronary stenosis to an adjusted OR of 36.59 (p=0.003). Patients with elevated telomerase activity and shorter mTRF increased an adjusted OR of coronary stenosis to 21-fold, as compared with those without activated telomerase and with longer mTRF (p=0.012, N=66).

Conclusion:

Our study suggests the regulation of telomerase in mononuclear cells, alone or in combination with the cellular age, can predict the angiographic severity of human coronary stenosis.

P167

Study of the association between elastin fibers degradation and tendinopathy

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Acute tendonitis and chronic overuse tendinopathy comprise the majority of tendon injuries. However, the intrinsic pathogenic mechanism underlying the development of tendinopathy is largely unknown, and debate continues as to whether inflammation processes play a prominent role in the disease process. Previously studies have shown that a switch expression of extracelluer matrix during tendinopathy, including collagen arrangement while elastic fibers decreased during pathogenesis of tendinopathy. Tendons are composed mostly of parallel arrays of collagen fibers and these fibers are closely packed together. The mechanical properties of the tendon depend on the collagen fiber diameter and elastic fiber which provide elasticity. However, the role of elastic fiber in tendinopathy is unknow. Thus, we hypothesize that degradation of elastic fiber promotes progression of tendinopathy. The objective of this study is to investigate whether degradation of elastic fiber is one of the risk factors of tendinopathy. The experimental group (12 rats) received elastase injection in paratendon of Achill's tendon. The sham operational control (10 rats) received PBS injection only. Focal lesion was detected occur near or at the enthesisat 28 days after injection. Hypevascularity、hypercellularity、acquisition of chondrocyte phenotype were detected in hematoxylin-and-eosin stained cross sections. Elastin fibers exist at high tension site of tendon, while these fibers are absent in area with disrupted collagen. In addition, infiltrated leukocytes were prominent in the vessel wall. The significance of this study is to understand the causal relationship between elastic fiber and tendinopathy , and provide a new treatment strategy for tendinopathy.

P168

Pnn mutant mice develop muscular dystrophy with centronuclear myopathy

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Backgrounds:

To investigate the functional role of truncated pinin(pnn), a nuclear speckle-associated SR-like protein in cultured cells and skeletal muscle morphogenesis of transgenic(Tg) mouse.

Materials and Methods:

We used immunofluoresce and Western blotting to study the expression patterns of pnn N-terminal coiled-coil domain(CCD) mutant in a variety of cell lines. In the animal model, overexpression of pnn CCD mutant in the skeletal muscle was achieved by creating mice carry a promoter of human skeletal actin and a pnn CCD gene behind the promoter. The resultant pnn mutant mice were examined by Southern blotting, histological and immunohistological approaches.

Results:

Overexpression of pnn CCD could reduce the endogenous pnn expression in different cell lines, but not those of other speckle-associated SR proteins. To evaluate the effect of pnn CCD in vivo, we generated transgenic mouse specifically overexpressing pnn CCD in skeletal muscle. Overexpression of N-terminal pnn in skeletal muscle cells not only decreased muscle mass, caused deformation of muscle fibers with nuclei localized at the center of muscle cells, but also retard mouse provement in Rota-Rod performance. RT-PCR analysis demonstrated certain genes associated with muscle fiber differentiation were altered in splicing pattern.

Conclusion:

Overexpression of pnn CCD mutant represses endogenous pnn expression, but could not affect other SR protein expression or disrupt general structure of nuclear speckle. Tg mouse overexpressing pnn mutant develop centronuclear myopathy with apparent muscle dystrophy. Molecular mechanism underlying pnn CCD's role in myopathy remains further studies.

P169

Increased nerve regeneration by intramuscular injection of human amniotic fluid mesenchymal stem cells in a muscle denervation model

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Purpose:

Neurotrophic factors provide the basis for neurotrophic signaling within muscle compartments essential for muscle regeneration in muscle denervation. It is well known that human amniotic fluid mesenchymal cells (AFS) have the potential to secrete various neurotrophic factors mandatory for nerve regeneration. In this study, we assess the outcome of nerve regeneration by intramuscular injection of AFS in a muscle denervation and nerve anastomosis model.

Materials and methods:

Sprague-Dawley rats weighting 200-250 gm were enrolled in this study. Muscle denervation was conducted by transverse resection of sciatic nerve with proximal end sutured into the gluteal muscle. The nerve anastomosis model was performed by transverse resection of sciatic nerve followed by 4 stitches suture. AFS with 5x106 cells were intramuscularly injected to gastrocnemius muscle with infusion pump. .

Results:

CNTF, BDNF, and NT-3 were remarkably expressed in AFS cells. Intra-muscular injection of AFS exerted significantly expression of several neurotrophic factors over nerve and innervated muscle. AFS caused high expression of Bcl-2 in denervated muscle with reciprocal decrease of Bad and Bax. AFS preserved the muscle morphology paralleling with high expression of desmin and acetylocholine receptors. AFS injection produced the significant improvement in neurobehavior such as SFI and CatWalk gait analysis as well as nerve conduction latency and CMAP. Significant perseveration of anterior horn cell and increased nerve myelination was line with muscle morphology.

Conclusion:

Intramuscular injection of AFS protects muscle apoptosis by the secretion of various neurotrophic factors. This protection furthermore improves the nerve regeneration in long term nerve anastmosis model.

P170

Prenatal Infection Induces Cognitive Deficits and Neuronal Alterations in the Offspring

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Backgrounds:

Accumulating evidence suggests maternal infection during pregnancy is a risk factor for neurodevelopment psychiatric disorders, such as schizophrenia and autism. Recent studies linked prenatal immune activation with behavioral abnormalities and deficits in the dopaminergic system. Therefore, we wanted to investigate the cellular and morphological alterations in the mesocortical dopamine system by using a maternal infection model.

Materials and Methods:

C57BL/6J pregnant female mice received either a single injection of viral mimic, polyriboinosinic: polyribocytidilic acid (Poly I:C) (20mg/kg, i.p.) or vehicle on gestation day 9. Adult male offspring of Poly I:C- and saline-treated mothers were used. The locomotor activity was examined in a novel open field and the short-term memory was evaluated using a novel object recognition task. The density of dopamine-producing neurons in the ventral tegmental area (VTA) was examined using immunohistochemical method. Golgi-Cox impregnation procedure was used to reveal the morphology of II/III pyramidal neuron in the mPFC. The morphometric parameters of dendritic architectures were analyzed using Stereo Investigator and Neurolucida system.

Results:

The adult male Poly I:C offspring exhibited hypo-locomotor activity in a novel open field and demonstrated short-term memory function impairment in the novel object recognition task compared to age-matched controls. The number of dopaminergic neurons in the VTA was increased in Poly I:C-offspring. Morphologically, the layer II/III pyramidal mPFC neurons in poly I:C offspring exhibited extensive dendritic arbors with greater segment length yet fewer branches and dendritic spines, compared to those in control mice.

Conclusion:

Together, our data thus revealed increased dopamine neurons in the VTA, hypo-locomotor activity, impaired short-term memory function and altered mPFC neuron structure in Poly I:C offspring. Our results validated this prenatal infection paradigm as a model for neurodevelopment psychiatric disorders.

P171

The Effects of Endothelial Progenitor Cells Derived from the Umbilical Cord on Ischemia-Induced Hind Limb Injury in Diabetic Mice and Their Related Mechanisms

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Backgrounds:

Peripheral arterial diseases, the major complication of diabetes, can result in lower limb amputation. Since endothelial progenitor cells (EPCs) are involved in neovascularization, the study was to examine whether EPCs isolated from Wharton's jelly (WJ-EPCs) of the umbilical cord, a rich source of mesenchymal stem cells, could reduce ischemia-induced hind limb injury in diabetic mice.

Materials and Methods:

We evaluated the effects of WJ-EPC transplantation on hind limb injury caused by femoral artery ligation in mice with streptozotocin (STZ)-induced diabetes.

Results:

We found that the ischemic hind limb in mice with STZ-induced diabetes showed decreased blood flow and capillary density and increased cell apoptosis and that these effects were significantly inhibited by injection of WJ-EPCs. In addition, hypoxia-inducible factor-1α (HIF-1α) and interleukin-8 (IL-8)were highly expressed in transplanted WJ-EPCs in the ischemic skeletal muscle tissues and were present at high levels in hypoxia-treated cultured WJ-EPCs. Moreover, incubation of the NOR skeletal muscle cell line under hypoxic conditions in conditioned medium from EPCs cultured for 16 h under hypoxic conditions resulted in decreased expression of pro-apoptotic proteins (p53 upregulated modulator of apoptosis and Bax) and increased expression of anti-apoptotic proteins (Bcl-x and Bcl-2). Knockdown of HIF-1α or IL-8 expression by EPCs using HIF-1α siRNA or IL-8 siRNA, respectively, prevented this change in expression of apoptotic-related proteins.

Conclusions:

Wharton's jelly in the umbilical cord is a valuable source of EPCs and transplantation of these EPCs represents an innovative therapeutic strategy for treating diabetic ischemic tissues. The HIF-1α/IL-8 signalling pathway plays a critical role in the protective effects of EPCs in the ischemic hind limb of diabetic mice.

P172

Eupafolin reduces COX-2 and iNOS Expression by down-regulating NF-κB, AP-1 and MAPK Pathway in LPS-Induced RAW264.7 Cells and Mice

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Eupafolin, a flavonoid isolated from Artemisia princeps, has been used traditionally to treat disease such as inflammation, cancer and infections by fungi, bacteria, and viruses. However, its mechanism remains unknown. The aim of this study was to evaluate the anti-inflammatory effects and underlying the regulatory mechanism of the eupafolin on LPS-stimulated RAW 264.7 cells *in vitro* and mouse paw edema *in vivo*. In *in vitro* study, eupafolin significantly decreased LPS-induced NO production and expressions of iNOS and COX-2 in RAW 264.7 cells. Furthermore, eupafolin suppressed NF-κB p65 translocation and activator protein-1 (AP-1) activation as well as p38 and NH(2)-terminal kinase (JNK) mitogen-activated protein kinases (MAPKs) phosphorylation, whereas the extracellular signal –regulated kinase (ERK) was not affected. In *in vivo* assay, eupafolin reduced the neutrophil infiltration and edema in LPS-induced mouse paw. The present results showed that eupafolin exerts its anti-inflammatory effects through the decrease of inflammatory mediators expression by suppressing P38, JNK, NF-κB and AP-1 signaling pathways. Taken together, our results indicate that eupafolin may provide a useful therapeutic approach for inflammation-associated diseases.

P173
Isolated Mitochondria Infusion Mitigates Ischemia-Reperfusion Injury of the Liver in rats

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Background:

A recent study showed that the injection of mitochondria isolated from a non-ischemic region mitigated myocardial injury. We tested the protective effects of infusing isolated mitochondria on the reperfusion injury in the liver of rats.

Materials and Methods:

A partial liver ischemia-reperfusion model in male Wistar rats was used. At 45th minute of liver ischemia, the recipient's spleen was infused with vehicle (I/R-Vehicle group) or vehicle containing isolated mitochondria (7.7 × 106 ± 1.5 × 106 /ml, I/R-Mito group). After a 240 minutes' reperfusion, the serum and livers were collected to assess tissue injury.

Results:

Our results show that the elevation of serum ALT (414.3±67.1 U/Lv.s208.8±30.2 U/L), the necrosis of hepatocytes on H&E staining, increase of positive counts in TUNEL staining (59.5 ± 4.4% vs 24.6 ±9.1%), the expression of cytosolic cytochrome c, cleaved caspase 9 and 4-HNE were all reduced in the I/R-Mito group, compared to the I/R-Vehicle group. The membrane potential of the isolated mitochondria measured by JC-1 fluorescence remained high, and the infused mitochondria were distributed in the liver parenchyma 240 minutes after reperfusion.

Conclusions:

These results demonstrate that an intra-splenic infusion of viable mitochondria isolated from the donor prior to reperfusion significantly reduced ischemia-reperfusion injury in the liver.

P174
Sleep deprivation-induced short-term memory deficit is preventable

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Backgrounds:

In our daily life, sleep disturbance or deprivations are inevitable. However, it is well documented that sleep loss impairs cognitive functions such as learning and memory. Reductions of neurotrophic factors, such as BDNF, subsequent to sleep deprivation have been proposed to be the key.

Materials and Methods:

In this study, we tested if voluntary exercise, a well known practice that increases the BDNF level, can prevent sleep loss-caused cognitive decline. Adolescent male mice were assigned into control and exercise groups. Mice in exercise group were allowed for voluntary exercise on running wheels for four weeks. Two groups of mice were then subjected to normal sleep or 72-hour sleep deprivation conditions. The locomotor activity of mice was then measured and a novel object recognition test was used to evaluate their short-term memory function.

Results:

The activity level was not affected by either exercise or sleep deprivation. The performance of short-term memory was impaired in sleep-deprived control mice but not in exercised mice($p<0.05$).

Conclusion:

Our results demonstrated a preventive effect of voluntary exercise on 3-day sleep loss-produced short-term memory deficit. To elucidate the underlying mechanism, further study is required.

P175
N-myc Downstream Regulated Gene 2: an Antitumor Gene is Upregulated by Phosphatase and Tensin Homolog and p53 in Bladder Carcinoma Cells

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Backgrounds:

N-myc downstream regulated gene 2 (NDRG2) belongs to the NDRG family genes (NDRG1-4) whose expression is down-regulated by N-myc/Max complexes and is shown to be negatively correlated with tumor metastasis. In this study, we determined the expression, function, and regulatory mechanisms of NDRG2 genes in the human bladder carcinoma cells.

Materials and Methods:

The expression of NDRG2, AKT, pAKT, PTEN, p53, MMP9 and cyclins were determined by real-time reverse transcription-polymerase chain reaction (RT-qPCR), immunoblotting, or transient gene expression assays. Effects of NDRG2-knockdown on cell proliferation, invasion, and migration were determined by 3H-thymidine incorporation, flow cytometry, matrigel invasion, and transmembrane migration assays.

Results:

Results indicated that knockdown NDRG2 enhanced cell proliferation by increasing DNA synthesis at S-phase of human bladder carcinoma HT-1376 cells and human bladder papilloma RT-4 cells. Knockdown NDRG2 enhanced migration and matrigel invasion by inducing MMP9 expression. Transient overexpression of p53 downregulated gene expression of MASPIN and NDRG2 in HT-1376 cells. Studies using the PTEN-overexpressed T24 cells indicated that overexpression of PTEN downregulated cell proliferation by arresting cell cycle at G1 phase. Results of immunoblot assays revealed that overexpression of PTEN upregulated the protein expression of NDRG2 and cyclin A, while dowregualated pAKT, cyclin D1, cyclin E, and MMP9. Treatments of PTEN inhibitor, VO-OHpic, enhanced AKT phosphorylation, while downregulated NDRG2 in RT-4 and PTEN-ovexpressed T24 cells.

Conclusion:

Our results suggested that NDRG2 is a p53- and PTEN-upregulated gene and to be regarded as the tumor suppressor genes in the human bladder carcinoma cells.

P176
An Acidic Extracellular pH Promotes Cell Migration of HepG2 through the ROS-ERK-FAK Signal Pathway.

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Backgrounds:

An acidic extracellular pH (acidic pHe) contributes to the behavior of migration and invasion of tumor cells. Our previous reports have demonstrated that acidic pHe enhanced cell migration in HepG2 cells. However, the inside mechanism for this cell migration remains to be explored.

Materials and Methods:

The human hepatocellular carcinoma cell line HepG2 (ATCC, HB8065) was purchased from the American Type Culture Collection (Rockville, MD). For the preparation of acidic growth medium, growth medium was adjusted to pH 6.6 by addition of 1 N HCl. This study used Western blotting for kinase activation, cell migration assay, flow cyotmery for intracellular ROS detection, and siRAN for protein knockdown. All results are expressed as the mean±SD for three replicate experiments. Statistical differences between means were evaluated using Student's t-test with P value of less than 0.05 considered significant.

Results:

In this study, we investigated whether reactive oxygen species (ROS), a signaling messenger, initiated signaling for acidic pHe-induced cell migration. pH 6.6 treatment stimulated ROS production, which was relative to cell migration since an antioxidant dithiothreitol (DTT) prevented this effect. Also, ERK and FAK were activated by pH6.6 treatment. DTT blocked pH 6.6-induced phosophorylation of ERK and FAK as well as the migratory behavior of HepG2 cells. It suggests that ROS generation may be the upstream event of this signal. Inhibition of ERK by U0126 decreased pH6.6- induced phosphorylation of FAK and retarded cell migration, supporting the presence of ERK-FAK pathway. Furthermore, FAK played a key role in cell migration since inhibition of FAK by a pharmacological inhibitor or knockdown technique effectively prevented pH6.6-induced cell migration.

Conclusion:

An acidic pHe enhanced the migratory behavior of HepG2 cells via the ROS- ERK- FAK signaling.

P177
SCUBE3 Modulates Fibroblast Growth Factor (FGF) Signaling for Muscle Development

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Backgrounds:

SCUBE3 [signal peptide-CUB-EGF (epidermal growth factor)-like-containing proteins 3] belongs to the newly-identified secreted and cell membrane-associated SCUBE family, which is highly conserved in vertebrates. SCUBE3 is expressed predominantly in a variety of developing tissues in mice, e.g. neural tubes, somites, and limb buds. However, its function in development remains unclear.

Methods and results:

In this study, we showed that knockdown of SCUBE3 in C2C12 myoblasts inhibited fibroblast growth factor receptor-4 (FGFR-4) expression and FGF signaling, resulting in decreased myogenic differentiation. Furthermore, we identified and characterized zebrafish *scube3* gene, and investigated its function during zebrafish embryonic development by using antisense morpholino (MO) knockdown approach. Whole-mount *in situ* hybridization revealed that zebrafish *scube3* mRNA is maternally expressed and widely distributed during early embryonic development. Knockdown of *Scube3* in zebrafish embryos led to decreased expression of the myogenic marker *myod1* in presumed fast muscle precursors in the lateral stripes of somites, whereas *myod1* expression in the adaxial cells which are precursors of slow muscle cells was unaffected in *Scube3*-morphants. In addition, immunofluorescent staining which labels fast-muscle myosin was reduced in *scube3*-morphants as compared to control embryos. Furthermore, we identified *fgf8* as a major factor in *scube3*-medeated fast-muscle differentiation. In *scube3*-morphants, the *fgf8* expression in somites was down-regulated and forced expression of *fgf8* RNA restored *myod1* expression in somites. Biochemical and molecular analysis showed that Scube3 may act as a FGF co-receptor to enhance FGF8 signaling.

Conclusion:

Our results suggest that *scube3* plays an important role in fast muscle development via regulating FGF signaling.

P178
Investigation of the Antimicrobial Activity of the Ethanol Extracts of *Sophora flavescens* against Nosocomial Antibiotic Resistant Pathogens

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Backgrounds:

In recent years, many different kinds of antibiotics have been wildly discovered. However, the widespread use and misuse of antibiotics has encouraged the development of microorganisms that resist to formerly effective drugs; exploring new antimicrobial components to substitute for currently used antibiotics have become a highly important issue. In this study, an indigenous herbal medicine, *Sophora flavescens*, was tested for the antibacterial activities against the clinically antibiotic resistant strains.

Materials and Methods:

The dry roots of *Sophora flavescens* were extracted with 95% alcohol and then further subject to the process of partition extraction with ethyl acetate and n-hexane. The antimicrobial activities and synergy effect of the various extracts from *Sophora flavescens* were examined via the disk diffusion method. The minimum inhibitory concentration (MIC) was measured by serial agar macrodilution.

Results:

The *in vitro* test indicated that the ethyl acetate extracts displayed significant antimicrobial activity against *Staphylococcus aurens* with the inhibition zone diameter range of about 16.67 ~ 18.00 mm. The active fractions did not show any antagonism effect with 10 commercial antibiotics base on the synergy effect test. According to the time-kill assay, the alcoholic extract was proved to possess bactericide effect.

Conclusion:

Overall, the extracts of *Sophora flavescens* present antimicrobial activity indeed the active components from *Sophora flavescens* have the valuable to be developed as an antibiotic substitute.

P179
Structural Folding, Purification and Functional Characterization of the Presumed Ring Contraction Enzyme for Indolocarbazole Glycoside Biosynthesis

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Backgrounds:

Indolocarbazole natural products are an emerging class of bioactive compounds holding potential therapeutic applications in the treatment of cancer and neurodegerative disorders. K-252a, for example, exhibits potent neuroprotective and cytotoxic activities against numerous cancer cells, and is a structurally unique indolocarbazole alkaloid containing a rare furanose moiety. We have recently cloned the entire biosynthetic gene cluster for K-252a, where the key enzyme presumably responsible for the conversion of pyranose to furanose has been identified. This key enzyme for the hexose ring contraction is valuable in that little is known in the enzyme of this class in terms of molecular mechanism and structure-and-activity relationship in natural product biosynthesis. Availability of functional form of this enzyme would greatly facilitate the synthesis of furanose-containing natural products for drug development, which has attracted us to carry out heterologous expression, purification and functional characterization of this enzyme. Therefore, our study has been to get access to large quantity of this enzyme and subsequently to investigate its catalytic function and mechanism.

Materials and Methods:

The enzyme has been cloned for expression as a His-tagged recombinant protein in *Escherichia coli*. For functional characterization and future X-ray structural determination, we aimed to obtain a large quantity of soluble protein by protein refolding techniques. Upon optimization of expression condition, the expression was induced to give substantial amount of protein, which was further treated with high concentration of denaturants (such as urea and guanidinium HCl). The protein refolding was carried out by dialysis against various buffers to allow a transformation of the enzyme from unfolded state to the folded state.

Results:

Throughout examinations with a series of expression and refolding conditions, a sufficient amount of soluble protein has been obtained for functional characterization. The refolded enzyme was further examined for enzymatic activity on several hexose nucleotide diphosphates as potential substrates. The hexose nucleotide diphosphates were synthesized by a combinatorial biosynthetic approach developed in our lab using a combination of various NDP-hexose biosynthetic enzymes. As a result, the enzyme has successfully displayed catalytic activity as analyzed by high performance liquid chromatography, showing a time-dependent consumption of NADPH cofactor. Interestingly, further enzymatic coupling reaction of the refolded form of the enzyme with the NDP-sugar epimerase from the K-252a biosynthetic pathway also generates unanticipated products.

Conclusion:

In summary, this study has for the first time developed a method to gain soluble form of the key enzyme presumed for ring contraction in indolocarbazole glycoside biosynthesis. Unprecedentedly, the enzyme has also been revealed to possess substrate promiscuity on the given sugar substrates. The information gained here has provided critical insights into molecular mechanism of this class of catalyst, as well as materials useful to carry out combinatorial biosynthesis of unusual NDP-sugars and indolocarbazole glycosides for development of potential therapeutic agents.

P180
Curcumin effect on prion misfolding

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Misfolding and aggregation into amyloids of the prion protein (PrP) is responsible for the development of fatal transmissible neurodegenerative diseases. Various studies on curcumin demonstrate promise for the prevention of Alzheimer's disease. To evaluate the effect of curcumin on prion misfolding, we investigate the interaction between curcumin and mouse prion protein in a cell-free system. Curcumin assists prion proteins to resist denaturation from guanidine hydrochloride. Significantly, curcumin stabilizes the α-helical structures of prion and inhibits the prion fibril formation. Furthermore, we monitor the change of apoptosis and reactive oxygen species (ROS) level upon the curcumin treatment in mouse neuroblastoma cell (N2a). Curcumin effectively rescues the cell viability and decrease ROS level caused by subsequently added prion amyloid fibrils. The function of curcumin in mechanistic view is discussed.

P181
Mast Cells Activation by Endogenous Danger Signals from Injured Cells

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Backgrounds:
Dying cells release intracellular components, so-called damage-associated molecular patterns (DAMPs), which induce an acute sterile inflammatory response. The physiological function of the sterile inflammatory response is to clear cell debris and restore tissue homeostasis. However, leukocytes recruited to the inflamed site may also cause damage to healthy tissue, which is thought to contribute to the pathogenesis of many diseases. The underlying mechanisms for how cell death triggers inflammation have yet to be elucidated. In this study, we investigated the role of mast cell as a cellular sensor for cell injury.
Materials and Methods:
Bone marrow-derived mast cells (BMMCs) were differentiated from the bone marrow cells of C57BL/6 mice using IL-3. Necrotic cell lysate was prepared from the EL4 cell line after three freeze-thawing cycles and centrifugation to remove the debris. BMMCs were treated with necrotic cell lysate, rIL-1α, rIL-33, or IgE/DNP and measured for degranulation and cytokine production. Mast cell degranulation was analyzed by staining for cell surface phosphatidylserine with Annexin V-FITC, and by measuring the release of β-hexosaminidase.
Results:
Necrotic cell lysate could stimulate BMMCs to undergo degranulation and produce proinflammatory cytokines IL-6 and TNF. We next investigated whether two DAMPs, IL-1α and IL-33, contribute to the activation of BMMCs. Treatment of BMMCs with rIL-33 resulted in IL-6 and TNF production, but did not induce the degranulation of BMMCs. In contrast, BMMCs did not respond to the stimulation of rIL-1α.
Conclusion:
Our results indicate that upon cell injury, the DAMPs released necrotic cells could stimulate the degranulation and cytokine production of mast cells, which may mediate the following inflammatory response. IL-33 may serve as a DAMP and stimulate mast cells to produce proinflammatory cytokines, while other unidentified DAMP(s) should be responsible for stimulating mast cell degranulation. Further studies using mast cell-deficient animals are underway to investigate the role of mast cells in mediating the sterile inflammatory response in vivo.

P182
The Anti-cancer Effects of BPR0L075 on DNA-PK-proficient Glioblastoma Cells

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Backgrounds:
Glioblastoma is a primary malignant tumor of brain, which devastates fast and has higher recurrent rate. Presently, combination of radiotherapy and chemotherapy following surgery is one of standard cancer treatments, however the prognosis remains poor in most patients. BPR0L075, is a novel synthetic indole compound which has anti-tumor and antiangiogenic activities both *in vitro* and in vivo that inhibits tubulin polymerization through binding to the colchicine-binding site of tubulin. Therefore, to investigate the cytotoxicity induced by BPR0L075 in glioblastoma cells and elucidate the molecular mechanisms which involve in cell death of glioblastoma after BPR0L075 treatment is beneficial for development of effective strategy for anti-glioblastoma therapies.
Materials and Methods:
We used M059K cells with DNA-PK and securin expression, which involve in DNA repair, and M059J cell which lack of DNA-PK and low expression of securin. Both M059K and M059J cells were treated with BPR0L075 in dose- and time-dependent manners. The cells cytotoxicity was performed by MTT. Western blot analysis was performed to explore the pathways induced by BPR0L075 in both M059K and M059J cells.
Results:
BPR0L075 efficiently induced cell death of M059K cells which have normal expression levels of DNA-PK and securin. On the other hand, M059J with lower expression of DNA-PK and securin exhibited higher cell viability compared to M059K cells after treatment with BPR0L075, suggesting that M059J cells is more chemo-resistant than M059K cells after treatment with BPR0L075. To further explore whether BPR0L075 induces cell death through DNA damage response in both M059J and M059K cells, western blot analysis was used to examine the expression of DNA damage response related proteins. Our results revealed that BPR0L075 induced phosphorylation of γH2AX, chk1 and chk2 in M059K and M059J cells. We found that BPR0L075 induced higher activation of Mitogen-activated protein kinase (MAPK) signaling pathways through phosphorylation of p38, JNK and ERK in M059J than M059K cells. Therefore, this could explain the higher chemo-resistance of M059J to BPR0L075 as compared to M059K.
Conclusion:
We found that BPR0L075 induced higher cytotoxicity in M059K than M059J human glioblastoma cells lack of DNA-PK and with low expression of securin, but triggered the DNA damage response in both M059K and M059J cells. Furthermore, BPR0L075 induced higher activation of MAPK signaling pathways in M059J than M059K cells. Our results suggest that BPR0L075 treatment is more beneficial for the patients with glioblastoma which expresses DNA-PK and securin.

P183
Phenethyl isothiocyanate (PEITC)-mediated generation of reactive oxygen species causes DNA damage, cell cycle arrest, mitochondria dysfunction and apoptosis in human oral cancer OC2 cells

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Backgrounds:
Phenethyl isothiocyanate (PEITC), a member of the isothiocyanates family, have been shown to exhibit antineoplastic ability against many human cancer cells.
Results:
In this study, we found that PEITC was investigated for its cytotoxicity against OC2 oral cancer cells, as was the underlying mechanisms by which PEITC might induce DNA damage and apoptotic cell death through reactive oxygen species (ROS). In OC2 cells, PEITC increased the generation of intracellular ROS and NO, followed by induction of DNA damage activation of the ATM-p53 pathway and checkpoint-related signals Chk2 and decrease of Cyclin B1 with Cdc2, which led to increased numbers of cells in the G2/M phases of the cell cycle. Furthermore, PEITC induced apoptotic cell death through mitochondrial membrane potential decrease and activation of cytochrome c, caspase-3, and PARP. The above effects were all prevented by the antioxidants N-acetylcysteine (NAC), glutathione (GSH) and nitric oxide synthase (NOS) inhibitor dexamethasone (DEX).
Conclusion:
These findings suggest that PEITC-induced DNA damage and mitochondria-dependent cell apoptosis in OC2 cells are mediated via ROS generation.

P184
A family-based filtering strategy to find cancer-causing genetic variants from exome sequencing data

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Background
Cancer is a worldwide leading cause of death. Only a few mutations were found to be hereditary. The next generation sequencing has provided a tool to discover the common variants of cancer patients among family members. Although exome sequencing may narrow down the search space, a pipeline is still needed to identify the candidate variations from huge amount of variants.
Materials and Methods:
The method contains three parts: preprocessing (mapping and quality assessment), filtering and consequence prediction. Preprocessing: we used Burrows-Wheeler Aligner (BWA) and Samtools to align reads and pick up variants. A quality assessment is then undertaken on the basis of exome probe coverage. Filtering: candidate variants found in common population were marked as background. Such variants are unlikely to cause hereditary disease. Consequence prediction: Ensembl perl APIs were used to annotate consequences of each candidate variants. We also use PolyPhen, SIFT and Condel to predict the severity of protein function damaging of for each candidate variants.
Results and discussion
This method has been used to analyze a hereditary cancer. More than 95 % of the variants were filtered out by this pipeline. The candidate genes were found to be involved in the proliferation, apoptosis, cell adhesion, *etc*. Our method effectively revealed candidate variants which might cause this rare hereditary cancer by using the exome sequencing data. This pipeline could be used to look for hereditary factors that might cause other types of cancers.

P185
Development of a Multiplex Bead-Based Assay for Detection of CCYV Virus

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Backgrounds:

Cucurbit chlorotic yellows virus (CCYV) causes chlorotic yellows on cucumber (*Cucumis sativus*) and melon (*Cucumis melo*) and is transmitted by *Bemisia tabaci* biotype B and Q whiteflies.

Materials and Methods:

The beads were conjugated with nucleic acid against the CCYV nucleoprotein, which is conserved in all the CCYV. The bead-captured virus probe was detected by reverse transcriptase– polymerase chain reaction (RT-PCR) without electrophoresis analysis and effective removal of RT-PCR inhibitors. The developed bead-based assay showed a more higher detection limit comparable to the RT-PCR reaction. To measure the sequence specificity of the capture DNA for the intended target DNA, we prepared synthetic target DNA, specificity target DNA, and noncomplementary target DNA. Instead of target DNA, hybridization buffer was added to meet as a negative reaction.

Results:

We designed a multiplex PCR test for the detection and identification of CCYV virus and, including a plant internal control gene. Hybridization behaviors were investigated in terms of specificity of DNA probes for target DNA when optimized condition and sensitivity of target DNA were individually applied.

Conclusion:

Using ready-to-use nucleic acid -conjugated bead, the method requires less than 5 h. Ferthermore, the method has potential to integrate into a Lab-on-a-chip system for rapid detection and identification of CCYV.

P186
Development of a RT-PCR Assay for the Detection and quantification of CGMMV

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Backgrounds:

Cucumber green mottle mosaic virus (CGMMV), a member of Tobamovirus genus, has a narrow host range confined to the family Cucurbitaceae. CGMMV is transmitted mechanically and through seeds, causing severe diseases primarily in cucumber and watermelon.

Materials and Methods:

To design appropriate primers and probe, the nucleotide sequence flanked by the specific CGMMV primers and probe were selected. Taqman assays for real-time RT-PCR were performed. To determine the theoretical sensitivity and the reliability of the real-time, repetitions of the assay were undertaken using the 10 fold serial dilution of the prepared transcripts.

Results:

The first assay was a one-step multiplex RT-PCR test for CGMMV and plant internal control. The relative sensitivity of the multiplex real-time RT-PCR assay was compared with that of the one-step multiplex RT-PCR for the detection of CGMMV and plant internal control. In this study, a specific and sensitive CGMMV one-step RT-PCR assay was developed as a tool for the diagnosis of CGMMV as well as a rapid indicator of infection by quantifying viral load. This study also showed that a simple viral RNA release during the reverse transcription step constituted an alternative to the conventional RNA extraction method.

Conclusion:

The TaqMan RT-PCR assays were compared to the conventional RT-PCR assays for the detection of viruses using purified total RNA as well as crude extract. In addition this study showed that TaqMan RT-PCR was more sensitive than conventional one-step RT-PCR for testing different isolates of these viruses either using RNA or crude tissue extract.

P187
TaiwaninC down-regulats COX2-EGFR and up-regulates P27 pathways to suppress Arecoline-induced oral cancer cell proliferation via ERK1/2 inactivation.

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Background:

Oral cancer is one of the most common cancers reported in Taiwan and other Southeast Asian countries, caused due to the betel nut chewing habit that is prevalent in their population. Arecoline, the most abundant alkaloid in betel nut is known to induce abnormal proliferation of epithelial cells by facilitating the activation of epidermal growth factor receptor (EGFR) and its downstream mechanisms and there by promotes the expression of a crucial downstream protein cyclooxygenase-2 (COX2) in oral epithelial cells. EGFR is a vital growth factor receptor, which can functionally activate cell differentiation and proliferation. TaiwaninC, a naturally occurring lignan extracted from *Taiwania cryptomerioides*, has been reported to potentially inhibit the expression of COX2. However, the potential of TaiwaninC to inhibit the oral cancer cell proliferation and the related upstream mechanism responsible for COX2 inhibition are not clear yet.

Materials and Methods:

The goal of this experiment is to examine the effect of TaiwaninC on abnormal proliferative of T28 oral cancer cells by MTT assay and further by western blot analysis to examine whether TaiwaninC could modulate the expression of COX2 and their possible up-stream proteins.

Results:

The results show that COX2 is expressed only in the T28 oral cancer cells and not in the N28 normal oral squamous cells. However, TaiwaninC could inhibit the expression of COX2 in T28 oral cancer cells. Further, by MTT assay, TaiwaninC was found to inhibit oral cell proliferation and by western blotting, TaiwaninC was found to inhibit the COX2 protein expression by regulating the RAS/RAF/MEK/MAPK pathway.

Conclusion:

In conclusion our results indicate that TaiwaninC down-regulates COX2-EGFR and up-regulates P27 pathways to suppress Arecoline-induced oral cancer cell proliferation via ERK1/2 inactivation.

P188
Designing primers for polymerase chain reactions based on next generation sequencing data to identify virulence factors of pathogens

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Background

In the previous study, we showed that candidate genes for virulence factors of pathogens could be identified by using a NGS-based approach. Once there are candidate genes, polymerase chain reaction (PCR) can validate if they exist in new isolates. For this purpose, PCR primers should be designed at genomic regions that contain as few variations as possible, ensuring their high efficiency in PCR amplification. Besides, a pair of good primers should guarantee that the product is unique. In this study, we create an automatic computational pipeline that can take these issues altogether into consideration.

Materials and Methods

First, suitable reference genomes for high-virulence isolates are identified. To achieve this, the NGS data of high-virulence isolates are mapped to various reference genomes. The targeted regions (*i.e.* candidate genes) on reference genomes with high depths (sequencing coverage) are regarded as the anchored gene references (AGR). Second, genomic variations for each AGR are collected from NCBI reference strains as well as from high-virulence isolates (*i.e.* the NGS data). Third, Primer3 is used to design PCR primers with the consensus sequence of each AGR. To avoid producing multiple PCR products, only primers uniquely mapped to the reference genomes are preserved.

Results

Taking a case of a type of nosocomial infections as an example, we tried our pipeline to design PCR experiments to detect if the candidate genes exist in other high-virulence isolates. Our pipeline proves to be effective for automatically designing the PCR primers for the validation of candidate virulence factors.

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Caffeine regulates osteogenic differentiation and mineralization of primary adipose-derived stem cells and a bone marrow stromal cell line

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Backgrounds:

Caffeine consumption reportedly influences bone mineral density and body weight. However, the effects of caffeine on bone metabolism are still controversial, and whether the dosage of caffeine influences osteogenic differentiation has yet to be clarified.

Materials and Methods:

In the present study, we cultured primary adipose-derived stem cells (ADSCs) and a bone marrow stromal cell line (M2-10B4) in osteogenic differentiation media containing varying concentrations of caffeine.

Results:

Caffeine had biphasic effects; 0.1 mM caffeine significantly enhanced mineralization and alkaline phosphatase (ALP) activity. Consistent with these observations, a caffeine concentration of 0.1 mM upregulated the osteogenic differentiation marker genes ALP and osteocalcin (OCN), and elevated osteoprotegerin (OPG), Runt-related transcription factor 2 (Runx2), and Sirtuin 1 (Sirt1) levels. However, a concentration of caffeine greater than 0.3 mM suppressed the differentiation of both cell types.

Conclusion:

These findings indicate that caffeine has a beneficial effect on adipose-derived stem cells and bone marrow stromal cells, enhancing differentiation to osteoblasts; this effect, which is mediated via Runx2 activation at low doses, is significantly suppressed at high doses.

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The Preventive Effect of Biochanin A on Bone Loss in Ovariectomized Rats: Involvement in Regulation of Growth and Activity of Osteoblasts and Osteoclasts

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Backgrounds:

Biochanin A (BCA) is a major isoflavone abundant in red clover (*Trifolium pretense*). The protective effect of BCA on bone loss in an ovariectomized (OVX) animal model has never been clarified.

Materials and Methods:

The objective of this study was to investigate the biological effects of BCA on bone loss in OVX rats *in vivo*, and on the development of osteoblasts and osteoclasts *in vitro*.

Results:

Ovariectomy resulted in a marked increase in body weight, and a decrease in femoral bone mineral density and trabecular bone volume that was prevented by BCA or 17β-estradiol (E2) treatment. However, an increase in uterine weight was observed in E2-treated OVX rats, but not in response to BCA treatment. Treatment with BCA increased the mRNA expression of osterix, collagen type I, alkaline phosphatase (ALP), and osteocalcin and decreased the mRNA expression of tartrate-resistant acid phosphatase (TRAP), and the receptor activator of nuclear factor-κB ligand (RANKL) / osteoprotegerin (OPG) ratio in the femur of OVX rats. Treatment with BCA or E2 prevented the OVX-induced increase in urinary deoxypyridinoline (DPD) and serum tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). *In vitro*, BCA induced preosteoblasts to differentiate into osteoblasts and increased osteoblast mineralization. BCA inhibited preosteoclasts and osteoclast proliferation and decreased osteoclast bone resorption.

Conclusion:

These findings suggest that BCA treatment can effectively prevent the OVX-induced increase in bone loss and bone turnover possibly by increasing osteoblastic activities and decreasing osteoclastic activities.

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miR-524 Regulates Proliferation and Migration Through MAPK Signaling Pathway in Melanoma

Szu-Mam Liu

MicroRNAs (miRNAs) have emerged as key post-transcriptional regulators of gene expression in metazoans. The mitogen-activated protein kinase (MAPK) signaling pathway occupies an essential role in many cancer progressions, including melanoma and colon cancers. However, there is little identified miRNAs information linked to MAPK signaling pathway. It will very interesting and important to study what miRNAs involve in MAPK signaling pathway and how they mediate MAPK pathway.

In order to resolve these questions, we performed a miRNAs array screen by treated the MAPK signaling inhibitor in five different but containing common *V600EBRAF* mutation tumor cell lines with hyperactive MAPK pathway.

MicroRNA array data revealed that the expression of mir-524 has increased after 24 hours treatment with MEK inhibitors comparing to control samples. We verified the expression of mir-524 by qRT-PCR. Mir-524 is down regulation in activated (*BRAF* mutated) MAPK pathways cells. In addition, mir-524 is specifically down regulation in *BRAF* mutated cell lines but not in wild type *BRAF* cells. Here, we found that 3' UTR of *BRaf* contain mir-524 targeting sequence. Therefore, we demonstrated *BRaf* as the direct target of mir-524. Furthermore, overexpansion of mir-524 resulted in down regulation of MAPK signaling pathway and induced apoptosis protein cPARP expression in activated MAPK pathway cells.

MiR-524 might inhibit tumor highly proliferation and migration through MAPK pathway and further apply in cancer treatment and the diagnosis biomarker.

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Neuroprotective Effects of Antrodia cinnamomea fruiting body alcohol extract on Middle Cerebral Artery Occlusion Induced Focal Cerebral Ischemic Stroke In Rats

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The involvement of reactive oxygen species has been implicated in the pathogenesis and/or progression of Parkinson's disease, ischemic stroke and postischemic brain cell damage. *Antrodia cinnamomea* fruiting body alcohol extract (AC-AE), one extracts of the *Antrodia cinnamomea* (AC). It's to have a good scavenging potency on free radicals, as well as enhance the activities of GSH-PX, SOD and reduce the content of malondialdehyde (MDA). In order to elucidate the neuroprotective functions, we used hydrogen peroxide (H_2O_2) and cobalt chloride ($CoCl_2$) to mimic hypoxic/ischemic conditions 18 hr in neuron cell line of pheochromocytoma 12 (PC12) with pre-incubation of AC-AE. The results showed that of AC-AE possess reducing ROS and an increase viability of PC12 cells stressed via H_2O_2 and $CoCl_2$. We also use middle cerebral artery occlusion (MCAO) induced focal cerebral ischemic stroke animal model to investigate the mechanism of neuroprotective effects of AC-AE 385 mg/kg (1X), 770 mg/kg (2X) and 1540 mg/kg (4X). However, we also can find reduction of MDA, inflammatory cytokine TNF-α and also activity of antioxidant enzymes in serum and brain tissue. According to above results, *Antrodia cinnamomea* fruiting body alcohol extract might reduction infarct volume and neuroprotective to prevention of stroke.

keyword:

Antrodia cinnamomea (AC), Neuroprotective, Antioxidative, Middle cerebral artery occlusion (MCAO)

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Two Unique Ligand-Binding Clamps of Rhizopus Oryzae Starch Binding Domain for Helical Structure Disruption of Amylose

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The *N*-terminal starch binding domain of *Rhizopus oryzae* glucoamylase (*Ro*SBD) has a high binding affinity for raw starch. *Ro*SBD has two ligand-binding sites, each containing a ligand-binding clamp: a polyN clamp residing near binding site I is unique in that it is expressed in only three members of carbohydrate binding module family 21 (CBM21) members, and a Y32/F58 clamp located at binding site II is conserved in several CBMs. Here we characterized different roles of these sites in the binding of insoluble and soluble starches using an amylose-iodine complex assay, atomic force microscopy, isothermal titration calorimetry, site-directed mutagenesis, and structural bioinformatics. *Ro*SBD induced the release of iodine from the amylose helical cavity and disrupted the helical structure of amylose type III, thereby significantly diminishing the thickness and length of the amylose type III fibrils. A point mutation in the critical ligand-binding residues of sites I and II, however, reduced both the binding affinity and amylose helix disruption. This is the first molecular model for structure disruption of the amylose helix by a non-hydrolytic CBM21 member. *Ro*SBD apparently twists the helical amylose strands apart to expose more ligand surface for further SBD binding. Repeating the process triggers the relaxation and unwinding of amylose helices to generate thinner and shorter amylose fibrils, which are more susceptible to hydrolysis by glucoamylase. This model aids in understanding the natural roles of CBMs in protein-glycan interactions and contributes to potential molecular engineering of CBMs.

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Temperature-dependent structural changes of Parkinson's alpha-synuclein reveal the role of pre-existing oligomers in alpha-synuclein fibrillization

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Backgrounds:

Amyloid fibrils of α-synuclein are the main constituent of Lewy bodies deposited in substantial nigra of Parkinson's disease brains. α-Synuclein is an intrinsically disordered protein lacking compact secondary and tertiary structures. To enhance the understanding of its structure and function relationship, we utilized temperature treatment to study α-synuclein conformational changes and the subsequent effects.

Methods:

Recombinant human α-synuclein were expressed from *E. coli* and purified by osmotic shock procedure. We employed α-synuclein with a short treatment of different temperatures (20, 40, 60, 80, and 95°C) at the early stage and studied their conformational changes by various biophysical tools such as size exclusion chromatography (SEC), circular dichroism (CD), analytical ultracentrifugation (AUC), dynamic light scattering (DLS), nuclear magnetic resonance (NMR), and transmission electron microscopy (TEM).

Results:

We found that after 1 hr of high temperature pretreatment, > 80 °C, α-synuclein fibrillization was significantly inhibited. However, the temperature melting coupled with circular dichroism spectra showed that α-synuclein was fully reversible and the NMR studies showed no observable structural changes of α-synuclein after 95 °C treatment. By using cross-linking and analytical ultracentrifugation, rare amount of pre-existing α-synuclein oligomers were found to decrease after the high temperature treatment. In addition, a small portion of C-terminal truncation of α-synuclein also occurred.

Conclusions:

The reduction of pre-existing oligomers of α-synuclein may contribute to less seeding effect that retards the kinetics of amyloid fibrillization. Overall, our results showed that the pre-existing oligomeric species is a key factor contributing to α-synuclein fibrillization. Our results facilitate the understanding of α-synuclein fibrillization.

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The Roles of Src and PKC in Radiation-induced Senescence in Breast Cancer Cells, and Migration, Invasion and Proliferation Inhibition of Unirradiated Cells

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Backgrounds:

Cellular senescence is a permanent cell cycle arrest that cannot be stimulated to revert back by growth factors. Our previous report showed that depletion of securin induced senescence after irradiation (IR) and enhanced radiosensitivity in human breast cancer cells. However, the molecular mechanism of radiation-induce senescence in securin-knockdown MDA-MB-231-2A human breast cancer cells remain unclear. In addition, senescent cell is known to secrete senescence-associated secretory phenotype (SASP) factors. We aim to explore whether the SASP factors in conditioned medium (CM) from radiation-induced senescent cells can promote proliferation, cell migration and invasion of unirradiated MDA-MB-231 cells. It has been showed that Src phosphorylation was increased in senescent cells. In addition to Src, protein kinase C (PKC) plays pivotal roles in cell growth, apoptosis, differentiation, malignant transformation and metastasis. Therefore, it is important to investigate the roles of Src and PKC in unirradiated cell migration and invasion induced by radiation-induced senescent MDA-MB-231-2A cells.

Materials and Methods:

MDA-MB-231-2A breast cancer cells were exposed to 6 Gy radiation. The levels of phospho-Src, -PKC family and -STAT3 were analyzed by Western blot analysis. In addition, radiation-induced senescent MDA-MB-231-2A cells were pretreated with or without saracatinib before radiation, followed by SA-β-gal staining. CM- was collected from MDA-MB-231-2A cells 2 days after irradiation treatment. MDA-MB-231 breast cancer cells were treated CM for various periods. MDA-MB-231 cells were pretreated with Src inhibitor (Saracatinib) or PKC inhibitor (Rottlerin) and incubated with CM for Western blot analysis. The levels of cell migration and invasion were examined by wound healing assay and Boyden chamber assay, respectively.

Results:

We found that radiation induced senescence through activation of Src/STAT3 and PKC pathways in irradiated MDA-MB-231-2A cells. Radiation-induced senescence in MDA-MB-231-2A cells was significantly reduced after pretreatment of saracatinib and rottlerin. In addition, CM from radiation-induced senescent MDA-MB-231-2A cells inhibited proliferation of cancer cells through up-regulation of p27 (Kip1) and down-regulation of cyclin D1. Moreover, CM from radiation-induced senescent cells promoted expression of Src and PKC proteins. MDA-MB-231 cells pretreated with saracatinib or rottlerin reduced activation of Src and PKC, respectively. We also found that saracatinib and rottlerin inhibited MDA-MB-231 cells' migration and invasion promoted by CM from radiation-induced senescent MDA-MB-231-2A cells.

Conclusion:

Taken together, radiation induced senescence through Src/STAT3 and PKC pathways and inhibited proliferation of MDA-MB-231-2A cells via regulation of p27 (Kip1). Besides, CM from radiation-induced senescent MDA-MB-231-2A cells promoted migration and invasion of unirradiated MDA-MB-231 cells through activation of Src and PKC signaling pathways. Our results may provide promising strategies to improve the treatment on breast cancer patients and reduce the risk for recurrence of breast cancer patients after radiotherapy.

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Glucose Metabolism Involve in NME1-Suppressed Cell migration and Chemoresistance of Human Oral Squamous Cell Carcinoma SAS Cells

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Backgrounds:

Our previous studies revealed that NME1 is an important prognosis factor in oral squamous cell carcinoma (OSCC). Low NME1 expression was correlated with the higher metastasis potential and disease-free survival of OSCC patients. Besides, accumulative evidences support that glycolytic addiction provides cancer cells the advantage for cell proliferation and cancer progression in human cancers. Therefore, this study investigates the effect and mechanism of glycolysis inhibition on NME1-regulated cell migration and chemoresistance in OSCC cells.

Materials and Methods:

NME1 knockdown (SAS-NME1) and overexpression (SAS+NME1) stable clones were derived from a human OSCC cell line named SAS. First, XF Extracellular Flux Analyzers was used to simultaneously measuring the two major energy producing pathways of mitochondrial respiration and glycolysis. Then, effects of glycolytic inhibitors on chemoresistance and migratory activity were evaluated by trypan blue exclusion assay and transwell assays, respectively.

Results:

SAS+NME1 cells showed higher oxygen consumption rate and lower extracellular acidification rate compared to the mock cells when the availability of ATP is reduced. The result suggested NME1 knockdown enhanced glycolysis dependency which might lead to cancer malignancy of OSCC. Besides, SAS-NME1 cells were more sensitive to glycolytic inhibitors as compared to mock cells. Furthermore, pretreatment with glycolytic inhibitor improved the cytotoxicity of anticancer drugs and attenuated cell migration in SAS-NME1 cells.

Conclusion:

These results suggested that NME1 is involved in glycolytic regulation and its responsible to cancer progression in OSCC cells. The detailed mechanisms of NME1 on bioenergetic regulation, chemosensitivity, and cell migration/invasion are under investigation.

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Combination Treatments with Fisetin Potentiate the Cytotoxic Effects of Chemotherapy Drugs in Glioblastoma Cells

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Backgrounds:

Glioblastoma multiforme (GBM; World Health Organization astrocytoma grade IV) is the most frequent and malignant primary brain tumor in adults. Brain tumors are exceptionally resistant to both radio- and chemotherapy regimens and need novel approaches for treatment. Fisetin (3, 3', 4', 7-tetrahydroxyflavone) is a natural flavonoid commonly found in fruits and vegetables. In addition, fisetin is also a promising natural agent against cancer in several *in vitro* and in vivo studies. Cisplatin is one of the most potent antitumor agents and an effective chemotherapy agent against several malignancies. Etoposide, a semi-synthetic derivative of podophyllotoxin, is also an anti-tumor agent and arrests cell cycle at the late S phase and early G2 phase. It has been shown that multiple-drug treatment is more efficiently than single treatment. Therefore, we aim to combine cisplatin or etoposide with fisetin to enhance the efficacy of chemotherapy for glioblastoma cells.

Materials and Methods:

M059K and M059J brain cancer cell lines were exposed to fisetin alone or in combination with etoposide or cisplatin, respectively. Cell viability was evaluated by MTT assay after treatments. Western blot analysis was used to examine the expression of γ-H2AX, Chk2, Securin in M059K cells and caspase-3, caspase-9 in M059J cells. The caspase inhibitor z-VAD-fmk was used to suppress apoptosis induced by combination treatments of drugs on M059J cells.

Results:

We found that the treatment of etoposide or cisplatin combined with fisetin enhanced cytotoxicity induced by etoposide or cisplatin treatment alone in glioblastoma cells. M059K cells viability was significantly lower with fisetin-etoposide combination treatment than fisetin treatment alone. Furthermore, fisetin-cisplatin co-treatment exhibited higher cytotoxicity than fisetin treatment alone on M059J cells. Then, we investigated the molecular mechanisms involved in cell death of M059K and M059J after treatments with fisetin combined with etoposide or cisplatin, respectively. Our results showed that combination treatment with fisetin and etoposide on M059K increased expression of DNA damage response protein, such as γ-H2AX. Furthermore, combination of fisetin and cisplatin treatments on M059J cells enhanced expression of apoptotic proteins caspase-3 and caspase-9. Treatment with z-VAD-fmk (Caspase inhibitor) significantly inhibited expression of caspase-3 and -9 induced by combination treatment of fisetin and cisplatin on M059J cells.

Conclusion:

Our present study reveals that combination treatments with fisetin and chemotherapy drugs can increase cytotoxicity of glioblastoma in cancer chemotherapy. Our results show that combination treatment with fisetin and etoposide induced cell death in M059K cells and fisetin-cisplatin treatment induced apoptosis in M059J cells. Our results suggest that combination treatment strategy may improve the response of glioblastoma to chemotherapy drugs.

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Modulation of antitumor functions by tryptophan catabolism

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Backgrounds:

Tryptophan degradation by Indoleamine 2,3-dioxygenase (IDO) contributes tumor cells to cancer-associated inflammation, immune escape and tumor outgrowth. The tumor progression and antitumor efficacy of IDO-expressing tumor cells during chemotherapy is unclear.

Materials and Methods:

Murine B16/OVA melanoma cells were genetically modified to express mouse *Indo* cDNA. C57BL/6 mice were inoculated s.c. with 5×10^5 IDO or pcDNA plasmids transfected-B16/OVA melanoma cells on right flank. IDO inhibitor-Methyl-tryptophan (400 mg/kg) and paclitaxel (10 mg/kg) were injected intraperitoneally each 3 or 4 days. Tumor growth was measured by caliper weekly. To address whether IDO-expressed tumor cells affected the quantity, phenotype, and function of tumor-associated T cells, the numbers of tumor-infiltrating $CD8^+$ and $CD4^+CD25^+$ T cells were monitored for a 4-wk period.

Results:

Tumor weight per mouse increased in C57BL/6 mice implanted with IDO-overexpressed B16/OVA melanoma cells compare to B16/OVA melanoma cells. Tumor areas in MT/paclitaxel combination therapy groups were smaller compare to paclitaxel group. Flow cytometric analysis of tumors in mice treated with MT/paclitaxel and paclitaxel group showed a significant increase of $CD8^+$ T cells. Additionally, a synergistic effect of MT and paclitaxel was found to affect a decreased tumor-associated $CD4^+CD25^+$ T cells.

Conclusion:

The above results suggest that inhibition of tryptophan catabolism by IDO inhibitor-MT may restore cytotoxicity of antitumor activity. The augmented antitumor effect of MT/paclitaxel is supposed to be the result of the immunomodulating antitumor effect of MT. These results give hints that regulation of tryptophan catabolism by MT may be used as an adjuvant in medicinal applications of paclitaxel in IDO positive tumors.

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Telomere-associated TERRA in the form of DNA-RNA hybrid induces type II telomere recombination to bypass senescence

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Telomeres are transcribed into non-coding telomere repeat-containing RNA (TERRA) that associates with telomeres and may be involved in telomere function. In human somatic cells or telomerase deficient cells in yeast, telomeres are gradually shortened into senescence with cell divisions. Very few survivors can bypass the senescence through alternative recombination pathway to lengthening of telomeres. Our lab has employed two mutants of THO complex, *hpr1 and tho2* cells form premature survivors to bypass senescence in the absence of telomerase. Mutants in THO components markedly decrease the amount of GC-rich transcripts and caused co-transcriptional DNA:RNA hybrid accumulation (R-loop) to induce transcription-dependent hyper-recombination phenotypes. The premature survivor formation in THO mutants was also transcription dependent and co-appeared with decreasing TERRA transcripts and accumulation R-loop at telomeres. Overexpressing *RNH1*, which encodes RNase H, effectively alleviated premature senescence and telomere recombination phenotypes. Our results strongly suggested that the co-transcriptional stalled telomere R-loops induced premature senescence by activating DNA damage checkpoint Rad53-phosorylation and recruited recombination repair factors Rad51p and Rad52p to form premature survivors of senescence in *tho* mutants.

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Full-Length Human TDP-43 in Frontotemporal Lobar Dementia Forms Toxic Amyloid-Like Oligomers

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Backgrounds:

Proteinaceous inclusions are general hallmarks in many neurodegenerative diseases. TDP-43 proteinopathies, consisting of several neurodegenerative diseases like Frontotemporal Lobar Dementia (FTLD) and Amyotrophic Lateral Sclerosis (ALS), are characterized by formation of TDP-43 inclusion bodies, in which TDP-43 are aggregated with the presence of truncated forms, ubiquitination, and hyperphosphorylation. Little is known about the toxicity and structural properties of TDP-43 aggregates.

Materials and Methods:

Recombinant human full-length TDP-43 with N-terminal His-tag was expressed and purified from E. coli and characterized by analytical size exclusion chromatography, dynamic light scattering, dot-blotting, transmission electron microscopy, and atomic force microscopy. The cytotoxicity assay of full-length TDP-43 was performed in Neuro2A cells, mice primary cortical neurons and mouse brain via intracerebroventricular injection. A polyclonal antibody was generated by purified TDP-43 and used to identify TDP-43 inclusions in FTLD-TDP patients' brain tissues.

Results:

We demonstrated that the recombinant full-length human TDP-43 formed structurally stable, ring-shaped oligomers that shared common epitopes with anti-amyloid oligomer antibody. These oligomers had exposed hydrophobic surface and possessed reduced DNA binding capability. The TDP-43 oligomers are capable of cross-seeding and converting amyloid-β to amyloid oligomers rather than fibrils. The oligomers of TDP-43 were demonstrated to be neurotoxic *in vitro* and in vivo. The TDP-43 oligomer antibody raised by the recombinant TDP oligomers can recognize only TDP in the cytoplasmic inclusion bodies but not endogenous nuclear TDP-43 in the FTLD-TDP patient tissue.

Conclusion:

Our results demonstrated possible amyloid-like structure and toxicity mechanisms for TDP-43 proteinopathies.

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Application of Algae Samples With High Solar-To BioH2 Conversion Efficiency To Bio-Solar-Fuel Cell

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Backgrounds:

Hydrogen is a highly promising energy carrier to offer many advantages for environment-friendly fuel with a high energy density per mass without releasing pollutants due to its final production as water. The hydrogen fuel cells thus draw attention of most workers in this energy demanding era. The solar to bio-hydrogen have been developed to yield efficiently hydrogen in the last decades by green alga. The conversion of solar energy to biological H_2 by algae should be the cheapest and cleanest than other green energy technologies.

Materials and Methods:

The silicon-based cell of nano-/micro-array structure with electrodes would be integrated by the polymer membrane as micro proton exchange membrane fuel cells (μ-PEMFCs). Three-dimensional electrode chips were carried out by using the MEMS fabrication process, including lithography and deep silicon reactive ion etching technique. The micro-structured electrodes incorporated in micro-hole array with Platinum nano-catalysts supported multi-wall carbon nano-tubes (MWCNTs) inside to significantly enhance the electro catalytic reaction area. Furthermore, to reduce the charge transfer and oxygen diffusion impedances, the paths of fuel and oxidant diffusion inside the reaction hole array could be implemented by forming the effective three-phase zone with Nafion® solution. The green alga *Chlorella* with high desiccation-tolerance has been isolated from a dry surface of a power-transmitting cable at a mountain. It was cultured with initial chlorophyll concentration of 4 μgml−1 in a 200 ml Chlorella medium. Finally, μ-PEMFCs would be triggered by infusing H_2 from *Chlorella* as a Bio-Solar-Fuel Cell.

Results:

With MEMS technology, the high-surface-area electrodes in a single chip base on would be made up. The diameter and deep distance of channel in chip of fuel cell are 50 and 100 μm, respectively. The Bio-Solar-Fuel Cell has best performance of 8.2 nW/cm^2.

Conclusion:

The system devised in this study directly generates hydrogen for highly efficient fuel cell. This cell would be more convenient to be carried and applied as portable power source and a marvelous device for biomedical research.

P202

Long-term Low Dose Exposure of Human Urothelial Cells to Sodium Arsenite Induces DNA Methylation Profile Change and Activates Lipocalin-2 via Promoter Hypomethylation

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Backgrounds:

In our previous study, we demonstrated that long-term low-dose sodium arsenite exposure induced cellular transformation and aberrant gene expression in human urothelial cell line (HUC). Since epigenetic alterations play critical roles in the regulation of gene expression, we performed the system analysis to identify the DNA methylation profiles in arsenic-exposed human urothelial cells (iAs) in this study.

Materials and Methods:

By aid of the Infinium Assay, we selected 63 genes with promoter methylation differences between HUC and iAs cells over 75%. As compared to HUC cells, 40 genes showed hypomethylated in iAs cells, while 23 genes hypermethylated. The gene expression profiles after treatment with 5-aza-dC was analyzed by quantitative real-time PCR. In addition, bisulfite sequencing analysis was performed to confirm the methylation status in arsenic-exposed cell lines and bladder cancer samples.

Results:

The results showed that 80% of hypomethylated gene expression levels in iAs cells are higher than HUC. However, only 35% hypermethylated genes consistently expressed lower levels of transcripts in iAs cells than HUC. Among the selected 9 genes, the relative methylation level of these genes which is consistent with the methylation array. Among these genes, we demonstrated that LCN2 played crucial roles to resist serum deprivation as well as mediated through activation of NF-κB signaling to increase the expression of pro-inflammatory genes. In addition, we found that LCN2 promoter were significantly hypomethylated in bladder tumors compared to adjacent normal tissues.

Conclusion:

Taken together, our present results showed that long-term and low dose arsenic exposure mediates through epigenetic mechanism to reprogram the gene expression profile and activates genes which are of essential to promote oncogenic progression.

P203

VEGF Links Glucose Uptake in Inflammatory Bowel Disease Pathogenesis

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Backgrounds:

Vascular endothelial growth factor (VEGF) plays a central role in physiologic angiogenesis and chronic inflammation. Recent reports have documented increased serum and tissue levels of VEGF in inflammatory bowel disease (IBD) patients. Na+/glucose cotransporter 1 (SGLT-1) is the main apical transporter for active glucose uptake in small intestine. It was also shown that SGLT1 is inhibited in the villus cells in the rabbit model of IBD and the inhibition is due to a decrease in the number of cotransporters. Despite of many studies, the effect and mechanism of VEGF on SGLT1- expression and its implications in IBD pathogenesis has not been understood.

Materials and Methods:

The specific substrate of SGLT-1, α- methylglucopyranoside (AMG), was used in glucose uptake assay. The mechanism involved in the regulation of VEGF-modulated down-regulation of SGLT-1 gene expression were performed using promoter reporter assay, nuclear translocation assay and CHIP assay in differentiated human intestinal Caco-2 cells. The effect of *in vivo* VEGF on glucose uptake and SGLT-1 expression was carried out using the trinitrobenzene sulfonic acid (TNBS)-induced IBD mouse model. The histopathology of IBD was examined by H&E staining. In addition to the IBD mice, the increased *in vivo* VEGF level has also been introduced directly by Intraperitoneal injection. Targeted gene silencing was performed by lentiviral delivery of shRNA .

Results:

The expression levels of VEGF and VEGFR2 are significantly increased in mice with TNBS-induced colitis. The overexpressed VEGF plays an essential role in the decreased glucose uptake and SGLT-1 expression in the intestinal of IBD mice. Further confirmation of the role of VEGF was obtained from overexpression of VEGF antagonist Cyclo-VEGI in colitic mice. Western blot and Q-PCR analysis demonstrated that the effect of VEGF on glucose uptake was due to the decreased level of SGLT-1. Transient transfection study of the SGLT-1-luciferase reporters demonstrated that the down-regulation occurred primarily at the transcriptional level. In addition, nuclear translocation assay and CHIP assay demonstrated that the increased binding of Sp-3 and the decreased binding of Sp-1 and CBP are responsible for the VEGF-mediated inhibition of the SGLT-1 transcriptional activity. Furthermore, PI3K and JNK signaling pathways were found to be involved in the inhibitory effect of VEGF.

Conclusion:

Our results demonstrated the molecular and signaling mechanisms involved in VEGF-mediated inhibition of intestinal SGLT-1 gene expression. The VEGF appears to play an important role in the IBD-elicited pathogenesis by inhibition of intestinal glucose uptake. This finding suggests that blockade of VEGF may represent a new strategy in patients with IBD. In summary, our finding provides a mechanistic link between intestinal glucose uptake and IBD pathogenesis.

P204

7-Hydroxydehydronuciferine Induces Human Melanoma A375.S2 Cell Death via Apoptosis and Autophagy

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Backgrounds:

To confirm that there were dual cell death machineries via apoptosis and autophagy inductions in 7-Hydroxydehydronuciferine treated A375.S2 cells to be a potential effective chemotherapeutic agent. Materials and Methods: We identified that 7-hydroxydehydronuciferine (7-HDNF) isolated from the leaves of Nelumbo nucifera Gaertn cv.rosa-plena to be a bio-active agent against human melanoma A375.S2 cells.

Results:

Via cell proliferation assay demonstrated the strongest anticancer effect of 7-HDNF on A375.S2 cells to exhibit a dose-dependent behavior. The apoptotic cell death ratio was measured via two-dimensional flow cytometry to confirm the cellular membrane asymmetry losing. One-dimensional flow cytometric analysis showed that 7-HDNF increased cellular DNA population in cell cycle at G2/M phase. With acridine orange staining, we found that 7-HDNF induced patterns of autophage. Unexpectedly, such as the formation of intracellular vacuoles and the augmentation of acidic vesicular organelles. Through western blot assay, protein expressions were discovered to verify apoptosis and autophage response mechanisms sharing some common associated pathways. In addition to, 7-HDNF presented the high-quality anti-migratory activity in wound healing assay.

Conclusion:

In vitro test, we confirm that there were dual cell death machineries via apoptosis and autophaginductions in 7-Hydroxydehydronuciferine treated A375.S2 cells to be a potential effective chemotherapeutic agent. In vivo research, we have co-culture model organisms, mouse and it represent a related system to test with pharmacological product.

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P205
Aquaporin-2 Serine 269 Phosphorylation Reduces Its Internalization by SIPA1L1 in mpkCCD Cells

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Backgrounds:

Renal water excretion is regulated by the collecting duct cells in response to the peptide hormone vasopressin. Vasopressin induces translocation of water channel protein aquaporin-2 (AQP2) from intracellular vesicles to the apical plasma membrane of the cells where AQP2 transports water from the forming urine to the interstitium thereby reducing water excretion. Vasopressin changes AQP2 phosphorylation at four sites in the COOH terminus. Among them, serine 269 phosphorylation (pS269) is strongly up-regulated by vasopressin and results in apical localization of AQP2. However, the molecular basis for pS269 AQP2 apical localization is largely unknown. Because serine 269 is located in the PDZ motif, we systematically analyzed PDZ domain-containing proteins expressed in rat and cultured mouse collecting duct cells (mpkCCD). SIPA1L1 was identified and shown to enhance pS269 AQP2 apical localization via a reduced PDZ interaction.

Materials and Methods:

Surface biotinylation followed by streptavidin affinity purification was coupled to LC-MS/MS to profile apical membrane proteins in the mpkCCD cells. Apically expressed PDZ domain proteins were identified in published transcriptome and proteome databases of rat collecting ducts and mpkCCD cells (http://helixweb.nih.gov/ESBL/Database/index.html). shRNA-based gene knockdown was conducted to examine their functions in vasopressin-induced AQP2 apical localization using immunofluorescence confocal microscopy. Phosphorylation-mimicking (AQP2-DKA) and phosphorylation-ablated (AQP2-AKA) AQP2 mutants at serine 269 were constructed to verify the roles of pS269 in AQP2 apical localization. An AQP2 polyclonal antibody was raised against a synthetic peptide corresponding to amino acid residues 241-254 of AQP2. Co-immunoprecipitation was used to examine protein-protein interactions between AQP2 and PDZ domain proteins. The SIPA1L1 polyclonal antibody (V-20) was purchased from Santa Cruz.

Results:

One hundred and ninety-one proteins were identified in the apical plasma membrane of the mpkCCD cells. Four apically expressed PDZ domain-containing proteins (SIPA1L1, GOPC, MPP5, and PDZRN3) were identified based on published transcriptome and proteome databases of rat collecting duct cells and mpkCCD cells. SIPA1L1 knockdown resulted in apical localization of wild type AQP2 (AQP2-WT) in the mpkCCD cells in the absence of the vasopressin analog dDAVP, suggesting a role of SIPA1L1 in AQP2 internalization. Compared to pS269-mimicking AQP2 mutant (AQP2-DKA), pS269-ablated AQP2 mutant (AQP2-AKA) had a higher binding preference for SIPA1L1, suggesting that SIPA1L1 facilitates AQP2 internalization when serine 269 is not phosphorylated. Indeed, SIPA1L1 knockdown resulted in apical localization of pS269-ablated AQP2 in the absence of dDAVP.

Conclusion:

Using systems approaches, we identified a PDZ domain protein SIPA1L1 that mediates AQP2 internalization. Vasopressin induces AQP2 serine 269 phosphorylation, resulting in a reduced interaction with SIPA1L1. As a result, vasopressin increases pS269 AQP2 retention in the apical plasma membrane.

P206
Immunomodulatory Effect of Fucoidan Isolated from Sargassum Cristaefolium in Balb/c Mice and RAW264.7 cells

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Backgrounds:

Fucoidan refers to a type of polysaccharide which contains substantial percentages of L-fucose and sulfate ester groups, mainly derived from brown seaweed. In this study, fucoidan isolated from *Sargassum cristaefolium* was investigated for its immunomodulatory potential using Balb/c mice and RAW264.7 cells.

Materials and Methods:

Mice were orally administrated with fucoidan at the doses of 820, 1640 and 3280 mg/kg body weight for 8 weeks. The effects of serum antibodies (IgG, IgA, IgM and IgE) level, splenocyte proliferation, production of T helper 1/T helper 2 cytokines from splenocyte, splenic nature killer (NK) cell activity and phagocytosis of monocytes were measured. During the cell experiment, FBS-free DMEM was used and RAW264.7 cells was cultivated with different concentrations of fucoidan for 24 h. Production of nitric oxide (NO) and Nitro-Blue tetrazolium chloride (NBT) induced by lipopolysaccharide (LPS) were measured.

Results:

In animal experiment, the results showed that fucoidan significantly increased proliferation of splenocyte proliferation, induced the secretion of Th1 cytokines including interleukin-2 and Interferon-γ, significantly enhanced NK cell activity and phagocytic capacity in monocytes. In serum, the results demonstrated the level of IgG, IgM and IgA were higher than control, and a significant decrease in levels of IgE. In RAW264.7 cells, fucoidan treatment significantly inhibited excessive production of NO and NBT in LPS-induced pro-inflammatory.

Conclusion:

This study suggested that fucoidan isolated from *Sargassum cristaefolium* possesses immunomodulatory properties and can be used as potential natural reagent for immune regulation.

P207
Novel Biodegradable Porous Scaffold for Skin Wound Healing

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Backgrounds:

Burn, abrasion or injury wounds, substantial loss of dermal tissues, heal with wound contractures and the formation of scar tissues. To enhance the growth of skin cell is a world-wide issue and costly procedure for each age range.

Materials and Methods:

A novel porous scaffold with collagen, hyaluronic acid (HA) and gelatin was fabricated for skin wound repairing.

Results:

The water absorption capacity of air-dried sponge-like collagen/HA/gelatin scaffold was over 20 g water/g dried scaffold. The *in vitro* degradation rates of this scaffold by lysozyme, hyaluronidase, or collagenase I showed good biodegradation abilities. The average pore diameter of the dried scaffold estimated by SEM image was 132.5 ± 8.4 m. After FBs seeded 14 days, the SEM image illustrated surface fractures inside the scaffold, indicating the material biodegradable properties. We investigated the therapeutic effects of scaffold on an *in vivo* rat model of excision skin wound on the back. The quantitative image analysis of the area of the excision wound was performed and indicated that the scaffold promoted the wound healing rate. From histological observations, treatments with scaffold ameliorated wound healings, including increasing neutrophils infiltrates and higher density of new generate epidermis and thickening of the epidermis.

Conclusion:

We made the sponge-like scaffold from suitable biomaterials, collagen, HA and gelatin, which are biodegradable and biocompatible in the human body and adjusted the mechanical strength by cross-linking with EDC. Our co-culture model represented an alternative system for testing pharmacological products in the place of laboratory animals, including mouse, rat or rabbits.

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P208
Autophagy Inhibition Sensitizes The Growth Inhibitory Effects of Propolin C But Not Propolin G on Human Lung Adenocarcinoma A549 Cells

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Backgrounds:

Propolins, prenylflavanones are isolated and characterized from Taiwanese propolis, have been shown inhibits certain cancer cell lines growth and induced apoptosis. The inhibitory effects of propolin C and propolin G on human lung adenocarcinoma A549 cells through autophagy and apoptosis pathway.

Materials and Methods:

A549 cells treated with propolin C for 72hr and cell viability was assessed by MTT assay. The PI3K/Akt/mTOR pathway and MEK/ERK pathway were detected by western blot. Cell morphology were observed with acridine orange or annexinV stain. Finally, autophagy inhibitor chloroquine (CQ) or the 3-methyladenine (3MA) combined with propolin C were assay to evaluate the effects of the A549 cells.

Results:

Propolin C could promote LC3B increased within 6 hours treatment, moreover, and AVOs (acidic vesicular organelles) significantly increased by AO (Acridine orange) staining. The autophagy induced by propolin C is accompanied by the inhibition of the PI3K/Akt/mTOR signaling pathway. Flow cytometry assay showed that sub-G1 phase doesn't change in propolin C treatment. Autophagy pharmacological inhibitors, 3-methyladenine and Chloroquine were used to test whether autophagy blockade could lead to enhance cell death. The results showed that only autophagy inhibitor chloroquine effectively enhanced apoptosis induced by propolin C in A549 cells. The expression of pERK and pAkt was suppressed dramatically by autophagy inhibitor in combination with propolin C. Ectopic expression of mutant Akt (constitutive active) in A549 cells abolished chloroquine sensitization effect of propolin C.

Conclusion:

Autophagy serves a protective role in propolin C-mediated cytotoxicity, and autophagy modulators may be used as adjunctive therapeutic agents for propolin.

P209
YPetri – Domain Model And Simulator of Functional Petri Nets for Cell-Biological System

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Background:

Nowadays, Petri nets (PNs) are commonly employed to describe cell-biological systems. PNs consist of places, representing system state (chemical concentrations, gene activation etc.) and transitions, representing temporal behavior (biochemical reactions etc.). Functional Petri nets (FPNs) allow one to specify operation of the transitions as an arbitrary function of the system state. To provide alternative to the existing FPN software, such as Cell Illustrator, we developed a new model and simulator of the FPN domain – YPetri.

Materials and Methods:

YPetri is written in Ruby and provides textual domain-specific language (DSL), that can be used either interactively, or as a script. It is generally understood, that graphical user interfaces (GUIs) cannot fully encompass cell-biological modeling. In all extant software, users must learn "formula language" – a scripting language describing the system behavior. Rather than using "formula language", YPetri provides powerful Ruby-based DSL. A DSL can be thought of as a user-friendly API.

Results:

We have used YPetri DSL to describe the pathway maintaining the cytoplasmic pool of deoxythymidine triphosphate (TTP). TTP is a nucleotide tied specifically to DNA replication, whose pathway is a frequent target of anti cancer drugs. During S phase, consumption of deoxyribonucleotides by the replicating DNA is very high compared to their cytoplasmic pools. TTP pool in particular requires highly responsive regulation of its synthetic pathway in order to maintain homeostasis. With YPetri, we were able to find and validate the parameter set, that exhibits robustness under the changing load of TTP consumption, as the cell cycle progresses from G1 hase, DNA replication starts in S phase, and TTP consumption decreases again as the replication forks gradually close towards G2 phase.

Conclusion:

The keywords of YPetri DSL are few in number and easy to learn, making simple modeling tasks easy with YPetri. The main design consideration; however, is ergonomy. YPetri does not sandbox the user inside its command interface. Rather, YPetri DSL extends the Ruby language, whose full power remains at user's disposal, if needed, thus making tasks of arbitrary complexity tractable.

P210
Serum N-glycans Profiling for the Discovery of Potential Oral Cancer Markers

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Backgrounds:

Glycosylation is a common and complex form of protein post-modification which plays important roles in protein folding, stability, activity, trafficking, and molecular recognition. Aberrant glycosylation in cells, which has been demonstrated to be potential cancer biomarkers, is reported to be associated with tumorigenesis and metastasis. Oral cancer is the tumor grows on the lips, tongue, cheeks, floor of the mouth, hard and soft palate, sinuses, and pharynx. The high prevalence and mortality rate of oral cancer in Taiwan makes it important to investigate new biomarkers for the surveillance of high-risk population. Recent years, mass-spectrometric (MS) glycomic measurements which can determine the molecular mass and predict the composition and structure of the analytes have been pursued as a powerful adjunct for biomarker discovery.

Materials and Methods:

In this study, serum *N*-glycomes from normal populations and oral cancer patients (obtained from tissue bank of NCKUH) were analyzed. Total serum proteins were lyophilized and subjected to chemical reduction, alkylation and trypsin digestion. The N-glycans were released by treated glyco-peptides with PNGase F and purified by Sep-Pak C18 cartridge. After permethylation, the purified *N*-glycans were analyzed using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) in positive ion mode.

Results:

The mass spectrum obtained from 20 μl of serum sample indicated that the primary structures of serum *N*-glycans could be predicted by GlycoWorkbench software. The relative intensity of each proposed N-glycan structure was calculated by expressing the intensity of each glycan ion as percent of the total intensity of all glycan ions.

Conclusion:

By analyzing the relative intensity of different *N*-glycan subclasses or specific *N*-glycan structure, we may find some differences between oral cancer patients and normal people. The differences may be a potential target for the discovery of oral cancer biomarkers. We are now expanding the sample sizes of normal and oral cancer patient serums to identify potential and novel carbohydrate tumor markers.

P211
Developing Specific Antibodies against Biomarkers of Gastric Cancer for Early Diagnosis

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Backgrounds:

Gastric cancer is the fourth frequent cancer and the second leading cause of cancer related death worldwide. There is some geographical variation in the distribution of gastric cancer, which is considered to have something to do with the dietary risks due to the local stomach-stimulating food culture in these countries. Understanding the progression of gastric cancer is important for the following cancer therapy and drug treatment strategies. However, the most common diagnosis methods used nowadays all require various kinds of invasive ways in the diagnosis, exploiting expensive instruments and complicated steps in sample analysis. Therefore, it is necessary to develop a specific, accurate and easy-operating diagnosis method for gastric cancer.

Materials and Methods:

The processes of my work start from gene cloning of the biomarkers, further protein expression and purification. Then, exploit these biomarkers as antigens for mice immunization to produce specific antibodies. With these antibodies as detecting materials, I would develop a diagnosis system for gastric cancer detection of serum samples. The brief idea of the system is exploiting antibody, which are initially immobilized on a nanogold/thionine/DNA-modified gold nanoparticles. With a sandwich-type immunoassay format was employed for the detection of biomarkers.

Results:

We have succeeded in producing four recombinant proteins of biomarkers and also the monoclonal antibodies of these biomarkers including TIMP1, OPN, CXCL1, and SPARC. Specificity and selectivity identification tests are under progressing so far. The sensitivities of these antibodies is around 50ng/mL so far, which are expected to be lower in the diagnosis system.

Conclusion:

Nowadays, the blood sample is the most general kind among all. Most of the targets for gastric cancer detection including biomarkers and genes expression, which are usually some gastric cancer-induced controlling cytokines or chemokines. Therefore, the strategy of my work is to develop specific antibodies against biomarkers of gastric cancer in early diagnosis.

P212
The influence of hyaluronic acid on UVA treated dermal fibroblast

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In this research, we prepare the UVA, which can penetrate into dermal layer of skin to treat fibroblasts, the most cells in dermal. We explore the damage of skin such as ECM degradation, wrinkles, and elasticity losing cause by UVA irradiation and photoaging. Then the research is focused on “The influence of hyaluronic acid on UVA treated dermal fibroblasts”. Hyaluronic acid can effectively ease the degradation of ECM, as well as restore the elasticity of fibroblasts. Fibroblasts get harm after UVA irradiation treatment, and led to low cell viability. Besides, UVA can induce MMP-1 and MMP-3 expression, which degrade ECM. UVA can also induce IL-1β expression, resulting inflammation. IL-1β promote MMP-1 and MMP-3 further, making the ECM losing more over. In addition, UVA reduce the synthesis of collagen and elastin. In the high degradation and low synthesis of ECM, obviously, the elasticity of fibroblasts reduced down analyzed by AFM. Fibroblasts led to photoaging by treating UVA. We find out the damage fibroblasts treated by hyaluronic acid can be down- regulated the gene expression of MMP-1, MMP-3, and IL-1β, and slow down the degradation of ECM. In the other hand, hyaluronic acid can little recover the regeneration of elastin but collagen in damage cell. Because the low degradation rate of ECM and recovery of elastin synthesis, the elasticity of fibroblasts are effectively restored. The sum of these results, the hyaluronic acid only has the ability to ease the photoaging fibroblasts caused by UVA, but not return to its normal state.

P213

Pseudomonas aeruginosa Colonization Increased the Ventilator-Associated Pneumonia in Mice through the TNF-α and JNK signal pathways

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Although mechanical ventilator (MV) is a life-saving medical equipment of patients with acute lung injury/acute respiratory distress syndrome (ALI/ARDS), longer ventilation time contributes to lung injury and increases pathogenic bacterial infection which lead to higher mortality of patients. Pseudomonas aeruginosa is the most common frequent causative microorganism of ventilator-associated pneumonia (VAP). C57BL/6 mice (WT mice) with *P. aeruginosa* nasal instillation 2 days before receiving 3-hr ventilation were used to study the pathogenesis mechanism of VAP induced by *P. aeruginosa* colonization. The data show that the levels of neutrophil sequestration, nitrite concentration and pro-inflammatory cytokines, and number of alveolar macrophages in bronchoalveolar lavage fluid (BALF) are significantly increased after ventilation in mice instilled with *P. aeruginosa*. The results suggest that *P. aeruginosa* instillation prior to ventilation increases the severity of inflammation in lungs. When ex vivo alveolar macrophages were stimulated with live *P. aeruginosa*, the concentration of tumor necrosis factor-α (TNF-α) in supernatants is increased 3-fold, indicating that TNF-α is a critical regulator of VAP. Furthermore, WT mice and $JNK^{-/-}$ mice were instillated with supernatants of *ex vivo* alveolar macrophages stimulated with live P. aeruginosa 1 hr before ventilation. The instillation caused more severe lung injury in WT mice than $JNK^{-/-}$ mice. $JNK^{-/-}$ mice instillated with supernatants showed lower levels of neutrophil sequestration and pro-inflammatory cytokines than WT mice, suggesting that JNK signaling is involved in the pathogenesis mechanism of VAP.

P214

Coup TF1b is important for vasculogenesis and angiogenesis in zebrafish

牟育正

Genetic programs and signaling pathways are required for proper growth and patterning of blood vessels, however, little known about the transcription factors functioning in vein identity and intersegmental vessels (ISV) patterning in zebrafish. The orphan nuclear receptor Chicken ovalbumin upstream promoter transcription factor II (CoupTFII) positively regulates vein identity in mice. We previously identified the first transcription factor Islet2 (Isl2) is required for specification of the vein and ISV growth in zebrafish. Here we show that the CoupTFIb is also important for vein and tip cell identity.

Comparison of amino acids sequence of and phylogenetic analysis of CoupTFIb orthologs in different species suggesting the CoupTFIb is conserved among vertebrates. *CoupTFIb* mRNA has a spatiotemporal expression pattern in ventral lateral mesoderm consistent with a role in early vascular specification. Morpholino knockdown of coupTFIb results in a decrease in both venous fluorescent signals and expression of the vein specific marker *flt4* and *mrc1*. These data suggest that coupTFIb has novel role in promoting vein identity. In addition, we show loss of coupTFIb impairs ISV growth, suggesting that coupTFIb also has an important role in controlling ISV growth. We have confirmed those vascular defects are CoupTFIb specific by using two different types of morpholinos knockdown strategies (ATG and splicing morpholinos). To address whether the decrease in vein signals and the growth defect in ISV result from cell death, we performed TUNEL assay. We showed that an increase in non-specific cell death after morpholino injection is not the cause of the observed vascular phenotype. We further test CoupTFIb likely interact with the Notch signaling pathway to control vascular development. Together, we show coupTFIb plays an indispensable role for vascular development in zebrafish.

P215

Con A-Affinity-Based Serum Glycoproteomic Analysis for Patients Before and After Hemodialysis

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Backgrounds:

Chronic kidney disease has emerged as a global public health issue. Taiwan has the highest incidence and prevalence rates of end-stage renal disease (ESRD) in the world, resulting in increased medical costs mainly from hemodialysis. In this study, in order to understand and control the dialysis incidence, we investigate serum protein contents, especially glycoproteins of patients before and after hemodialysis, using Concanavalin A (Con A)-affinity chromatography-based glycoproteomic approaches.

Materials & Methods:

Serum specimens collected from twenty patients (aged 31-61 years) before and after hemodialysis were obtained from Taipei Medical University-Shang Ho Hosptial. Pre-dialysis and post-dialysis serum specimens from ten patients were each pooled and treated with agarose-bound Con A for glycoprotein extraction and purification. Glycoprotein samples were then separated on SDS-PAGE. Protein bands were excised and subjected to in-gel trypsin digestion. Tryptic peptide mixtures were then subjected to LC-ESI-Q-TOF MS/MS analysis and protein identification was performed using an in-house Mascot program. The statistical analyses were done by Student's t tests to reveal protein expression levels significantly altered before and after hemodialysis. A p value of less than 0.05 was considered statistically significant.

Results:

The results were obtained in triplicates from three independent experiments. From hundreds of proteins indentified, there were seven proteins with a p value of less than 0.05. Three proteins, hemoglobin subunit alpha, hemoglobin subunit beta and fibrinogen gamma chain were up-regulated, while alpha-1-acid glycoprotein 1, N-acetylmuramoyl-L-alanine amidase, monocyte differentiation antigen CD14 and desmoglein-1 were down-regulted. Among them, fibrinogen gamma chain only appears in post-dialysis serum, while desmoglein-1 only exists in pre-dialysis serum. Western blot analysis will be applied to verify the proteomics results.

Conclusion:

A detailed survey of these seven proteins will be performed to further delineate their relationships with hemodialysis and renal functions. These findings may reveal the key protein markers before and after hemodialysis, thus facilitate prevention of ESRD and control of dialysis incidence.

P216

Modification of Phenylboronic Acid on Gold Surface and the Study of Antibody Immobilization

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Backgrounds:

Although ELISA is very stable, but the experiment is waste a lot of time. One after another the new biosensor was found now, like SPR QCM etc. So we choose the QCM because it is too small , easy to carry and the most important one is very fast for the experiment.

Materials and Methods:

First we use α-lipoic acid and 3-aminophenylboronic acid to synthesize the linker. One of the side is sulfide and another side is phenylboronic acid. We modification the linker on the QCM chip. And then modification of antibody on the linker which is on the QCM chip. After modification of

antibody on the linker, we can use second antibody which in different concentration. Then we add TMB to QCM chip to look the change of the TMB's color by the wavelength absorption of ELISA.

Results:

We can sure the linker is right by NMR. By the CV test, we can know the

Change of electric current. Further speculated that we know the linker and the antibody one by one modification o the QCM chip. Then through the different color's change, we sure the QCM chip is modified by second antibody.

Conclusion:

The future, we will put the QCM chip which is modified by linker, antibody and second antibody in the different PH buffer to find the safe range. Also we use the same QCM chip to check the number of the antibody modification on QCM chip. After that we add the real sample to QCM chip for the result.

P217
Anti-NLRP3 Inflammasome Activity of Stigmasterol

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Backgrounds:

Stigmasterol is an unsaturated plant sterol showing anti-inflammation, anti-cancer, anti-microbial, and anti-osteoarthritic; however, the effect of stigmasterol on inflammasome activation is unclear.

Materials and Methods:

Effect of stigmasterol on NLRP3 inflammasome activation in LPS- and ATP-activated macrophages was monitored by detecting IL-1β secretion and caspase-1 activation using ELISA and western blot respectively. LPS- and ATP-mediated signaling associated with NLRP3 inflammasome activation were measured by western blot.

Results:

Stigmasterol reduced IL-1β secretion and caspase-1 activation by inhibiting both priming signal and activation signal of NLRP3 inflammasome in LPS- and ATP-activated macrophages. The stigmasterol also reduced LPS-induced protein expression levels of NLRP3 and IL-1β precursor. The underlying mechanisms for the anti-NLRP3 inflammasome activity were demonstrated as reducing ATP-induced phosphorylation of ERK1/2, but not JNK1/2, PKC-α, and PKC-δ. Stigmasterol also inhibited the activation of NLRP1and NLRC4.

Conclusion:

These results demonstrate that stigmasterol inhibited NLRP3 inflammasome activation through reducing not only the priming signal, but also the activation signal, of NLRP3 inflammasome in LPS- and ATP-activated macrophages.

P218
Identification of IRES trans-acting factors (ITAFs) Involved in the Hypoxia-induced Up-regulation of FGF9 Protein Expression

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Background:

Hypoxia is a reduction in the normal level of tissue oxygen tension, and involved in many human diseases including acute and chronic vascular diseases, pulmonary diseases and cancers. Under hypoxia, canonical cap-dependent translation is generally inhibited, many hypoxia-responding genes initiate their translations with an alternative mechanism known as internal ribosome entry site (IRES)-mediated translation. The IRES-mediated translation requires RNA sequence to form a complex tertiary structure in the 5'untranslated region (5'UTR). It has been demonstrated that some proteins, named IRES trans-acting factors or ITAFs, are interacting with IRES-containing mRNA and recruiting ribosomes to the IRES element. Thus, these ITAFs directly involves in the initiation of IRES-dependent translation. Our previous study showed that FGF9 5'UTR contains an IRES element and FGF9 protein expression is up-regulated during hypoxia through this IRES-mediated mechanism.

Material and method :

Both cytosol and nuclear fractions were isolated from hypoxia-treated HEK293 cells and incubated with biotinylated-RNA probes, which were in vitro transcribed from different regions of FGF9 5'UTR. After pulled-down by streptavidin-agarose beads, the captured proteins were separated by SDS-PAGE and followed by silver stain. Specific bands were excised from the gels and subjected for protein identification by MALDI-TOF analysis. The results of mass analysis were confirmed by Western blot.

Result :

The results of RNA pull-down followed by mass analysis revealed that NF90 and hnRNP M bind to FGF9 IRES region. These results were confirmed by Western blot. In addition, screening the association between FGF9 mRNA 5'UTR and common ITAFs also identified hnRNP C1/C2 and HuR interacting with FGF9 mRNA 5'UTR.

Conclusion:

Our results demonstrated that NF90 and hnRNP M specifically bind to the FGF9 IRES region, while hnRNP A1,C1/C2, PTB, HuR bind to the other regions of FGF9 5'UTR. These proteins may form a protein network to regulate FGF9 hypoxia-induced, IRES-mediated translation. In this study, we have identified FGF9 IRES interacting ITAFs and illustrate their role of this interaction in the regulation of FGF9 expression during hypoxia.

P219
The biological function of endothelial cells to natural polymers comprised of nanogold composites and the associated signaling pathway

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Backgrounds:

The biological function of endothelial cells (ECs) on two novel nanogold composites (fibronectin-nanogold composites, FN-Au) and (collagen-I-nanogold composites, Col-Au) containing smaller amount of nanogold (≈ 43.5 ppm) were used as a model system to study the molecular mechanisms that influenced endothelial cell migration and proliferation on biomaterial surfaces.

Materials and Methods:

The cellular behavior and signaling pathway of ECs on the FN-Au and Col-Au was evaluated by a series functional assay (such as flow cytometry, western blot, MMP zymography and migration rate test).

Results:

It was found that ECs had the highest migration rate on the FN-Au and Col-Au while containing 43.5 ppm of AuNPs. The high ECs migration rate was associated with increased levels of endothelial nitric oxide synthase (eNOS) and phosphorylated-Akt (p-Akt) expressed by ECscultured on FN-Au and Col-Au. The higher expression of α5β3 integrin for ECs on FN-Au and Col-Au was also demonstrated. Phalloidin staining showed that actin appeared as a circumferential band surrounding each cell on control group (glass), whereas on FN-Au and Col-Au , the cells had their margin spread out and extend processes with stress fibers in the protruding lamellipodia. The higher MMP-2 Protein expression on FN-Au and Col-Au was also observed.

Conclusion:

It was concluded that FN-Au and Col-Au activated α5β3 integrin/MMP-2 signaling pathway in ECs, leading to better proliferation and migration effect of ECs on these surfaces.

P220
The Effect of Novel Histone Deacetylase Inhibitors in Human Pancreatic Carcinoma

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Backgrounds:

Pancreatic carcinoma is one of the leading causes of cancer mortality. In the worldwide, either the prevalence or incidence of pancreatic cancer is increasing. Despite recent progress in chemoradiation therapy and improved systemic chemotherapeutic agents, the 5-year survival rate is still less than 10%. In many cancers, histone acetylation plays an important role in the epigenetic regulation of gene expression. Recent studies have suggested that histone deacetylase inhibitors could induce histone acetylation and resulted in chromatin remodeling as well as repression of tumor suppressor genes.

Materials and Methods:

The cell viability was measure by MTT assay. Caspase-3 activation was measure by flow cytometry and analyzed by WinMDI analysis software. The expression of apoptosis-related proteins, including members of Bcl-2 family and survivin, was determined by western blot. The expression of histone acetylation and p21 were also determined by western blot.

Results:

We investigated the effect of a novel phenylbutyrate-derived histone deacetylase inhibitor (HDACi) in human pancreatic cancer cell lines. Novel HDACi induced pancreatic cancer cells cytotoxicity and apoptosis. Novel HDACi also suppressed the expression of survivin. Conversely, the members of Bcl-2 family, including anti-apoptotic proteins Bcl-2, Bcl-xL, and pro-apoptotic proteins including Bak, Bid, were unchanged upon novel HDACi treatment. p21, which is a cyclin-dependent kinase inhibitor, was significantly induced by novel HDACi.

Conclusion:

In summary, novel HDACi induced cell apoptosis through repressing survivin expression as well as up-regulating the expression of p21, but not via Bcl-2 mitochondrial apoptotic pathway. Our results demonstrated that novel HDACi is a potential drug for cancer therapy.

P221
從缺

P222
The Metabolic Pathway of Polyamine Participates in Lymphoma Apoptosis

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Backgrounds:
The metabolites of polyamine play important roles in cell growth, differentiation and cell survival. Studies have reported that increased polyamine levels were found in cancer cells suggesting the regulation of polyamine might be changed in cancer cells. This study was designed to investigate the effect of spermine on lymphoma proliferation.
Materials and Methods:
Three lymphoma cell lines of U937, Raji and Jurkat with different degree of differentiation were treated with 0,10, or 20 μM spermine for 24 h, and then detection of cell viability, apoptosis, cell cycle progress, mitochondrial membrane potential and apoptosis-related proteins expressions.
Results:
Results showed Jurkat cell line was more sensitive to spermine than the other two cell lines. Treatment of Jurkat cells with 20 μM spermine, DNA fragmentation was found. The cell death ratio reached 20 %, or 50 % as treatment with 10 μM, or 20 μM spermine, respectively. Western blotting showed cytochrome c release and caspase-9 and caspase-3 expressions were increased by spermine treatment.
Conclusion:
These results demonstrate spermine can induced apoptosis of Jurkat cells, but not of U937 and Raji cells. The precise mechanisms of spermine effects on Jurkat cells are worth further exploration.

P223
Characterization of Serine/Threonine Kinase from Red Sea Bream Iridovirus on Nucleus Fragmentation-mediated Cell Death

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Backgrounds:
Virus induced cell death, including apoptosis and nonapoptotic cell death, plays a critical role in the pathogenesis of viral diseases. Red sea bream iridovirus (RSIV), a iridovirus of genus megalocytivirus, is a causative agent of lethal infections in many cultured marine fish species in Taiwan, and causes high mortality and heavy economic losses in aquaculture. RSIV is belonging to a large ds-stranded DNA virus that it replicated in nucleus.
Materials and Methods:
In previous study showed that RSIV induced apoptosis in fish cells. Here, we characterize serine/threonine (S/T) kinase of RSIV involved in cell death. The kinase gene was cloned and fused with EGFP, which green fluorescent GFP protein can tracer the fusion protein localization within cells.
Results:
The S/T kinase fusion protein was transiently expression in fish GF-1 cells. The S/T kinase fusion protein was located in nucleus that induced nucleus fragmentation and caspase-8 activation.
Conclusion
This is a first report to examine the RSIV S/T kinase can target into nucleus and induced nucleus fragmentation. This finding may contribute to understanding the pathogenesis mechanism of iridovirus.

P224
Acetaminophen Inhibitions of Ethanol Metabolism with Human Alcohol and Aldehyde Dehydrogenases

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Backgrounds:
Acetaminophen, i.e. *p*-acetamidophenol, is widely used over-the-counter analgesic, antipyretic medication. The drug's hepatotoxicity can be significantly enhanced by chronic excessive alcohol consumption. ADH and ALDH are the principal enzymes responsible for metabolism of ethanol in humans. The goal of this study was to investigate the acetaminophen inhibitions of ethanol oxidation with recombinant human Alcohol and Aldehyde Dehydrogenases.
Materials and Methods:
Recombinant human alcohol dehydrogenases, ADH1A, ADH1B1, ADH1B2, ADH1B3, ADH1C1, ADH1C2, ADH2, and ADH4, and recombinant human acetaldehyde dehydrogenases, ALDH1A1 and ALDH2 were assessed the inhibitions of oxidation with acetaminophen at near physiological pH 7.5 and a cellular coenzyme concentration, 0.5 mM NAD. For comparison, S-hydroxymethylglutathione and benzaldehyde were used as substrates for human ADH3 and ALDH3A1, respectively, which are virtually inactive in ethanol metabolism due to their very high K_m for ethanol or for acetaldehyde.
Results:
Acetaminophen acted as a noncompetitive inhibitor (I) for all of the ADH family members studied with the slope inhibition constants (K_{is}) ranging from 0.90 to 20 mM, and the intercept inhibition constants (K_{ii}), 1.4 to 19 mM, suggesting that two abortive ternary-complex intermediates, E-NAD-I and E-NADH-I, can be formed during catalytic reaction. This is consistent with that dissociation of E-NADH being rate-limiting step in catalysis for ADH family. Acetaminophen was a noncompetitive inhibitor for ALDH2 (Kis = 3.0 mM and Kii = 2.2 mM), but a competitive inhibitor for ALDH1A1 (Kis = 0.96 mM).
Conclusion:
Kinetic simulations using the experimentally determined numerical steady-state rate equations of human ADH and ALDH families show that the ethanol-oxidizing activities of ADH1C1, ADH1C2, ADH2 and ADH4, and the acetaldehyde-oxidizing activity of ALDH1A1 can be significantly inhibited in ethanol metabolism at therapeutically attainable doses of acetaminophen, thus potentially reducing hepatic and gastric first-pass metabolism of ethanol.

P225

Curcumin nanoparticles ameliorate ICAM-1 expression in TNF-α-treated lung epithelial cells through p47 phox and MAPKs/AP-1 pathway

李江文

Up-regulation of intercellular adhesion molecule-1 (ICAM-1) involves adhesions between both circulating and resident leukocytes and the human lung epithelial cells during lung inflammatory reaction. Previously, we have demonstrated that curcumin-loaded polyvinylpyrrolidone nanoparticles (CURN), improve the anti-inflammatory and anti-oxidative properties of curcumin in hepatocytes. In the further study, we focused on CURN effects on the expression of ICAM-1 in TNF-α-treated lung epithelial cells compared to the curcumin water preparation (CURH). TNF-α induced ICAM-1 expression, ROS production and cell-cell adhesion were significantly attenuated by pretreatment with antioxidants (DPI, APO or NAC) and CURN, but not by CURH, revealed by western blotting, RT-PCR, promoter assay, ROS detection and adhesion assay. In addition, CURN and antioxidants also inhibited activation of p47 and phosphorylation of MAPKs upon TNF-α-treated cells comparable to the effects of CURH. Moreover, phosphorylation of MAPKs may eventually lead to activate of transcription factors. Thus, we found that binding activity of AP-1 was significantly increased by 30min treatment with TNF-α, as well as phosphorylation of c-jun and c-fos, such effects were reduced by CURN. Furthermore, in vivo studies revealed that CURN improve anti-inflammation activities of CURH in the lung epithelial cells of TNF-α-treated mice. Our results indicate that system of curcumin-loaded polyvinylpyrrolidone nanoparticles may be useful as a potential anti-inflammation drug for treatment of respiratory disease.

P226

Frequency And Pattern of Chinese Medicinal Prescriptions for Dysmenorrhea in Taiwan: A Nationwide Cross Sectional And Analgesic Evaluation

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Backgrounds:

Dysmenorrhea still has a significant impact on quality of life among women nowadays. Chinese medicinal prescription (TCMP) is the clinic application to disease therapy by Chinese medicinal doctors. The aim of this study is to investigate the dysmenorrhea efficacy and pain release from TCMP by scientific proof.

Materials and Methods:

The top 5 high frequency TCMP surveyed from National Health Insurance Database. The pain release effects were evaluated from the acetic acid induced abdominal pain on normal female mice and oxytocin induced dysmenorrhea pain on estrogenized mice. Inhibitory effects of ROS, which may cause uterus damage, was evaluated from the ferrous ion induced lipid peroxidation (LPO) on female mice uterus homogenates model.

Results:

The top 5 high frequency traditional Chinese medicine prescription in National Health Insurance Database are Danggui shaoyao san(當歸芍藥散), Jiawei xiaoyao san(加味逍遙散), Wenjing tang(溫經湯), Guizhi fuling wan(桂枝茯苓丸) and Xuefu zhuyu tang(少腹逐瘀湯). They exhibited a significant dysmenorrhea pain release on estrogenized ICR mice. Otherwise, only wenjing tang shows analgesic effect. LPO inhibitory TCMP were Wenjing tang, Guizhi fuling wan , and Xuefu zhuyu tang.

Conclusion:

From above evidence based experiment and NIHDB survey, we suggested that Wenjing tang the most potential TCMP.

P227

The roles of anti-apoptotic Bcl-2 family proteins Bcl-2 and Bcl-xL on betanodavirus-induced autophagy in fish cell

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Backgrounds:

Autophagy is a metabolic process to remove damaged protein or organells for recycling when cells were under the stresses. The autophagic response can be induced by viral infection.

Betanodavirus (RGNNV) cause viral nervous necrosis (VNN, ssRNA virus), which causes mass mortality in larval grouper fish and other economic fishes, but its pathogenesis is still uncover.

In our studies, we found that betanodavirus infection can induce host cell death and autophagy, which downregulate either Bcl-2 or Bcl-xl proteins in fish cells. Whether the Bcl-2 family proteins can involve in regulating of RGNNV-induced autophagy is still unknown.

Materials and Methods:

The grouper cell line (GF-1) was obtained from Dr.Chi. Virus collected from nature infected red grouper larvae in the Tainan prefecture were the source of redspotted grouper nervous necrosis virus Tainan No.1(RGNNV TN1), which was used to infect GF-1 cell.

GF-1 cells were seeded in 60 nm diameter petri dishes for 20 h and infected with RGNNV TN1 (MOI=5) for 0, 24, 36, 48 h. At the end of each incubation times, the culture medium was aspirated; the cells were washed with PBS. Then viral expression level or cell death assay used by western blot analysis or PI staining.

Results:

In the results, with overexpression of EGFP-Bcl-2 or EGFP-Bcl-xl in fish GF-1 cell, could prevent RGNNV-induced necrotic cell death. Then, we found that Bcl-2 and Bcl-xL can block the autophagic process, which correlate to reduce autophagic marker, p62 degradation. And blockage of autophagy can reduces virus protein expression such as protein A (RdRp) and capsid protein (protein α) and slightly decreased the viral titers at 36 h and 48 h p.i.

Conclusion:

Taken our results, we found that zebrafish Bcl-2 family proteins either Bcl-2 or Bcl-xl also can regulate the RGNNV-induced autophagic process, but have shown a little different result in regulating viral expression. Our studies may provide some insights into the pathogenesis of RGNNV infection and treatment.

P228

The Insulin Signaling Cascade Modulated Aβ Induced Cell Death by The Generation of Human ES-like Mir302-induced Pluripotent-stem-cell-like State

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Backgrounds:

Alzheimer's disease (AD) is the most common reason of dementia among elderly population over 65. In the matter of fact, the risk of onset of AD was doubled every half decade after age 65 and consequently reaches nearly 50% at age of 85. Clinical characterizes of AD including progressive cognitive deterioration such as lost of long-term memory, poor reasoning, and retarded language ability. The onset of AD due to senile plaques produced from beta-amyloid (Aβ) aggregation by the pattern of cleavage from APP by β, and γ secretases which triggered neuronal dysfunction, cell toxicity and cell death. Increasing evidence indicates that critical aging pathologies including AD were associated with diabetes. Insulin receptors are abundantly distributed in synaptic membranes in the brain. Furthermore, Insulin signaling cascade has proven to be important for neuronal survival and signal transduction. Therefore, insulin signaling cascade may plays a pivotal role in the cognitive function and the development of several neurodegenerative diseases. The potential contribution to insulin signaling cascade in aging and AD are not clear yet. Therefore, we designed present study to demonstrate a possible mechanism of delaying aging in AD pathogenesis.

Materials and Methods:

For the certain purpose, a custom designed neuronal cell line SK-N-MC with over-expression of mir-302 (mirPS cell) was applied to mimic the delaying aging condition.

Results:

For the mir-302s was one of the key factors essential to maintenance of embryonic stem cell self-renewal and pluripotency through inhibition of glycogen synthase kinase 3 (GSK3β) and suppresses residual differentiation. In present study we discovered that Aβ decreased the activation of PI3K/Akt/GSK3β pathway in SK-N-MC but not in mirPS cells.

Conclusion:

According to this result, we assume that activation of insulin signaling cascade plays a critical role in neuronal survival.

P229
The inhibitory effects of propolin G on human hepatocellular carcinoma cell line Hep3B through autophagy

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Backgrounds:
The prenylflavanone propolin G (PPG) were isolated and characterized from Taiwanese propolis, a honeybee product used extensively in medicine for anticancer effects. Human hepatocellular carcinoma cell line Hep3B was used to investigate the anticancer activity of PPG.
Materials and Methods:
Hep3B cells were treated with different concentrations of PPG to investigate the cytotoxicity effects using MTT assay. The expression of pERK and pAkt were suppressed in western blot analysis. Apoptosis were assay by flow cytometry and western blot. Finally, autophagy inhibitor either chloroquine (CQ) or the 3-methyladenine (3MA) combined with PPG was used to evaluate the effects of the Hep 3B cells.
Results:
PPG could inhibit Hep3B cells growth by dose-dependent manner. The expression of pERK and pAkt were overexpression by western blot analysis. PPG treatment Hep3B cells could increase autophagy specific protein LC3 expression and these were time- and dose-dependent increase. Autophagy pharmacological inhibitor, 3-methyladenine was effectively enhanced apoptosis induced by PPG in Hep3B cells.
Conclusion:
The combination of PPG with autophagy inhibitor was able to induce Ras-dependent proliferation and phosphorylation of Akt in hepatocellular carcinoma. Popolin G causes cell death through upregulation of the MEK/ERK pathway and induces the autophagic mechanism to protect themselves from the chemical stress.

P230
Role of Transmembrane Helice 3 in H+-translocating Pyrophosphatase

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Ching-Hung Lee, Yen-Wei Chen, Yun-Tzu Huang, Yih-Jiuan Pan, Chien-Hsien Lee, Shih-Ming Lin, Lin-Kun Huang, Yueh-Yu Lo, Yu-Fen Huang, Yu-Di Shiu, Ya-Yun Liao, Rong-Long Pan
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Backgrounds:
Vacuolar H^+-pyrophosphatase (H^+-PPase; EC 3.6.1.1) exists primarily in plants, algae, prokaryotes, and some protozoa. By hydrolyzing pyrophosphates, H^+-PPase is capable of pumping protons across membrane to build up an electrochemical gradient for transporting substances, like ions, metabolites, and even toxicants, into vacuole. Sequence alignment suggests that the amino acids along TM3 are highly conserved in plants. Besides, hydrophobicity of TM3 is relatively low among the 16 TMs. Therefore, TM3 is comprehensibly involved in the enzymatic function of H^+-PPase.
Materials and Methods:
Alanine-scanning mutagenesis was used in this study to investigate the role of TM3 in H^+-PPase. Each amino acid along TM3 was individually replaced with alanine, and alanine residue with serine, if any. A set of site-specific constructs were generated, over-expressed in *Saccharomyces cerevisiae*, and then used to determine their hydrolysis activities and proton translocation.
Results:
The function of each residue on TM3 was examined and the results indicate that K133, T138, I140, S142, T143 and the $G_{149}xxxS_{153}$ and $G_{160}xxxA_{164}$ motifs, which function as GxxxG-like motifs are involved in maintenance of the expression and function of H^+-PPase.
Conclusion:
The two GxxxG-like motifs in TM3 are indispensable for H^+-PPase. Besides, G149 at the $G_{149}xxxS_{153}$ motif in TM3 is critical for the expression of H^+-PPase. T138, S142, and T143 along TM3 are involved in efficient coupling of H^+-PPase.

P231
Effect of Penconazole, Propiconazole and Triadimefon on Thyroid Receptors Expression with Whole Rat Embryo Culture

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Backgrounds:
Penconazole, propiconazole, and triadimfefon were most common triazole pesticides in Taiwan. They were developed to inhibit the ß-tubulin assembly in mitosis, it is likely to disturb the synthesis of steroid hormone in mammals. A few reports showed that triazole chemicals antagonized the aromatase, which transfer testosterone into 17ß-estradiol in mammals. We want to know if the thyroid receptor is related to the AR and ER expressions.
Materials and Methods:
This study aimed to investigate the effect of these three pesticides on thyroid receptor (TRα, TRß) expression in whole rat embryo culture (WREC) on gestation day (GD) 10.5. The concentrations of WREC were pencoazole, 0.004, 0.008, 0.016 ppm; propiconazole, 0.005, 0.01, 0.02 ppm; triadimefon, 0.005, 0.009, 0.019 ppm. The culture period was 48 hours. After evaluation of embryo development it was fixed in formalin or kept in HBSS for immunohistochemistry (IHC) and Western blot (WB), respectively.
Results:
The positive control of triiodothyronine (T3) showed significant expression for its receptors. Results showed that these three triazoles induced expressions of TRß but not in TRα with WREC. Based on the principle that triazoles were designed to disrupt the synthesis of steroid hormone and inducing expressions of AR and ER, what is the relationship between TRß and AR and ER still need to be investigated. We will further detect these effects by Western blot both in WREC. Also, we need to study the antagonistic effects by adding the antagonists for the receptor expression.
Conclusion:
Penconazole, propiconazole, and triadimfefon significantly induced expression of TRß but not TRα. It seems that WREC can be used as a robust method of endocrine disrupting screening for thyroid hormone receptors.

P232
Risk Assessment for the Widely Used and Hormone-like Pesticides with Benchmark Dose Approach

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Backgrounds:
Benchmark analysis is a widely used tool in public health risk analysis. Therein, estimation of minimum exposure levels, called Benchmark Doses (BMDs), that induce a prespecified Benchmark Response (BMR) is well understood for the case of an adverse response to a single stimulus.
Materials and Methods:
Based on JMPR reports, this study collected the subchronic and chronic toxicological data of widely used hormone-like pesticides acephate, carbaryl, carbofuran, chlorothalonil, glyphosate, chlorpyrifos, methomyl, terbufos, carbendazim and fenitrothion for benchmark dose analysis and compare with corresponding NOAEL values.
Results:
In terms of dose-response relationship, some of these ten pesticide data were not significant and not quite appropriate for the BMD analysis. Conventionally, NOAEL values, safety factor (SF) and RfD of acephate, carbaryl, carbofuran, chlorothalonil, glyphosate, chlorpyrifos, methomyl, terbufos, carbendazim and fenitrothion were 0.25, 10, and 0.03, 15, 2000 and 0.008, 0.22, 100 and 0.002, 3, 100 and 0.03, 175, 100 and 0.175, 1, 100 and 0.01, 3, 100 and 0.03, 0.06, 100 and 0.0006, 2.5, 100 and 0.03, 0.5, 100 and 0.005, respectively. The study showed that BMDL ($BMDL_{10\%}$), SF and RfD of acephate, carbaryl, carbofuran, chlorothalonil, glyphosate, chlorpyrifos, methomyl, terbufos, carbendazim and fenitrothion were 7.61, 100 and 0.08, 104.43, 500 and 0.209, 0.61, 100 and 0.006, 192.4, 100 and 0.2, 6001, 1000 and 6, 0.0036, 100 and 0.00004, 0.121, 100 and 0.0012, 0.171, 100 and 0.0017, 70.9, 1000 and 0.07, 0.433, 100 and 0.0043, respectively.
Conclusion:
The results indicate that BMD analysis is better than that with NOAEL method when the dose-response relationship is significant but NOAEL method is better than BMD analysis when the dose-response relationship is insignificant.

P233

Histone Methyltransferase G9a Promotes the Oral Cancer Cells Recovery from Drug-Induced DNA Damage

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Backgrounds:

Oral cancer is one of the serious life-threatening diseases in the world. In addition to surgery, the conventional treatment for oral cancer includes radiation and anti-cancer drugs, which usually act by induction of DNA damage. However, approximately one-third of treated patients will experience local or regional recurrence. G9a is a histone methyltransferase responsible for mono- or dimethylation of lysine 9 in histone H3. Recent works had demonstrated that G9a level is highly correlated with aggressiveness and poor prognosis of several cancer patients in Taiwanese population. However the role of G9a in drug-induced DNA damage is unclear.

Materials and Methods:

SAS human oral cancer cells were stably transfected of shRNA-G9a or shLuc-control. These cells were treated with hydroxyurea (5μM) or etoposide (12.5 μg/ml) for 1 hour, and then we used immunoblotting to analysis of phosphorylation of histone H2AX (γ-H2AX) which provides information on unrepaired DNA damage.

Results:

We found that knockdown of G9a resulted in decrease cell survival and cologenic capacity following DNA damage induced independently by hydroxyurea and etoposide. Depletion of G9a caused sustained phosphorylation of γ-H2AX after drug treatments.

Conclusion:

Our data suggest that a novel function of G9a may increase the DNA repair efficiency and promote the oral cancer cells recovery and survival from the DNA damage agents. We suggest that G9a may contribute to oral cancer proliferation by improvement of homologous recombination repair of DNA double break. Our study paves the way for exploring the blockade of G9a overexpression as a novel approach for the prevention and treatment of oral cancer.

P234

Complexation of curcumin with pea protein and its implications solubility and stability of curcumin

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Backgrounds:

Curcumin, a natural polyphenolic compound, has low solubility and instability in aqueous solutions and poor bioavailability leading to limit of applicability in human as a health promoting agent. The aim of this study was to investigate the potential of pea protein isolate as a carrier for curcumin.

Materials and Methods:

Curcumin was added to 0.3% (w/v) pea protein solution in water and homogenised using a homogeniser to disperse curcumin into the solution. After stirring overnight, the pea protein-curcumin complex was centrifuged to remove free curcumin and the supernatant was spray-dried to get the dry pea protein-curcumin complex powder. We determined the stability of pea protein-curcumin complex in stimulated gastric and intestinal fluids. The antioxidant activities of pea protein-curcumin complex were also examined by reducing power assay and TEAC.

Results:

Our study demonstrates that the pea protein can form a complex with the curcumin. Upon complexation, curcumin showed increased water solubility. Stability studies showed that the pea protein-curcumin complex was stable when dissolved in water, simulated gastric and intestinal fluids for 12 h. Pea protein-curcumin complex exhibits enhanced antioxidant activities in the assays of reducing power and TEAC when compared with curcumin.

Conclusion:

In the present study, pea protein- curcumin complex can enhance the residence time in the gastrointestinal tract and improve the curcumin stability in aqueous solution. Additionally, pea protein-curcumin complex also has the excellently antioxidant capacity. This study suggests that pea protein could be used as a material to encapsulate water-insoluble bioactive compounds in functional food.

P235

Research of Dioxins Toxicogenomics with Biological Network Analysis

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Backgrounds:

To investigate the important genes/receptors by analyzing toxicogenomics data of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in human and use biological networks to calculate the nodal centrality describing the network topology.

Materials and Methods:

More than 70 chemicals have been found in dioxin family, and TCDD is the chemical compound that has the greatest number of gene-interactions. Retrieving TCDD toxicogenomics data from Comparative Toxicogenomics Database (CTD). Using Gene Set Enrichment Analysis (GSEA) method to analyze gene sets by pathways, GeneOntology (GO) and networks. To analyze array data of TCDD effect of human using Affymetrix GeneChip Human Genome U133 Plus 2.0 platform (HG-U133_Plus_2), we use CLC genomics workbench software to execute statistical analysis. After mining feature ids at false discovery rate (FDR) p-value less than 0.05, we add annotations of their gene symbols. The visualization software Cytoscape could construct biological network with gene list, and its plugin CentiScaPe can compute specific nodal centrality parameters in the biological networks analyze.

Results:

The curated interactions between TCDD and genes/interactions were obtained from CTD, and 2234 unique human genes/proteins were found. The GO and pathways of these 2234 genes/proteins were fully analyzed. The top 20 genes/proteins may serve as molecular biomarkers of TCDD toxicity. The top 10 diseases included pathologic processes, female urogenital, stomach, skin, adnexal and ovarian disease. The high nodal centrality nodes, MAPK14, MAP3K7 and SP1 are retrieved by CentiScaPe from TCDD related toxicogenomics data. These nodes with high nodal centrality value could be more important than other nodes in each network.

Conclusion:

These results from TCDD toxicogenomics data analysis provide how TCDD may cause many effects in different diseases, cell components, molecular functions and biological processes. In array data analysis of TCDD effect of human with high nodal centrality value, we find out what are the most important nodes in each network with statistical differences. These nodes could be support their importance with animal model testified. Or, they could explore another new effect of TCDD. Furthermore, potentially these findings could be used as a biomarker in health check or human biomonitoring system.

P236

Role of TLR4/NADPH Oxidase/ROS-Activated p38 MAPK in VCAM-1 Expression Induced by Lipopolysaccharide in Human Renal Mesangial Cells

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Backgrounds:

In bacteria-induced glomerulonephritis, Toll-like receptor 4 (TLR4) activation by lipopolysaccharide (LPS, a key component of the outer membranes of Gram-negative bacteria) can increase oxidative stress and the expression of vascular cell adhesion molecule-1 (VCAM-1), which recruits leukocytes to the glomerular mesangium. However, the mechanisms underlying VCAM-1 expression induced by LPS are still unclear in human renal mesangial cells (HRMCs).

Materials and Methods:

We demonstrated that LPS induced VCAM-1 mRNA and protein levels associated with an increase in the promoter activity of VCAM-1, determined by Western blot, RT-PCR, and promoter assay.

Results:

LPS-induced responses were inhibited by transfection with siRNAs of TLR4, myeloid differentiation factor 88 (MyD88), Nox2, Nox4, $p47^{phox}$, c-Src, p38 MAPK, activating transcription factor 2 (ATF2), and p300 or pretreatment with the inhibitors of reactive oxygen species (ROS, edaravone), NADPH oxidase [apocynin (APO) or diphenyleneiodonium chloride (DPI)], c-Src (PP1), p38 MAPK (SB202190), and p300 (GR343). LPS induced NADPH oxidase activation, ROS production, and $p47^{phox}$ translocation from the cytosol to the membrane, which were reduced by PP1 or c-Src siRNA. We observed that LPS induced TLR4, MyD88, c-Src, and $p47^{phox}$ complex formation determined by co-immunoprecipitation and Western blot. We further demonstrated that LPS stimulated ATF2 and p300 phosphorylation and complex formation via a c-Src/NADPH oxidase/ROS/p38 MAPK pathway. Up-regulation of VCAM-1 led to enhancing monocyte adhesion to HRMCs challenged with LPS, which was inhibited by siRNAs of c-Src, $p47^{phox}$, p38 MAPK, ATF2, and p300 or pretreatment with an anti-VCAM-1 neutralizing antibody.

Conclusions:

In HRMCs, LPS-induced VCAM-1 expression was, at least in part, mediated through a TLR4/MyD88/c-Src/NADPH oxidase/ROS/p38 MAPK-dependent p300 and ATF2 pathway associated with recruitment of monocyte adhesion to kidney.

P237
Cytotoxicity Evaluation of Fe3O4 Nanoparticles on Hep G2 Cell

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Superparamagnetic iron oxide nanoparticles can be used as contrast agents in clinic use to enhance diagnostic resonance imaging. Our current studies have demonstrated that Fe3O4 nanoparticles cause the cell growth inhibition in human hepatocarcinoma cells Hep G2 and Huh-7. In this Study, use the immunofluorescence assay (IFA), we employed Hep G2 cell to evaluate the cell survivability treated by 10 nm of iron oxide and general iron oxide (0.4 μm size), respectively. The results indicated that nano-scaled iron oxide can induced reactive oxygen species (ROS) appeared after 5 min at the concentration of 31.25 μg/ml. The cell self-antioxidant substance (reduced glutathione, GSH) was also produce to resistant the production of ROS for 60 mins, but the micro size iron oxide could maintain for 3 hrs. The results indicated that the cytotoxicity caused by nano iron oxide was abundant production of ROS to trigger the apotosis. When the apotosis level was compared with nano-scaled iron oxide and micron-scaled iron oxide, nano-scaled iron oxide were found significantly higher than micron-scaled iron oxide. In summary, our results show that high concentration of iron oxide induce cytotoxicity in Hep G2 cells from cell growth inhibition toward apotosis.

P238
Development of Polyclonal Antibody and Its Application in Enzyme-Linked Immunosorbent Assay (ELISA) for Detection of Malachite Green and Leucomalachite Green

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Backgrounds:
Malachite green (MG) is a toxic triphenylmethane dyes, which possesses sterilization function, can be effective in the treatment of fish parasites, fungi and protozoa diseases. MG is easily absorbed by fish during waterborne exposure and is rapidly metabolised into the lipophilic compound leucomalachite green (LMG). Both MG and LMG are potential carcinogens, teratogens and mutagens ,and respiratory toxicity in animals.Therefore, we hoped that polyclonal antibody (pAb) against the Malachite green were produced.
Materials and Methods:
The LMG-NHS ester linkage group conjugated to γ-globulin as the immunogen to immunize mice and rabbit respectively. Antibodies are generated against this MG and LMG, purified and used to develop the competitive direct enzyme-linked immunosorbent assay (cdELISA) and a competitive indirect enzyme-linked immunosorbent assay (idELISA) for detection of MG and LMG.
Results:
Rabbit antibody is generated against this MG and LMG, the concentration causing 50% inhibition (IC50) of malachite green and leucomalachite green was 39 ng/ml and 69 ng/ml respectively by cdELISA and mice antibody is generated against this MG, the concentration causing 50% inhibition (IC50) of malachite green was 62 ng/ml by cdELISA. In the cdELISA, the concentration causing 50% inhibition (IC50) of binding of LMG-NHS-horseradish peroxidase (HRP) to the antibody by malachite green was detected to be 4.6 ng/mL from immunised rabbit.
Conclusion:
We have developed convenient and fast detection assay for MG and LMG and successfully generated ploYclonal antibody for MG.The rabbit antibody-based ELISA established in the present study is sensitive enough for MG analysis. The IC_{50} values of MG in cdELISA is 4.6 ng/mL.

P239
The Mechanisms of Premature Cellular Senescence Induced by Chidamide (Epidaza) in Non-Small Cell Lung Cancer Cells

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Backgrounds:
Telomerase activity is detected in 90% of all tumor cells, which plays an important role in carcinogenesis. Chidamide is a novel benzamide-type histone deacetylase inhibitor. We investigate the mechanisms of premature cellular senescence that induced Chidamide in A549 lung cancer cells.
Materials and Methods:
Cell viability was analyzed by MTT assay and clonogenic assay in A549 human non-small cell lung cancer cells (NSCLC). hTERT expression was quantified by RT-PCR and Western blot. Telomerase activity was detected by Telomere Repeat Amplification Protocol (TRAP assay). The hypermethylation of hTERT promoter was performed on methylaion specific-PCR(MSP) and real-time methylaion specific-PCR(qMSP). The DNA contents and reactive oxygen species (ROS) were analyzed by flow cytometry. Using D2DCFDA, DHE, APF and HPF as indicators for hydrogen peroxide, superoxide anion and hydroxyl radicals respectively. Cellular senescence was detected by senescence-associated β-galactosidase activity (SA-β-gal) using cytochemical staining and flow cytometric determination of C12FDG. Acidic vesicular organelles development was detected by acridin orange (AO) using cytochemical staining and flow cytometry.
Results:
Chidamide inhibits growth of A549 lung cancer cells, telomerase activity and hTERT mRNA expression. Chidamide down-regulates DNMT1 and DNMT3b mRNA expression. Chidamide also increases in ROS production including hydrogen peroxide, superoxide anion and hydroxyl radicals. A549 cells were shown G1 arrested and cellular senescence under Chidamide treatment. Furthermore, Chidamide increased the expression p21 and p27, but decreased the expression of p-RB, p53 and thymidylate synthase.
Conclusion:
We first demonstrated that Chidamide induced premature cellular senescence via epigenetic regulation of hTERT and ROS production in non-small cell lung cancer cells.

P240
Ni-induced Epithelial-mesenchymal Transition via Down-regulation of MicroRNA-519c

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Backgrounds:
Epithelial-mesenchymal transition (EMT) has been considered as a transient and reversible event that leads to tissue fibrosis and tumor progression. MicroRNAs (miRNAs) are small non-coding RNA molecules that bind to their mRNA targets, thereby leading to transcription repression. We previously reported that Ni-induced EMT by promoter hypermethylation of E-cadherin via ROS generation. HIF1A is up-regulated by nickel chloride ($NiCl_2$) and is known to be a target gene of microRNA-519c (miR-519c).
Materials and Methods:
The aim of this study is to explore whether miRNA is involved in Ni-induced EMT in human bronichial epithelial cells. The miR-519c expression levels were examined by quantitative real-time PCR. Western blot analysis shows protein lystates form miR-519c overexpression stable cells line treated with $NiCl_2$ for 72 h. In addition, 3'UTR Luciferase reporter assay were performed to demonstrate that Ni-induced HIF1A expression through miR-519c. ROS scavengers were used to determine which mechanisms of Ni regulated miR-519c in BEAS-2B cells.
Results:
Our results showed that expression of miR-519c is reduced following $NiCl_2$ treatment. In addition, overexpression of miR-519c restores the down-regulation of E-cadherin induced by $NiCl_2$. Ni-induced HIF1A expression through miR-519c was determined by 3'UTR Luciferase reporter assay. BEAS-2B cells were pretreated with different antioxidants or ROS inhibitors/scavengers, including NAC (antioxidant), Tiron (superoxide anion scavenger) and SOD (superoxide dismutase) all abolished $NiCl_2$-induced EMT. Therefore, these ROS scavengers partly restore miR-519c expression in the presence of $NiCl_2$.
Conclusion:
These results shed new light on the contribution of $NiCl_2$ to carcinogenesis for miR-519c. This study provides evidence that down-regulation of miR-519c is involved in Ni-induced EMT.

P241

The Essential Role of Angiotensin II Receptor AT1R at Rostral Ventrolateral Medulla in the Cardiovascular Responses Elicited by the Binge Administration of Methamphetamine

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Background:

Illicit drug use is a serious health concern throughout the world. Methamphetamine (METH; N-methyl-1-phenylpropan-2-amine) is a potent central nervous system stimulant and is one of the most common drugs used by drug abusers in Taiwan. A majority of the hitherto investigations on METH focuses on the behavioral alterations, physical dependence or psychopathology of withdrawal syndrome, however much less studies are devoted to the mechanisms of METH-induced cardiovascular responses. Concerning the important roles of angiotensin II (Ang II) and rostral ventrolateral medulla (RVLM) in central regulation of cardiovascular responses, the present study aimed to delineate the role of Ang II type I receptor (AT1R) in RVLM of adult, male Sprague-Dawley rats contributing to the cardiovascular responses elicited by binge administration of METH, which is a common method used by METH abusers.

Materials and Methods:

Binge METH (5 or 10 mg/kg) via four injections at 2-hour intervals was intraperitoneally (i.p.) administered to Sprague-Dawley rats. Radiotelemetry method was applied to monitor the blood pressure (BP), heart rate (HR) and behavioral activities of conscious rats. 84 key genes regulating increase or decrease of BP in RVLM was determined by hyperteison PCR array. The concentration of METH, the protein level of AT1R and the level of superoxide in RVLM were determined by ELISA, Western blot and chemiluminescence method. Pretreatment of AT1R antagonist candesartan (10 mg/kg, i.p.) or peripheral AT1R antagonist valsartan (5 mg/kg, i.p.) or local microinjection of candesartan into bilateral RVLM was applied before binge METH administration.

Results:

Binge METH elicited a dose- and time-dependent elevation of BP, HR, behavioral activities, mortality and METH concentration in RVLM. Agtr1a and Agtr1b were two of the most upregulated mRNA involving in BP elevation in RVLM and the protein expression of AT1R was also dose-dependently increased after METH. METH elicited oxidative stress in RVLM indicated by an increase of superoxide levels. We further found that candesartan, rather than valsartan, significantly antagonized METH-induced oxidative stress in RVLM and elevation of BP and HR.

Conclusion:

These results suggested that the AT1R-mediated oxidative stress in the RVLM is responsible for binge METH-induced elevation of cardiovascular responses.

P242

The Inhibitory Effect of Hibiscus Sabdariffa Leaf Extract on UVB-Induced Expression of Matrix Metalloproteinases in Human Dermal Fibroblast Cells

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Background:

Hibiscus sabdariffa leaf, the edible part of H. *sabdariffa* Linne (*Malvaceae*), is usually ignored and discarded. Previous studies have indicated *H. sabdariffa* L. leaf extract (HLE) has antioxidant and anticancer activities. Ultraviolet (UV) radiation is one of the main factors of skin aging. Long-term exposure to the UV leads to skin collagen breakdown, and generate wrinkles in the skin. Recently, it has been known that excessive matrix degradation by UV-induced matrix metalloproteinases (MMPs) secreted by fibroblasts cells during photoaging. In this study, we evaluated the effect of HLE on MMPs expressions in human dermal fibroblast WS-1 cells exposed to UVB irradiation.

Materials and Methods:

We utilized trypan blue assay to analyze the effect of HLE on WS-1 cell viability. In a cellular model, WS-1 cells were exposed to 25 mJ/cm^2 UVB in the in the presence or absence of different concentrations of HLE for 24 h. To highlight the mechanisms of anti-UVB effects of HLE, the mRNA and protein expressions of MMPs were measured by real time-PCR, Western blotting and EMSA.

Results:

Non-cytotoxic doses of HLE and its main compound ellagic acid (EA) abolished the UVB-induced the expressions of MMP-1/2/9/13. The HLE-inhibited UVB-induced MMPs expressions appeared be a consequence of activator protein-1 (AP-1) inactivation, because its DNA binding activity was suppressed by HLE.

Conclusion:

These findings suggest that HLE inhibits UVB-induced MMP-1/2/9/13 expressions by suppressing of AP-1 activation in WS-1 cells. Thus, HLE might be used as a potential agent for treatment of UV-induced skin photoaging.

P243

Cytotoxic and Lethal Activity of Venom from Crown-Of-Thorns Starfish (Acanthaster planci) Spine

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Backgrounds:

The crown-of-thorns starfish *Acanthaster planci* is an Echinoderm from the class Asteroidea, the order Valvatida, and the family Acanthasteridae. The spine venom of *A. planci* (ASV) seems to contain numerous types of toxins. In order to understand the cytotoxicity of the ASV, influence of ASV on viability of different cell lines was studied.

Materials and Methods:

The cytotoxic effects of the crown-of-thorns starfish *Acanthaster planci* spine venom (ASV) in five cell lines, including human neuroblastoma (SH-SY5Y), human hepatocellular carcinoma (HepG2), human melanoma (A375.S2), human skin fibroblast (CCD-966SK) and mouse macrophage-like cell (RAW264.7) were assayed.

Furthermore, we assayed the stability of cytotoxicity of ASV. In mouse bioassay, male mice weighing about 16-18 g were intraperitoneal injected with 1 ml of serial dilutions of ASV. SDS-PAGE was performed by using 5% stacking gel and 12.5% separating gel.

Results:

The results indicated that ASV showed cytotoxic effects depending on dose in these five cell lines. Specifically, ASV significantly inhibited the proliferation of human melanoma cell line A375.S2 at 10 μg/ml, indicating *A. planci* spine venom could be utilized as potential chemotherapeutic agent in the treatment of cancer. The cytotoxicity of ASV to A375.S2 cell line was sharply lost at temperature more than 80°C. The cytotoxicity of ASV also sharply lost at extreme pH environments. The cytotoxicity of ASV was attenuated when treated with Cu^{2+} and anti-oxidant N-acetylcysteine. The ASV was lethal to mice when administered intraperitoneal injection and the LD50 was determined to be 3.47 mg/kg. After SDS-PAGE analysis, ASV showed the major protein components ranging from 10 kDa to 37kDa.

Conclusion:

In conclusion, our results showed that ASV has strong cytotoxicity, which might be the proteinous venom. The most effective cytotoxicity of ASV was on human melanoma A375.S2 cell line. The cytotoxicity of ASV was inhibited by divalent cation Cu^{2+}, extreme acid or alkali environment and high temperature. Anti-oxidants NAC attenuated cell death induced by ASV. In this result, we may suggest that the cytotoxicity of ASV was relative to oxidative stress induced in cells. The lethal activity was observed in this study, the dose-death time curve was drawn and LD50 value determined.

P244

N-acetylcysteine Suppresses the Enhancement of 5-HT2A Receptor-mediated Responses By Adolescent Toluene Exposure

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Backgrounds :

Adolescent toluene exposure causes behavioral dysfunction and enhancement of the behavioral and molecular responses to hallucinogenic 5-HT_{2A} agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) in mice, mimicking the schizophreniform psychosis observed in toluene abusers. N-acetylcysteine (NAC), a glutathione precursor, modulates glutamatergic transmission via activation of cystine-glutamate antiporter. Several lines of evidence demonstrated that hallucinogens-induced responses involve serotoninergic and glutamatergic transmission. The present study investigated the effects of NAC on the alterations in the 5-HT_{2A} receptor-mediated responses by adolescent toluene-exposure.

Materials & Methods:

Male NMRI mice received injection per day of either toluene (750 mg/kg) or oil at postnatal day P35-P39, and P42-P46. One week after drug withdrawal, NAC was administered for 14 days. Thereafter, DOI-induced behavioral, molecular and electrophysiological responses were measured by head-twitches, c-Fos and egr-2 expression, and field potentials in the medial prefrontal cortex.

Results :

Toluene exposure significantly increased the head twitch response and c-Fos and egr-2 expression induced by DOI, and enhanced the DOI-evoked field potential activity. Subsequent NAC treatment attenuated the alterations in 5-HT_{2A} receptor-mediated responses in toluene-exposed mice.

Conclusion:

These findings further support NAC as a novel therapeutic approach in the treatment of neuropsychiatric syndromes related to toluene abuse.

P245

Antioxidant Activity and Decreasing Oxidative Stress of Water Extract from Shells of Freshwater Clam (*Corbicula fluminea*) and Hard Clam (*Meretrix lusoria*) in Human Skin Fibroblast Cells

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Backgrounds:

Freshwater clam (*Corbicula fluminea*) and hard clam (*Meretrix lusoria*) are the most abundant freshwater bivalve and seawater bivalve in Taiwan, respectively, and the weight of both shells is more than 50% of total weight. The mechanisms of bivalve shell formation are similar to pearl formation. Therefore, the antioxidant activities of water extract from shells of freshwater clam (FSE) and hard clam (HSE) were investigated.

Materials and Methods:

Freshwater clams and hard clams were obtained from Renai market, Keelung. After clean-up pretreatment, clam shells were crushed to a powder. An equal weight of deionized water was added to the powder and extracted at 130 ℃ and 1.5 atm for 60 min. The extract solution was filtered and dialyzed. Finally, the solution was lyophilized and employed as the water extract of clam shells. Antioxidant activities including DPPH radical scavenging activity, iron (II) chelating activity, and reducing power were examined. The human dermal fibroblast cells (CCD966SK) were used as cell model to evaluate the ability of decreasing oxidative stress by the extract.

Results:

The IC_{50} value of DPPH scavenging activity of FSE is 0.25 mg/ml. HSE has no DPPH radical scavenging activity. The IC_{50} values of Fe^{2+}-inhibition of FSE and HSE are 0.49 and 0.25 mg/ml, respectively. The results of reducing power show that the absorbencies are 1.786 and 0.318 (10 mg/ml FSE and HSE), which is equals to 0.40 and 0.06 mg/ml vitamin C. The growth rate of CCD966SK cells are slightly increased by FSE and HSE treatment and the cell viability is about 110%. Furthermore, H_2O_2-induced oxidative stress in CCD966SK cells was reduced by FSE and HSE treatments.

Conclusion:

The DPPH scavenging activity and reducing power of FSE are higher than HSE. The iron (II) chelating activity of HSE is higher than FSE. Either FSE or CSE have no cell toxicity. On the contrast, the cell viability was enhanced by FSE or CSE treatment even with the presence of H_2O_2-induced oxidative stress.

P246

Methionine Adenosyltransferase II Alpha Regulates Histone Methyltransferase G9a Expression in Colorectal Cancer Stem Cells

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Backgrounds:

Histone modification is critical for regulation of chromatin structure and gene transcription in cells and recent evidences have characterized that histone methylation may contribute to tumorigenesis and cancer malignancies. We have previously demonstrated the expression of histone methyltransferase G9a, which is responsible for histone H3 lysine 9 methylation, not only correlates to tumor aggressiveness but also plays an important role in regulation of colorectal cancer stem cells (CCSCs). However, the upstream regulator involves in G9a enrichment in CCSCs is still unclear.

Materials and Methods:

Non-CCSCs ($CD133^-$) and CCSCs ($CD133^+$) are isolated by flow cytometry and the expression and the location of MATIIα are examined by immunoblot. Non-CCSCs are transiently transfected with MATIIα plasmid or combined with G9a shRNA. Oppositely, CCSCs are transfected with MATIIα shRNA or combined with vector containing G9a. The marker of CCSCs, differentiation genes and self-renewal ability of transfected cells are examined by immunoblot, real-time PCR analysis and sphere formation assay. S-adenosylmethionine (SAM), which serves an inhibitor of MATIIα, is also used for investigation of G9a enrichment mechanism in CCSCs.

Results:

MATIIα expression is specifically elevated in the nucleus and may form the complex with G9a in CCSCs. G9a expression is increased in MATIIα-overexpressed non-CCSCs and is decreased in MATIIα-inhibited CCSCs. Inhibition of G9a expression in MATIIα-overexpressed non-CCSCs will attenuate the stemness, promote differentiation and inhibit the self-renewal ability. In contrast, re-expression of G9a in MATIIα-inhibited CCSCs will significantly restore the characteristics of CCSCs, suggesting that MATIIα controls the function of CCSCs mainly through regulation of G9a. Moreover, after SAM treatment, the expression of MATIIα and G9a and the self-renewal ability of CCSCs are dramatically decreased.

Conclusion:

We first demonstrate that MATIIα maintains G9a expression, which serves as a crucial role in CCSCs. These findings provide a new insight into understanding of the link between the metabolic enzyme and epigenetic regulator in CCSCs, which may be a therapeutic target in the future.

P247

Nanomaterials Enhance Antimicrobial Activities of Cefmetazole against Multidrug-Resistant Neisseria Gonorrhoeae

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Backgrounds:

The antibiotic-resistant *Neisseria gonorrhoeae* has led to difficulties in treating patients, and novel strategies to prevent and treat this infection are urgently needed. Herein, several novel nanomaterials for their potential activity against *N. gonorrhoeae* were examined.

Materials and Methods:

Five different multidrug-resistant clinical isolates from patients and a standard *N. gonorrhoeae* strain (ATCC 49226) were used in this study. Anti-bacterial activity of nanomaterials was determined by colony formation and minimum inhibitory concentration (MIC). The change of morphology was observed by electronic microscope and additive effect was determined by checkboard assay. Interaction between Ag NPs and cefmetazole was assayed by spectrophotometer.

Results:

We examined 21 different nanomaterials for their potential activity against *N. gonorrhoeae* (ATCC 49226) and found silver nanoparticles (Ag NPs, 120 nm) showed the greatest potency for reducing *N. gonorrhoeae* colony formation (MIC: 12.5 μg/ml) within a concentration range that did not induce cytotoxicity in human fibroblasts or epithelial cells. Electron microscopy revealed that the Ag NPs significantly reduced bacterial cell membrane integrity. Furthermore, the use of clinical isolates of multidrug-resistant *N. gonorrhoeae* showed that combined treatment with 120 nm Ag NPs and cefmetazole produced additive effects. The interaction data showed Ag NPs might complex with cefmetazole to deliver over twice of the dose into the bacteria and cause the additive effects.

Conclusions:

This is the first report to screen the effectiveness of nanomaterials against *N. gonorrhoeae*, and our results indicate that 120 nm Ag NPs within less toxicity to human epithelial cells and could be used as an adjuvant with antibiotic therapy, either for topical use or as a coating for biomaterials, to prevent or treat multidrug-resistant *N. gonorrhoeae*.

P248

Cooperation of TLR4 with MyD88/TRAF6/c-Src/$p47^{phox}$/Rac1 Complexes through PI3K/Akt and ASK1 in LPS-induced ICAM-1 Expression in Pulmonary Alveolar Epithelial Cells

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Backgrounds:

Lipopolysaccharide (LPS) has been shown to induce the expression of adhesion molecules on airway resident cells and contributes to inflammatory responses. Here, we attempted to investigate the mechanisms of LPS-induced intercellular adhesion molecule (ICAM)-1 expression and its effects on human pulmonary alveolar epithelial cells (HPAEpiCs).

Materials and Methods:

HPAEpiCs were used in this study. We applied Western blot, RT-PCR, real-time PCR, selective pharmacological inhibitors of PI3K (LY294002), MEK1/2 (U0126), JNK1/2 (SP600125), p38 MAPK (SB202190), and AP-1 (Tanshinone IIA), transfection with respective siRNAs, promoter assay, and THP-1 monocytes adhesion assay to investigate the mechanisms by which LPS-induced ICAM-1 expression and monocytes adhesion. Moreover, male ICR mice aged 6-8 weeks were used to investigate the mechanisms of LPS-mediated responses in the study.

Results:

We demonstrated that LPS-induced PI3K/Akt, ASK1, or MAPKs activation is mediated through the TLR4/MyD88/TRAF6/NADPH oxidase-dependent pathway, which in turn initiates the activation of Elk1 and AP-1 (ATF2, c-Jun, and c-Fos) and ultimately leading to ICAM-1 expression in HPAEpiCs. The results obtained with lung tissue and bronchoalveolar lavage showing that the lung morphologies displayed inflammation and hematoma phenomena induced by LPS. The lung tissue sections stained by immunohistochemical (ICH) staining showed that ICAM-1 expression in alveolar epithelial cells and count of leukocytes in BAL were increased by LPS administration.

Conclusion:

Our results suggested that in both *in vitro* and *in vivo* studies, LPS could augment ICAM-1 expression associated with leukocytes adhesion and transmigration in alveolar epithelial cells and exaggerated lung inflammatory diseases.

P249

Taurine Promotes Cell Cycle Progression Via Restoring Neurotrophic Gene Expression In Arsenite-treated SH-SY5Y Cells

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Backgrounds:
This study investigated the effect of taurine on neurotrophic gene expression in arsenite-treated human neuroblastoma SH-SY5Y cells.

Materials and Methods:
Arsenite was used to simulate neurodegenerative progression that raised intracelllular reactive oxygen species (ROS) and interrupted cell cycle in SH-SY5Y cells. In addition, arsenite reduced mitochondria membrane potential (MMP) and down-regulated neurotrophic gene expression such as n-myc downstream-regulated gene 4 (NDRG-4), brain derived neurotrophic factor (BDNF) and sirtuin-1 (SIRT-1) observed in SH-SY5Y cells examined by qPCR and flow cytomertry.

Results:
When co-treated with taurine in arsenite-treated SH-SY5Y cells, taurine promoted cell cycle progression, restored MMP and reduced intracellular ROS level. Simultaneously, taurine recovered NDRG-4, BDNF and SIRT-1 gene expression. The results indicated that taurine not only reduces oxidative stress but also regulates neurotrophic gene expression in arsenite-treated SH-SY5Y cells.

Conclusion:
In summary, the results in this study provided that 1) taurine reduced ROS level, 2) restored MMP and recovered NDRG-4, BNDF and SIRT-1gene expression and 4) promoted cell cycle promotion in Ars-treated SH-SY5Y cells. Furthermore, we fist revealed that Ars inhibited neurotrophic and memory-related gene expression. This study suggested the regulatory effect of taurine on neurotrophic gene in Ars-treated SH-SY5Y cells.

P250

Long-term exposure to low concentration of nonylphenol exacerbates the severity of hyperglyciemia and macrovascular endothelium dysfunction in male diabetic rats.

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Backgrounds:
Increasing evidences have shown that long-term exposure of nonylphenol, one of endocrine disrupting compounds, increases reactive oxygen species (ROS) level in various tissues, such as aorta, pancreas, liver, and kidney. Our recent studies also demonstrate that chronic exposure to nonylphenol aggravates adenine-induced chronic renal dysfunction. According to these observations, it is reasonably to suggest that chronic exposure to nonylphenol exacerbates pancreatic toxin-induced diabetic situation and hyperglycemia-related macrovascular dysfunction.

Materials and Methods:
In order to examine this hypothesis, we used male Sprague-Dawley rats divided into four groups: control group (n=10), nonylphenol-treated group (NP, n=10), streptozotocin-treated group (STZ, n=30), and rat nonylphenol plus streptozotocin-treated group (NP+STZ, n=30). NP at the dosage of 2 mg/kg/day was treated for 15 weeks followed by STZ (20mg/kg, i.p) treatment for 4 times during the period of 8 weeks. In the end of study, thoracic aorta of rats was isolated for evaluation of vascoconstriction by phenylephrine and vasorelxation by acetylcholine, clonidine or insulin, and various tissues such as aorta, fat, skeletal muscle, were collected for assessment of basal reactive oxygen species (ROS) production and NADPH-related oxidase activity.

Results:
We found that: (1) Although the STZ-induced morbidity of diabetic rats was similar between NP+STZ group and STZ group, the severity of hyperglycemia is significantly higher in NP+STZ group compared with STZ group; (2) Acetylcholine, a NO-cyclic GMP pathway stimulator, -mediated endothelium-dependent relaxation were significant lower in NP+STZ group than that in STZ group, and similar results were observed in vascular response to clonidine, a α2-adrenoceptor agonist and PI3K/Akt pathway stimulator. However, insulin, another PI3K/Akt pathway stimulator, -induced vasorelaxation of aortic rings was comparable between these two groups; (3) Basal endothelium-derived nitric oxide release was significantly decreased in NP+STZ group than that in STZ group, but the basal ROS production was comparable between these two groups; (4) NADPH-related oxidase activity was affected only in aorta, not in fat or skeletal muscle, of NP+STZ group comparing to STZ group.

Conclusion:
Chronic exposure to low concentration of nonylphenol could aggravate the severity of hyperglyciemia induced by low-dose of pancreatic toxin, and hyperglycemia-related macrovascular endothelium dysfunction was also enhanced in NP-treated diabetic rats. We suggest that decreased nitric oxide production could play an important role in this higher severity of elevated blood glucose and its related macrovascular dysfunction.

P251

Fisetin Inhibits Reactive Oxygen Species Production and Up-regulates Anti-inflammatory Cytokines in Microglia

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Microglia, is the resident innate immune cell in the central nervous system (CNS). Microglia has been implicated as active contributor to neuronal damage. In active form, microglia cause reactive oxygen species (ROS) production, and neuroinflammation result to cytotoxicity. Fisetin, one of the major flavonol that found in strawberry, has direct antioxidant and anti-inflammatory activity. However, fisetin causes the anti-inflammatory cytokines expression in microglia still poorly understood. In this study, fisetin significantly inhibited cell viability, nitric oxide (NO) and ROS production in microglia. Moreover, fisetin also up-regulates the anti-inflammatory cytokines production, such as heme oxygenase-1 (HO-1), forkhead box P3 (FOXP3), suppressor of cytokine signaling-3 (SOCS-3). Moreover, fisetin also increased p38 phosphorylation time-dependetly. Our results indicate that fisetin potentially to inhibits ROS production and cell death, and regulates anti-neuroinflammatory cytokine expression in microglia.

P252

2-Methoxystypandrone ameliorates brain function by inhibiting inflammatory responses and preserving BBB integrity in mice with acute ischemic stroke

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Backgrounds:
2-Methoxystypandrone (2-MS), a naphthoquinone, has been shown to display an immunomodulatory effect in an inflammatory cellular model. To explore whether 2-MS can protect mice against cerebral ischaemic/reperfusion (I/R)-induced brain injury.

Materials and Methods:
We evaluated 2-MS's protective effects on an acute ischemic stroke by inducing a middle cerebral artery occlusion/reperfusion (MCAO) injury in mice, and using lipopolysaccharide (LPS)-induced activation of microglial cell BV2 to elucidate the mechanisms of action by 2-MS.

Results:
Treatment of mice that have undergone I/R injury with 2-MS (10 and 50 μgkg-1, i.v.) at 2 h after MCAO enhanced survival rate and ameliorated neurological deficits, brain infarction, neural dysfunction and massive oxidative stress, due to an enormous production of free radicals and breakdown of blood-brain barrier (BBB) by I/R injury; this primarily occurred with extensive infiltration of CD11b-positive inflammatory cells, upexpression of NADPH oxidase (gp91phox) and inducible nitric oxide synthase (iNOS) by activation of nuclear factor-kappa B (NF-κB). All of these pathological changes were diminished by 2-MS and pyrrolidine dithiocarbamate (PDTC, an inhibitor of NF-κB). In BV-2 cells, 2-MS and PDTC suppressed LPS-induced production of free radicals and nitric oxide, and upexpression of iNOS and cyclooxygenase 2 by interfering with IκBα degradation to impair activation of NF-(B.

Conclusion:
2-MS impedes inflammatory responses by impairing IκB/NF-κB signaling. This compromises the activation/infiltration of microglial and/or inflammatory cells, which then, limits the oxidative stress and, in turn, preserves BBB integrity that mediates 2-MS's protective effect in the ischemic stroke mice.

P253
Experimental study on the neuron protective effect of Shen-Yu-Tang on an acute ischemic stroke mice

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Backgrounds:

To study the neuron protective effect of a traditional Chinese medicine Shen-Yu-Tang (SYT, 聖愈湯) on an acute ischemic stroke mice model.

Materials and methods:

Male ICR mice were subjected to an acute ischemic stroke by inducing middle cerebral ischemic/reperfusion (CI/R) injury to examine whether oral administration of SYT (1.0 g/kg) twice daily starting from 2 h after ischemia could extend the lifespan of mice with a stroke as compared with treatments of vehicle control and recombinant tissue-type plasminogen activator (rt-PA, 10 mg/kg, i.v.). An integrative neurofunctional and genomic approach was performed to elucidate the underlying molecular mechanisms for SYT.

Results:

More than 70% of the mice died within 2 days after stroke induction with vehicle treatment. Treatments of SYT and rt-PA both significantly enhanced the survival rate and extend lifespan as compared to vehicle-treated CI/R group, with SYT (1.0 g/kg) being more effective one. SYT successfully restored brain function, ameliorated cerebral infarction, and significantly improved neurological deficits in mice with a stroke that paralleled to the reduction of inflammation, oxidative stress, and apoptosis, as well as neurogenesis. Molecular impacts of SYT by a genome-wide transcriptome analysis using brains from CI/R mice showed a total of 162 out of 2081 ischemia-induced probe sets were significantly influenced by SYT. Mining functional modules and genetic networks of these 162 genes revealed a significant upregulation of neuroprotective genes in Wnt-associated neurogenesis & cell communication, and down-regulation of destructive genes in the induction of inflammation, stress, wounding, angiogenesis, and blood vessel development by SYT.

Conclusions:

Our results suggest that SYT could protect mice against stroke and extend lifespan primarily through significantly down-regulating genes involved in inflammation, stress, wounding, angiogenesis, and blood vessel development, as well as up-regulating genes mediating neurogenesis and cell communication that confers SYT to be beneficial for ischemic stroke.

P254
Aspirin Inhibited Hyperlipidemia-Induced Adhesion Molecules and Chemokines in Sprague-Dawley Rat

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Backgrounds:

This study examined the effects of aspirin on adhesion molecules of platelet, lymphocyte and aorta, and chemokines of aorta in a hyperlipidemic rat model.

Materials and Methods:

The six-week-old Sprague-Dawley (SD) rats received either a regular diet or high fat diet (HFD), and the treatment group was fed HFD with 5 mg/kg aspirin for a 10-week period.

Results:

As compared with the regular diet group, the HFD group had markedly higher body weight. The HFD group had lower levels of high-density lipoprotein, higher concentrations of insulin, triglyceride, total cholesterol and low-density lipoprotein, but no differences in blood glucose and HbA1c. The prothrombin time and activated partial thromboplastin time of HFD group were decreased obviously. The HFD group had increased expression of adhesion molecules: P-selectin, ICAM-1, ICAM-2, ICAM-3, VCAM and PECAM in platelets, and ICAM-1, ICAM-2, ICAM-3 and VAP-1 in lymphocyte, and E-selectin, ICAM-1, ICAM-2, ICAM-3, VCAM and PECAM in aorta, and chemokines (MCP-1 and CCR2) in aorta. The PKCα, MAPKs (p38, JNK1, ERK1)- and p65 were also enhanced by feeding HFD. In the treatment group, aspirin improved HFD-induced hyperinsulinemia and hyperlipidemia. The prothrombin time and activated partial thromboplastin time were recovered. Aspirin inhibited the up-regulation protein levels of adhesion molecules and chemokines. It also reduced the PKCα, MAPKs and p65 expression.

Conclusion:

Taken together, hyperlipidemia enhanced the expression of adhesion molecules and chemokines protein, and that those increases were ameliorated by aspirin. We suggested that the mechanism of aspirin decreased adhesion molecules and chemokines could be through inhibiting the PKCα, MAPKs- and p65-mediated NF-κB pathway.

P255
Effects of Melatonin on Lipopolysaccharide-Induced Disseminated Intravascular Coagulation in Rats

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Backgrounds:

Disseminated intravascular coagulation (DIC) is a common complication of sepsis and leads to multiple organ failure and death. Cytokines and oxidative stress were responsible for coagulation activation by expression of tissue factor and platelet activation.

Melatonin, an indolamine, is an endogenous circadian neuro-hormone produced during darkness by the pineal gland. It is a powerful free radical scavenger. Many studies also suggest that melatonin has anti-inflammatory and anti-coagulation effects. Therefore, this study tried to evaluate the effect of melatonin on lipopolysaccharide (LPS)-induced DIC defined by ISTH score system in Wistar rats.

Materials and Methods:

Rats were divided into four groups: (1) Sham, (2) Sham + Melatonin, (3) LPS (10 mg/kg i.v. infusion for 40 min), (4) LPS + Melatonin (3 mg/kg for i.v. infusion at 40 min and 180 min after administration of LPS). Whole blood samples were collected at 0, 1, 2, 4, 6 h after LPS and immediately measured platelet account, blood glucose, organ function index, hemodynamics and the other sample were stored at -80℃ for measurement of IL-6, prothrombin time, D-dimer, fibrinogen, tissue factor pathway inhibitor. Blood flow in liver, kidney and mesenteric artery were measured at 6 h before the animal sacrificed. In addition, superoxide levels in lung, liver and kidney were detected. Finally, we calculated the survival rate for each groups.

Results:

Our preliminary results showed that melatonin have the beneficial effects on LPS induced hypotension, the lower reactivity of NE response and hypo-perfusion of organs. It seem to improve the injuries of liver and kidney on the animals with LPS induced DIC.

Conclusion:

Future studies are needed to clarify the relationship between generation of microvascular thrombosis by hyper-activation of coagulation and the hypo-perfusion of organs on the group of LPS. Evaluate the effect of melatonin on lipopolysaccharide (LPS)-induced DIC defined by ISTH score system in vitro study.

P256
Role of High Glucose in the Metastasis of Colon Cancer Cell

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Background:

Colorectal cancer is one of major causes of cancer death in clinics. One-third of colorectal cancer patients show metastasis during initial diagnosis and more than 70% of these patients have metastasis eventually. Many epidemiological studies have showed that diabetic patients have a higher colorectal cancer risk. Meanwhile, diabetic patients with colorectal cancer are less effective in treatment and poor prognosis than patients without diabetic disorders. However, the relationship between of diabetes and colorectal cancer metastasis is still unclear.

Materials and Methods:

Colorectal cancer cell line (CT-26) incubates in different glucose concentration environment (10, 20 and 30 mM). The wound healing assay is used to evaluate CT-26 cells migration. The in vitro invasion assays were carried out using a transwell chamber to evaluate CT-26 cells invasion ability. Immunoblot analysis by primary antibodies against STAT3 and MMP-2 is used to detect the expressions of MMP-2 and STAT3. Sttatic, a nonpeptidic small molecule, is used to inhibit the action of STAT3 in cells.

Results:

In wound healing assay and transwell in vitro invasion assays, more numbers of CT-26 cells express migration and invasion in high glucose environment. The numbers of moved CT-26 cells are glucose-dose dependent. The MMP-2 and STAT3 also have higher expression in high glucose environment. Sttatic can inhibit the activity of migration and invasion in high glucose environment obviously. The expressions of MMP-2 and STAT3 also decrease when incubated with Sttatic in high glucose environment.

Conclusion:

The high glucose environment enhances CT-26 cells metastasis that is mainly via activation of STAT3 and MMP-2 pathway.

P257

The Study of Inhibitory Effect of mabc2333211 on GPVI-mediated Platelet Activation

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Backgrounds:

Platelet dysfunction is a major risk factor of cardiovascular diseases such as atherosclerosis, acute coronary syndrome and stroke. The anti-platelet therapy is a well-established part of the treatment of cardiovascular arterial disease. However, there remain considerable side effects of current anti-platelet drugs. GPVI is collagen receptor, which plays a critical role in initial platelet activation. The screened out natural product, mabc2333211, was extracted from a plant. In this study, the inhibitory mechanism of mabc2333211 on GPVI-mediated platelet activation was investigated.

Materials and Methods:

Platelet activations were measured by aggregation assay and adhesion assay. Briefly, washed rabbit platelets were incubated with vehicle or different concentrations of mabc2333211, and then convulxin (GPVI specific agonist) or collagen was added as agonist. The platelet aggregation was measured by using an aggregometer. The fluorescent dye-loaded rabbit platelets were incubated with vehicle or different concentrations of mabc2333211 on collage coated plate, after washing with phosphate buffered saline (PBS), the fluorescence was determined by using a spectrofluorometer. In addition, the cytotoxic effect was measured by lactate dehydrogenase (LDH) activity assay.

Results:

The mabc2333211 can inhibit not only the convulxin-induced but also the collagen-induced platelet aggregation in a dosage-dependent manner with IC50 of 72.33±2.92 and 21.63±4.03 μM, respectively. Meanwhile, mabc2333211 exhibits little cytotoxicity to platelets even incubated at concentration of 100 μM for 30 minutes. In addition, mabc2333211 can concentrate-dependently inhibit the initial platelet adhesion on collagen surface.

Conclusion:

The adhesion assay indicates that mabc2333211 can suppress the interaction between collagen and platelet GPVI. Whether mabc2333211 also affects the GPVI signaling pathway during collagen- or convulxin-induced platelet aggregation? The research is being investigated continuously.

P258

4-Acetylantroquinonol B Isolated From Antrodia Cinnamomea Inhibited Prostate Cancer Cells Growth And Metastasis In Vitro And In Vivo.

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Backgrounds:

To investigate the *in vitro* and *in vivo* anti-proliferation and anti-metastasis effects of 4- acetylantroquinonol B (4AAQB) isolated from *Antrodia Cinnamomea.*

Materials and methods:

We first used MTT assay to determine the effect of 4AAQB on PC3 cell viability. Further, we used LDH release assay to verify the inhibition effect either causing cytotoxicity or approving apoptosis. Then, cell scratch motility assay was used to evaluate whether the 4AAQB would inhibit motility of PC3 cell line. To investigate the possible mechanisms involved in the inhibitory effect of 4AAQB, the phosphorylation of Akt, ERK and IκB was analysis by western blot. 4AAQB IP administration was used to examine the anti-tumor and anti-metastasis effect *in vivo* model.

Results:

4AAQB dose-dependently inhibited the PC3 cell viability and there were no cytotoxicity at doses less than 3μM. In non-toxic concentration, 4AAQB dose-dependently inhibited the migration of PC3 cells *in vitro*. Furthermore, 4AAQB dose and time-dependently affected the Akt, ERK and IκB phosphorylation in PC3 cells. 4AAQB also significantly inhibited tumor growth and metastasis in PC3 *in vivo* model.

Conclusion:

Taken together, these findings suggested that 4AAQB could reduce the growth and metastasis of PC3 cells *in vitro* and *in vivo*. 4AAQB may be a promising therapeutic agent for prostate cancer.

P259

The C825T polymorphism of the gene encoding the G protein beta3 subunit is associated with individual variability of platelet response to epinephrine

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Backgrounds :

Epinephrine is one of endogenous inducers that can cause platelet aggregation. This platelet reactivity is considered to be exclusively mediated by Gi protein after activation of the alpha2A adrenergic receptor. In Caucasian, the 825T allele of the gene (GNB3) encoding the beta 3 subunit of G protein was recently shown associated with enhanced epinephrine-induced platelet aggregation and higher risk of myocardial infarction. This useful information remains lacking in the population of Taiwan. We thus examined the response of platelets to epinephrine and its relation to the C825T polymorphism of the GNB3 gene, aiming to establish genetic markers that can tell individuals with increased platelet reactivity.

Methods :

The citrated platelet-rich plasma prepared by low-speed centrifugation was subjected to light transmittance aggregometry (LTD) with epinephrine (10 mM) stimulation for 10 min. A response with secondary aggregation following addition of epinephrine was defined as good response. The C825T polymorphism of the GNB3 gene was determined by sequencing the corresponding DNA fragment amplified by PCR using the leukocyte genomic DNA as the template.

Results :

One hundred and nice healthy adults (20-45 year-old), including 45 males and 64 females, were enrolled in this study. A total of 49 subjects (44.9%) were good responders and no gender difference was found. The genotype frequencies of the good responders were 14% (n=7) for CC, 45% (n=22) for CT and 41% (n=20) for TT. The genotype frequencies of the poor responders were 20% (n=12) for CC, 62% (n=37) for CT and 18% (n=11) for TT.

Conclusion:

There is a high prevalence of GNB3 TT genotype in the epinephrine good responders as compared with the poor responders (40.8% vs 18.4%) in Taiwanese population. This information might be useful in distinguishing between patients who need more intensive antithrombotic treatment.

P260

The Interaction Of Dengue Core Protein And Positive Transcription Elongation Factor B

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Backgrounds:

Dengue virus (DENV) is mosquito-borne illness and infects 50 to 100 million people worldwide per year, of which 500,000 develop severe life-threatening disease without any effective drugs. Previous study shows that IL-8 gene expression is induced by dengue core protein which is associated with positive transcription elongation factor b (P-TEFb) , composed of cyclin T1 and cyclin dependent kinase 9 (CDK9). In addition, the study also found the inhibition of CDK9 decreased the level of virus RNA. Therefore, the interaction between dengue core protein and P-TEFb is important for host immune response and virus replication. We want to determine the directly interaction between dengue core protein and P-TEFb and binding domain of dengue core protein to P-TEFb.

Materials and Methods:

First, we examined the interaction between dengue core protein and the cyclin T1 of P-TEFb by treating DSP crosslinker. Then, we used anti-cyclin T1 with Atto-550 dye and HeLa cell transfected EGFP-dengue core plasmid and determine FRET effect to strengthen the result. Next, we co-transfected full length and truncated dengue core plasmid in NF-kB luciferase transfected HeLa cell and test the luciferase activity to determine the binding domain of dengue core protein to P-TEFb. Finally, we determine the level of IL-8 in co-transfected full length dengue core and binding domain of dengue core plasmid.

Results:

In DSP crosslinker treatment, flag-dengue core band shifts to 34 kDa and 95 kDa in western blotting. On the other hand, we also found cyclin T1 band shifts to 95 kDa. According to this result, we considered that dengue core protein interact to cyclin T1 of P-TEFb directly. Furthermore, we used 488 nm laser to excite Atto 550 dye, and then accepted the 532nm emission of EGFP-core protein. So, we can more confirm the result of western blot. Next, we determined the luciferase activity in co-transfected HeLa cell and found that the the 85-100 amino acids of dengue core protein are possible binding domain to P-TEFb. And we also found similar result in determining the level of IL-8.

Conclusion:

Our data indicated that the interaction between dengue core protein and P-TEFb is directly. Moreover, we also find possible binding domain of dengue core protein to P-TEFb. Based on the result, we will find the important residues for the interaction between dengue core protein and P-TEFb in the future.

P261

The Database of Anti-Cancer, Anti-Platelet, and Anti-Tuberculosis Phytochemicals from Indigenous Plants in Taiwan

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Backgrounds:

The unique geographic features of Taiwan attribute to the rich of indigenous and endemic plant species in Taiwan. These plants serve as resourceful bank for biologically active phytochemicals. Most databases of plants in Taiwan lack a link between the chemical structures and their known pharmacological activities in a quantitative manner. Given these plant-derived chemicals are prototypes of potential drugs for diseases, databases connecting the chemical structures and pharmacological activities may facilitate drug development. Owing to the accessibility of data, we aim to construct a database containing the anti-cancer, anti-platelet, and anti-tuberculosis activities of the chemical compounds from indigenous plants in Taiwan.

Materials and Methods:

Indigenous plants and corresponding chemical compositions were extracted and manually curated from published literatures. Taxonomy classifications were manually assigned to the indigenous plants. Experimental information of cytotoxicity against cancer cells, anti-platelet activities, and anti-tuberculosis activities of the chemical compounds was also manually curated from published literatures. For cytotoxicity data, the curated information includes assay and cell type, activity value and assay variation. For anti-platelet compounds, both chemical and inducer with their concentrations are curated in addition to activity value and variation. The information curated for anti-tuberculosis compounds includes testing strain, assay type, and activity value and variation. Chemical structure files were either manually created or downloaded from PubChem database. MySQL Server Edition 5.1 was utilized to implement databases. Web user-interface and functions were implemented using PHP and JavaScript languages.

Results:

A database of anti-cancer, anti-platelet, and anti-tuberculosis phytochemicals from indigenous plants in Taiwan was constructed. It provides a standardized resource of published anti-cancer, anti-platelet, and anti-tuberculosis phytochemicals from indigenous plants in Taiwan. Currently, there are 114 indigenous plants in Taiwan curated in the database with 1720, 1671 and 265 anti-cancer, anti-platelet, and anti-tuberculosis records, respectively. A browse function was implemented for users to browse the database in a taxonomy-based manner. Search functions can be utilized to filter records of interest by botanic name, part, chemical class or compound name.

Conclusion:

Phytochemicals from indigenous plants in Taiwan could be potential drugs. A structured and searchable database was constructed to serves as a comprehensive and standardized resource for anti-cancer, anti-platelet, and anti-tuberculosis compounds search. The chemical structures are also curated in the database that provides a great opportunity to develop quantitative structure-activity relationship models for the high-throughput screening of potential anti-cancer, anti-platelet, and anti-tuberculosis drugs.

P262

Oxytocin Promotes Long-Term Potentiation by Enhancing Epidermal Growth Factor Receptor-Mediated Local Translation of Protein Kinase Mζ

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In addition to triggering the birthing process and milk release, the hypothalamic neuropeptide oxytocin (OXT) plays an important role in the regulation of complex social cognition and behavior. Previous work has shown that OXT can regulate hippocampal synaptic plasticity and improve hippocampus-dependent cognitive functions during motherhood, but the underlying mechanisms remain largely unclear. Here, we demonstrate that OXT promotes the maintenance of long-term potentiation (LTP) induced by one train of tetanic stimulation (TS) in the CA1 region of hippocampal slices from both nulliparous female and male rats through a previously unknown mechanism involving OXT receptor (OXTR)-dependent and epidermal growth factor receptor (EGFR)-mediated local translation of an atypical protein kinase C isoform, protein kinase Mζ (PKMζ). Using pharmacological and biochemical approaches, we show that both the conventional OXTR-associated signaling pathway (Gq/11-coupled phospholipase C) and the transactivated EGFR downstream signaling pathways (phosphatidylinositol 3 kinase and extracellular signal-regulated kinase 1/2) are involved in OXT regulation. In addition, OXT stimulates local dendritic PKMζ mRNA translation via activation of a mammalian target of rapamycin-regulated mechanism. Furthermore, blockade of OXTR results in a modest decrease in the ability to maintain late-phase LTP induced by three trains of TS. These results reveal a novel OXTR-to-EGFR communication to regulate the new synthesis of PKMζ, which functions to promote the maintenance of LTP at hippocampal CA1 synapses.

P263

Reactive oxygen species protection of Rhus semialata var. roxburghiana stem on mice liver mitochondria

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Backgrounds:

Oxidative stress (OS), the imbalance of the reactive oxygen species (ROS) with antioxidant enzymes, will damage the cellular molecule, like lipid, protein, and all components of the cell to cause diverse disease. *Rhus semialata* var. *roxburghiana* stem (*RSRS*) is a Taiwan native folk medicine for anti-inflammatory, diarrhea, and liver disease. In the study, the bioactivity of *RSRS* was investigated.

Materials and Methods:

According chemical polarity, the *n*-hexane, ethyl acetate (EA), acetone, ethanol, and water extracts of *RSRS* to evaluated the liver lipid peroxidation (LPO) inhibitory capability, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, superoxide anion radical ($O_2^{\cdot-}$)-scavenging, hydrogen peroxide (H_2O_2) scavenging activity, ferrous ion chelating activity, ferric reducing antioxidant power (FRAP) and total phenol contents (phenolic, flavonoid, falvonol).

Results:

Five extracts and main constituent-gallic acid elucidated inhibitory effects of ferrous ion-induced LPO on mice liver mitochondria (IC_{50} were 0.78±0.74~55.24±0.63 and gallic acid: 36.13±3.07 μg/ml). Otherwise, all of tested sample were no induced LPO production.

Furthermore, antioxidant activities (IC_{50}) were 1) DPPH scavenging: 1.53±0.08~56.25±1.13 μg/ml; 2) superoxide anion radical-scavenging: 53.48±0.75~226.56±2.07 μg/ml; 3) hydrogen peroxide-scavenging: EA and acetone extracts (264.25±4.75 and 208.03±1.87 μg/ml, respectively); 5) ferric reducing antioxidant power: EA extract (equivalent trolox 116.40±0.34 μM). Total phenolic content of extracts were 81.98±2.07~455.17±9.29 μg gallic acid equivalent/mg. The higher total flavonoid and flavonol content of extracts were acetone extract (30.94±2.66 μg rutin equivalent/mg) and water extract (10.92±0.05 μg epicatechin equivalent/mg). Then, gallic acid of active extract was quantitative analyzed by HPLC. It was a main peak and consisted of 123.62 μg gallic acid/mg EA extract.

Conclusion:

Overview of above evidences supporting *RSRS* and gallic acid are high potential antioxidant, LPO inhibitor and safety for developing a liver protective phytochemical functional food.

P264

Comparison of the antioxidant and lipid peroxidative inhibition from different collected location, used part and extract solvents of Rhus semialata var. roxburghiana

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Backgrounds:

Rhus semialata var. roxburghiana (RSR) (Anacardiaceae) is salt alternative and widely folk medicine for diarrhea, anti-inflammatory and liver disease by the aborigines in Taiwan.

Materials and Methods:

RSR was collected from the middle Taiwan (MT) and north Taiwan (NT), divided into three used parts (stem: S, branch: B and leaf: L) and extracted by five solvents, respectively. The scientific evidences of its antioxidant ability and liver lipid peroxidation (LPO) inhibitory effect were established by the free radical scavenging, polyphenol (phenolic, flavonoid, flovonol) contents, ferric reducing antioxidant power (FRAP), and liver mitochondria ferrous-induced LPO. To test the effect of the factors (location, used part and extract solvent), the general linear model (Univariate) was applied.

Results:

RSR extracts elucidated inhibitory effects of ferrous ion-induced LPO (IC_{50} were 8.40±0.35~88.98±7.42μg/ml) and the best was observed in NTS acetone extract. Almost all of the tested samples were no induced LPO production except MTB ethyl acetate (EA), MTB ethanol, MTL ethanol and NTS water extracts. Besides, the highest antioxidant activities (IC_{50}) were detected in 1) DPPH scavenging and superoxide anion radical-scavenging: MTS EA extract (1.53±0.08 μg/ml, even greater than positive control and 53.48±0.75 μg/ml); 2) hydrogen peroxide-scavenging: MTS acetone extract (208.03±1.87 μg/ml); 3) FRAP: MTS EA extract (equivalent trolox 116.40±0.34 μM). Total phenolic content of extracts was higher in MTS and the greater total flavonoid and flavonol contents were observed in leaf hydrophilic extracts.

Conclusion:

In conclusion, the extract solvent (27.62%) and used part (25.77%) are more influential factors in antioxidant and LPO inhibitory effects than location (5.22%). *RSR* exertes high antioxidant activity, LPO inhibitory effect and safety for liver protector development.

P265
Protective Effect of Loganin Against MPP+-Induced Neurotoxicity

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Backgrounds:

Loganin is an active compound isolated from *Corni Fructus* against inflammation, oxidative stress and hyperglycemia. In this study, we investigated the protective effect of loganin against 1-methyl-4-phenylpyridinium (MPP^+)-induced neurotoxicity, and further explored its underlying mechanisms

Materials and Methods:

MPP^+-treated primary cortical neurons were used to study the neuroprotective effect and mechanism of loganin. Cell viability was measured by MTT assay and protein expression was analyzed by western blot. Morphological changes of neurite lengths were observed by an inverted microscope.

Results:

Loganin increased cell viability, upregulated Bcl-2 and p-Akt expression, and decreased apoptotic signal in MPP^+ (100 μM)-treated primary cortical neurons. Moreover, loganin inhibited RhoA activation and promoted neurite outgrowth against MPP^+ (10 μM)-induced retraction of neurite lengths.

Conclusion:

Our findings demonstrated that loganin attenuated MPP^+-induced neuronal death and neurite damage, which suggests that loganin is a potential candidate in neuroprotection.

P266
BDNF regulates VEGF production and angiogenesis in human chondrosarcoma cells

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Backgrounds:

Vascular endothelial growth factor (VEGF) is a major regulator of tumor angiogenesis, it is occurs during development and vascular remodeling as a controlled series of events leading to neovascularization. Because of angiogenesis is essential for tumor growth and metastasis, controlling tumor-associated angiogenesis is a promising targeted therapy in limiting cancer progression. Brain-derived neurotrophic factor (BDNF) is commonly up-regulated in a variety of tumor angiogenesis. However, the role of BDNF in chondrosarcoma cells has not been unknown. The aim of the present study was to examine the mechanism involved in BDNF-mediated VEGF expression and angiogenesis in human chondrosarcoma cells.

Materials and Methods:

The VEGF expression was examined using ELISA and qPCR assay. The PLCγ, PKCα, and HIF-1α activation was examined by using Western blot method. A transient transfection protocol was used to examine HRE activity.

Results:

We found that BDNF increased VEGF production by using qPCR, western blotting, and ELISA assay. Pretreatment of cells with TrkB receptor, PLCγ, PKCα, and HIF-1α inhibitor reduced BDNF-induced VEGF expression. In addition, transfection of cells with TrkB receptor, PLCγ, PKCα, and HIF-1α siRNA also abolished BDNF-increased VEGF production. Incubation of cells with BDNF also enhanced HIF-1α activation.

Conclusion:

BDNF plays a critical role in tumor angiogenesis. We investigated the potential role of BDNF in VEGF expression in human chondrosarcoma cells. We found that BDNF increased VEGF expression. In addition, TrkB receptor, PLCγ, PKCα, and HIF-1α signaling pathways are involved.

P267
bFGF increases VEGF expression in human chondrosarcoma cells through the p38, c-Src, NF-κB signaling pathway

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Background:

Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and metastasize. In normal tissue, basic fibroblast growth factor (bFGF) is present in basement membranes and in the subendothelial extracellular matrix of blood vessels. It has been found that bFGF increased the formation of new blood vessels and angiogenesis during tumor growth and metastasis. However, the effect of bFGF in VEGF expression in human chondrosarcoma is largely unknown. In this study, we examine the effect of bFGF in VEGF expression in human chondrosarcoma cells.

Materials & Methods:

The qPCR and ELISA was used to examine the mRNA expression of VEGF. The p38, c-Src, and NF-κB phosphorylation was examined by using Western blot method. A transient transfection protocol was used to examine NF-κB activity.

Results:

We found that bFGF increased mRNA and protein expression of VEGF in a time- and concentration-dependent manner in human chondrosarcoma cells by using qPCR and western blotting. Pretreatment of cells with p38, c-Src, or NF-κB inhibitor antagonized bFGF-induced production of VEGF. Incubation with bFGF induced p38, c-Src and NF-κB phosphorylation.

Conclusion:

Our results indicated that bFGF enhances the VEGF expression of chondrosarcoma cells. One of the mechanisms underlying bFGF-directed VEGF expression was through the p38/ c-Src/NF-κB signal transduction pathway.

P268
The effect of estrogen on lipopolysaccharide-induced myocardial dysfunction in ovariectomized rats

林鈺儒

Sepsis, the systemic inflammatory response syndrome to infection, is the leading cause of death in the critically ill, predominantly as a consequence of multiple organ failure. Myocardial depression is a well-recognized manifestation of organ dysfunction in sepsis. Cardiac dysfunction in sepsis is characterized by decreased contractility. Furthermore, female had a better survival rate and lighter gravity in organ failure than male in sepsis. In this study, we want to investigate whether estradiol (E2) will safeguard myocardial function to improve the condition of organ failure and prolong survival in rats with sepsis.

P269
Preparation and Toxicity of Recombinant Human Granulysin

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Backgrounds:

Granulysin is a cationic protein that is expressed in human natural killer cells and cytotoxic T lymphocytes. It has been reported that granulysin involves in many different types of human diseases. Granulysin is synthesized as a 15 kDa precursor, and then cleaved to a 9 kDa form. 9 kDa granulysin have been well studied, however 15 kDa is not. In this study, we generate recombinant 15 kDa granulysin and characterize its antimicrobial activity.

Materials & Methods:

The expression vectors pET-22b contained the genes encoding maltose-binding protein, PreScission™ protease cutting site, and 15 kDa granulysin. The soluble recombinant fusion protein expressed in Escherichia coli was treated with 2-mercaptoethanol, purified by phosphocellulose and HisTrap™ HP chromatographies. After PreScission ™ protease digestion, the precipitated 15 kDa granulysin was denatured and refolded, and then purified by SP Sepharose chromatography. The antimicrobial activity of 15 kDa granulysin against Pseudomonas aeruginosa was assessed in vitro by colony forming unit assay.

Results:

We expressed and purified the recombinant 15 kDa granulysin from Escherichia coli which has potent antimicrobial activity against Pseudomonas aeruginosa, causing a large magnitude reduction in colony forming unit in a dose-dependent manner.

Conclusion:

In this study, we provided a new method to generate recombinant 15 kDa granulysin which possesses antimicrobial activity against Pseudomonas aeruginosa, although some studies showed that 15 kDa granulysin, not like 9 kDa granulysin, lacks cytotoxic activity.

P270
Alleviation of proinflammatory cytokine-induced adhesion molecule and endothelin-1 expression by Sporopollenin

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Backgrounds:

Vascular adhesion molecules such as intercellular adhesion molecules (ICAM)-1 and vascular cell adhesion molecules (VCAM)-1, are thought to play an important role in the inflammatory response of blood vessels. In addition, endothelin-1 (Et-1) has been implicated in regulation of vascular tones and progression of atherosclerosis. Inflammatory cytokines are markedly induced in the development of atherosclerosis. We have previously shown that Chlorella possess strong anti-inflammatory effect. In this study, we aimed to explore the possible role of Sporopollenin, derived from Chlorella, on pro-inflammatory cytokine-induced adhesion molecule.

Materials and methods:

RAW 246.7 macrophages were stimulated by LPS with and without Sporopollenin. NO production was measured with Griess reagent. SVEC endothelial cells were treated with 50% RAW conditioned media (normal SVEC culture media contains 50% of LPS-activated macrophage culture media) with and without Sporopollenin. Indomethacin was used as a positive control. Productions of ICAM-1 and VCAM-1 were measured by ELISA assay kits. Et-1 gene expression was evaluated by PCR.

Results:

Sporopollenin did not induce any cytotoxicity in RAW 267.4 macrophages. LPS-induced NO production was suppressed by Sporopollenin in a dose-dependent manner. ICAM-1 and VCAM-1 concentrations were increased in 50% RAW conditioned media-treated SVEC endothelium. The inductions of ICAM-1 and VCAM-1 were markedly suppressed by Sporopollenin. Whereas Indomethacin, a known anti-inflammatory drug, inhibited 50% RAW conditioned media-induced VCAM-1 but not ICAM-1. Sporopollenin also significantly inhibited Et-1 expression.

Conclusions:

Taken together, Sporopollenin can be a potential material to alleviate the development of atherosclerosis by inhibiting the adhesion molecules and Et-1 expressions.

P271
Antihyperglycemic Effect of Glossogyne tenuifolia in Normal and Diabetic Mice

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Backgrounds:

All over the world the diabetes mellitus (DM) is one of the most common metabolic disease of people's health. Therapeutic medicine of diabetes develop the increase that demand stands opposite each other at in new medicine. Glossogyne tenuifolia (GT) is a traditional Chinese herb. GT has been shown to possess antioxidative and anti-inflammatory. The aim of this research is to experiment GT relevance to antihyperglycemic.

Materials and Methods:

In this study, we used streptozotocin (STZ) 75mg/kg intravenous (i.v.) injections in the lateral tail vein and high fat diet (HFD) fed mice for 4 weeks induced diabetic mouse models. The effects of GT (p.o.) on animals showing blood glucose levels and release insulin. In C2C12 cell, the effects of GT on glucose uptake, activity of glucose transporter type 4 (Glut4) and glycogen synthesis were examined.

Results:

The experimental result of this study is found in acute treatment with GT (p.o.) produced a hypoglycemic effect in a dose-dependent manner in normal and diabetic mice, GT significantly lowered the plasma glucose at 90 min after oral treatment. Four-week treatment of diabetic mice with GT improved the expression of the Glut4 in the skeletal muscles. In C2C12 cell were treated GT with different concentrations of 0.01 (mg/ml), 0.05 (mg/ml) and 0.1 (mg/ml) in 60 minutes. Treating with GT resulted increased of Glut4 expression in C2C12 myotube concentration-depende-ntly, Glut4 expression was significantly increased at 0.1 (mg/ml).

Conclusion:

Found by the preliminary results, GT does have the effect of the regulation of blood glucose. Hence, GT have potential as an antidiabetes medicine for the treatment of DM.

P272
Genome-wide Study in a Methadone Maintenance Cohort Reveals Associations between Treatment Dose and GRK5 Genetic Polymorphisms

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Backgrounds:

Methadone maintenance treatment has been applied in clinical for heroin dependence patients. The drug was suggested to be administered with an initial low dose and then gradually increase to a maintenance dose in considerance for the tolerance effect. In this study, we tested the hypothesis that genetic polymorphisms in human genome may harbor susceptibility gene for the use of methadone maintenance dosage.

Materials and Methods:

A total of 366 patients under methadone treatment were recruited with records of dosage, plasma methadone and metabolite concentrations, and treatment outcome profiles. The genomic DNA were genotyped using the Axiom® Genome-Wide CHB 2 Array, which was population-optimized to have a better genomic coverage of common alleles (MAF >5%) of the Han Chinese genome.

Results:

Gene-based association analysis identified a candidate gene G protein-coupled receptor kinase 5 (*GRK5*) showing genome-wide significance with methadone dose ($p=2.37x10^{-6}$). Further fine mapping was performed through selecting 36 SNPs in considerance the ethnic group of minor allele frequency and functions within the genetic region of *GRK5* and the SNPs were genotyped on the same patients. The SNPs rs11819686, rs10886472, rs11198907, rs4752300, and rs1537576 of both genotype (GLM $p<0.0038$, FDR<0.027) and allele types (GLM $p<0.0035$, FDR<0.021) within the intron 1, intron 3, and intron 4 regions showed significant associations with methadone dose. The allele type of rs11198898, rs10787959, rs1268947, and rs2275036 at intron 2 were significantly associated with the adverse reaction symptom scores of constipation (GLM, $p<0.0046$, FDR<0.041). SNP rs17098759 was associated with the symptom score of weakness (GLM, p=0.0002, FDR, p=0.0072).

Conclusion:

We concluded that *GRK5* may be an important candidate gene which harbors biomarkers for methadone maintenance dose and adverse reactions.

P273

Long Term Electroacupuncture at The Zusanli Acupoints(ST36) of Effect Blood Glucose in Diabetic Mice

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Backgrounds:

In consequence of addition modern metabolic syndrome, will lead to chronic complications, such as diabetes and cardiovascular disease. Acupuncture is a traditional medicine has many different roles, for instance, reduce pain or weight loss. Animal studies have shown that acute treatment electroacupuncture (EA) at bilateral Zusanli acupoints decrease plasma glucose levels effect through β endorphins. Further study long-term treatment electroacupuncture (EA) at bilateral Zusanli acupoints points may be through other pathway regulation of blood glucose.

Materials and Methods:

In this study was Streptozotocin (STZ) (75mg/kg, i.v.) induce type I diabetic mice by intravenous injection of after one week measured plasma glucose and type II diabetic mice fed high sugar and high fat diet maintained for four weeks. These were randomly divided into four groups: Zusanli, non-acupoint, Zusanli combine Naloxone (1mg/kg, i.p.) and Zusanli combine Naloxone (1mg/kg, i.p.) and Pirenzepine. (7mg/kg, i.p.) The plasma glucose each groups in acute, two weeks and four weeks of variations.

Results:

The experimental results indicate that long-term treatment EA at bilateral Zusanli acupoints significant hypoglycemic effect. Naloxone (1mg/kg) blocked the plasma glucose reducing effect of acute EA at bilateral Zusanli acupoints. Existence of Naloxone (1mg/kg) long-term treatment EA at bilateral Zusanli acupoints significantly reduced plasma glucose effect. In the Zusanli combine Naloxone (1mg/kg) and Pirenzepine (7mg/kg) of acute, two weeks and four weeks is not significant hypoglycemic effect.

Conclusion:

Diabetic mice have long-term treatment EA at bilateral Zusanli acupoints treatment can be effective regulation of plasma glucose. According to the experimental results displayed long-term treatment EA at bilateral Zusanli acupoints have other pathway involved of regulation in plasma glucose. In the future may be able to integrating traditional chinese medicine and western medicine can application to diabetes care.

P274

The Antioxidant Activity of Economically Important Tree Species in Taiwan

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Backgrounds:

This research study important economic species of antioxidant activity for directly application in our daily lives and increase the value in use.

Materials and Methods:

This study selected Taiwan's northern, central and southern portion of 20 years old trees of *Pasania konishii, Pasania cornea, Bischofia javanica, Cinnamomum camphora , Swietenia mahagoni, Swietenia macropnylla* and *Melia azedarach* as tested material .

Results:

In the radical scavenging ability assay , crude extract of *P. konishii* cupule had better antioxidant activity , free radical median inhibitory concentration (IC_{50}) of about 5.52 ± 0.74 μg / mL , Ethyl acetate soluble part IC_{50} approximately 4.18 ± 0.88 μg / mL . The *B. javanica* of 5% EtOH leaves crude extract , IC_{50} approximately 17.06 ± 1.0μg/mL , Butanol soluble fraction approximately 8.78 ± 2.07 μg / mL . *S. macropnylla* leaves MeOH crude extract IC_{50} of 12.83 ± 1.70 μg / mL. Total phenolic content of the *P.konishii* of approximately 994.03 ± 0.05μm of Trolox. Ethyl acetate fraction content of about 989.92 ± 0.05 mg of GAE / g. Total antioxidant capacity test , DPPH, is proportional to the total phenolic content . The content of flavonoids in the *S.macropnylla* new leaf were content higher flavonoid content .

Conclusion:

Overall, *P. konishii* of crude extract had more activity. The *B.javanica* and *S.macropnylla* folk medicinal plants had antioxidant capacity , less than a *P.konishii* cupule , but can be directly applied to human life, it was worth we continue to explore.

P275

Effects of Furosemide on Lipopolysaccharide-Induced Disseminated Intravascular Coagulation in Rats

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Hao-Yuan Hung[1], Mei-Hui Liaw[2], Chih-Chin Shih[1], Wei-Ming Lue[1], Rui-Ling Chen[1], Shiu-Jen Chen[3], Chin-Chen Wu[1]
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Backgrounds:

Disseminated intravascular coagulation (DIC) is a common complication of sepsis and leads to multiple organ failure and death. A considerable body of recent evidence suggests that oxidant stress and inflammation response play a major role in DIC. A growing body of evidence shows that furosemide is a powerful anti-oxidant and anti-inflammatory reagent in addition to its diuretic effect. Thus, we tried to evaluate effects of furosemide on lipopolysaccharide (LPS)-induced DIC in Wistar rats.

Materials and Methods:

Rats were divided into four groups: (1) sham operation (SOP), (2) SOP + furosemide (3 mg/kg for 10 min i.v. infusion), (3) LPS (10 mg/kg for 40 min i.v. infusion) and (4) LPS + furosemide (3 mg/kg for 10 min i.v. infusion at 1 hr after LPS). Whole blood sample was collected at 0, 1, 2, 4 and 6 h. The changes of hemodynamics, blood glucose, fibrinogen (FIB), prothrombin time (PT), platelet, lactate dehydrogenase (LDH), hepatic (ALT) function, renal (BUN and CRE) function, and survival rate were monitored over 6 h. The vital organs including lung, liver and kidney were harvested at 6 hr after LPS challenge to perform Western blot and histo-pathological studies. The survival rate in each group was calculated at the end of studies.

Results:

Our results showed that the LPS group matched the diagnostic crriteria of the ISTH for overt-DIC. The administration of furosemide increased the concentration of FIB and platelet count, decreased the level of plasma LDH, ALT, BUN and CRE, improved prolongation of the PT and the LPS-induced delayed hypotension, and increased survival rate in LPS-induced DIC rats. In addition, furosemide not only attenuated the inducible nitric oxide synthase (iNOS) expression in the lung and liver but also attenuated the superoxide in the aorta, lung and kidney.

Conclusion:

These data suggest that furosemide has benefit effects on the LPS-induced consumptive coagulopathy occurred in late endotoxemia.

P276

Resveratrol-Induced $[Ca^{2+}]i$ Rise and Apoptosis in Human Oral Cancer Cells

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Backgrounds:

This study examined whether the natural product resveratrol altered $[Ca^{2+}]i$ and viability in OC2 human oral cancer cells.

Materials and Methods:

The Ca^{2+}-sensitive fluorescent dye fura-2 was applied to measure $[Ca^{2+}]i$. Cell viability was measured by detecting reagent WST-1. Apoptosis and cell cycle were detected by flow cytometry.

Results:

Resveratrol at concentrations of 5-20 μM induced a $[Ca^{2+}]i$ rise in a concentration-dependent fashion. The response was decreased partially by removal of extracellular Ca^{2+}. Resveratrol-induced Ca^{2+} signal was not altered by nifedipine, econazole, SK&F96365, and protein kinase C activator phorbol myristate acetate (PMA), but was inhibited by the protein kinase C inhibitor GF109203X. When extracellular Ca^{2+} was removed, incubation with the endoplasmic reticulum Ca^{2+} pump inhibitor thapsigargin or 2,5-di-tert-butylhydroquinone (BHQ) partially inhibited resveratrol-induced $[Ca^{2+}]i$ rise. Incubation with resveratrol abolished thapsigargin or BHQ-induced $[Ca^{2+}]i$ rise. Inhibition of phospholipase C with U73122 abolished resveratrol-induced $[Ca^{2+}]i$ rise. At concentrations of 20-100 μM, resveratrol killed cells in a concentration-dependent manner. This cytotoxic effect was not changed by chelating cytosolic Ca^{2+} with 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid/acetoxy methyl (BAPTA/AM). Annexin V/propidium iodide staining data suggest that resveratrol (20 μM, 40 μM and 60 μM) induced apoptosis in a concentration-dependent manner. At concentrations of 20 μM, 40 μM and 60 μM, resveratrol caused cell cycle arrest.

Conclusion:

In human oral cancer cells, resveratrol induced a $[Ca^{2+}]i$ rise by inducing phospholipase C-dependent Ca^{2+} release from the endoplasmic reticulum and Ca^{2+} entry via protein kinase C-sensitive, non store-operated Ca^{2+} channels. Resveratrol induced cell death that might involve apoptosis.

P277

In vitro and Zebrafish Model to Study Anti-atherosclerotic and Anti-inflammatory Effects of Marine Compound

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Backgrounds:

According to the latest statistics of American Heart Association, the death rate of cardiovascular disease was 251.2 per 100,000. In particular, atherosclerosis is growing increasing common in the aging society of Taiwan. There are many risk factors associated with atherosclerosis including dyslipidemia, hypertension and smoking. Besides, recent studies indicated that inflammation is the major cause of atherosclerosis. However, the clinically used anti-atherosclerotic drugs do not focus on anti-inflammation. Therefore, it is imperative that we find new anti-inflammatory drugs for treating atherosclerosis.

Materials and Methods:

In the present study, we examined whether marine compounds have anti-atherosclerotic and anti-inflammatory properties by using the RAW264.7 cells as an *in vitro* model. Simultaneously, we used proteomic such as Western blot and 2-D electrophoresis to clarify the detailed molecular mechanisms of marine compounds. Based on the analysis of proteomic results, we apply siRNA, gene overexpression and inhibitors to verify suspected molecular targets of marine compounds. On the other hand, we established the Tg(fli1a:EGFP)^{y1} zebrafish model to examine the anti-atherosclerotic effect of bioactive compounds.

Results:

In our data, we found that C-10, which is a marine-derived compound isolated from soft coral, significantly inhibited the expression of the pro-inflammatory protein, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), in the lipopolysaccharides (LPS)-stimulated RAW 264.7 macrophage cell. Besides, the proteomics results showed that some targets may involve in anti-atherosclerotic and anti-inflammatory effects of C-10. Insterestingly, we found that the effects of C-10 would be inhibited by knockdowning the suspected targets. Conversely, the effects would be improved by overexpressing the targets or adding antibodes. As for zebrafish model, we have established the model to observe the accumulation of cholesterol.

Conclusion:

The present study demonstrateed that C-10 significantly inhibits expression of two pro-inflammatory proteins, iNOS and COX-2. Based on many positive results, we suggested that C-10 may become potent drugs in atherosclerosis. Besides, we found several targets which associated with atherosclerosis and inflammation. Therefore, we may develop novel protein drugs in treating atherosclerosis and inflammatory diseases in the future.

P278

4β-hydroxywithanolide E inhibits survivin expression in breast cancer MDA-MB-231 cells

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Backgrounds:

Worldwide, breast cancer is the most common cancer in women. Triple-negative (estrogen receptor, progesterone receptor, and HER2 negative) breast cancer (TNBC) is more aggressive and less responsive to standard therapies compared to other types of breast cancer. Therefore, discovery of new therapeutic agents for treating TNBC is an urgent need. Withanolides naturally from Nightshade family possess anticancer potential. In this study, 4β-hydroxywithanolide E (HW), a withanolide isolated from *Physalis peruviana*, was examined for its cytotoxic effect against a TNBC cell line MDA-MB-231. The possible mechanism of action of HW was also investigated.

Materials & Methods:

MDA-MB-231 cell lines were incubated with different doses of (HW). Cell viability was determined by MTT assay. Survivin mRNA expression was detected by real time RT-PCR. Protein levels and/or protein phosphorylation of survivin, XIAP, Bcl-2 family proteins, AKT, ERK, and NFκB were detected by Western blot.

Results:

After 48 h treatment, HW significantly reduced the viability of MDA-MB-231 cells with an IC_{50} value of 0.58 μM. HW inhibited protein levels of survivin, XIAP, Bcl-xL, which are related to antiapoptotic and cell survival. Among them, survivin was most susceptible to HW-induced loss of protein expression. Real time RT-PCR revealed that HW also repressed survivin mRNA levels. The activation of AKT, ERK, and NFκB, which are the upstream signaling molecules that regulate survivin expression, were inhibited by HW.

Conclusion:

Our results have demonstrated that HW potently inhibited the viability of TNBC MDA-MB-231 cells. Cell death induced by HW was possibly mediated by inhibition of survivin and other antiapoptotic proteins.

P279

Anandamide inhibits cell migration and angiogenesis through the regulation of MMP2/9 and VEGF.

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Yun-Hong Wong[1,2], Shih-Hua Chen[1], Chun-Chien Yang[1], Yu-Chiao Yang[1], Meng-Husan Cheng[2,3], Yu-Wen Chiang[1], Chia-Ming Chang[1], Chien-Yuan Chen[1], I-Ning Yang[3], Kuo-Hsiung Liao[1], Hsiao-Mei Kuo[4], Yuan- Chun-Lung[5], Show-Jen Hong[1], Hui-Fen Chiu[1,2#]

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Backgrounds:

Anandamide (AEA), the first endogenous canabinoid of arachidonic acid and ethanolamine linkage. AEA is implicated in a variety of physiological and pathological conditions: inflammation, analgesia and cancers; the anti- tumor effects of AEA have been widely study in recent years.

Materials and Methods:

Anadamide is an arachidonic acid derivative. After various concentration of AEA treatment for 72h and the anti-proliferation activity of AEA were assessed by XTT and methylene blue assay. Seeding cells as confluent and make a straight scratch wound by using 200ul tips. Then treat with AEA and the effect of anti-migration was observed. Extract upper layer medium after desire treatment in serum-free MEM and analyzed the activity of MMP2/9 by zymography assay. Moreover, whole cell lysates were be extracted and apoptotic protein were estimated by Western blotting analysis.

Results:

We demonstrate that AEA decrease cell proliferation and inhibit cell migration by wound healing assay as time and dose-dependent manner. MMP2/9 plays an important role in cell migration, AEA also reduce the activation of MMP2/9 and angiogenesis related proteins expression (MMP2/9, VEGF, VEGFR) with Western blot analysis. We found that the ability of cancer cell migration were down-regulation by increasing the concentration of AEA.

Conclusion:

Anadamide inhibited hepatoma cell migration and angiogenesis. The angiogenetic protein VEGF and VEGFR and MMP2/9 were decreased in AEA-treated hepatoma cell.

P280

The Effect of Naltrexone on Neuropathic Pain in Mice Locally Transfected with the Mutant Mu-opioid Receptor Gene in Spinal Cord

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Backgrounds:

The opioid antagonists, such as naloxone and naltrexone, exhibited agonistic properties at the mutated mu-opioid receptor, MORS196ACSTA, in which the conserved Ser^{196}, Thr^{327}, and Cys^{330} were mutated to Ala, Ala, and Ser, respectively. In our previous study, systemic naloxone (10 mg/kg, s.c.) elicited antinociceptive effect (determined by tail-flick test) without the induction of tolerance, dependence, and rewarding effect in mice 2 weeks after intrathecal administration of dsAAV2-MORS196ACSTA-EGFP. In the present study, we further investigated whether this antinociceptive paradigm could be effective in mice with neuropathic pain.

Materials and Methods:

The spinal nerve ligation (SNL) surgery was performed on mice 3 or 4 weeks after intrathecal injection of the lentivirus which carried the gene of MORS196ACSTA-EGFP or MOREGFP (for control). The von Frey tests were used to detect the anti-allodynic effect of systemic morphine or naltrexone before and after gene transfection. After 14 days of naltrexone or morphine treatment (10 mg/kg, s.c., q.d.) from day 1 or day 6 after surgery, the natural withdrawal signs were counted at 22 and 46 hours after the last drug injection.

Results:

The ipsilateral paw withdrawal pressure was significantly decreased one day after SNL surgery and persisted at least for 19 days. Naltrexone (10 mg/kg, s.c.) or morphine (10 mg/kg, s.c.) elicited significant anti-allodynia effects on both ipsilateral and contralateral hind paws. The paw withdrawal pressure after naltrexone or morphine treatment was significantly increased when compared to saline treatment. The SNL-induced allodynia was improved gradually and almost back to normal after chronic naltrexone or morphine treatment on day 19. However, chronic treatment of morphine (10 mg/kg, s.c., q.d.) but not naltrexone (10 mg/kg, s.c., q.d.) induced natural withdrawal signs.

Conclusion:

These data imply that systemic injection of naltrexone may have therapeutic potential for chronic neuropathic pain without the development of dependence after the gene therapy with the expression of MORS196ACSTA in the spinal cord.

P281

Amantadine Hydrochloride Improves Cognitive and Motor Deficits after Fluid Percussion Injury of Cerebral Cortex in Rats

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Backgrounds:

Amantadine hydrochloride, a weak NMDA receptor channel blocker, has been the subject of considerable interest and clinical use for patients with prolonged disorders of consciousness after traumatic brain injury (TBI). Preliminary studies have shown that amantadine hydrochloride accelerated the pace of functional recovery during active treatment in patients. To date, no studies have explored the potential for amantadine hydrochloride to provide behavioral recovery in chronic treatment.

Materials and Methods:

Using male Sprague-Dawley rats, we employed the 6 atm fluid percussion traumatic brain injury model to treat with saline or amantadine hydrochloride that was released 3.6 mg/kg per hour for each rat for 7 weeks by using subcutaneous mini-osmotic pump after a week of injury. Novel object recognition (NOR) and fixed-speed rotarod (FSRR) behavioral tests were used to determine whether the treatment enhanced cognitive and motor deficits recovery every week after injury.

Results:

Cognitive and motor behavior were impaired in NOR and FSRR behavioral tests after injury. Treatment with amantadine hydrochloride persistently improved the impairments in both NOR and FSRR behavioral tests after TBI.

Conclusion:

Persistent treatment of amantadine hydrochloride could ameliorate cognitive and motor deficits caused by traumatic brain injury.

P282

The protective effect of 5-lipoxygenase inhibitor in animal models of Parkinson's disease

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Background

Neuroinflammation and oxidative stress are the important factors inducing neurodegeneration in age-related neurological disorders. 5-lipoxygenase (5-LOX) is the enzyme that can insert oxygen into the molecule of arachidonic acid and thereby synthesize inflammatory leukotrienes or 5-HETE. 5-LOX is expressed in central nervous system neurons and may participate in neurodegenerative disease.

Materials & Methods:

In the present study, we studied the effect of pharmacologic inhibition of 5-lipoxygenase activating protein by MK-886 on the rat neuron glia co-culture and MPTP injected mice.

Results:

It was found here that 5-LOX was over-expressed in astrocyte after injection of MPTP to C57BL6 mice. We thus evaluated whether the inhibitors of 5-lipoxygenase is a possible neuroprotective agents in midbrain culture of rat. MK-886, a specific 5-LOX activating protein (FLAP) inhibitor, significantly increased the [^{3}H] dopamine uptake, which is a functional indicator of the integrity of dopaminergic neurons, in mesencephalic neuron-glia co-cultures after MPP^+ treatment. In addition we found that LTB_4, one of 5-LOX downstream product, enhanced MPP^+-induced neurotoxicity. However, LTD_4 and 5-HETE did not exert similar potentiating effect. Furthermore, MK-886 reduced the level of LTB_4 in MPTP-induced Parkinsonism mice and exert the neuroprotection.

Conclusion:

These experiments indicate that 5-LOX inhibitors may be useful to developed as a novel neuroprotective agent and LTB_4 may play important pathological role in Parkinson's disease.

P283

A study for the preparation of biodegradable microspheres

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Purpose:

In this study, we evaluated a new drug delivery system for cancer radiotherapy using a biodegradable PLGA microspheres consisting of therapeutic radionuclide.

Materials and Methods:

In this experiment, PLGA (50/50)and PVA(2wt%) were proved to have the better result for manufacturing the microsphere and the PLGA microspheres were conjugated with DOTA for the labeling of In-111. The following instruments were used to analyze the material and its particle size, including (1) GPC(2) SEM (3) FT-IR, (4) NMR and (5) XPS.

Result:

The selected particles size for the microsphere was ranged between 25±10μm~47±10μm. We modified DOTA-PLGA- microspheres by EDC/NHS mixed solution. By using the 0.2 N sodium acetate solution as environment buffer in In-111 labeling DOTA-PLGA-microsphere preparation and the labeling rate of 90%.

Conclusion:

In this experiment, PLGA (50/50) and PVA (2wt%) were proved to have the better result for manufacturing the microsphere and in the In-111 labeling rate of 90%. In the future, we would use In-111/Y-90-DOTA-PLGA-microspheres to evaluate the therapeutic potential in hepatoma animal model via transcatheter arterial embolization pathway.

P284

B7, a Novel Anthraquinone Derivative, Inhibit Telomerase and HDAC Activities in Hepatoma Cells Apoptosis

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Chia-Ming Chang[1], Yu-Chiao Yang[1], Yun-Hong Wong[1,2], Meng-Hsuan Cheng[2,3], Chun-Chien Yang[1], Yu-Wen Chiang[1] , Shih-Hua Chen[1], Chien-Yuan Chen[1], Kuo-Hsiung Liao[1], Chun-Lung Yuan[4], Hsiao-Mei Kuo[5], Show-Jen Hong[1], Hui-Fen Chiu[1,2#]

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Backgrounds:

After screening hundreds of anthraquinone derivatives, we found B7 possesses significant cytotoxic effects on different cell line including, HT29, HepG2 and A549. Increasing evidence indicates that HDAC inhibitors show anticancer activity in cell culture and animal models of carcinogenesis. Moreover, the intimate connection between telomerase regulation and cancer is now well established. Consequently, we exploring the role of DNA histone deacetylase(HDAC) and telomerase in B7 induced hepatoma cell apoptosis.

Materials and Methods:

The human hepatocellular carcinoma cell line (HepG2), human lung cancer adenocarcinoma cell line (A549) and human colon cancer cell (HT29) were cultured and seeded in 24-well plate for 24 h prior to B7 addition. The cell viability was estimated by XTT and methylene blue assay at indicated exposure time. Levels of specific proteins expression were detected by Western blotting analysis. DNA strand breaks were determined by alkaline comet single-cell gel electrophoresis and the stained with SYBR Green. The SYBR Green RTQ-TRAP assay was conducted with dilution of HepG2 protein extracts. The change in CT value related to fixed fluorescence intensity (ΔRn) can estimate telomerase activity.

Results:

Our data have demonstrated that B7 increased apoptosis signals, caused DNA damage and activated acetyl-histone H3 as a dose-dependent manner. Telomerase repeat amplification plot also showed that B7 cause significant inhibition on telomerase activity as dose-response manner.

Conclusion:

Our results have indicated that in addition to DNA damage, we found the activities of HDAC and telomerase were decreased in B7 induced hepatoma cells apoptosis. These outstanding anticancer activities made B7 as a potential compound to fight cancer.

P285

Effects of a Xanthone Derivative Induces Apoptosis on Human Cancer Cells.

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Backgrounds:

Since 1982, malignant tumors are the first of the ten leading causes of death in Taiwan according to the data from Department of Health, Executive Yuan. For the multiple drugs resistant of cancer chemotherapy, development of effect agents from natural products is one of the important issues to treatment malignant tumors in the future. YCX-1 is a xanthone derivative isolated from natural product. In our previous studies, we found evidence of anti-inflammatory, anti-tumor, and antioxidant activities in YCX-1.

Materials and Methods:

Cell viability was determined by MTT assay, and apoptotic effects in YCX-1-treated HepG2, HT-29, U-87 MG and GBM 8401 cells were investigated via Giemsa staining, PI staining, DCFDA assay and $DiOC_6(3)$ staining.

Results:

YCX-1 showed concentration- and time-dependent antiproliferative activities on HepG2, HT-29, U-87 MG and GBM 8401 cells. YCX-1 induced nuclear condensation and the appearance of apoptotic bodies, characteristics of apoptosis in YCX-1-treated cells. In addition, flow cytometry analysis showed increases of hypodiploid cells with an enhancement of intracellular peroxide production was detected in YCX-1-treated cells. These results supported that YCX-1 induce apoptosis in HepG2, HT-29, U-87 MG and GBM 8401 cells.

Conclusion:

It suggests that YCX-1 is an effective phytochemical with benefic activity to treat several different cancers include liver, colon and brain cancer, and may play as a potential lead compound for future anti-cancer drug development.

P286

A Gene-Gene Interaction between OPRK1 and OPRL1 is Associated with Liver Gamma-glutamyl transpeptidase in Methadone Maintenance Patients

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Backgrounds:

Combining alcohol use is commonly occurr in heroin dependence patients under methadone maintenance treatment (MMT). Opioid receptor has been reported association with alcohol use. In this study, we tested the hypothesis of whether the genetic polymorphisms in opioid receptors were associated alcohol use in MMT patients.

Materials and Methods:

The alcohol use was evaluated through liver gamma-glutamyl transpeptidase (γ-GT) enzymatic activity. Among 366 MMT patients, we selected and genotyped the single nucleotide polymorphisms (SNPs) through consideration of ethnic group minor allele frequency and functions on four opioid receptors; mu, delta, kappa (*OPRK1*) and nociceptin (*OPRL1*).

Results:

The SNPs genotypes on *OPRK1* and *OPRL1* genetic regions showed significant associations with the amount (days) of alcohol use (GLM, P<0.038) and γ-GT activities (U/L) (GLM, p<0.038). In gene-gene interaction analyses, we selected functional SNPs on the exons of both *OPRK1* and *OPRL1*. The rs702764 (exon 4) of *OPRK1* and rs2229205 (exon 3) of *OPRL1* were significantly associated with γ-GT (U/L) in interation (GLM, P<0.021) and in genotype model effect (GLM, P<0.0001).

Conclusion:

In summary, the genetic variants on *OPRK1* and *OPRL1* may be the major opioid receptors involved in combining alcohol use for methadone maintenance patients.

P287

Activated PAR-2 Regulates Pancreatic Cancer Progression through ILK/HIF-α-induced TGF-α Expression and MEK/VEGF-A-mediated Angiogenesis

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Backgrounds:

Tissue factor (TF) initiates the process of thrombosis and activates cell signaling through protease-activated receptor-2 (PAR-2). The aim of this study was to investigate the pathological role of PAR-2 signaling in pancreatic cancer.

Materials and Methods:

First, we used PAR-2 activating peptide to mimic the action of trypsin to trigger PAR-2 signaling pathway and effects of PAR-2 activation on gene expression in human pancreatic cancer cell line BxPC-3 investigated by microarray analysis. Then, PAR-2-mediated genes were confirmed by RT-PCR (for mRNA level) and western blot analysis (for protein level). VEGF-A levels were detected by a ELISA kit. For *in vitro* angiogenesis assay, HUVECs proliferation was measured by crystal violet staining; tube formation was examined by Matrigel capillary-like tube formation assay.

Results:

We first demonstrated that activated PAR-2 upregulated the protein expression of both hypoxia-inducible factor-1α (HIF-1α and HIF-2α), resulting in enhanced transcription of transforming growth factor-α (TGF-α). Downregulation of HIFs-α by siRNA or YC-1, a HIF inhibitor, resulted in depleted levels of TGF-α protein. Further, PAR-2 through integrin-linked kinase (ILK) signaling, including the p-AKT, promoted HIFs protein expression. Diminishing of ILK by siRNA decreased the levels of PAR-2-induced p-AKT, HIFs-α and TGF-α our results suggest that ILK is involved in the PAR-2-mediated TGF-α via a HIF-α-dependent pathway. Furthermore, the culture medium from PAR-2-treated pancreatic cancer cells enhanced HUVEC proliferation and tube formation, which was blocked by the MEK inhibitor PD98059. We also found that activated PAR-2 enhanced tumor angiogenesis through the release of vascular endothelial growth factor-A (VEGF-A) from cancer cells, independent of the ILK/HIFs-α pathways. Consistent with microarray analysis, activated PAR-2 induced TGF-α and VEGF-A gene expression.

Conclusion:

In conclusion, the activation of PAR-2 signaling induced human pancreatic cancer progression through the induction of TGF-α expression by ILK/HIFs-α, as well as through MEK/VEGF-A-mediated angiogenesis, and it plays a role in the interaction between cancer progression and cancer-related thrombosis.

P288

Blockade of Reactive Oxygen Species is Critical for Anti-inflammation and Growth inhibition of Insulin Sensitizers in Phosphatase and Tensin Homolog-Deficient RAW 264.7 Cells

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Backgrounds:

Insulin sensitizers are used for treatment of type 2 diabetes and have a potential application on the treatment of inflammation and cancer. How phosphatase and tensin homolog (PTEN) regulates insulin sensitizers against cancer and inflammation remains unclear.

Materials and Methods:

We have established PTEN knockdown in RAW264.7 murine macrophages (shPTEN cells) to observe PTEN deficit cell condition in response to inflammation by detecting levels of inflammatory mediators by commercial kits, reactive oxygen species (ROS) by flow cytometry, and cell growth by MTT assay. Effects of insulin sensitizers (i.e. rosiglitazone and metformin) on the signal molecules were examined by western blot.

Results:

The shPTEN cells had significant large amounts of iNOS/NO and COX-2/PGE2; also elevated production of ROS and increased cell proliferation. These effects were accompanied with the activation of Akt and p38 MAPK and the inactivation of AMPK and ERK 1/2. Pretreatment with a PPARγ agonist, rosiglitazone, did not alter the increased level of iNOS/NO and COX-2/PGE2, while metformin, an AMPK activator, blocked these inflammatory mediators. Inactivation of p38 MAPK by rosiglitazone and Akt by metformin were also observed. Rosiglitazone caused G1 arrest and lower proportion of S+G2/M phase, whereas metformin induced significant apoptosis. These agents against enlarged inflammatory mediators and/or cell growth in shPTEN cells through blocking ROS generation, but independent of AMPK activation.

Conclusion:

Our results indicated that macrophages with PTEN deficiency developed a continuous inflammatory microenvironment, where further aggravated tumor cell growth. Rosiglitazone and metformin affected the dependence of PTEN-deficient cells on ROS through differential pathways.

P289

The Role of Aryl Hydrocarbon Receptor in Lipopolysaccharide-induced Indoleamine 2, 3-Dioxygenase Expression in Primary Rat Astrocytes

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Purpose:

Aryl hydrocarbon receptor (AhR) is activated by environmental hormones and endogenous tryptophan metabolites, such as kynurenine (Kyn), the indoleamine 2, 3-dioxygenase (IDO)-catalyzed tryptophan metabolite that is involved in immune modulation. Here we investigated the role of AhR in the lipopolysaccharide (LPS)-induced IDO/Kyn pathway and proinflammatory cytokine expression in astrocytes.

Materials and Methods:

Primary rat astrocytes were used. AhR activity was assessed by transfecting dioxin response element-driven luciferase reporter constructs and analyzing the luciferase activity. Expressions of IDO, interleukin-6 (IL-6) and AhR target gene cytochrome P450 1A1 (CYP1A1) were measured by quantitative RT-PCR, Western blotting, and ELISA. AhR knockdown was performed by AhR siRNA transfection. The in vivo AhR deficiency on LPS effect was examined by intracerebroventricular (i.c.v.) injection of LPS in wild type, heterozygous, and homozygous AhR-knockout mice, and the cerebral cortex was harvested for gene expression analysis.

Results:

Kyn activated AhR and increased the expression of CYP1A1. Furthermore, LPS treatment also activates AhR, and markedly induced IDO and IL-6 expression, with the peak induction at 3-6h. AhR knockdown profoundly enhanced and prolonged the LPS-induced IDO and IL-6 expression. Importantly, LPS i.c.v. induced IDO expression in the mouse cerebral cortex, and the expression was significantly higher in the AhR-knockout mice than the heterozygous and wild type.

Conclusion:

AhR regulates LPS-induced IDO and proinflammatory cytokines in cultured astrocytes and the brain, and LPS may activate AhR via IDO/Kyn pathway to auto-regulates its proinflammatory and astrocyte activating effects to dampen the immune response in neuroinflammation.

P290

Gelsolin Controls the Caveolin Signaling Against H2O2-induced Oxidative Stress in H9c2 Rat Cardiomyoblasts

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Backgrounds:

Gelsolin (GSN), a Ca^{2+}-regulated actin severing and capping protein, has been found to be highly expressed in murine and human hearts after myocardial infarction. However, the biological function of GSN in cardiac cells associated with oxidative stress remains to be determined.

Materials and Methods:

The pcDNA6-GSN containing full-length GSN was transfected into H9c2 rat cardiomyoblasts, and their stable clones (GSN op) were used to study involvement of GSN in the caveolin signaling for H_2O_2-induced oxidative stress in cardiac cells. Phase contrast microscopy and MTT assay were used to determine changes in cell shape and cell proliferation, respectively. Intracellular Ca^{2+} levels, and reactive oxygen species (ROS) were measured by fura-2 F340/F380 fluorescence ratio, and by dichlorofluorescein diacetate fluorescence, respectively, in the cells. The expression of mRNA and protein for GSN, caveolins, SOD were measured by semi-quantitative RT-PCR and western blot, respectively.

Results:

The GSN op H9c2 cells showed a phenotypic transformation of the cell shape to a “shorter and broader” form, a decreased proliferation rate, and a 30% increased intracellular Ca^{2+} but no changes of intracellular ROS levels, as compared to controls. In addition, GSN overexpression caused an increase of mRNA expression and protein content for caveolins (cav-1 and cav-3). Caveolins (Cavs) has been shown to mediate multiple signal transduction events for cardiac protection in cardiac cells. Cav-3 is the muscle-specific isoform in cardiac myocytes, whiles Cav-1 and Cav-2 are present in other cell types in the heart. When exposed to H_2O_2, GSN op cells increased their anti-oxidative capacity by preventing the down-regulation of Cav-3 and by attenuating the H_2O_2-increased intracellular ROS levels, as compared with controls.

Conclusion:

These findings suggested that GSN might mediate the caveolin signaling for anti- oxidative stress in cardiac cells.

P291

Ceftriaxone Prevents and Reverses MPTP-Induced Behavioral and Neuronal Deficits in Animal Model of Parkison’s Disease Dementia

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Backgrounds:

A high percentage of patients with Parkinson’s disease (PD) suffer from not only motor dysfunction but also dementia that is Parkinson’s disease dementia (PDD). Hyperactivity of glutamatergic system has been observed in PD. Ceftriaxone (Cef), a beta-lactam antibiotic, shows neuroprotection by increasing the expression of glutamte transporter 1 (GLT-1). GLT-1, one of the major glutamate transporters on the astrocytes, is responsible for reuptake of synaptic glutamate. This study was aimed at clarifying whether Cef prevents or reverses behavioral and neuronal deficits in MPTP-induced PD animal model.

Materials and Methods:

Male Wistar rats were stereotaxically administered with MPTP that was bilaterally infused into the substantia nigra pars compacta to induce PD rat model. Cef (100 and 200 mg/kg/day, i.p.) was administered, starting either 5 days before or 3 days after the MPTP lesioning. All animals were subjected to behavioral tests and the brains were taken for histological evaluation.

Results:

One day after the MPTP lesioning, motor dysfunctions in bar test were observed. Such impairments were spontaneously recovered to control level 7 days after the MPTP lesioning. In addition, MPTP lesioning caused deficits in working memory and object recognition in the T-maze test and object recognitive task, respectively. However, these deficits were blocked by both pre- and after-treatment of Cef. Histologically, MPTP induced hyperactivity of glutamatergic system in the subthalamic nucleous, which were suppressed by Ceft treatment. Moreover, the increase of GLT-1 expression and GLT-1 colocalization with astrocytes were observed in the striatum and hippocampus. More interestingly, cell loss in the hippocampus at CA1 area was suppressed by Cef treatment.

Conclusion:

These results suggest that, by increasing GLT-1 expression, both pre- and after-treatment of Cef block PD-related deficits of cognitive functions and cell excitotoxicity. Thus, Cef may have clinical potential for prevention and treatment of dementia associated with PD.

Keywords:
Parkinson’s disease, dementia, ceftriaxone, GLT-1, neuroprotection

P292

梨形蟲感染小鼠排除後附加心理壓力下內臟高敏感性與腸道屏障功能失調之現象

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Backgrounds:

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by chronic abdominal pain and altered bowel habits without macroscopic abnormality and presence of pathogen. Symptoms of IBS may begin following stressful events or infectious gastroenteritis termed post-infectious (PI) IBS. A recent study in Norway showed that > 80 % of patients developed IBS symptoms after a parasite *Giardia* infection. However, the pathogenesis of PI-IBS remains obscure. Previous animal studies have shown that psychological stress increases abdominal pain and intestinal barrier disruption. The aim is to establish a PI-IBS model using post-giardiasis combined with water avoidance stress (WAS) to evaluate abdominal pain and gut barrier function.

Materials and Methods:

Mice were inoculated with 10e7 trophozoites of *Giardia lamblia*, and the trophozoites in the small intestine were enumerated. The number of colonized *Giardia* peaked on day 4-7 which was termed “colonization phase”; the parasites were cleared by day 14, and therefore, day 21-49 was denoted “post-clearance phase”. Further stress experiments were performed post-giardiasis on PI day 35.

Results:

Stress alone induced hyperalgesia while post-giardiasis alone induced allodynia; dual factors caused both hyperalgesia and allodynia without synergistic effects. Moreover, tight junctional disruption and increased epithelial permeability were seen in post-giardiasis. Despite normal gut morphology, presence of bacteria in lamina propria and increased endocytosed bacteria in mucosal cells were found in mice post-giardiasis, which was potentiated by stress.

Conclusion:

Post-infection combined with stress caused visceral hypersensitivity, intestinal barrier dysfunction and commensal bacteria invasion. The post-giardiasis animals may be a suitable model for future studies in pathogenesis of PI-IBS.

P293

Involvement of Mechanosensitive Channels TRPV1, TRPV4, and ASIC3 at Zusanli (ST36) Acupoint : Possible Acupuncture Responding Channels?

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Backgrounds:

Acupuncture is an ancient therapy used for over thousands of years and gained scientific approval recently. It involves mechanostimulation after needling at acupoints. Although many proposed theories as endomorphin release or neural inhibition by adenosine after local ATP release, the link between mechanostimulation and following biological response is limited. TRPV1, TRPV4 and ASIC3 are suggested as mechanosensitive channels. Therefore we seek if they are involved during acupuncture.

Materials and Methods:

We compared Zusanli (ST36) acupoint with non-acupoint using western blot. Effects of manual acupuncture and agonist injection at ST36 on CFA inducted mouse were tested by withdraw time of radial heat.

Results:

TRPV1 was abundant in muscle and connective tissue of ST36, whereas TRPV4 and ASIC3 showed abundance at connective tissue. Second, TRPV1 agonist injected show similar effect as manual acupuncture.

Conclusion:

Our results revealed TRPV1, TRPV4, and ASIC3 were anatomically abundant at ST36 and TRPV1 resulted in similar acupuncture analgesic effect when stimulated. Interestingly, TRPV1, TRPV4, and ASIC3 can ATP release after stimulation. Thus, we suggest neuronal response to acupuncture is mediated directly by TRPV1 activation or indirectly by ATP release from tissues nearby. We stress the possibility of TRPV1, TRPV4 and ASIC3 as acupuncture responding channels. This can lead to create a new therapy by agonist injection and to the physiological identity of acupoints.

P294

Effects of Advanced Glycation End Products (AGEs)-Stimulated Hypoxia Induced Factor 1alpha (HIF1α) on Diabetic Retinopathy

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Backgrounds:

To investigate the molecular mechanism underlying AGEs-stimulated HIF-1α expression in diabetic retinopathy.

Materials and Methods:

In vitro, we treatment the human retinal pigment epithelial (RPE) cells with different dose AGEs. The expression of HIF-1α, PHD1, PHD2, PHD3, VHL and RAGE were examined by western blotting in RPE cells. Both RAGE and HIF-1α gene expression were verified by q-RTPCR. Different kinases inhibitors were evaluated to figure out the downstream signaling pathway that AGEs-stimulated HIF-1α. *In vivo*, Deposition of AGEs and expression of RAGE and HIF1α will be examined by immunohistochemical/ immunofluorescence staining in the retina of *db/db* mice, a diabetic animal model.

Results:

Our preliminary data implied that AGEs could significantly promote human retinal pigment epithelial (RPE) cell migration but not alter the proliferation. Interestingly, in normoxia condition, AGEs can enhance the HIF-1α protein expression in a dose and time dependent manners in RPE cells. The phosphorylation of Akt and p70S6K are involved AGEs-induced HIF1α expression and the pretreatment with the PI3K inhibitor (LY294002) and the p70S6K inhibitor (Rapamycin) can significantly reverse the phenomenon. AGEs also induced RAGE expression in a dose dependent manner in RPE cells. However, AGEs do not alter the HIF-1α mRNA expression and the PHD1, PHD2, PHD3 and VHL protein expressions. We also examined different kinds of AGEs including *N*-(carboxymethyl)lysine (CML) as well as methylglyoxal (MGO) and found that both AGEs can markedly induced HIF-1α protein expression in RPE cells.

Conclusion:

Our study revealed the AGEs can enhance the HIF-1α protein expression through the RAGE and the PI3K/Akt/p70S6K pathways and it may provide an important pathogenic molecular mechanism for AGEs in diabetic retinopathy.

P295

Effects of Natural Herbal Extracts and Their Components on Cardiac Disorders of Metabolic Syndrome Animal Models

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Backgrounds:

To investigate whether anytrocyanin and *Andrographis paniculata* extracts could inhibit cardiac cell apoptosis in diabetes and obesity respectively.

Materials and Methods:

Diabetes was induced in five-week-old male wistar rats using streptozotocin, then progressed for 1 weeks, and the treatment of extract product from purple rice, anthocyanins were gavage for 4 weeks constantly. Obesity was induced of four-week-old male C57/BL6 mice, high-fat diet by 45 kcal% were fed for ten months, and then *Andrographis paniculata* extracts were gavage for 1weeks. Moreover, cardiac diastolic and systolic function was assessed using ecocardiogaphy, and heart weight, cardiomyocyte morphology, protein level were also assessed individually.

Results:

As a result, anthocyanins and *Andrographis paniculata* extracts both significantly inhibited Fas-dependent and mitochondria-dependent apoptotic protein activation, prevented cardiomyocyte disarray and even restored cardiac function of diabetes and obesity animal hearts. Moreover, the progression of heart failure is through pathological hypertrophy to cause cardiomyocytes apoptosis, then lead to cardiac fibrosis, finally cause cardiac contractile dysfunction in diabetes and obesity animal hearts. However, anthocyanins and *Andrographis paniculata* extracts both reversed the heart damage effects, all the results were identified by western blot assay and histopathological analysis in the hearts of diabetes and obesity animal.

Conclusion:

Metabolic Syndrome may cause cardiomyocytes apoptosis and lead to fibrosis and cardiac dysfunction, but gavage natural herbal extracts anthocyanins and *Andrographis paniculata* prevented all those unhealthy effect of diabetes and obesity hearts.

P296

Behavioral Effects on Cue-dependent Fear Conditioning-Extinction process and Pre-synaptically Monoaminergic Changes within Fear Circuit in an Animal Model of Posttraumatic Stress Disorder

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Purpose:

Posttraumatic stress disorder (PTSD) is characterized by dysfunction of fear conditioning-extinction mechanism and abnormality of monoaminergic systems within the areas of fear circuit. Correlated exploration of behavioral and monoaminergic changes after extreme stress may provide useful evidence toward more understanding of the neurobiology of PTSD. The present study aimed to investigate the changes of fear conditioning-extinction mechanism and clarify the relationship of pre-synaptic monoamine neurotransmitters concentration, release capacity, and reuptake mechanism after single-prolonged stress (SPS).

Materials and Methods:

Rats were randomly divided into SPS and control groups. Locomotor activity prior to SPS was used as an index of rat's baseline performance. The degree of freezing was used to assess the cue-dependent fear conditioning-extinction after SPS. Further, HPLC with electrochemical detection techniques was used to examine both tissue and extracellular levels of dopamine (DA), norepinephrine (NE), serotonin (5-HT), and their metabolites in infralimbic cortex (IL), ventral hippocampus (vHPC) and basolateral of amygdala (BLA). Finally, the expression of DA transporter (DAT), norepinephrine transporter (NET) and serotonin transporter (SERT) level

Results:

SPS in the present study increased the locomotor activity and stimulus sensitivity and impaired the fear extinction function in cue-dependent fear conditioning. Pre-synaptic DA releasing was found specifically reduced in IL and vHPC. Furthermore, the expression of several monoamine transporters was changed in fear circuit after SPS.

Conclusion:

Pre-synaptically monoaminergic disruptions were involved in the mechanism of Cue-dependent Fear Conditioning-Extinction process after SPS.

P297

Young Huntington Mice Produce Less Pain Behavior in Inflammatory Pain Model and Were Associated with Less Inflammatory Response in Spinal Cord

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Backgrounds:

Huntington's disease (HD) is a genetically neurodegenerative disease that affect the nervous system and can lead to mental and motor dysfunctions. Previous studies had well known that Huntington's disease was caused by the exon 1 region of the Huntington (HTT) gene with expanded CAG trinucleotide repeats and results an abnormally long polyglutamine tract at N-terminus. However, little research focuses on the relationship of HD and pain.

Materials and Methods:

We used a previous published transgenic HD mice (7-10 week of age, coming from Dr. Yang SH, NCKU) carrying GFP fused with mutant HTT exon 1 containing 84 CAG trinucleotide repeats to evaluate the relationship of HD and pain. Inflammatory pain models were induced by either formalin or Complete Freund's adjuvant (CFA) injection. Spinal cord and dorsal root ganglion (DRG) sections were harvested at the end of the inflammatory pain. Immunofluorescence assay and blotting were used to identify the change of cells and cytokines within tissues.

Results:

Our data showed that HD mice produced less pain behavior (both mechanical allodynia and heat sensitivity) in both inflammatory pain models. In hot water tail immersion test (for Morphine analgesia), morphine also produced better analgesia in HD mice. Immunohistological examination of lumbar spinal cord tissue and DRG also demonstrated HD mice produced less activation of glial cells and astrocyte in both inflammatory pain models. The level of inflammatory cytokine TNF-α was also less in HD mice spinal cord.

Conclusion:

Young HD over-expression mice produced less pain behavior in inflammatory pain models and better analgesia after morphine administration. Tissue in spinal cord also demonstrated less inflammatory reaction both in cells response and cytokine production. HD protein may be involved in the mechanism of inflammatory pain and morphine analgesia. Further studies were needed to investigate the possible role of HD protein within specific tissues and cells in various pain models.

P298

Dendritic Morphology of Hippocampal Neurons in *Cyp11a1* null mice

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Neurosteroids can be synthesized from cholesterol through the P450scc in the nervous system. Neurosteroids affect gene expression through the action at classic intracellular nuclear receptors or regulate neurotransmission through action at membrane ion-gated or other neurotransmitter receptors. Neurosteroids have a variety of neurological functions, such as neuroprotection, myelination, neurite growth and neurogenesis. They also play roles in many behavioral functions and diseases, including stress, anxiety, seizure disorders, and memory.

In this study, we used *Cyp11a1* null mice as an animal model to study the effect of steroids on neural development of hippocampus. *Cyp11a1* knockout mice lack the ability to synthesize steroids and would die at postnatal 6 days, as a result, we used the WT and KO mice at P5. In addition, we also maintained the lives of KO mice through hormone rescue. We started to inject hormone for both WT and KO mice at P4, and sacrificed both of them at P15.

To reveal the morphological features of dendrites, we use Golgi-Cox method to stain the CA1 and CA3 neurons in hippocampus. Pyramidal neurons in CA1 and CA3 were selected to morphological examination. The dendritic morphology of selected neurons was reconstructed and analyzed with Neurolucida software (Microbrightfield Bioscience). Branched structure analyses in the Neurolucida Explorer software toolbox were used to quantify the topological parameters and size-related parameters

Our results indicated that the deficiency of steroids resulted in reduced total dendritic length, dendritic branchings and orders, as well as dendritic arborization in the CA1 and CA3 pyramidal neurons.

P299

Transient Receptor Potential Vanilloid Subtype 1 Inhibition Reduces Brain Injury after Experimental Intracerebral Hemorrhage

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Backgrounds:

Intracerebral hemorrhage (ICH) induces a complex sequence of apoptotic cascades that lead to delayed brain damage. However, the molecules that contribute to neuronal apoptosis remain largely unknown. Transient receptor potential vanilloid subtype 1 (TRPV1), a nonselective cation channel activated by the vanilloids, has been reported to trigger apoptotic cell death in cultured neurons. The aim of this study was to investigate whether TRPV1 plays a deleterious role via regulating apoptosis in mouse ICH.

Materials and Methods:

Primary neuronal or mixed glial cultures from C57BL/6 mice were stimulated with hemin and exposed to capsaicin (CAP). In the mouse ICH model of bacterial collagenase (type VII-S, 0.075 U) injection into the striatum, C57BL/6 mice were intracerebrally injected with capsaicin (1 or 3μM) or vehicle 30 mins before ICH to investigate the effect of TRPV1 desensitization. TRPV1-knock out (KO) mice were also used to examine the effect of gene deletion.

Results:

TRPV1 was found in the peri-hematomal area predominantly in neurons, and occasionally in astrocytes or microglia. In hemin-stimulated cultured neurons, a brief exposure (5-min and 10-min) of CAP led to a 7.5% and 12.1% reduction of cell viability by the MTT test. In contrast, after a longer exposure (1-h and 24-h), the cell viability was increased by 15.7% and 25.9% compared with the vehicle control. These changes were associated with an increase of $[Ca^{2+}]i$ following a 10-min exposure of CAP and a reversed reduction of $[Ca^{2+}]i$ following a 24-h exposure using fluorescent Ca^{2+} images. However, CAP did not influence the increase in hemin-induced nitric oxide production. After ICH, deficiency or desensitization of TRPV1 following 24-h exposure of CAP resulted in reduced neuronal degeneration at 1 day and TRPV1 KO mice exhibited better motor performance at up to 28 days. Furthermore, TRPV1 desensitization attenuated brain injury volumes, BBB permeability and neutrophil infiltration at 1 day. These changes were accompanied by a decrease in cleaved caspase-3 at 1 day.

Conclusion:

These data suggest that TRPV1 plays a deleterious role in ICH-induced brain injury. Inhibition of TRPV1 may translate into a novel therapy for hemorrhagic stroke.

P300

Valpric Acid Induces Hepatic Steatosis by Regulating Lipid Transport and Fatty Acid Oxidation

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Backgrounds:

Valproic acid (VPA) is traditionally used to treat epilepsy, bipolar disorder and several mental diseases. Although VPA is successfully applied to clinics for many years, it has been reported that VPA treatment also leads to hepatic steatosis and hepatotoxicity. In this study, we aimed to explore the possible mechanisms of VPA-induced hepatic steatosis.

Materials and Methods:

To evaluate the effects of VPA on lipid accumulation in hepatocyte, FL83B cells were exposed to different concentrations of oleic acid (OA) or/and VPA for 24 hours. Cells were then stained by Nile Red and Hoechst 33342 in 96-well microplates. The fluorescent signal was detected using Biotek Synergy H1 microplate reader and fluorescence microscopy. Cell viability under treatments of different OA/VPA concentrations was evaluated by MTT assay. Combined with results of cell viabilities, VPA (1mM) and OA (100 μM) were determined for following experiments. To figure out the possible mechanisms of VPA induced hepatic steatosis, total RNA from BSA, OA and VPA single treated, and OA/VPA co-treated cells was isolated. After the RNA extraction, the total RNA was then reverse-transcribed into cDNA and analyzed by real-time PCR.

Results:

After 24 hours treatment, the fluorescence intensity and lipid droplets was increased in a dose-dependent manner in both OA and VPA treated cells. Besides, we found that in co-treatment with 100 μM OA and 1 mM VPA, lipid accumulation was significantly increased compare to OA and VPA single treatments. Furthermore, we found co-treatment of VPA increase *Adrp, Gpam* and *Vldlr*, but decrease *Cpt1a, Acca,* and *Fasn* mRNA levels.

Conclusion:

Our data showed that VPA co-treatment can significantly enhance OA induced lipid accumulation in FL83B cells. As for gene expression, *Cpt1a*, encoding a protein that is a rate-limiting enzyme of mitochondrial fatty acid oxidation, reduced by VPA co-treatment. Moreover, gene expression of VLDLR, a receptor to uptake VLDL from outside of cells, was up-regulated by VPA co-treatment. VPA co-treatment also significantly increased *Adrp*, which encodes a lipid droplet protein and is capable of inducing lipid accumulation in cells, as well as enhanced *Gpam* expression, a rate-limiting enzyme in triglycerides synthesis. However, genes related to fatty acid synthesis, such as *Acca* and *Fasn* were down-regulated compare to OA-treatment. Thus, from the results of mRNA expression analysis, we found VPA may increase lipid accumulation in hepatocyte through regulating lipid transport and β-oxidation.

P301
Down-expression Of CK2/DARPP-32/GAD67 Signaling Pathway In The Striatum Of MPP+-treated Rats

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Protein kinase CK2 is a heterotetrameric and serine/threonine protein kinase. Its protein levels and activity were found to be elevated in the striatum when compared to other brain areas. CK2 is known to involve in the neuroprotective effects of dopaminergic neurons, whether it also regulates the neuronal function relative to motor behaviors is still unclear. DARPP-32 protein is known as one of the substrates for CK2 and is highly expressed in the GABAergic medium spiny neurons (MSN) responsible for dopamine stimulation in the striatum. Furthermore, other studies have indicated that the expression of Glutamic acid decarboxylase 67 (GAD67) mRNA and protein was different in the striatum of MPTP vs naïve animals, which is one of the enzymes responsible for the synthesis of neurotransmitter GABA. In the present study, we observed that the parallel decrease in protein levels of CK2, DARPP-32 and GAD67 as well as the GAD67 mRNA in the striatum of MPP+-treated rats. Our preliminary results suggest that CK2/DARPP-32/GAD67 signaling pathway might involve in the cellular mechanism of motor-deficit in Parkinson's disease.

P302
Effects of Lipopolysaccharide on the Brain NMDA Receptor and GABAA Receptor Expression in the Valproic Acid-induced Autism-like Animal Model

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Purpose:
Neuroinflammation has been implicated in the neurocognitive abnormality. This study was to investigate whether bacterial endotoxin lipopolysaccharide (LPS) would change the levels of excitatory versus inhibitory neurotransmitter receptors in the autistic brain.

Materials and Methods:
Pregnant female Sprague Dawley rats at the 11.5th~12.5th day gestation received a single intraperitoneal injection of VPA (500 mg/kg). The 4-week old offspring rats were subjected to social interaction, and then killed with the coronal brain slices prepared and incubated with aCSF or LPS for 30 min or 1hr. The medial prefrontal cortex (mPFC) and hippocampus were collected from the brain slices and analyzed by Western blotting for NMDAR subunits and GABAAR subunits, and their respective postsynaptic scaffolding proteins PSD95 and gephyrin.

Results:
VPA-exposed offspring rats, with characteristic 'kink' in the tail, exhibited significantly shorter duration and lower frequency in social interaction. The VPA group showed lower GABAAR β3 subunit expression in the mPFC as compared with the saline-treated offspring. Short-term LPS treatment further decreased GABAARβ3 in both groups. In contrast, LPS treatment increased the NMDAR essential subunit NR1 and gephyrin in both groups. However, PSD95 was increased in the saline control but declined in the VPA group after the LPS treatment.

Conclusion:
Our results suggest that LPS has rapid effects on the protein levels of excitatory/inhibitory amino acid receptors and their synaptic anchoring proteins. The distinct effect of LPS on the PSD95 expression in the VPA offspring mPFC provides a clue for the postnatal susceptibility to inflammation-induced E/I imbalance in autistic children.

P303
Loss of CDKL5 alters striatal dopamine levels and impairs motor and cognitive functions in mice.

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Cyclin-dependent kinase-like 5 (CDKL5) is a X-linked gene encoding a putative serine-threonine kinase. Mutations of CDKL5 have been implicated in many neurodevelopmental disorders including atypical Rett Syndrome (aRTT) and autism spectrum disorders (ASDs). To understand the neural basis of disorders caused by CDKL5 mutations, we studied the brains and behaviors of mice lacking CDKL5.We found that the 4-5-week-old male Cdkl5-/y mice traveled faster through much longer distance in an open-field test compared with their wild-type littermates. In accelerating rotarod test, the Cdkl5-/y mice showed impaired motor coordination and motor learning. When we monitored their home-cage behavior, both the male and female Cdkl5 mutants kept digging the bedding matrix showing dramatically increased motor stereotypy. To further investigate whether CDKL5 involves pathogenesis of autistic features, we next studied ultrasound vocalization of the maternally isolated pups from postnatal day 4 (P4) to P10. The Cdkl5-/y pups called differently from the wild-type pups in both calling frequency and durations. In a three-chamber social test, we found that the Cdkl5-/y mice showed higher interest in sniffing non-social objects and spent less time in the chamber containing novel strangers. According to the motor and social deficits we found, we measured the dopamine levels in the striatum of Cdkl5-/y mice. We found significant increase of dopamine in the rostral striatum and reduction of dopamine in the caudal striatum.The molecules related to dopamine transmission are ongoing tested biochemically. Together, our findings suggest that CDKL5 is essential for dopamine-mediate motor control and underlies autistic-like behaviors.

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P304
Endothelial Cells and Smooth Muscle Cells Interactions in Response to Fluid Shear Stress: Role of High Glucose Plus Free Fatty Acid.

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Backgrounds:
Vascular fluid shear stress had always existed and played an important role in regulating vascular endothelial cells (ECs) and smooth muscle cells (SMCs) functions. High glucose (HG) and excessive free fatty acid (FFA) are well-known risk factors of ECs dysfunction and atherosclerosis. However, the effects of ECs response to shear stress and effects of SMC under HG plus FFA is still unclear.

Materials and Methods:
Co-culture was established by plating cells on the opposite sides of a 10-μm-thick membrane containing 0.4-μm pores. Human aortic endothelial cells (HAECs) were seeded onto the outer side of the membrane. After allowing 2 hours for adherence, the membrane was placed with the HAECs side down into a 6-well plate containing the culture medium, and the opposite (inner) side of the membrane was seeded with human aortic smooth muscle cells (HASMCs) to form EC/SMC co-culture. ECs were exposed to low shear stress (0.5 dyne/cm^2, LSS) or high shear stress (12 dyne/cm2, HSS) or a static condition for 24 hours. In addition, high glucose (25 mM, HG) and palmitic acid (300 mM, PA) were treated individually or in combination.

Results:
The EC side of EC/SMC co-culture, vaso-protective proteins such as heme oxygenase-1 (HO-1), prostacyclin synthase (PGIS), peroxisome proliferator-activated receptor alpha (PPARα), and peroxisome proliferator-activated receptor delta (PPARδ) were reduced under static HG plus PA treatment which were compared with the static control. ECs exposure to HSS could counter-induce these proteins, but LSS could not. LSS exposure would down-regulate HO-1, PPARα, PPARδ, and proliferating cell nuclear antigen (PCNA). The SMCs in EC/SMC co-culture, this study found that sheared ECs under HG plus PA condition induced HO-1 and PPARδ expression compared with the static control. In addition, in both co-cultures of ECs and SMCs, HSS induced phosphorylation of AMP-activated protein kinase (AMPK) and was abolished under additional HG plus PA treatment. Conversely, HSS induced HO-1 and retained its expression under additional HG plus PA treatment.

Conclusion:
In summary, HSS played a protective role under HG plus PA treatment such as inducing HO-1, PPARα, and PPARδ, however LSS could not. This may provide another aspect for investigating how shear stress regulates vascular function in early stages of diabetes induced atherosclerosis with hyperlipidemia.

P305
The Regulations of Kiss1 on Steroidogenesis and Cell Proliferation in Corpus Luteum

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Backgrounds:

Kiss1 and *Kiss1* receptor (*Kiss1r*) are essential gatekeepers of reproduction system. Previous studies showed that *Kiss1* control reproductive functions mainly through regulating GnRH secretion from hypothalamus. However, its expression in corpus luteum with uncertain functions was also discovered recently. To investigate the functions of *Kiss1* in corpus luteum, we performed analysis on caprine corpus luteum and tsCLC-D cells.

Materials and Methods:

In the first part of our study, we confirmed the presence of *Kiss1* and *Kiss1r* in corpus luteum by RT-PCR, Western blotting, and immunohistochemistry staining in caprine corpus luteum obtained from various estrual stages, and immunofluorescent staining on tsCLC-D cells. Next, we evaluated the cytotoxicity and stimulatory effect of Kp-10 (active form of Kiss1 peptide) on P_4 secretion in tsCLC-D cells by XTT assay and ELISA respectively. In addition, effects of Kp-10 on expressions of mRNA encoding steroidogenic genes (*Star, Cyp11a1 and Hsd3b*) in tsCLC-D cells were assayed by real-time PCR. In last part, we used Fluo-3, a fluorescent calcium probe, to monitor the cytoplasmic calcium level changes in tsCLC-D cells before and after Kp-10 treatment.

Results:

According to our results, *Kiss1* and *Kiss1r* are expressed in not only all-stage caprine corpus luteum, but also the tsCLC-D cells. In addition, we found the P_4 secretion and steroidogenic genes can be significantly decreased in tsCLC-D cells by Kp-10 (10 μM) with increased cell viability. Finally, we showed that Kp-10 (10 μM) can evoke intracellular calcium levels in tsCLC-D cells by real-time calcium monitoring.

Conclusion:

Locally produced *Kiss1* in corpus luteum might contribute to regulation of P_4 secretion and cell proliferation. *Kiss1* lowers P_4 secretion by inhibition of *Star, Cyp11a1* and *Hsd3b* mRNA expression. However, additional studies are needed to investigate whether the intracellular calcium increase is associated with cell proliferation or other cell physiological functions.

P306
The Involvement of Neuronal CCR5-mediated Signaling in the Regulation of Energy Homeostasis

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Backgrounds:

Hypothalamus is the integration center for body energy homeostasis. CC Chemokine 5 (CCL5/RANTES) signal in central nerve system has been reported to increase rat body temperature and decrease food intake with a direct hypothalamic injection. CC chemokine receptor 5 (CCR5), belongs to one of CCL5 receptors, up-regulated in human obesity adipose tissue has strong correlation in peripheral glucose utility. The role and contribution of CCL5/CCR5 signaling in hypothalamic remain unclear. The aim of this study is to investigate hypothalamic CCR5 signaling in energy homeostasis and adaptive thermogenesis.

Materials and Methods:

C57BL/6 mice were applied as control mice and global CCR5 knockout mice was conducted as linkage in the present study. Experimental mice fed with normal chow diet for 13 weeks were separately housed at 22°C or 4°C (24hr) for 1 week. Metabolic activity along with blood glucose level, daily food consumption, and body weight were measured in this study. Hypothalamus tissues were collected and RNA was extracted to preform quantitative reverse transcriptase PCR (qRT-PCR). Insulin signaling and cellular energy regulatory genes were analyzed to elucidate the possible effect of CCR5 on energy homeostasis.

Results:

After one week continuing cold exposure, body weight loss of $CCR5^{-/-}$ mice was significantly higher than that in C57BL/6 mice. Food consumption of $CCR5^{-/-}$ mice was lower than that in C57BL/6 mice. The blood glucose of C57BL/6 mice was elevated 32% in cold environment; which conversely was decreased 16% in $CCR5^{-/-}$ mice. Insulin receptor and its substrate protein - IRS1(Insulin receptor substrate 1) and mitochondria energy generating enzyme - UCP2 (Uncoupling protein 2) have decreased about 28% and 13% in C57BL/6 but increased 50% and 62% in $CCR5^{-/-}$ mice with qRT-PCR analysis.

Conclusion:

Our finding suggested that hypothalamic CCR5 might play a negative regulatory role in energy expenditure.

P307
Peroxisome Proliferator-activated Receptor delta Regulates Phenotypic Modulation in Vascular Smooth Muscle Cells Through the Activation of Heme Oxygenase-1

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Backgrounds:

Phenotypic modulation of vascular smooth muscle cells (VSMCs) is critical in regulating vascular function in health and disease. During development of atherosclerosis, VSMCs change from the physiological contractile phenotype to the pathophysiological synthetic phenotype. There is increasing evidence that peroxisome proliferator-activated receptors delta (PPARδ) plays significant roles in modulating vascular function. However, the molecular mechanisms of the action for the phenotypic modulatory effects of PPARδ remain unclear.

Materials and Methods:

Human umbilical artery smooth muscle cells (HUASMC) were obtained commercially and maintained in F12K medium supplemented with 10% FBS. Cells between passages 5 to 7 were used.

Results:

We found that when VSMCs were treated with various concentrations of the synthetic PPARδ ligand GW501516 for 24 h, there was a significantly induced expression of heme oxygenase 1 (HO-1) and contractile marker proteins, including calponin, h-caldesmon, and smooth muscle-myosin heavy chain (SM-MHC). As positive control, treating VSMCs with cobalt protoporphyrin (COPP), a HO-1 inducer also induced contractile marker protein expression. The knockdown of the endogenous expression of HO-1 by specific small interfering (si)RNA inhibits PPARδ ligand-induced contractile marker protein expression. On the basis of our preliminary results, we postulate that the synthetic-to-contractile phenotypic switching of VSMCs by the PPARδ ligand may be mediated through a HO-1-dependent mechanism. NF-E2-related factor 2 (Nrf2) is considered to play a major role in HO-1 expression. To investigate the mechanism underlying of HO-1 induction by PPARδ, we examined the localization of Nrf2. PPARδ ligand GW501516 increased the expression of Nrf2 translocation from the cytoplasm to the nucleus in VSMCs by using immunocytocheminal staining and Western blot. We also found that PPARδ ligand-induced serum response factor (SRF), a transcription that regulates SMCs phenotype. We will further investigate the interaction of the transcription factors SRF and Nrf2 contribute to the PPARδ-induced phenotypic modulation.

Conclusion:

We have demonstrated a significant role between PPARδ and HO-1. The HO-1 induction plays a cytoprotective actions of PPARδ to against synthetic phenotype formation. The results may provide new insights into the roles of PPARδ in VSMCs phenotypic modulation, and also may facilitate the identification of new therapeutic strategies for treating of progression disorders such as atherosclerosis and restenosis.

P308
Shikonin Suppress Proliferation of Ovarian Cancer by Inhibiting PKM2 Activity.

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Backgrounds:

Many studies demonstrate that pyruvate kinase (PK)-M2 (PKM2) plays an important role in regulating glycolytic flux to aerobic glycolysis, known as the Warburg effect in cancer cells. Shikonin, a natural naphthoquinone isolated from the traditional Chinese herb, has been reported to be an inhibitor of tumor-specific PKM2 and suppresses proliferation of breast cancer. In this study, we test that whether shikonin inhibits the cell proliferation or induces cell death in several ovarian cancer cell lines.

Materials and Methods:

We calculated the proliferation rate of ovarian cancer cell by MTS assay. And we used PI staining to observe the apoptosis and cell cycle of ovarian cancer cell. ATP production was analyed by ATP assay. PK activity was measured with PK activity assay and the detection of lactate production.

Result:

We found that shikonin induces cell cycle arrest and apoptosis in ovarian cancer cells. Shikonin treatment decreased the ATP production of ovarian cancer cells. PK activity and lactate production of ovarian cancer cells were reduced by shikonin treatment.

Conclusion:

Our results confirmed that shikonin inhibits PKM2 activities by detection of PK activities and lactate production assay in ovarian cancer cells. And shikonin inhibited effectively the proliferation of ovarian cancer cell *in vitro*. Animal model will be used to confirm the effects of shikonin *in vivo*.

P309
Comparison of Breathing Patterns Following High and Mid Cervical Spinal Cord Injury

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Backgrounds:
More than half of spinal cord injuries occur at the cervical level, and respiratory compromise is the primary cause of mortality and morbidity following cervical spinal cord injury. The purpose of present study was to compare breathing patters following high and mid cervical spinal cord injury during normal breathing and respiratory challenge.

Materials and Methods:
A left unilateral hemisection (Hx) at C2 or C4 spinal cord was performed in adult male Sprague-Dawley rats. Respiratory behavior of unanesthetized animals were measured during baseline (21 % O_2, balance N_2) and hypercapnia (21 % O_2, 7 % CO_2, balance N_2) by a whole body barometric plethysmography at 1, 2, 4 and 8 weeks after spinal cord injury.

Results:
Both C2Hx and C4Hx animals had a significant lower tidal volume and higher respiratory frequency compared with control animals at 1 and 2 weeks post-injury. Tidal volume remained lower in both injured group at 4 and 8 weeks post-injury, however, only C2Hx animals maintained a rapid breathing pattern. Although C4Hx animals usually had higher tidal volume than C2Hx animals during the baseline, this differential tidal volume between C2Hx and C4Hx animals was no longer observed during hypercapnic challenge.

Conclusion:
These results suggested that both high and mid cervical spinal cord hemisection induces a rapid shallow breathing pattern during the subchronic phase of injury (1-2 weeks). In addition, animals received C4Hx may have lower capability to increase tidal volume during hypercapnia due to loss of phrenic motoneuron following mid cervical spinal cord injury.

P310
The Role of Rantes and CCR5 in the Regulation of Adaptive Thermogenesis in Brown and White Adipose Tissues

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Backgrounds:
White adipose tissue (WAT) and brown adipose tissue (BAT) have been documented to play the important role in energy balance in human. The aim of this study was undertaken to investigate the physiology role of CCR5 (C-C chemokine receptor 5) and its ligand Rantes (also known as CCL5) in the regulation of adaptive thermogenesis in brown and white adipose tissue.

Materials and Methods:
Male CCR5 and CCL5 knockout and wild type (WT) mice were fed regular chow diet for 13 weeks. Body weight and food intake were measured every week. The physical activity of mice was measured at 11-week-old and then were further divided into two subgroups: control group stayed at 22 ℃ environment and experimental group were exposed to 4℃ for 1 week after 7-day 4℃ acclimation period. Inguinal WAT (iWAT), epididymal WAT (eWAT) and interscapular BAT obtained after euthanization at age of 13-week-old were used for weight measurement and HE staining. In addition, the thermogenic genes and UCP1 protein expression were analyzed by quantitative real-time PCR and Western blot.

Results:
Body weight and food intake were similar among experimental groups but the physical activity was significant decreased in CCL5 KO mice compared to those in WT mice. The blood glucose and weight of iWAT markably increased in CCR5 KO mice compared to the other two groups. After exposed to 4 ℃ , every experimental group had similar body weight loss but increase in food intake was significantly higher in CCR5 KO mice than those in other mice. Chronic cold stress induced similar increase in iBAT weight and decrease in eWAT among experimental groups except there was significantly decreased weight of iWAT in CCR5 KO group as compared to those in the other groups. UCP1 protein expression was significantly increased in iBAT, iWAT and eWAT of CCL5 KO mice, but it was only enhanced in iBAT of CCR5 KO mice. The brown–like adipocytes (brites) were significantly increased in iWAT and eWAT of CCL5 KO mice but only increased in iWAT of WT mice.

Conclusion:
It is suggested that Rantes/CCR5 signaling might play a negative regulatory role in adaptive thermogenesis of BAT. Additionally, Rantes might be via other receptors than CCR5 to suppress the brites formation in WAT under cold exposure.

P311
Effects of Up-regulation of Glutamate Transporter on Cognition Deficits in Parkinson's Disease Rat Model

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Backgrounds:
Around 30-50% of Parkinson's disease (PD) patients show impairments of recognition and working memory, known as Parkinson's disease dementia (PDD). Hyperactivation of the glutamatergic system is implicated in the pathophysiology of PD. Ceftriaxone, a β-lactam antibiotic, has long been used to cure bacterial infections. Recent studies have indicated that ceftriaxone can provide neuroprotection by up-regulation of glutamate transporter (GLT-1). Therefore, the aim of this study was to evaluate the effects of ceftriaxone on working memory and recognition in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD rat model.

Materials and Methods:
Male Wistar rats were stereotaxically infused with MPTP into the substantia nigra pars compacta (SNc) to induce PD rat model. Starting from the 3rd day after MPTP lesioning, the rats were treated daily with ceftriaxone (200 or 100 mg/kg/day, i.p.) for 12 days. The rats underwent a battery behavior test for detecting motor function, measuring working memory and object recognitive function. One day after the behavioral test (day 15), the rats were sacrificed and brains were taken for detecting the level of GLT1 by using Western blotting.

Results:
A week after MPTP lesioning, motor dysfunction was recovered to control levels. The impairments of working memory and object recognition were prevented by the treatment of ceftriaxone. Finally, the expression of GLT1 in the hippocampus of the groups treating with ceftriaxone was higher than that in the controls.

Conclusion:
These results suggest that up-regulation of glutamate transporter may have therapeutic potential for the treatment of cognitive dysfunction in PDD.

P312
The Role of Matrix Metalloproteinase 9 (MMP 9) in Development of Alcoholic Fatty Liver

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Backgrounds:
The aim of this study is to investigate whether MMP9 regulates hepatic fatty acid metabolism in response to chronic ethanol consumption.

Materials & Methods:
To determine the role of MMP9 in development of alcoholic fatty liver, total 51 male six-month old $mmp9^{-/-}$ and wild type C57BL/6J mice were used. The genotype, mRNA, protein expression and activity of *Mmp9* were verified by PCR, real-time PCR, Western blotting and Zymography respectively. After random grouping, mice were fed with Lieber-DeCarli liquid control or ethanol diet for 4 weeks. After 4 weeks, mice were sacrificed, and the liver tissues were collected for histological section analysis and triglyceride level measurement using enzymatic method. Also, expression of lipogenesis (SREBP-1c, ACC and FASN) and inflammation (TLR4, TGFβ, TNFα and IL6) related mRNA in livers was assessed by real-time PCR.

Results:
According to our results, partial deletion of *mmp9* exon 2 does not affect the protein synthesis, but the enzymatic functions. In ethanol-fed $Mmp9^{-/-}$ mice, the liver sections are characterized with remarkable steatosis, and the hepatic triglyceride content ($P < 0.05$) is higher compared to ethanol-fed wild type mice. Also, under chronic ethanol consumption, decreased ACC and FASN, and increased SREBP-1c, TNFα and TLR4 mRNA expression ($P < 0.05$) were found in $mmp9^{-/-}$ mice.

Conclusion:
Results in our study suggest that *Mmp9* may play a regulatory role in hepatic fatty acid metabolism and inflammation under ethanol consumption by its enzymatic functions.

P313
A Methodological Study for Longitudinal Manganese-Enhanced Magnetic Resonance Imaging

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Backgrounds:

Mn^{2+} is paramagnetic. It enters voltage-gated calcium channels of active neurons and transports through neural pathway. These characteristics allow manganese-enhanced magnetic resonance imaging (MEMRI) to use Mn^{2+} as a dynamic contrast agent for the study of functional connections in the brain. However, MEMRI so far has been limited to single injection in the brain. The present study was undertaken to explore methods for multiple application of Mn^{2+} in the brain of individual rat.

Materials and methods:

A cannula targeting the ventroposterior thalamic nucleus (VP) was chronically implanted. 7 days after implantation, a microfil needle (34G) was inserted into the cannula and MnCl2 solution was slowly injected with the aid of a Hamilton syringe. The animals underwent MR scan after various transportation time.

Results:

Several parameters were examined. These included reproducibility of Mn^{2+} injection volumes and doses, clearance rate of Mn2+ in the brain tissue and local neural toxicity. For reproducibility, we found in the range of 0.2-0.5 microliter, local Mn^{2+} signals linearly correlated with the volume injected. For dose selection, we examined receptive field evoked potentials for injection site functionality and found 40 mM as a suitable dose for repeated injection. Basically, Mn^{2+} injected was removed within a week. For toxicity, we used immunostaining of NeuN and GFAP to ascertain the viability of local neurons after 2 weeks.

Conclusion:

We found 40mM and 0.5μl of MnCl2 can be applied in chronic cannula implantation without acute neurotoxicity. By multiple injections, Mn^{2+} can be used to longitudinal trace the plasticity of functional neural circuits in the brain of the rat.

P314
Social instability stress differentially affects amygdalar neuron adaptations and memory performance in adolescent and adult rats.

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Backgrounds:

Adolescence is a time of developmental changes and reorganization in the brain, thus adolescents are likely to be more vulnerable than adults to the stress effects. However, scientific evidence in support of this hypothesis is still limited.

Materials and Methods:

We compared the stress effects on the amygdala experienced in adolescent (4-wk-old) rats to similar stress effects experienced in young adult rats (8-wk-old).

Results:

Chronic social instability for 5 wks reduced food consumption and body weight gain in both age groups. However, this stress paradigm exerted opposite effects on fear-potentiated startle, an amygdala-dependent learning and memory task, in two groups, i.e., hampered the performance in adolescent and improved it in adulthood. Using a single neuron labeling technique, we found that the stress applied in adolescent rats reduced dendritic field and spine density in basal and lateral amygdala neurons. Yet opposite stress effects on neuron morphology were observed in adult rats. Moreover, stress in adolescence suppressed the amygdala expression of synaptic proteins, i.e., TrkB, synaptotagmin I and SNAP 25. In adult rats, it enhanced TrkB expression in the amygdala.

Conclusion:

In summary, our results supported that chronic social instability exerted age-dependent effects on the fear-potentiated startle, possible via BDNF-TrkB signaling and neuroplasticity in the amygdala. While amygdalar neuron development in the adolescent brain was hampered by the stress, mature neurons in the amygdala were capable of adapting to the stress.

P315
Protective Effect of 7,8-dihydroxyflavone on Lipopolysaccharide-induced Acute Lung Injury in Rats

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Backgrounds:

To investigate the effects of the 7,8-dihydroxyflavone (7,8 DHF) on LPS-induced lung injury, and further elucidated the mechanism involved

Materials and Methods:

Male Sprague-Dawley rats were treated intraperitoneally with 7,8 DHF (20 and 50 mg/kg) or vehicle 3 hours after intratracheal (IT) instillation of LPS (5mg/kg). Six hours after LPS or saline installation, samples of lung tissues were collected for determination of myeloperoxidase (MPO) activity and superoxide (ROS) level. Histopathology was examined the pathological changes in lungs. The wet/dry (W/D) ratio, protein concentration and total cell count in bronchoalveolar lavage fluid (BALF) were also determined. BALF was carried out for quantification of tumor necrosis-alpha (TNF-alpha), Interleukin (IL)-6, IL-1β, cytokine-induced neutrophil chemoattractant (CINC)-3 and nitrate/nitrite (NOx) level. The expression of TrkB, phospho-TrkB and Caspase-3 were examined by western blotting. The pulmonary apoptosis was verified by TUNEL assay.

Results:

Our preliminary data showed that after treatment for LPS for 6 hours, post-treatment with 7,8 DHF significantly inhibited the levels of TNF-α, IL-6, IL-1β, CINC-3, protein leakage, neutrophil counts in BALF, and lung MPO activity accompanied by improved pathological changes. 7,8 DHF also decreased LPS-induced formation of superoxide and nitrite/nitrate in BALF. Meanwhile, 7,8 DHF diminished caspase-3 protein expression and TUNEL-positive cells, but increased phospho-TrkB protein expression in lung tissue.

Conclusion:

We demonstrate that 7,8 DHF has beneficial effect on LPS-induced ALI by decrease of pro- inflammatory mediators and pulmonary cells apoptosis, but enhancement of TrkB expression.

P316
Effect of Magnolol Inhibits HIF-1α Expression Through PEDF-mediated Pathway in Hypoxia-treated Human Colon Cancer Cells

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Backgrounds:

To investigate the effects of magnolol on PEDF-mediated signaling pathway to suppress hypoxia-treated HIF-1α/VEGF-induced angiogenesis in human colon cancer cells (HCT116).

Materials and Methods:

For hypoxic exposure, HCT116 cells were incubated in serum starved medium for indicated time, followed by placing in a sealed hypoxic incubator flushed with a gas mixture of 94% N2, 5% CO2 and 1% O2. Magnolol purchased from Medical and Pharmaceutical Industry technology and Development Center (Taipei, Taiwan), was solubilized in 100% DMSO at 10 mM, and then diluted as need and fixed the final concentration of DMSO at 0.1% (v/v). The angiogenesis function assay were evaluated by wound healing assay and invasion assay. The PEDF-mediated signaling mechanisms were examined by western blotting and activity assay.

Results:

Our preliminary results showed that treatment with magnolol dose-dependently inhibited hypoxia- induced ROS formation, p-ERK5, HIF-1α, VEGF expression accompanied by an attenuation of hypoxia-induced cell migration and invasion. In addition, magnolol up-regulated PEDF and increased PPAR-β activity and expression under hypoxia condition.

Conclusion:

We demonstrate that magnolol may be a potential anti-tumor drug in HCT116 human colon cancer by elevating PEDF expression and in turn diminishing ROS/ HIF-1α/ VEGF pathway.

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Multimodal Synergism on Spontaneous and Evoked Neuropathic Pain: Interaction among Tramadol, Ketorolac and Pregabalin

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Backgrounds:
Neuropathic pain is a highly prevalent clinical syndrome caused by either central or peripheral nervous system dysfunction. With its complicated pathogenesis involved, conventional pharmacological treatment focusing on selective receptor mechanism is proved to be ineffective while multimodal therapy is currently accepted as the best approach.
Materials and Methods:
In this study, we combined three drugs with distinct pharmacological mechanisms, i.e. opioid, NSAID, and α-2-δ on the rat spared nerve injury (SNI) model, in which two of the three major branches of the sciatic nerve were cut with the sural nerve left intact. We characterized the effectiveness of tramadol (2.5, 5, 10 mg/kg i.p.), ketorolac (2.5, 5, 10 mg/kg i.p.), their combination (tramadol 5 mg/kg + ketorolac 5 mg/kg i.p.) and pregabalin (3, 10, 30 mg/kg i.p.) on spontaneous and evoked pain behaviors, including mechanical and cold allodynia in SNI rats. The frequency of spontaneous hindpaw lifting was used for spontaneous pain index. The withdrawal threshold of the von Frey test was used as the index for mechanical allodynia; and the duration of hindpaw lifting after acetone to the hindpaw was used for the index for cold allodynia.
Results:
We found high dose tramadol and pregabalin had good analgesic effects on all three behaviors, whereas low dose tramadol and ketorolac alone had poor effect. However, low dose tramadol and ketorolac combination demonstrated powerful antinociceptive effects.
Conclusion:
Our data confirm the effectiveness of pregabalin in treating peripheral neuropathic pain. We demonstrate that combination of ineffective NSAID and weak opioid can provide sufficient analgesic effect on neuropathic pain.

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A Comparison of Cold Pressor Responses in Rats with and without Rapid-eye-movement Sleep Deprivation

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Aims:
To test that influence on sympathetic activity would change cardiovascular responses (CVR) of rats underwent stressful cold impact (CI) with and without 4 days of rapid-eye-movement sleep deprivation ($REMSD_4$), and the re-experiencing changes of those rats while re-exposed to a conditioned stimulus (CS) in $REMSD_4$ as well as in recovery.
Methods:
S-D rats were used to measure CI and $REMSD_4$ in different trials. During a CI trial the extremities of rat were immersed in 2 cm iced water (4 ± 2^0C) for 10 min. A tempo (72 dB, 6 Hz x 2 min) that precedes this CI trial was applied as CS. CVR was monitored continuously via a telemetric device before, after, and during the 10 min of CI trial. Changes of CVR-parameters were separately obtained, including systolic blood pressure (SBP), heart rate (HR), variance of SBP (SBPV), Dichrotic notch number ($Dn^{\#}$), and specific spectrum of the HR variability (HRV): very low frequency (VLF), normalized low frequency (nLF) and high frequency (nHF), LF/HF, and total power (TP). Drug interventions included peripheral administration of L-NAME, and guanethadine (GUA). Plasma NO and norepinephrine levels were measured by ELISA-kit and HPLC-ECD methods respectively.
Results:
Our findings indicated that firstly, the baseline CI resulted in significant pressor and tachycardia responses concomitantly HRV changes, mainly, a trend of rising SBPV and lowering VLF, nLF, LF/HF and TP. $Dn^{\#}$ was significantly decreased also. In contrast, the baseline CS did not constitute a significant change. Both drug interventions may lead to expansion of the TP and SBPV while L-NAME inverted and reached VLF ceiling that was contrary to the effects of GUA. Nevertheless, L-NAME raised but GUA lowered SBP, and both drugs may produce bradycardia accompanied by raising $Dn^{\#}$ responses. Secondly, the $REMSD_4$ alone resulted in pressor and tachycardia and a trend of rising VLF, LF/HF, TP, and SBPV. $Dn^{\#}$ was disappeared from $REMSD_4$ as compared with the baseline. $REMSD_4$ plus CI also resulted in CI-CVR changes but were different to the effects of REMSD4 alone. In general, $REMSD_4$ potentiated the CI-CVR, including pressor, tachecardia, VLF lowering, TP lowering, $Dn^{\#}$ vanishing, and SBPV rising. Finally, tempo showed conditioning by lowering nHF during $REMSD_4$, whereas re-exposed after REMSD4, the effects still were existed as showed by rising SBPV and lowering both nHF and nLF.
Conclusion:
These results indicated that both myogenic responses and autonomic regulations will be changed when animal is challenged with CI and/or $REMSD_4$. Tempo as a cue may induce conditioning in $REMSD_4$ as well as in recovery.

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Sympathetic Tone Overrides Prosocial Behavior in Rats

I-Te Hsieh

Prosocial behaviors have been found to be associated with emotional status, which also affects the autonomic nervous system (ANS). In the present study, we aimed to establish an animal model for quantitatively and objectively exploring the relationship between the level of prosocial behavior and ANS function. We hypothesized that high sympathetic and low vagal tone are related to a decreased level of prosocial behavior. Thirty male Wistar-Kyoto rats (observers) were trained to press a left or a right lever for water reward. After water deprivation for 12 or 24 hours, the observer rats were then tested in the presence of another rat (demonstrator), and this time, one of the two levers was set as the pain bar that would deliver a foot-shock to the demonstrator. The percentage of pressing the pain bar was defined as the suffering level, based on which observers in the two water-deprivation conditions were separated into a high-concern group (<50 %) and a low-concern group (>50 %). Heart rate variability was used to assess autonomic functioning. After water deprivation for 12 hours, the low-concern rats had significantly higher LF/HF than the high-concern rats. When water deprivation was extended to 24 hours, LF/HF and HF in the low-concern group were higher and lower than the high-concern group, respectively. In addition, the suffering level was inversely correlated with LF/HF, but not with HF, under both conditions. This animal model provides an objective quantification of prosocial behavior, and clearly demonstrates an inverse relationship between prosocial behavior and sympathetic tone.

Keywords:
prosocial behavior, animal model, autonomic nervous system, heart rate variability

P320

Aging reduces the IGF-I Compensated signaling and accelerate the cardiac apoptoic effects induced by Second-hand smoke exposure

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Background:
Exposure to secondhand smoke (SHS) increased the risk of heart diseases including atherosclerosis and coronary disease. Aging is a physiology process involving progressive impairment of normal heart functions, due to an increasing vulnerability, which reduces the ability of survive. However, it is not clear pathological condition in aging exposure to SHS. The aim of this study was to examin SHS exposure in aging-related death-survival imbalance of rat hearts.
Methods:
The young SD rats (3 months) and aging SD rats (24 months) were subjected into non-smoking and smoking exposure. All animals were divided into four groups: MYC (male-young-non-smoking group), MYS (male-young-smoking group), MOC (male-old-non-smoking group) and MOS (male-old-smoking group). The smoking groups were placed in SHS exposure chamber and exposed to 10 cigarettes for 30 min, twice a day, 5 days per week for 1 month. After 4 weeks secondhand smoke exposure, rats left ventricular (LV) underwent morphological and function study with echocardiography. Histopathologic of left ventricular sections were stained with Hematoxylin-Eosin staining and related death-survival protein expression levels evaluated by Western blot analysis.
Results:
After 4 weeks SHS exposure, LV weight showed significantly increas in MYS and MOC groups and showed greater synergistic effect in MOS group. Similarly results were observed from echocardiography analysis, The EF (%) and FS (%) were apparently decrease in young SHS exposure and aging group, and even synergistic enhanced in MOS group. The IVS, LVID and LVPW displayed the similar findings. Morever, we found the upregulation of Fas-dependent apoptosis pathway, TNFα-Fas-L-Fas-FADD-cleaved caspase 8 and mitochondrial-dependent apoptosis related proteins, cytochrome c, t-Bid, Bid, Bad, cleaved-caspase 9 in MYS and MOC groups and synergistically enhanced in MOS group. In addition, the IGF-I/IGFIR and p-PI3K/p-Akt survival signaling pathways were compensated increase in MYS and MOC groups, but totally suppressed in MOS group.
Conclusions:
Our study strongly suggest that aging and SHS synergistically enhanced apoptosis related pathways. However, aging under SHS exposure totally compromised the compensative survival signalings of rat hearts.

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Mechanisms of cardiomyopathy induced by second-hand smoke exposure in aging rats

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To determine the aging rats exposure to secondhand smoke exposure engenders changes to left ventricular remodeling due to the age- or disease-dependent alterations. Rats were divided into two age groups, young adult and old male which were divided into two subgroups and treated for 4 weeks secondhand smoke exposure as follows: Control(C), not exposed to cigarette SHS. Secondhand smokers(S), exposed to cigarette SHS. The rats were placed in whole-body exposure chambers and exposed to 10 cigarettes. Filtered air was introduced into the chamber at a low rate. Rats were exposed to cigarette smoke for 30 min, twice a day, 5 days/week for 1 month. After 4 weeks secondhand smoke exposure, rats underwent morphological study with trichome stain and left ventricular remodeling related protein analysis by western blot. From echrocardiography results, we found EF(%) and FS(%) were decreased in SHS exposure more than in old age heart on 4 weeks, however, in young age exposure to SHS was increased on 4 weeks compared with 2 weeks. LVID, LVPW and IVS were increased in systolic diameters in SHS exposure, but not in diastolic diameters. In addition, downregulation of survival signaling pathway (IGF-I-IGFIR-p-PI3K-p-Akt) and upregulation of apoptosis signaling pathway (TNFα-Fas-L-Fas-FADD-cleaved caspase 8) in SHS exposure were respresented by Western blot. Mitochondrial protein, t-Bid, Bid, cytochrome c and Bad/Bcl 2 ratio were increased in old age and old SHS exposure. Furthermore, caspase 9 and caspase 3 were increased in both young and old exposure to SHS and age rats. Protein expression levels of calcineurin/NFATc4, MEK1/ERK1/2, MEK5/ERK5, and JAK/STAT signaling pathways were detected by Western Blot. Results showed that in old age left ventricular (LV) mass was increased, left ventricular muscle fiber rangment was disorder. Calcineurin/NFATc4, MEK5/ERK5, MEK1/ERK1/2 and JAK/STAT3 signaling pathways were increased in old age(MOC), but also found in SHS exposure in old age(MOS), but not found in young age exposure to SHS(MYS). We suggested that ageing induced LV hypertrophy was through calcineurin/NFATc4, MEK5/ERK5, MEK1/ERK1/2 and JAK/STAT signaling pathways.

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Amp-activated Protein Kinase Mediates Erythropoietin-induced Activation of Endothelial Nitric Oxide Synthase

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Backgrounds:
We investigated whether AMP-activated protein kinase (AMPK), a multi-functional regulator of energy homeostasis, participates in the regulation of erythropoietin (EPO)-mediated activation of endothelial nitric oxide synthase (eNOS) in endothelial cells (ECs) and mice.
Materials and Methods:
Nitric oxide (NO) production was examined by Griess reagent assay. Protein expression and interaction were detected by western blotting and immunoprecipitation. Angiogenesis was determined by proliferation, migration; in vitro tube formation and in vivo Matrigel plug assays.
Results:
In ECs, treatment with EPO increased the phosphorylation of AMPK, acetyl-CoA carboxylase (ACC), and eNOS, as revealed by Western blot analysis. Inhibition of AMPK activation by compound C or dominant-negative AMPK mutant abrogated the EPO-induced increase in the phosphorylation of AMPK, ACC, and eNOS, as well as NO production. Additionally, suppression of AMPK activation abolished EPO-induced EC proliferation, migration and tube formation. Immunoprecipitation analysis demonstrated that AMPK mediated the EPO-induced increase in the phosphorylation of β common receptor (βCR) and the formation of a βCR–AMPK–eNOS complex. In mice, inhibition of AMPK activation by compound C markedly decreased EPO-elicited angiogenesis in Matrigel plugs. Furthermore, the phosphorylation of AMPK and eNOS was significantly higher in aortas from EPO transgenic mice than wild-type mice. Moreover, treatment with EPO neutralizing antibody greatly reduced the exercise training-induced increase in phosphorylation of AMPK and eNOS in aortas of wild-type mice.
Conclusion:
In conclusion, EPO may trigger AMPK-dependent signaling, which leads to enhanced phosphorylation of βCR and eNOS, increased βCR–AMPK–eNOS complex formation, NO production, and, ultimately, angiogenesis.

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Aloe-emodin Induces Ubiquitin-Dependent ERα Protein Degradation and Suppresses Breast Cancer Cell Proliferation

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Aloe-emodin, which belongs to anthraquinone, is abundant in both of plants Rhubarb (*Rheum palmatum*) and Aloe vera. Several lines of evidence indicate that aloe-emodin and its analogue, emodin, contain estrogenic activity as phytoestrogens. However, their effects on estrogen receptor α (ERα) activation and breast cancer cell proliferation were still controversial. Therefore, the goal of this study is to investigate the effects and their molecular mechanisms of aloe-emodin and emodin on breast cancer cell proliferation. The results showed that both aloe-emodin and emodin were capable of inhibiting breast cancer cell proliferation by down-regulating ERα protein levels. Furthermore, both nuclear distribution and transcriptional activation of ERα were suppressed by treatments of these two drugs. Importantly, ERα protein degradation was achieved through the elevations of heat shock protein 90 dissociation and ubiquitin-conjugated levels by treating with aloe-emodin but not emodin. In conclusion, the data demonstrates that aloe-emodin might specifically suppress breast cancer cell proliferation through targeting ERα protein. This finding may be important to the future treatment of breast cancer.

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從缺

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Mechanism of Dilong and lumbrokinase to protect second-hand smoke induced fibrosis in rat heart

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Background:

Second-hand smoke (SHS) exposure has been reported to cause deterioration of lung function and increase risk of cardiovascular mortality. Acute exposure to environmental tobacco smoke strongly reduces the high density lipoprotein (HDL) level that plays a role in blood vessel protection. The traditional Chinese medicine Dilong is considered as a treatment for Blood stasis, and lumbrokinase from Dilong is been administered as the most effective oral thrombolytic agent.

Materials and Methods:

In this study Sprague Dawley (SD) rats were used as experimental models to examine the effects of SHS on the healthiness of rat heart and further to determine potential of Dilong and lumbrokinase to inhibit SHS related cardiac fibrosis. Six week old rats were divided into four groups- control, SHS exposed (10 cigarettes smoke within 30 min), SHS exposed then treated with Dilong and SHS exposed then treated with lumbrokinase. After 4 weeks of respective treatments, proteins from the left ventricular tissues were collected and the modulations in the levels of cardiac fibrosis marker proteins were determined by western blot assay. The left ventricular heart tissue and the degree of cardiac fibrosis were examined by Hematoxylin and eosin (HE) staining and Masson's trichrome staining

Results:

HE staining revealed that the SHS exposure increases cardiomyocyte disorders in rat heart and causes the formation of abnormal shaped nuclei. But in the experimental groups treated with Dilong and lumbrokinase cardiomyocyte disorders were reduced and nuclei were normal. Masson's trichrome staining of left ventricular heart tissue revealed that SHS exposure increases cardiac fibrosis which was significantly reduced in Dilong and lumbrokinase treatment groups. Western Blot analysis of cardiac fibrosis marker protein showed that SHS exposure increases the expression of fibrosis related MEKK p-ERK1 / 2, uPA, SP1, CTGF, MMP proteins whereas Dilong and lumbrokinase treatment either reduced or suppressed their expression

Conclusion:

In conclusion our results indicate that Dilong and lumbrokinase can significantly protect heart tissue from SHS exposure

P326
Suppression of seizure by optogenetic stimulation in reticular thalamus

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Backgrounds:

Seizure is a common neurological disorder which affects 1 % of population worldwide, and 30 % among them suffered from drug-resistant epilepsy. Several alternative therapeutic approaches were adapted in clinical studies, these include transcranial magnetic stimulation, deep brain stimulation (DBS) and electrical field stimulation. One of the targets for stimulation is thalamus. Previous study indicate that thalamus play an important role in modulating cortical seizure. However, the roles of thalamocortical system during the cortical seizure still need further elucidation. The thalamocortical activities were controlled by reticular thalamus nucleus (nRT). nRT contains GABAergic neurons that innervate to thalamus and provide synaptic inhibition on excitatory thalamocortical relay eurons. Previous studies showed that enhancing GABAergic inhibitory strength in nRT reduces the duration and power of absence seizure. However, previous studies using traditional approach might lead to unwanted side effects. Because nRT also receives inputs from the cortex, and thalamocortical and corticothalamic pathway lies in vicinity, electrical or pharmacological stimulation of nRT will impact these passing fibers.

Materials and Methods:

In present study we use optogenetic method to study the role of nRT and cortical seizure. We use transgenic mice in which only Channelrhodopsin-2 (ChR2) will be expressed in parvalbumin (Pv) positive interneurons. No functional ChR2 expression was found except in nRT and cerebellum of these Pv-ChR2-EYFP transgenic mice. To study the effect of nRT activation to cortical seizure, cortical seizure-like activities were induced by corpus callosum after i.p injecting of GABAA antagonist pentylenetetrazol. One Michigan electrode was inserted into the somatosensory cortex to record the seizure, and one optoelectrode was inserted into the corresponding thalamic regions to stimulate and record nRT activities.

Results:

Light stimulation could cause burst firing in nRT and thalamic activities were suppressed during light stimulation. Rebound burst firing was observed after light was turned off.

Conclusion:

Our results showed that high frequency light stimulation is more effective in suppressing seizure. Locations of optoelectrode are crucial. Histological examination verified that those areas near the nRT and thalamus are more likely to suppress cortical seizure.

P327
Regulation of Cyclooxygenase-2 by Dual-specificity Phosphatase-2 in Cancer Cells

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Cyclooxygenase-2 (COX-2) plays a crucial role in the development of cancer via its enzymatic product, prostaglandin E2 (PGE2). Clinical studies have demonstrated that COX-2 is overexpressed in numerous types of cancers and enhances cancer progression. However, the underlying mechanism responsible for COX-2 upregulation remains largely unknown. We have previously demonstrated that the expression of dual-specificity phosphatase-2 (DUSP2) is markedly reduced in many kinds of cancers. Large scale screening revealed that COX-2 may be regulated by DUSP2. In vitro studies confirmed that knockdown of DUSP2 significantly increased COX-2 expression and PGE2 production while overexpression of DUSP2 reduced those in cancer cells. Treatment of cancer cells with hypoxia suppressed DUSP2 and resulted in an increase of COX-2 expression. Re-expression of DUSP2 under hypoxic condition prevented COX-2 upregulation. Moreover, hypoxia pretreatment sensitized cancer cells to cytokine stimulation in terms of COX-2 overexpression. Because many cytokines are upregulated in cancer region due to inflammation and infiltration of tumor-associated macrophages, this finding provides a molecular clue to explain why COX-2 is overexpressed in many cancer cells. In xenografted mice model, we demonstrated that cancer cells with DUSP2 knockdown expressed greater levels of COX-2 and grew faster and bigger. Furthermore, treatment with COX-2 inhibitor, NS-398, significantly inhibited shDUSP2-induced tumor growth. These results indicate that DUSP2 is an upstream effector for COX-2 overexpression during cancer progression and hypoxia may be the initiating factor for all these phenomena. Our findings suggest that DUSP2 may be an ideal molecular target for the treatment of COX-2 overexpressed cancers.

P328
Activity-Dependent Regulation of Feed-Forward Inhibition in the Dentate Gyrus

Yu-Chao Liu & Cheng-Chang Lien

The dentate gyrus (DG) serves as a gateway to control information transfer from the cortex to the hippocampus. Cortical afferent inputs provide direct excitation with subsequent feed-forward inhibition (FFI) onto granule cells (GCs) of the DG. GABAergic (γ-aminobutyric acid-releasing) inhibitory interneurons, which comprise two distinct classes of interneurons: fast-spiking (FS) and non-fast spiking (non-FS) cells, mediate FFI onto GCs. Detailed morphological analysis revealed that FS cells are soma-targeting interneurons, whereas non-FS cells are dendrite-targeting interneurons. These two types of interneurons are preferentially recruited by the specific activity patterns of their inputs and exert distinct spatial inhibition onto GCs. However, it is unclear how FS and non-FS interneurons transform their activities to inhibitory output. Here, we show that FFI is dominated by reliable somatic FFI during sparse afferent inputs, whereas dendritic FFI is rapidly switched on during high-level bursting activities. This state-dependent GABA release is reversible and sensitive to presynaptic activities. Such dynamic regulation of dendritic FFI may act as an activity-dependent filter to prevent over excitation to the DG and set the balance of excitation and inhibition onto the GCs.

P329
Implementation of Cloud-Computing Healthcare based on Xenon uploading System and Hilbert Huang Transform

Yu-Cheng Lin

With the advance of technology, cloud-computing healthcare is rapidly growing. Current methods for data acquisition and analysis, however, are not fully optimized. In the data acquisition, the wired transmission has been gradually phased out. The current wireless protocols are complicated, power consuming and hard to use. Therefore, they are not broadly applied in healthcare devices after years of promotion. With regard to data analysis, because of the non-linear and non-stationary nature of the physiological signals, traditional methods can not provide satisfactory analysis. To optimize data acquisition, we developed “Xenon” uploading system. It is seamlessly compatible with existing healthcare devices and automatically uploads data to the cloud server. To optimize data analysis, we constructed cloud-computing Hilbert Huang Transform (HHT) platform, which is suitable to analyze the non-linear and non-stationary physiological data. This analysis platform is expected to isolate confounding factors and measurement noises, therefore, the adaptive trend can correctly responded to a pathophysiologic mechanism. By an integration of the Xenon uploading system and cloud-computing HHT platform, the cloud-computing healthcare becomes more realistic. Users would have better opportunity enjoy a high performance yet affordable automatic cloud-computing healthcare service.

Keywords:
Ultra low power, Radio frequency, Data communication, Data analysis, Hilbert Huang Transform

P330
Role of Plzf plays in limb patterning and male germ cell renewal

Yung-Hao Ching

The zinc finger and BTB domain containing 16 *Zbtb16* (also called Plzf, Zfp145 or Green's luxoid) belongs to the POZ/zinc-finger family of transcription factors. It contains a BTB/POZ domain that mediates epigenetic transcriptional repression. ZBTB16 is essential for proper skeleton patterning and male germ cell renewal. Two alleles have been reported that display similar phenotypes: a targeted knock-out, and the spontaneous nonsense mutation luxoid. We describe a new ENU induced mis-sense allele of *Zbtb16* called seven toes (Zbtb16 ^{7t}). Homozygous animals exhibit hindlimb and axial skeleton abnormalities. Whereas the skeletal abnormalities are similar to those of the other alleles, Zbtb16 ^{7t} differs in that it does not cause spermatogonial depletion and male infertility. Positional cloning revealed a point mutation changing the evolutionarily conserved amino acid Glu44 to Gly, possibly altering the BTB domain's activity. Therefore, Zbtb16 ^{7t} is a separation-of-function allele that reveals differential requirements for domains of ZBTB16 in different developmental milieus.

P331
Bi-effectiveness of targeting $P2X_7$ in treatment of rat central post stroke pain

管永惠

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Background and aims:
P2X7 receptor has been highly postulated associated with nociceptive transmission and inflammatory pain. Aim to dissect the role of P2X7 involved in pain perception, we utilized an acute pain and a central post-stroke pain (CPSP) rat models to investigate if targetingP2X7 may alter these pain conditions.
Methods:
The anterior cingulate cortex (ACC) field potentials were recorded simultaneously with multichannel-electrodes upon electrical stimulation of the sciatic nerve (SNS) with or without site direct microdialysis of P2X7 agonist or antagonists into medial-thalamus(MD). CPSP was generated by injecting callagenase into the right ventral posterior-lateral/medial nucleus(VPL/VPM) of the thalamus. Behavior tests were examined to ensure the occurrences of hyperalgesia up to 5 weeks post induction of CPSP. Intravenous P2X7 antagonist BBG(50mg/kg) was applied 3 times during 12~72 hours post CPSP induction. Ipsilateral lesion and contralateral unaffected CPSP rat brain tissues were collected for qRT-PCR analysis of potentially involved neuronal inflammatory factors. Collected cryo brain slices were subjected to multiple immune-detections.
Results:
MD application of selective P2X7 antagonists reveal inhibition of ACC neuron activity in response to SNS stimulation. P2X7 receptor exhibit highly elevated pattern overlapping with microglia maker CD11b in the brains of CPSP rats around the lesion site. Quantative RT-PCR analysis reveal that TNFα, IL-6, IL-1β, and BDNF are strongly increased at the lesion site in comparison with that in the contra-lateral unaffected site. CPSP rats with early BBG treatment shows reduction of their hyperalgesia condition, the hyper-excitability of ACC and MD neurons, and reduction of the aberration on tested cytokines.
Conclusions:
The ATP receptor P2X7 expressed in MD is directly involved in mediating acute pain transmission. Targeting P2X7 reduces the hyper pain sensitivity and inflammatory conditions on CPSP rats indicating that elevated P2X7 receptor in CPSP may serve an important factor involving in the neuro-inflammatory progression and modulating central chronic pain perception.

P332
Disruption of TGF-β/SMAD signaling induces demethylation of E-cadherin promoter and reverses mesenchymal phenotype in ovarian cancer

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Backgrounds:
The TGF-β signaling pathway plays a critical role in controlling cell growth and differentiation. Frequent TGF-β-induced metastasis or epithelial-mesenchymal transition (EMT) can be observed in advanced ovarian cancer. This phenomenon is often accompanied with epigenetic silencing of epithelial marker, E-cadherin such that demethylation treatment can restore E-cadherin expression in ovarian cancer. We therefore hypothesize that long term activation of TGF-β signaling may induce EMT phenotype by epigenetic silencing of E-cadherin and that inhibition of the signaling may restore E-cadherin and reverse EMT in ovarian cancer.
Materials and Methods:
we disrupted the TGF-β/SMAD signaling by over-expression of an inhibitory SMAD, SMAD7 in a mesenchymal ovarian cancer cell, CP70 in which E-cadherin is silenced by complete promoter methylation. we perform methylation capture using MBD binding protein coupled with next generation sequence (NGS) technology (MethylCap-Seq) to investigate the global methylation changes of all SMAD target loci in the knockdown SMAD4 ovarian cancer cells. Used a statistical approach MEDIPS to analyze the MethylCap-Seq data.
Results:
Cells over-expressing SMAD7 showed a decrease in SMAD2 phosphorylation while the control cells maintained a hyperphosphorylation of SMAD2 thus suggesting that TGF-β signaling is disrupted in SMAD7-overexpressing cells. We further examined the expression of E-cadherin from passage 5 up to 30 of the stable transfectants. Surprisingly, stable restoration of E-cadherin can only be observed from passage 20 in the SMAD7-overexpressing cells. Additionally, one of the SMAD7 stable expression clones with highest restoration of E-cadherin showed decreased migration and invasion ability as determined by wound healing and invasion assay. We perform the saturation analysis , the coverage analysis and showing the RPM signals, CpG density and AMS signals.
Conclusion:
Taken together, disruption of TGF-β signaling can induce demethylation of E-cadherin promoter and reverse EMT phenotype in ovarian cancer. MEDIPS analysis demonstrated a increase in global methylation level after SMAD4 knock down in CP70 cells. Interestingly, SMAD4 knock down demonstrated a decrease in promoter CpG methylation in CP70 cells.SMAD4 may play an important role in controlling DNA methylation in ovarian cancer.

P333
Effect of cantharidin and its derivatives on inhibiting murine CT26 colorectal cancer cells

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Backgrounds:

Cantharidin and norcantharidin are potential anti-cancer drugs, but may caused severe side effects. The chemical derivatives modified by norcantharidin, including NC15 and NOC15, were assessed the inhibitory effect on CT26 colon cancer cells.

Materials and Methods:

Murine CT26 cells were treated with norcantharidin and its derivatives. Then, cell viability were determined by trypan blue dye exclusion test, cell morphology were observed by Liu's stain, cell migration were assayed by wound healing method, and β−catenin expression were analysis by western blotting.

Results:

Norcantharidin, NC15, and NOC15 significantly decreased cell viability with IC50 values of 34, 78, and 49 μM, respectively for 24 h's treatment. Cells treated with norcantharidin and NOC15 for 24 and 48 h also reduced cell adhesion, migration ability, and β−catenin expression. However, NC15 didn't have such effects, suggesting that the inhibitory effect of NC15 on CT26 cells may be regulated through different mechanisms.

Conclusion:

The present study demonstrated that norcantharidin and its derivatives NOC15 and NC15 decreased the viability of CT26 colon cancer cells. Norcantharidin and NOC15 showed similar effects on cell adhesion, migration, and β−catenin expression. Future investigations were going on for clarifying their regulatory mechanisms on anti-cancer and anti-metastasis activities.

P334
G6PD Deficiency Affects Aflatoxin B1 Metabolsim in A549/HepG2 Cells Revealed by iTRAQ Based Quantitative Proteomics Analysis

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Backgrounds:

Glucose-6-phosphate dehydrogenase (G6PD) plays a key role in the pentose phosphate pathway by regenerating NADPH and thus maintaining the cellular redox homeostasis. Many cellular activities such as cellular signaling, proliferation, differentiation, immune response and cell death may be altered by imbalance redox status. How G6PD-knockdown cells with impaired redox status may affect protein expression has not been fully explored.

Materials and Methods:

We have used G6PD-knockdown A549 cells as cell models to investigate the effects of G6PD status on global protein expression by applying twice iTRAQ-labeling LC-MS/MS; The iTRAQ ratio validated by qRT-PCR and western blot. Cell viability evaluated by MTT assay and Annexin/PI staining.

Results:

In the iTRAQ analysis, 34 dysregulated proteins (20 proteins down-regulated; 14 proteins up-regulated) involved in metabolic pathway, protein folding, apoptosis and cellular component movement were identified in G6PD-knockdown A549 cells compared to control ones. Following iTRAQ ratio validated by qRT-PCR and western blot, Epoxide hydrolase 1 (EPHX1) was found to be down-regulated in G6PD-knockdown cells. In addition, G6PD-knockdown A549 /HepG2 cells was found to have higher susceptibly toward aflatoxin B1 induced cytotoxicity compared to that in control cells. This finding is consistent with previous report that EPHX1 is involved in aflatoxin B1 detoxification. Furthermore, G6PD-knockdown A549 cells was found to display lower expression level of nucleus Nrf2 upon aflatoxin B1 treatment than that in control cells.

Conclusion:

These findings reveal that G6PD knockdown A549 cells exhibit lower expression level of EPHX1 due to impaired Nrf2 activation pathway and provide support to the notion that cellular redox status in cells is an important factor in modulating cellular activities such as aflatoxin B1-detoxification.

P335
Novel Role of ARHGAP22 in the Development of Type 2 Diabetic Retinopathy

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Backgrounds:

We previously identified *ARHGAP22*, which is implicated in endothelial cell angiogenesis and increased capillary permeability, as a novel diabetic retinopathy (DR) gene. Here, we would like to investigate if the expression of ARHGAP22 and its related mechanism proteins increases or decreases *in vitro* and *in vivo* in response to exposure to different glucose concentrations.

Materials and Methods:

To determine the expression level of ARHGAP22 and its mechanism of action, including Rac1, VEZF1 and EDN1, we used a Western blot assay in human retinal endothelial cells (HRECs) under different glucose concentrations (5 mM or 30 mM D-glucose). In addition, we used intraperitoneal injection of streptozotocin (STZ)-induced diabetic mouse model to examine the expression of ARHGAP22 and Rac1. Total protein and RNA from mouse retina were subjected to Western blot analyses and real-time PCR.

Results:

The results showed that high levels of D-glucose (30 mM) decreased the expression of the ARHGAP22 protein in HRECs for 24 h compared with normal glucose (5 mM). However, the expression of VEZF1 and EDN1 at high levels of D-glucose was increased for 24 h compared with normal glucose. The total protein expression of Rac1 increased under high glucose levels for 5 min compared with low glucose levels. In contrast, the protein expression of active Rac1-GTP protein increased at high glucose levels. At an mRNA level, the mRNA expression of ARHGAP22 at high glucose levels increased for 24 h and 48 h, whereas the mRNA expression of Rac1 at high glucose levels was similar to that observed at low glucose levels. Furthermore, the results of STZ-induced diabetic mouse model have shown that the protein expression either in ARHGAP22 or in VEZF1 was decreased in STZ-induced diabetic mouse compared with saline control mouse. The mRNA expression level of ARHGAP22 was decreased in STZ-induced diabetic mouse compared with saline control mouse, but the mRNA expression level of Rac1 was increased in STZ-induced diabetic mouse.

Conclusion:

In summary, the protein expression of ARHGAP22 was decreased and the expression of VEZF1 and EDN1 was increased under high levels of glucose condition. ARHGAP22 is the only reported regulatory cell signal for VEZF1, which participates in a direct protein-protein interaction with VEZF1. VEZF1 specifically bound to the EDN1 promoter and suggested that the VEZF1 binding site was responsible for endothelial cell dependent EDN1 expression. Therefore, it is suggested that ARHGAP22 inhibits VEZF1 transcriptional activation of the EDN1 expression in high glucose condition may increase to DR development.

P336
The role of microRNAs in ET-1 and Thrombin–induced CTGF expression and fibroblast differentiation

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Backgrounds:

Fibrosis is an important cause of morbidity and mortality in the lung, and the resident fibroblast has been reported as the primary cells in causing pulmonary fibrosis. A class of small cellular RNAs, termed microRNAs (miRs), acting as agents of the RNA interference pathway, have been implicated in the regulation of a variety of cellular processes, and even the pathogenesis process of human diseases. CTGF, which belongs to the highly conserved acronym of Cyr61/CEF-10, CTGF/Fisp-12, Nov (CCN) family of immediate early genes, is a potential target for intervention of lung fibrosis, and recent studies indicated that CTGF is indispensable for persistent lung fibrosis. However, role of miRs in regulation of CTGF expression and myofibroblast formation is still unclear.

Materials and Methods:

WI-38 cell, the fibroblast derived from normal embryonic lung tissue, was treated with ET-1 or Thrombin.. Expression of CTGF protein and mRNA, and characteristics myoblast differentiation including SMA and collage expression were detected in WI-38 cells under ET-1 or thrombin stimulation.

To predict which miRNAs target to CTGF gene using target-prediction programs. Transfection of miRs to identify the role of miRs in ET-1 and Thrombin–induced CTGF expression and myofibroblasts formation was performed.

Results:

Our results revealed that CTGF protein was able to be induced by ET-1 and thrombin stimulation in WI-38 cells. Accordingly, increased SMA and collagen expression by ET-1 and thrombin were detected in WI-38 cells. Activation of MAPKs including ERK, JNK, and p38 via induced phosphorylation was observed. Bioinformatic analysis showed that miR-26b, miR-19a and miR-19b possessed ability to bind CTGF 3'UTR, and decreases in miR-26b, miR-19a and miR-19b expression by ET-1 and thrombin were examined by RT-PCR. Overexpression of miR-26b, miR-19a and miR-19b to identify their role in regulation of CTGF expression and myofibroblast formation is involved.

Conclusion:

Data of the present study provide scientific evidence to support that miRs may participate in regulation of CTGF expression, and contribute to pulmonary fibrosis.

P337
Tumor Suppressive Profiling in Rhenium-188 Embedded PEGylated Liposome Treated NSCLC Animal Model

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Backgrounds:

Non-small cell lung cancer (NSCLC) is documented to be insensitive to chemotherapy and external beam radiotherapy (EBRT). Here we developed PEGylated liposome carrying embedded ^{188}Re to determine if this theragnostic agent could be used for both imaging and internal radiotherapy. Additionally, the molecular expression was investigated to better understand the tumor suppressive mechanisms raised by ^{188}Re-liposome.

Materials and Methods:

Human NSCLC NCI-H292 cells were inoculated subcutaneously or orthotopically in nude mice. The tumor growth and localization was confirmed by IVIS system. Bio-distribution analysis and nanoSPECT/CT system were used for confirming the accumulation of ^{188}Re-liposome. Real-time PCR was performed for measuring the expression of let7i and miR-182 microRNA.

Results:

According to bio-distribution, ^{188}Re-liposome efficiently accumulated in xenograft lung tumor. Although the nanoSPECT/CT imaging showed apparent signals in subcutaneous tumor lesion, it was barely detected in orthotopic tumor because of the resolution limitation. However, we evaluated the therapeutic efficacy in orthotopic tumor bearing mice and displayed that the growth of tumor was suppressed by ^{188}Re-liposome. Additionally, the life span of treated mice was 2 fold longer than that of untreated control. We dissected the tumors from mice and extracted the total RNA for further analysis of microRNA expression. Intriguingly, it revealed that Let-7i, a well-characterized microRNA with tumor suppressive phenotype, was up-regulated after treatment of ^{188}Re-liposome. Whereas a potential oncogenic microRNA, miR-182, was down-regulated. To the best of knowledge, this is the first report demonstrating that ^{188}Re-liposome can affect the expression of microRNA for tumor suppression in vivo.

Conclusion:

PEGylated 188Re-liposome is a potent therapeutic agent for human NSCLC. Change of microRNA expressive profile may be the putative mechanism to mediate the therapeutic efficacy of this nanoradiopharmaceutical agent.

P338
TP53 Mutation Screening Method And Related Database

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Backgrounds:

TP53, a well-known tumor-suppressor gene maintaining genome integrity, is mutated in ~50% human cancers. Most *TP53* mutations are single-base missense substitutions and they distribute across the coding region, especially in the DNA binding domain. *TP53* gene has been considered a prime target for cancer drug development; therefore, mutation detection is essential for clinical investigation as well as cancer genomics research.

Materials and Methods:

To routinely investigate the *TP53* gene mutation profiles in clinical samples of various cancer types, we applied mass spectrometry to develop a high-throughput procedure to screen 126 mutation hotspots, technology transferred from National Health Research Institutes (NHRI) and Industrial Technology Research Institute (ITRI). A total of 987 tumor samples of eight cancer types, including 351 oral, 250 breast, 130 liver, 95 gastrointestinal stromal, 55 colorectal, 48 bladder, 36 lung and 22 bone cancers, were screened to identify cancer-common and –specific mutation sites.

Results:

The frequency of *TP53* mutation differs between various cancer types. More than 35% of the liver and colon cancers had a *TP53* mutation, while only 15% of the bladder cancer was found to be positive by this method. Most mutations was found in multiple cancer types, but some were recurrent for specific cancer types, for example, 747C>T in 9.2% of the liver cancer samples.

Conclusion:

This cost-effect protocol should facilitate collecting *TP53* mutation information for investigating cancer etiology and managing patients on individual basis.

P339
For Better Senior Sex – insights from *Drosophila*

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Backgrounds:

Better senior sex has long been an important objective in healthy aging research. This is a big question about the declination of sexual activity and sexual desire along with age has been proven in various clinical studies. Dopamine has been proven to participate in the sexual desire control of mammals. The involved cellular and molecular mechanisms of sexual problem in senior have not been clarified.

Materials and Methods:

The *GAL4/UAS* gene expression system is a precise means of targeted gene expression employed to study sexual activity in *Drosophila*. *Drosophila* also provides a research advantage of large-scale analyses that the analyses and quantification of courtship behavior have been clearly confirmed. In this study, we using tissue-specific GAL4 drivers resulted in tyrosine hydroxylase (TH) expression that was restricted, and expected dopamine level will be boost to those specific tissues for large amount screening dopamine-mediate sexual activity circuits.

Results:

More compelling evidence was shown when increasing dopamine level in dopaminergic neurons, the declination of courtship strength of *Drosophila* along with ageing could be slowed down. It increases the DA level in different types of dopaminergic neurons. Interestingly, we demonstrate the existence of critical circuit; in VPL neurons which innervate the calyx of mushroom bodies, through DA, may boost the male courtship strength.

Conclusion:

VPL neurons are necessary and sufficient, through dopamine, enhance male sexuality; and age-related declines in sexual activity also leads to alterations of dopamine in VPL neurons. As such a complicated behavior could be controlled by this simply neural circuitry, it is expected that the outcome would become an effective platform for relevant research on sexual activity.

P340
The Effect of Cigarette Smoke Components in Human Normal and Adenocarcinomic Lung Cells

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Backgrounds:

Cigarette smoke (CS) is a complex mixture of the gas and particulate phases, in which contain more than 4000 chemical compound. It is generally recognized that the development of cancer in lung is affected by duration of smoking, and the risk factor also increases with the number of cigarettes smoked. Lung cancer is the leading cause of cancer-related mortalities in both genders and this malignancy accounts for 15% of all cancer deaths globally and this malignancy is associated with cigarette smoking. In addition, the development of lung cancer is also accompanied with tumoral hypoxia, which has been shown to play important role in disease progression. The purpose of this study is to investigate the effects of known cigarette smoke toxicants components on human normal and malignant tumor lung cells and whether hypoxia augments these observed changes.

Materials and Methods:

Initially, normal human fetal lung fibroblast cell line MRC5 and adenocarcinomic human alveolar basal epithelial cell line A549 were co-cultured with heterocyclic aromatic components identified in cigarette smoke and cell proliferation was examined under normoxia and hypoxia. In addition, AnnexinV-FITC were used to detect cells apoptosis, and DCFH reagent was used to measure cell oxidative stress when treated with cigarette smoke components.

Results:

Our data showed that a reverse dose-dependent reduction in normal lung cells while the smoke components induced cell proliferation in malignant tumor lung cells. When A549 cells were maintained in hypoxia, the stimulatory effect on cell proliferation was enhanced. Based on our results, the lowest observed adverse effect level (LOAEL) was determined to be at 10-10M. When cells were administrated with cigarette smoke components at LOAEL, the percent of apoptotic cells detected in cultures was comparable to that of control group in both normal and malignant, suggesting that these smoke constituents impair normal cell proliferation via mechanism(s) other than cell program death. Our oxidative stress analysis data showed that cigarette smoke components caused a significant induce in the generation of intracellular H2O2, evidencing oxidative stress induction in both MRC5 and A549 cells.

Conclusion:

Cigarette smoke components decreased cells proliferation in MRC5 while induced cell proliferation in A549 cells. These effects were enhanced under hypoxic since cell proliferation was elevated in control groups and smoke components were more potent under this condition. Cigarette smoke components also caused a significant induce in the generation of intracellular H2O2. However, these heterocyclic aromatic components did not induce apoptosis in MRC5 and A549 cells. Taken together, the present finding reveal adverse effects of cigarette smoke components on human respiratory system and cigarette smoking promotes the progression of lung cancer.

P341

Endoplasmic Reticulum Stress regulates Bim mediates Avian Reovirus S1133-induced Apoptosis

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Backgrounds:

To investigate Avian Reovirus (ARV) S1133 infected Vero and DF1 cell whether induced endoplasmic reticulum stress link to apoptosis.

Materials and Methods:

ARV S1333 inoculation of SPF chickens. Eighteen-day-old specific-pathogen-free (SPF) White Leghorn chicks were purchased from the Animal Health Institute, Council of Agriculture, Executive Yuan. The chicks were randomly divided into three groups and fed under a positive-pressure environment at the Southern Taiwan Disease Diagnostic Center of National Pingtung University of Science and Technology. Each group was inoculated *via* the footpad and nostril with PBS or 10^6 $TCID_{50}$ in 100 µl (low dose) or 10^7 $TCID_{50}$ in 100 µl (high dose), and the infected chicks were housed in separate Horsfall-Bauer cages under negative pressure. At 6 days post-infection, and every 3–4 days thereafter, chicks were sacrificed and their organs harvested, including the bursa, intestines, liver, heart, kidney, tendons, pancreas, proventriculum and gastrocnemius. Tissues were stored under liquid nitrogen for protein extraction.

Results:

The UPR often elevates the expression level of ER-resident proteins. Compared to uninfected control, ARV S1133 infected Vero and DF1cells resulted in augmented expression of certain ER response-related genes in a time-dependent manner, including chaperons Bip, protein disulfide isomerase (PDI), C/EBP homologous protein (CHOP) and PKR-like ER kinase (PERK). Although the upregulated kinetics differs between individual genes, this result confirmed the induction of ER-stress mediated signalings in the presence ARV S1133. The expression level of ER chaperon calnexin, ER stress sensor inositol-requiring enzyme 1α (IRE1α) and disulfide bonds formation promoting protein Ero1-Lα were not altered after ARV S1133 infection. Additionally, in ARV S1133-infected intestine, heart and bursa also revealed upregulation of certain ER-stress response genes after different days post infection. Under higher infection dose, the expression level was more evident.

Conclusion:

Our analysis indicated that ARV infected Vero and DF1 cells could induce ER stress and elevate ER-resident proteins expression, and the Bip could regulate Bim translocate to ER and mitochondrial and induce apoptosis.

P342

Muscle Differentiation Requires RICAP, A Novel Component in mTOR Complex 2

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Background:

Many diseases are involved in the defects of muscle metabolism and functions such as diabetes and myopathy. At the molecular level, mammalian target of rapamycin (mTOR) plays a role in muscle differentiation. Especially, tissue-specific knockout mice of rictor have revealed novel insights on one of mTOR complex 2 (mTORC2) functions. Depletion of rictor blocks terminal myoblast differentiation, suggesting that rictor plays a vital role in the regulation of muscle differentiation. Utilizing mouse embryonic fibroblast (MEF) cells in which rictor gene was knocked out and immunoprecipitation (IP), we uncovered a novel rictor associated protein, temporarily named RICAP.

Materials and Methods:

Lysate of HEK 293 cells were IPed with IgG, rictor, and raptor. Western blotting analysis was performed by using antibodies against rictor, RICAP, mTOR, and RICAP. Viral particles of rictor and RICAP RNAi were prepared and used to infect C2C12 myoblasts. After virus infection, the cells were differentiated for five days. The cells were fixed by paraformaldehyde and blotted by using tropomyosin antibody in immunocytochemistry (ICC).

Results:

The association of endogenous RICAP and endogenous rictor was indeed verified by using immunoprecipitation. The efficacy of C2C12 differentiation was initially certained by using a muscle specific protein, tropomyosin. Utilizing such a specific bio-marker, both rictor and RICAP RNAi caused the impairment of C2C12 myoblasts differentiation.

Conclusion:

Taken together, RICAP is crucial for myocytes differentiation. Its molecular mechanism might also be related to mTORC2.

P343

Human Gingival Fibroblasts Cultured On Chitosan Film Crosslinked By Glutaraldehyde Could Inhibit Bacterial Invasion And IL-8 Relative Inflammatory Responses.

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Chitosan, which is a nature polymer, has been fabricated into various forms such as antibiotic additives in bone cements, micro/nano particles or reservoir devices for drug delivery and membranes or matrices for tissue engineering. However, there is no research indicating chitosan anti-bacterial abilities after cell culture. In this study, we have fabricated chitosan solutions, partices and films by glutaraldehyde crosslinking reaction to study their effect on bacterial killing and the prevention of bacterial invasion on human gingival fibroblasts (HGF). The results show that all chitosan forms have anti-bacterial abilities on E. coli, Staphylococcus Epidermidis and Streptococcus mutans. In comparison with chitosan particles, chitosan films and solutions demonstrated higher antibacterial ability and no bacterial invasion into HGF. Interestingly, chitosan films crosslinked by glutaraldehyde present hydrophobic surface and which could not support cell adhesion. Therefore, various proteins were added into chitosan films for modifying their hydrophobicities. After bacterial invasion, HGF cultured on chitosan films could inhibit bacterial invasion and gene expressions of inflammation markers such as IL-8, ICAM and VCAM but not COX-2 gene. Based on these results, we conclude that chitosan films could not suppress inflammation responses on COX-2 pathway but could prevent cell adhesion and gathering in immune system through the inhibition of IL-8, ICAM and VCAM expressions. Chitosan films crosslinked by glutaraldehyde showed good cell attachment and proliferation and can inhibit bacterial invasion and further immune responses. Therefore, chitosan films crosslinked by glutaraldehyde could be potential in tooth and bone biomaterial applications.

P344

Characteristics of Presenilin 1 G206D Mutation with Familial Alzheimer's Disease on Cellular Level.

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Backgrounds:

Presenilin-1 (PS-1) plays an important role in Alzheimer's disease (AD) as a part of γ-secretase complex to process amyloid precursor protein (APP), but PS1 itself also have normal physiological functions. In this study, we aimed to investigate the possible mechanisms of PS1 G206D mutation, which was identified from familial AD members in Taiwan, through its impact on both γ-secretase dependent and γ-secretase independent manners.

Materials and Methods:

We generated constructs expressing $PS1^{wt}$ and $PS1^{G206D}$ and transiently transfected them into different cell lines. In γ-secretase dependent manner, we monitored the interaction among components of γ-secretase by co-immunoprecipitation. By ELISA and Western-blotting, we measured products of γ-secretase's two substrates- APP and Notch. In γ-secretase independent manner, PS1 itself may impacts calcium homeostasis by forming a calcium leakage channel, so we used Fura2-AM to monitor intracellular calcium level in life cells under different drugs treatment. We used Western-blotting, immunofluorescence microscope, TUNEL assay and MTT to identify markers of cell protection, autophgy, apoptosis and cell survival rate under different stresses.

Results:

In γ-secretase dependent manner, PS1 CTF, PS1 NTF and Notch cleaved product NICD level showed no significant difference between $PS1^{wt}$ and $PS1^{G206D}$. However, the ratio of $Aβ_{42}$ /$Aβ_{total}$ in cells expressing $PS1^{G206D}$ was significantly higher than in $PS1^{wt}$ group ($p<0.05$). In γ-secretase independent manner, we preliminarily found that $PS1^{G206D}$ disrupt calcium homeostasis by altering total calcium pool in Endoplasmic reticulum. After treating with H_2O_2 , cell protective signal phospho-AKT/total AKT ratio was lower in cells expressing $PS1^{G206D}$. Finally, both MTT and TUNEL result showed $PS1^{G206D}$ expressing cells had lower survival rate under stress.

Conclusion:

Our study showed that $PS1^{G206D}$ mutation affected both on γ-secretase dependent and independent manner. In γ-secretase dependent, $PS1^{G206D}$ altered γ-secretase activity towards APP, increasing $Aβ_{42}$ /$Aβ_{total}$ ratio and may eventually caused further damage to cell. In γ-secretase independent manner, $PS1^{G206D}$ affected cellular calcium homeostasis and apoptosis signaling, directly making cells vulnerable to oxidative stresses and triggering cell death.

P345
The Functional Roles of Prothymosin Alpha in Chemoresistance of Hepatocellular Carcinoma

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Backgrounds:

Hepatocellular carcinoma (HCC) is one of the most frequent and devastating malignancies in Taiwan, representing 83% of all cases, and early diagnosis is very difficult, therefore with poor prognosis and high mortality. HCC most often exhibits a poor response to present drug therapies. Early studies and GEO dataset (GSE1898) both revealed that prothymosin alpha (PTMA) was overexpressed in HCC patients with poor survival. PTMA promoted anti-apoptosis through inhibition of anti-apoptosome formation and Caspase 9 activity, but the mechanism is not well known.

Materials and Methods:

By using GEO dataset (GSE1898) analysis, we found PTMA was overexpressed in HCC patients with poor survival. Then we used loss- and gain-of-function to detect the difference of apoptotic, pro-apoptotic, and anti-apoptotic signals. We also used pathway inhibitors to detect the expression mechanism of PTMA.

Results:

In this study, we found that PTMA may act as a potential chemoresistant gene via inhibiting Caspase activity in liver cancer cell lines and could resist Sorafenib. PTMA knockdown can increase the expression of Bax and Cytochrome c and decrease the expression of Bcl-2. Our results also showed PTMA may be regulated through JNK and PI3K/AKT pathways, and may be down-regulated by treatment of Sorafenib through wnt/β-catenin pathways.

Conclusion:

Furthermore, we will verify that activator protein 1 (AP-1), CCAAT-enhancer-binding protein β (C/EBPβ), and c-myc may regulate the transcription of PTMA through JNK and PI3K pathways. HuR may regulate the mRNA stability of PTMA by binding the AU-rich element (ARE) of 3' UTR.

P346
The matricellular CCN1 protein suppresses TGFbeta1-induced EMT in lung carcinomas.

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Background:

CCN1, a secreted matrix-associated molecule, is involved in multiple pathological conditions. Expression level of CCN1 has been shown to be inversely correlated with malignancies of human non-small-cell lung carcinomas (NSCLCs). However, the molecular mechanism underlying CCN1-mediated suppressing effects on NSCLCs is largely unknown. The tranforming growth factor (TGFβ1) signaling pathway can induce tumor-promoting effects by triggering the epithelial-mesenchymal transition (EMT). Yet, paradoxically, long-term treatments of TGFβ also exert growth-suppressing effects on tumor cells. The present study investigates effects of the matricellular CCN1 protein on the transforming growth factor β1 (TGFβ) signaling pathway in lung cancers.

Materials and Methods:

We used three representative lung cancer cell lines including human NSCLC, A549 (adenocarcinoma) and H520 (squamous-cell carcinoma), as well as murine LLC-1 (Lewis lung carcinoma) to elucidate the CCN1 effects. TGFβ1-induced EMT and cell migration were prominent in lung cancer cells, while these tumor-promoting effects were inhibited in the presence of CCN1 protein.

Results:

CCN1 could reduce TGFβ1-induced morphological changes, vimentin expression, cell migration, and invasion. Consistently, the CCN1 alone also induced partial reversion of EMT process. By knocking down the endogenous CCN1 gene, the prolonged effects of TGFβ1 on NSCLC growth suppression could be alleviated. The long-term combined treatments of TGFβ1 and CCN1 accelerated the growth arrest of lung cancer cells.

Conclusion:

Taken together, our data indicate that CCN1 protein can redirect the TGFβ1 signaling pathway to suppress lung tumorigenesis by reducing EMT process as well as reinforcing induction of cell growth arrest.

P347
Screening UVB-Inducible Marker Genes in zebrafish

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Backgrounds:

UVB irradiation can induce skin damages. The specific aim of this research is to screen molecular markers that sensitive to UVB irradiation and later use its gene promoter to construct highly sensitive reporter that can be used to screen potential sun protection drugs in vitro or in vivo.

Materials and Methods:

To induce photo-damages, we exposure zebrafish embryos aged 3dpf to UVB. After UVB irradiation, the treated larvae were collected to perform microarray analysis, RNA deep sequencing and antibodies screening in order to screen the UVB-inducible markers.

Results:

We irradiated zebrafish embryos at dosages of 300 J/m2 and later quantified the UVB induced apoptosis by Acridine Orange staining. UVB irradiation induces skin shrinkage, DNA condensation, reactive oxygen species (ROS) generation, p53 activation and inflammatory response in zebrafish embryos. All this phenotypes are similar with those detected in mammals. By marker gene screening, we indeed identified several interesting markers related to inflammation and DNA repair showing elevated expressional levels after UVB irradiation.

Conclusion:

The action of UVB irradiation induces cell apoptosis, DNA damage, p53 activation, production of ROS and inflammation that similar to those findings detected in higher vertebrates. The fundamental findings obtained in this study benefit us to clone the upstream regulatory region of those UVB-inducible marker genes and construct the expressional vectors in the future.

P348
Establishment of Rabbit Embryonic Stem Cell Lines Using Small Molecule Inhibitors

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Backgrounds:

Unlike naïve mouse ES cells, all established rabbit embryonic stem (rES) cells are still at their primed pluripotent status with a flat colonial morphology. To investigate whether rES cell colonies can be isolated and maintained in a potentially naïve status with a dome-shaped morphology, small molecule inhibitors were used to block the differentiation pathways.

Materials and Methods:

Female rabbits were superovulated and the uterine horns were flushed with DPBS + 1% FBS to collect early blastocyst embryos, which zona pellucidae were removed using pronase E before seeding on the feeder cells. Media used to isolate ES cells were supplemented with leukemia inhibitory factor (LIF) along with various concentrations of GSK3β (CHIR99021), ERK1 (PD98059) and/or ERK1/2 (PD0325901).

Results:

Culture media supplemented with LIF and various concentrations (0.5, 0.75 or 1 μM) of ERK1 inhibitor (PD98) were tested. The percentages of acquiring dome-shaped colonies were low and showed no differences among treatment groups ($P>0.05$). Under different concentrations (2, 3 or 4 μM) of GSK3β inhibitor (CHIR), the percentages of deriving dome-shaped colonies increased in the 3 μM CHIR group rather than in the 2 μM and the 4 μM groups ($P<0.05$). With a combined treatment of GSK3β and ERK1 inhibitors, the percentages of dome-shaped colonies increased under 0.5 μM PD98 + 3 μM CHIR group as compared to other treatments ($P<0.05$). When culture media were supplemented with LIF, GSK3β and/or ERK1/2 inhibitors (PD03), formation of dome-shaped colonies improved in the 1 μM PD03 + 3 μM CHIR group than with 0.5 μM PD98 + 3 μM CHIR ($P<0.05$). With the presence of one or two inhibitors, the acquired rES cells expressed pluripotency markers (Oct4, Nanog and Sox2), and the induced embryoid bodies also expressed three germ layer markers (MAP2, Desmin and GATA4).

Conclusion:

The present study has demonstrated that dome-shaped colonies of rES cells can be established with the use of one or two inhibitors, and those cells were capable of self-renewal and expressing pluripotency markers, as well as the formation of three germ layer cell lineages of embryoid bodies. Nevertheless, the naïve status of the isolated rES cells remains to be determined

P349

The Activities of Yakuchinone A, a Compound from *Alpiniaoxyphylla*, on Antioxidant Activity, Anti-Adipocyte Differentiation, Induces Apoptosis by Bcl-2 Pathway and Anthelmintic Activities Against *Hymenolepis* nana and *Anisakis simplex*

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Background :
This investigation demonstrated antioxidant and antitumor characteristics of yakuchinone A in skin cancer cells as well as elucidates novel mechanisms for the inhibition of adipocyte differentiation, cestocidal activities against *Hymenolepis* nana adults, and nematocidal activities against *Anisakis simplex* larvae.

Materials and Methods:
Inhibition of DPPH and ABTS free radical activities and anthelmintic activities of yakuchinone A was determined. The level of peroxisome proliferators-activated receptor γ (PPARγ) and leptin mRNA was assayed by reverse transcription PCR. Cytotoxic effects in skin cancer cells used for MTT reagent and the apoptosis pathway also explored.

Result:
Yakuchinone A exhibits an antioxidant capacity, inhibited intracellular lipid accumulation during adipocyte differentiation and suppressed the expressions of PPARγ and leptin. Yakuchinone A induced apoptosis and inhibited cell proliferation in non-melanoma skin cancer. Treatment with yakuchinone A in BCC cells down-regulated Bcl-2, up-regulated Bax and increased cleavage poly ADP ribose polymerase (PARP). The anthelmintic activities of yakuchinone A against *A. simplex* are better than *H. nana*.

Conclusion:
Yakuchinone A had antioxidant, anti-adipocyte, and anthelmintic activities as well as induced apoptosis by Bcl-2 pathway in skin cancer cell.

P350

Electrospun technology could replace the cell-based feeder system in cultureing pluripotent stem cells.

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Backgrounds:
Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) were able to self-renewal and differentiation into ectodermal, endodermal, and mesodermal derivatives. Both of two cells have highly potential to clinical application. Traditionally, ESCs were cultured on mouse embryonic fibroblast (MEF) feeder layer in serum containing media. The concern over xenogeneic contaminants from the mouse feeder cells may restrict stem cell based therapy. For this reason, we wanted to find out a novel biomaterial to replace the cell-based feeder layer.

Materials and Methods:
In the study, we used poly-acrylonitrile (PAN) with biocompatibility and used electrospun technology to make up the feeder layer. We used ESCs culture with different density of electrospun (replaced the feeder layers) to test whether it was useful to maintain the pluripotency of ESCs. The stem cell markers staining, gene expression, microarray, and embryoid body formation was used to tests pluripotency of ESCs on electrospun-based feeder layer.

Results:
The results indicated higher expression levels of several stem cell markers in electrospun-cultured ES cells that compared with controls (feeder-free), including alkaline phosphatase, SSEA1, and Nanog. At the KEGG pathway analysis from microarray, the data showed ESCs turn on TGF-beta pathway that culture on electrospun. By Q-PCR, the gene expression levels of TGF-beta receptor, smad3, smad4 and Nanog increased.

Conclusion:
We demonstrated that electrospun-based feeder layer could maintain the pluripotency of stem cells. Furthermore, nano-fibrous scaffold could be a good candidate for feeder-free culture of ESCs and iPSCs for clinical application.

P351

α-Solanine Inhibits Invasion of Human Prostate Cancer Cells through Suppressing the Expression of Matrix Metalloproteinases

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Backgrounds:
α-Solanine is a glycoalkaloid found in Solanaceae. α-Solanine possesses anti- carcinogenic properties, such as inhibiting proliferation and inducing apoptosis of tumor cells. Recently, we demonstrated that α-solanine suppressed migration and invasion of human melanoma cells. In the present study, we examined the inhibitory effect of α-solanine on metastasis of prostate cancer cell PC-3 in vitro.

Materials & Methods:
The effect of α-solanine on viability of prostate cancer cell PC-3 was determined by MTT assay. Then the effect of non-toxic doses of α-solanine on cell migration and invasion was examined by in vitro wound healing assay and Boyden chamber assay, respectively. The expression of metalloproteinase-2 (MMP-2), MMP-9, tissue inhibitor of metalloproteinase-1/-2 (TIMP-1/-2), extracellular inducer of matrix metalloproteinase (EMMPRIN) and reversion-inducing-cysteine-rich protein with kazal motifs (RECK) were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR).

Results:
Data indicated the α-solanine can inhibit the viability of human prostate cancer PC-3 cell in a dose-dependent manner. When treated with non-toxic doses of α-solanine, cell invasion was inhibited significantly. The mRNA level of MMP-2, -9, and EMMPRIN were also suppressed, while RECK and TIMPs were increased by α-solanine.

Conclusion:
The results suggested that α-solanine inhibited invasion of PC-3 cells by reducing expression of MMP-2, -9, EMMPRIN and increasing expression of TIMPs and RECK. These findings reveal new therapeutic potential for diosgenin in anti-metastatic therapy.

P352

The Role of TGF-β1/pSmad3L Oncogenic Pathway in the Initiation of ICC Formation in Zebrafish

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Backgrounds:
Intrahepatic Cholangiocarcinoma (ICC) is the second most common type of primary liver cancer after hepatocellular carcinoma (HCC). Our previous results show that the liver of one-month-old HBx+HCP transgenic zebrafish exhibite several predominant feature of hepatobiliary disorder.

Materials and Methods:
To investigate the initiation mechanism of ICC, we histologically and genetically analyzed the liver of one-month-old zebrafish by IHC and next generation sequencing (DGE), respectively.

Results:
The results show that the different gene expression profiles in the liver include genes involved in cell cycle initiation, development and cytoskeletal remodeling among the top 10 GeneGo pathways at one-month-old zebrafish. In which, TGF-β-dependent epithelial-mes- enchymal transition (EMT) and cell proliferation occurred among the top 10 rankings. QRT-PCR and Western blotting confirmed the activation of TGF-β/smad3L pathway. Using an antibody against PCNA, we found that liver of HBx+HCP zebrafish with dilated bile ducts showing increased proliferation.

Conclusion:
Taken together, these data revealed the involvement of TGF-beta/smad3L pathway in ICC initiation.
to cause ICC initiation and promotion.

P353
Effect of Sesamin On OVA-Induced Asthma Model

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Backgrounds:

Allergic asthma is a xhroic airway inflammatory disease, which is characterized by pulmonary inflammation, epithelial hypertrophy, goblet cell hyperplasia and excessive mucus secretion in the airway. Sesamin is a flavonoid compound found in Asarum sieboldii, sesame seeds and other herbs, and anti-inflammatory, anticiral, anticarcinogenic, and antihypertensive effects. However, the effect of sesamin as an anti-asthma treatment is poorly understood. The purpose of this study is to investigate whether sesamin can inhibit airway mucus overproduction and the related asthma symptoms.

Materials and Methods:

The in vivo asthma model was established by sensitizing 6 to 8-week male BALB/c mice with i.p. injection of 50 μg of ovalbumin (OVA, adsorbed in 2mg aluminium hydyoxide in 200 μl PBS) on day 0, 7, and 14, followed by challenging the mice with i.t. instillation of OVA (100μg in 40μl of saline) on day 21, 22, and 23. Survived mice were divided into groups of vehicle and 10 mg/kg/day sesamin (i.p. administration). The animals of sham and vehicle groups received i.p. saline administration once daily for 6 days.

Result:

The inflammation assays showed that sesamin inhibited the increase of eosinophils and macrophage/lymphocyte in the bronchoalveolar lavage fluid (BALF) and lung. The mucus hypersecretion assays indicated that the periodic acid-Schiff (PAS) positive cells were significantly lower in the sesamin-treated group compared with control, suggesting that sesamin attenuates mucusproduction.

Conclusion:

Sesamin effectively attenuates pulmonary inflammation and mucus overproduction in asthma.

P354
Detection of Microsatellite Instability in Colorectal Cancers Using High-Resolution Melting Analysis

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Backgrounds:

Microsatellite instability (MSI) is a form of genetic lesion that caused by genetic mutation or epigenetic silencing of genes involved in DNA mismatch repair system. MSI is a well-established marker for screening of patients with hereditary nonpolyposis colorectal cancer (HNPCC), as well as the prognostic marker for sporadic colorectal cancer. The most common technologies used for MSI detection are fluorescence-based capillary electrophoresis and denaturing HPLC. However, these approaches are not sensitive enough, relatively time-consuming and expensive. These drawbacks substantially limit their usage as a mass screening method for CRC. This study aimed to establish and optimize a high throughput platform with improved sensitivity and cost-effectiveness for MSI detection.

Materials and Methods:

Regions of interest were amplified with PCR and followed by high resolution melting analysis (HRM). Both PCR reaction and melting analysis were conducted in HT7900 fast real-time PCR system. Fragment analysis of fluorescence-labeled PCR products was performed in ABI Prism 3100 genetic analyzer and results were analyzed with GeneScan Analysis software. Genomic DNA extracted from FFPE sample of CRC patients were used to evaluate the performance of HRM method for MSI detection.

Results:

A panel of five mononucleotide repeats was selected as the targets for MSI detection. HRM primers were designed using LightCycler Probe design software. PCR conditions were optimized using genomic DNA prepared from CRC cell lines with known MSI status. Lower detection limit was evaluated by mixing different ratios of genomic DNA from CRC cells with MSI and MSS. The HRM platform can detect as low as 5-10% of MSI cells among the wild-type cells. The detection sensitivity is similar or slightly better than fragment analysis method. We implemented this assay into a 384-well plate format for high throughput MSI screening and detected ~ 7-8% MSI positive CRC samples in a cohort containing 80 pairs of CRC samples.

Conclusion:

We have established a high-throughput HRM platform for MSI detection. This platform allows us to detect five mononucleotide repeat regions commonly used for MSI detection with high sensitivity and specificity. This method may be adapted as a massive screening method for early CRC detection.

P355
The Role of *Alpinia Galanga* Extracts on Mitochondrial Dynamics

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Backgrounds:

Alpinia galanga is traditionally cooked as food and spice but it can be used as folk medicine. Moreover, 4'-hydroxycinnamaldehyde (4'-HCA) purified form *A. galanga* has cytotoxic effects on human leukemic cells and induces apoptosis through a combination of mitochondria and endoplasmic reticulum stress pathways. Mitochondria are highly dynamics organelle controlled by continuous fission and fusion process. More and more evidences indicate that the maintaining proper mitochondrial dynamic balance is critical for cellular physiology. The effect of *A. galanga* on the regulation of mitochondrial dynamics is still unclear. Therefore, we intend to clarify whether the extracts from *A. galanga* rhizomes affects mitochondrial dynamics.

Materials and Methods:

In this study, we treated cells with two different extracts purified from *A. galanga* rhizomes and dissolved in DMSO. These two components were known to have antioxidative and anti-inflammatory property. Cell viability assay was examined by dehydrogenase-based method using MTT. JC-1 (5', 6, 6'-tetrachloro-1, 1', 3, 3'-tetraethylbenzimidazolylcarbocyanine iodide) was used for mitochondrial membrane potential analysis by flow cytometry. MitoSOX™ Red mitochondrial superoxide indicator was used for highly selective detection of superoxide in mitochondria of live cells. We further examined whether the effects of these extracts were depends on conventional fusion and fission proteins by RNAi.

Results:

A. galanga extracts lead to fragmented mitochondria. In addition, flow cytometry shows that *A. galanga* extracts result in loss of mitochondrial membrane potential and significant increased the production of mitochondrial reactive oxygen species.

Conclusion:

Our results demonstrate that *A. galanga* extracts do affect mitochondria dynamics and cell physiology. We will further clarify how the mitochondrial dynamics influence cellular functions. Our researches are aim to clarify how *A. galanga* affects mitochondrial dynamics regulation. The results will lead to new horizon in therapeutic approach

P356
Development of Near-Infrared Nanoscissor for Down Regulation of Target Genes

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Backgrounds :

Change the expression of the gene is involved not only in the initiation of cancers, but also in tumor progression and treatment response. Therefore, a lot of researches are developing a novel gene therapy. However, most of current approaches in gene silencing act in the mRNA levels such as siRNA, ribozyme and anti-sense strategies. These approaches only gave partial and temporal gene expression suppression. Besides, delivery system of such approaches is also challenging. This study aimed to develop a Near-Infrared, Artificial, Targeted, Light-Activated Nanoscissor (NIR-ATLANS) to perform gene sequence specific double strand scission to permanently silence target cancer genes.

Materials and Methods :

The NIR-ATLANS comprised gold nanorod core with a monolayer of cypate-modified triplex-forming oligonucleotides (TFOs) that targets EGFP as a model system. The NIR-ATLANS activation specific wavelength is around 808 nm. After laser exposure at 808 nm peak wavelength, we observed and analyzed the EGFP expression of the model cell to check the gene cleavage efficiency.

Results :

We discovered that the NIR-ATLANS could selectively target gene through electrophoretic mobility shift assay. When activated the nanoscissor by near-infrared laser, we observed decreased EGFP expression at protein level in the NIR-ATLANS combined lasing group, while not as significant in all other groups.

Conclusions :

Our results showed a permanent gene silencing at the genomic level by combined NIR-ATLANS and laser activation. The gold nanorod could act as a quencher to prevent non-specific NIR-ATLANS cutting through free radical attack, thus protects non-target DNA from collateral damage. In our study, NIR-ATLANS successfully down regulate EGFP expression. Both reported gene and functional gene were able to be down regulated in cancer cell through our approach. We anticipate such technology hold a great potential in the future cancer gene therapy.

P357

Pituitary Adenylate Cyclase-Activating Polypeptide affected Protein Expression in the Gonad of Male Tilapia (Oreochromis mossambicus) *in vitro*

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Backgrounds:

Reproductive function including gonadal development, gametogenesis, emergence of secondary sex characteristics and sex reversion are regulated through hypothalamus-pituitary-gonad axis in most fish and mammals. Tilapia is one of the most common commercial fish in Taiwan, but literature concerning the mechanism of proteins regulated in whose reproductive process is still scant. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide and regulates the physiological function of the organisms. Our previous results showed that PACAP and its receptor expressed in tilapia (*O. mossambicus*) gonads and PACAP was involved in the cAMP-protein kinase A (PKA) signaling pathway and could play important roles in the regulation of reproduction system as an autocrine/paracrine factor in bony fish. In present study effect of PACAP on the protein expression profiles in tilapia testes *in vitro* was analyzed by proteomic approaches.

Materials and Methods:

Protein extracted from tilapia testes was divided into 7 groups including fresh tissue, Pre-culture (cultured in DMEM for 8 hours), 4 experimental groups (PACAP, Forskolin [adenylate cyclase activator], H89 [PKA inhibitor], and PACAP+Forskolin, all groups with 10 μM) for additional 2 hours after Pre-culture, and control (no chemical supplemented) and were analyzed by two-dimensional gel electrophoresis. Spot detection and matching were performed using the Phoretix 2D software (Nonlinear Dynamics, UK). Significance values ($P < 0.05$) between groups were compared by a Duncan multiple range test after one-way analysis of variance.

Results:

The results showed that PACAP treated group had significantly higher protein spot number than those of control and H89 treated groups, but no significant difference was observed when compared with other groups. The results revealed that the expression of tilapia testicular protein *in vitro* was affected by PACAP. When compared with those previously treated by steroid (testosterone) and gonadotropins, 6 and 3 spots were identical and identified to present result, respectively.

Conclusion:

The above data provided the reproductive information to clarify the relationship among gonadotropins, steroids, neuropeptide, and related proteins in tilapia gonads, and a fish testis protein database could be established, and further could be applied in reproductive endocrine and fishery fields.

P358

The autophagic effect of 16-hydroxy-cleroda-3,13-dine-15,16-olide (HCD) on Doxorubincin resistant Anti-Dox-A549 cells and A549 in vitro

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Lung adenocarcinoma is the top one cause of death that is the ten fatalities due to cancer in Taiwan. Chemotherapy treatment often causes lung adenocarcinoma to metastasis and resistance to the drug; subsequently reduces the survival rate of patient. 16-hydroxy-cleroda-3,13-dine-15,16-olide (HCD) isolated from *Polyalthia longifolia* possesses some medicinal values, hence in this study aimed to investigate the beneficial effect of HCD on Doxorubincin resistant lung adenocarcinoma cell (Anti-Dox-A549) and lung adenocarcinoma cell (A549). HCD could inhibit both Anti-Dox-A549 and A549 cells by MTT assay. HCD could arrest Anti-Dox-A549 and A549 at G0/G1 without altering Sub-G1 phase using flow cytometry, implying that HCD induced apoptosis through autophagy pathway. In addition, Prodigiosin (PG) isolated from *Serratia marcescens* can inhibit cancer cell by autophagy signaling pathway, therefore PG was taken as a positive control. Accordingly, the protein levels of mTOR protein kinase PI3K-Class III, Beclin-I, PARP and Akt decreased and LC3-B protein (autophagy marker) increased in Anti-Dox-A549 and A549 after PG treatment. The protein levels of mTOR protein kinase, PI3K-Class III, Beclin-I, PARP and Akt were inhibited and the LC3-B protein (autophagy marker) increased after HCD treatment. This result is evident that the anti-cancer effect of HCD and PG could be through the autophagy-dependent or -independent pathway.

P359

Inhibition of p38 MAPK–dependent MutS homologue-2 (MSH2) expression by metformin sensitize human lung squamous cancer cells to gefitinib

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Gefitinib, a quinazoline-derived tyrosine kinase inhibitor shows anti-tumor activity in vivo and in vitro. Human MutS homologue-2 (MSH2) plays a central role in promoting genetic stability by correcting DNA replication errors. In this study, the effects of p38 mitogen-activated protein kinase (MAPK) signal on the MSH2 expression induced by gefitinib in two human non-small cell lung squamous cancer cell lines was investigated. Exposure of gefitinib increased MSH2 protein and mRNA levels, which was accompanied by MKK3/6-p38 MAPK activation in H520 and H1703 cells. Moreover, SB202190, a p38 inhibitor, or knockdown of p38 expression by specific small interfering RNA (siRNA) significantly decreased the gefitinib-induced MSH2 expression through increasing the mRNA and protein instability. Enhancement of p38 activation by constitutively active MKK6 (MKK6E) increased MSH2 protein and mRNA levels. Specific inhibition of MSH2 by siRNA significantly enhanced the gefitinib-induced cytotoxicity. Moreover, metformin, an antidiabetic drug, may reduce cancer risk. In human lung squamous cancer cells, metformin could decrease the gefitinib-induced p38 MAPK-mediated MSH2 expression and augment the cytotoxic effect and growth inhibition by gefitinib. Transient expression of MKK6E or HA-p38 MAPK vector could abrogate metformin and gefitinib-induced synergistic cytotoxic effect in H520 and H1703 cells. Together, our results first suggested that down-regulation of p38 MAPK-mediated hMSH2 expression enhances the sensitivity of gefitinib to human lung squamous cancer cells.

P360

Wiring Specificity in Lateral Subdivision of Amygdala Central Nucleus

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Backgrounds:

The amygdala is a brain structure associated with fear learning and memory. The lateral subdivision of central amygdala (CeL), which comprises 90% of GABAergic neurons, provides tonic inhibition to the medial subdivision of central amygdala (CeM), the output of the amygdala - and thus controls the fear expression. However, the CeL contains diverse neuronal subtypes, whose role is poorly understood, and the intra-circuitry connections within the CeL remains unclear. To understand how information is processed within the CeL, it is essential to unravel the functional connectivity between different neuronal types. Here, we performed multi-neuron whole-cell recordings to determine the wiring principle within the intra-CeL.

Materials and Methods:

300 μm thick coronal sections of acute brain slice from C57BL/6 male mouse (3-6weeks) were prepared. We performed patch-clamp whole-cell recordings from multiple neurons to determine functional connections within the CeL. We filled the cell with biocytin and performed post-hoc morphological reconstruction of using the software Neurolucida.

Results:

At least three different types of neurons within the CeL were identified on the basis of the electrophysiology properties: late spiking (LS), regular spiking neurons (RS) and low-threshold bursting (LTB). The synaptic connections between intra-CeL neurons are all GABAergic. Our preliminary studies reveal that temporal dynamics of GABA transmission within the CeL is target cell-specific. Specifically, we found that synaptic transmission at the late spiking (LS) to regular spiking (RS) neuron synapse exhibits strong activity-dependent depression whereas synaptic transmission at LS to LS or RS to RS neuron synapse is relatively insensitive to presynaptic activity or exhibits use-dependent facilitation.

Conclusion:

1.In the CeL, LS and RS neurons are the two major types of neurons.
2.Depending on target cell types, presynaptic neurons shows different release probability and short-term plasticity, indicating target-specific transmission in the CeL.
3.In addition to chemical synapses between neurons, LS and RS neurons exhibit autaptic transmission, which may provide the feedback nhibition, thus enhancing spike timing precision.

P361
Elastase Induced Lung Epithelial Cells Apoptosis and Emphysema through Placenta Growth Factor

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Background:

Chronic pulmonary obstructive disease (COPD) is the fourth leading cause of death in the world, but the pathogenic factors and mechanisms are not fully understood. Pulmonary emphysema is a major component of COPD, and is considered resulting from oxidative stress, chronic inflammation, protease-antiprotease imbalance, and lung epithelial (LE) cells apoptosis. Previously, we demonstrated patients with COPD had higher levels of placenta growth factor (PlGF) in serum and bronchoalveolar lavage (BAL) fluids than controls. The exposure of LE cells to PlGF led to apoptosis. Transgenic mice overexpressing PlGF developed pulmonary emphysema. Intratracheal instillation of porcine pancreatic elastase (PPE) onto PlGF wild-type mice induced emphysema, but not in PlGF knockout mice. Therefore, we hypothesized that PPE generates pulmonary emphysema and LE cells apoptosis through the upregulation of PlGF expression.

Materials and Methods:

Mouse and human LE cells, MLE-15 and S-cells, were treated with PPE. PPE-induced PlGF promoter activity, expression and secretion were evaluated by luciferase activity assay, RT-PCR, Western blot and enzyme-linked immunosorbent assay (ELISA). LE cells were treated with PlGF and quantified the apoptotic level by trypan blue inclusion, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), flow cytometric and caspase-3 activity assay. The PlGF-induced c-jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38 MAPK) pathways were determined by Western blot. 8-week-old B6 mice were intratracheally instillated with saline, PPE or PPE with kinase inhibitor. In lung tissue section and BAL from treated mice, the PPE-induced PlGF expression and activation of JNK and p38 MAPK were validated by immunohistochemistry stain and ELISA.

Results:

In this study, we proved that PPE increases the PlGF secretion in vivo and in vitro. PPE increased PlGF promoter activity and the expression of PlGF mRNA and protein in LE cells. Moreover, PlGF induced LE cells apoptosis in a dose dependent manner and chronic treatment of PlGF increased LE cells apoptosis. PlGF activated JNK and p38 MAPK pathways. We then proved that both pathways were involved in PlGF-induced LE cells apoptosis and PPE-induced pulmonary emphysema in mice.

Conclusion:

Given these findings, we suggest the increase of PlGF and downstream activated JNK and p38 MAPK pathways contribute to PPE-activated LE cells apoptosis and emphysema. Regulatory control of PlGF and agents against its downstream signals may be potential therapeutic targets for COPD.

P362
Design of an Intelligent Anti-caries Particle for Oral Preventive Care

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Backgrounds:

Caries is one of the most popular oral diseases derived from acid challenge produced by cariogenic bacteria. Streptococcus mutans (BCRC10793) is the major pathogenic bacteria involved in caries. Not all bacteria in oral cavity contribute the development of caries. Instead, many oral bacteria have protective effect to caries development through counter-balance of the cariogenic flora. Therefore, it is important in the design of effective anti-carious strategy that integrates selectivity for cariogenic pathogens inhibition and non-cariogenic bacteria preservation.

Materials and Methods:

Hydroxyapatite (HAp), as the main mineral component of bones and teeth, has applications as biomaterials, tissue enginnering, and drug delivery, etc. In previous study, organic molecules and polymers play an important role in the formation of these spherical HA particles through forming a specific structure of micelles in solution. However, a solution method to prepare fluoride-doped HAp (FAp) spheres with hierarchical structure via an ion-assisted and surfactant-free mineralization process has been employed. The morphology and particle size was observed by the high resolution scanning electron microscopy. To identify the crystalline phase and structure, X-ray diffraction (XRD) patterns were recorded on X-ray. Fourier transform infrared (FTIR) spectra were recorded using Spectrometers. The cytotoxicity was tested by 3T3 cells and cell viability was evaluated using MTT assay. Further, the antibacterial activity was evaluated against liquid cultures of S. mutants by minimal inhibitory concentration (MIC) broth microdilution assay and real-time PCR.

Results:

The result showed that the designed spheres have biocompatibility. In addition, fluorapatite (FAp) could selectively inhibit the growth of the cariogenic bacteria.

Conclusion:

It is important to design effective anti-caries strategy that integrates selectivity for cariogenic pathogens inhibition and non-cariogenic bacteria preservation. The results showed the great potential in development of such smart designed nano spheres in the prevention of tooth demineralization through pathogenic challenges. It may provide a new approach in intelligent anti-caries strategy for oral preventive care.

P363
Resveratrol Induces Apoptosis and Autophagy in HHV8 Harboring Primary Effusion Lymphoma Cells.

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Backgrounds:

Resveratrol (3,4',5-trihydroxy-trans-stilbene), a polyphenolic natural product, shows chemopreventive properties against several cancers, heart diseases, inflammation, and viral infections. Primary effusion lymphoma (PEL) is an aggressive neoplasm caused by human herpesvirus 8 (HHV8). Conventional chemotherapy has limited effect on PEL, and the prognosis is poor. In this study, we utilized the HHV8 harboring PEL cells to examine whether resveratrol treatment would induce apoptosis and autophagy in PEL cells.

Materials and Methods:

Cell viability and ROS generation were determined by trypan blue exclusion assay and a fluorogenic 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) probe respectively.. Apoptosis were evaluated using caspase-3 activity assay. Autophagy activation was determined using acridine orange staining and Beclin 1 expression. ROS scavenger (N-acetyl cysteine, NAC), autophagy inhibitor (3- Methyladenine, 3MA) and caspase inhibitor were used to investigate the interplay among resveratrol induced ROS, autophagy and apoptosis.

Results:

We observed that resveratrol induced caspase-3 activation and the formation of acidic vacuoles in the HHV8 harboring PEL cells. NAC could attenuate both the resveratrol induced caspase-3 activity and the formation of acidic vacuoles, but enhanced resveratrol induced cell death in the HHV8 harboring PEL cells. 3-MA also increased the cytotoxicity of resveratrol in PEL cells and caspase inhibitor failed to alleviate resveratrol induced cell death.

Conclusion:

Resveratrol could induce both apoptosis and autopahgy in HHV8 harboring PEL cells. Inhibition of apoptosis or autophagy did not revert resveratrol induced toxicity in PEL cells, suggesting resveratrol may induce the other cell death pathway except for apoptosis and autophagy.

P364
Evaluate Rat Liver Fibrosis Using Molecular Images

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Backgrounds:

Use in-vivo molecular imaging to evaluate liver fibrosis in a thioacetamide(TAA) -induced rat model and Xiao Chai Hu Tang (XCHT) chemoprevention formula.

Materials & Methods:

Sprague-Dawley rats receiving thioacetamide (TAA, 200mg/kg) intraperitoneal injection were divided into three groups with XCHT in drinking water for 6 weeks: 0 g/kg (n=3), 0.25g/kg low dose XCHT (n=3) and 1g/kg high dose XCHT (n=3). Sono-elastography (Supersonic), liver specific MRI contrast agent Primovist (Shering), diffusion-weight MRI were applied to evaluate liver fibrosis at week 3 and week 6. Core needle biopsy at week 3 and liver specimens after sacrifice at week 6 were done to evaluate to liver fibrosis. We compared the accuracy of these methods for staging the degree of liver fibrosis with liver tissue pathology with H&E stain, Sirius red-stain, and hepatic transporters OATP1,Mrp2 immunohistochemistry staining .

Results:

Liver fibrosis Ishake scores of rats with TAA alone, TAA+ low dose XCHT and TAA+ high dose XCHT were 3, 2 and 1, respectively. Primovist enhancement was lower in TAA alone group than the other two groups at 10 minutes after contrast agent Primovist injection ($p<0.05$). On week 6, the liver elasticity ultrasound of TAA alone , TAA+ low dose XCHT and TAA+ high dose XCHT were 16.51±0.91kPa.13.83±0.43kPa.9.37±0.24kPa.Chemoprevention effect of XCHT reduced the fibrosis degree of liver specimen and liver sonoelasticity at weeks 3 ($p<0.05$) and week 6 ($p<0.001$). We also found that XCHT downregulate TAA-related Mrp2 overexpression but no change on OATP1 and alpha-smooth muscle actin expression.

Conclusion:

We can use non-invasive medical imaging tools to access the liver fibrosis and anti-fibrosis drug effect in rat.

P365
Investigate the Relationship Among Perfusion MRI Parameter, Circulating Angiogenic Factors and Endothelial Progenitor Cell in Patients with Breast Cancer

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Backgrounds:
Breast cancer is characterized by high vascularity on MRI, increasing circulating endothelial precursor cells (EPC) counts and elevated levels of serum angiogenic factors, implying active tumor neovascularization. We used MRI scanning to evaluate the relationships among clinical breast cancer staging, tumor enhancement on MRI, circulating EPC and angiogenic factors.
Materials & Methods:
Fifty women with suspicious breast malignancy underwent MRI exams, circulating EPC calculation and serum angiogenic factors measurement before breast biopsy. Eleven women without clinical breast disease were chosen for controls. The circulating angiogenic factors in our study included VEGF (vascular endothelial growth factor), IL-6 (Interleukin-6), IL-8 (Interleukin-8), Macrophage migration inhibitory factor (MIF), Transforming growth factor beta (TGF-β) and sVCAM-1 (vascular cell adhesion molecule 1). The MRI perfusion data and perfusion parameters including: initial percent enhancement (PE) and signal enhancement ratio (SER).
Results:
Thirty-four (68%) women with breast cancer were identified. Other women had fibrocystic disease (n=13, 26%) or breast pre-malignancy (n=3, 6%). Breast malignancies have higher PE than non-malignant breast lesions ($p<0.0001$). Qualification of circulating angiogenesis factors of breast disease patient can be easily done by multiplex bead array assays (Bio-plex). Circulating angiogenesis factors (IL-6, IL-8 and MIF) are higher in patients with breast cancer ($p=0.004$). Women with benign or malignant breast disease had higher circulating EPC than healthy control ($p=0.001$), but no significant correlation with MRI perfusion parameters.
Conclusion:
Angiogenesis of breast benign and malignant breast disease has complex mechanisms and can be observed using circulating angiogenic factors, circulating EPC and dynamic contrast MRI.

P366
Fibrillar Human Serum Albumin Suppresses Cancer Growth and Metastasis by Targeting β1 Integrin to Down-regulate Akt and Erk Phosphorylation

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Backgrounds:
As prognosis for patients with metastatic cancer is generally poor, advances in treatment are needed.
Materials and Methods:
Recently, we have discovered a chromatographical process which converts native protein, human serum albumin (HSA), regardless of its sequence, into a water-soluble nano-fibrillar protein structure. Fibrillar HSA (F-HSA) inhibits in vitro growth of cervical cancer (CaSki), prostate cancer (PC-3 and 22Rv1) and lung cancer (A549 and CL1-5). Herein, we studied the mechanism of action of F-HSA and explored its effect against ovarian and breast tumor growth and metastasis in vitro and *in vivo*.
Results:
Key results were demonstrated F-HSA promoted apoptosis and decreased invasion and migration of human ovarian/breast cancer cells (SK-OV-3/MDA-MB-231) cancer cells and the murine breast cancer cell TS/A *in vitro*. Under the various therapeutic strategies, orthotopic and metastatic xenograft/allograft mouse models demonstrated that F-HSA prolonged survival and attenuated metastasis of human/murine ovarian/breast cancer SK-OV-3, MDA-MB-231, or TS/A by alone or combined therapies (F-HSA and/or cisplatin) as shown by bioluminescence imaging of mice (BALB/cAnN-Foxn1). This effect of F-HSA was accompanied by activation of PTEN as well as down-regulation of FAK, Akt, Raf, Erk, and MMP-2. Anti-β1 integrin antibody reversed the cellular effects of F-HSA.
Conclusion:
Taken together, these results indicated that negative regulation of Akt/Erk signalling and MMP-2 by F-HSA may have the potential to suppress ovarian and breast tumor growth and metastasis, therefore, it will be serve as a potential anti-cancer drug in the future.

P367
Naringenin Reverse Epithelial-Mesenchymal Transition and Suppresses Prostate Cancer Metastasis

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Backgrounds:
It is well recognized that the majority of cancer related deaths is caused by metastasis. Therefore, there is an urgent issue for the development of therapeutic intervention specifically targeted to the metastatic process. Growing evidence indicate that epithelial-mesenchymal transitions (EMT) is associated with carcinogenesis, cancer invasion and metastasis. Recently, Chinese medicinal herbs have been reported to have various anti-carcinogenesis properties, including inducing cell apoptosis and suppressing cell proliferation. Naringenin, which is plentiful in citrus fruits, has been shown to exhibit anti-inflammatory, anti-oxidant and hypolipidemic effects. However, limited studies are available concerning the potential of Naringenin in reversing epithelial-mesenchymal transition (EMT). Thus, we want to investigate whether naringenin might suppress cancer migration and invasion via reverse EMT of prostate cancer cells.
Materials and Methods:
The PC3 androgen-independent prostate cancer cell line was used in this study. The effect of naringenin on prostate cancer cell viability was measured by MTT assay. Protein expression level was monitored by Western blot through specific antibodies against epithelial phenotype and mesenchymal phenotype markers. Finally, Boyden chamber method was used to detect the effect of naringenin treatment on cell mobility of PC3 cell line.
Results:
The cell viability was significantly decreased in a time and dose-dependent manner during naringenin exposure for 48 and 72 h. Immunoblot data revealed that naringenin increased the expression of the epithelial phenotype marker, E-cadherin, and repressed the level of the mesenchymal phenotype markers such as Snail and Vimentin in proportion to concentration. Naringenin also inhibited EMT-inducing transcription factors including Twist and Slug and subsequently inhibited the motility of PC3 cells in a dosage-dependent manner.
Conclusion:
In our result, we demonstrated that naringenin inhibited proliferation and suppressed prostate cancer cell metastasis *in vitro* by reversing EMT progression.

P368
Characterization and Functional Studies of Staufen1 in EV71-Infected SF268 Cells

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Backgrounds:
In this study, we focus on the characterization and functional relevance of Staufen1 in EV71-infected SF268 cells. Upon EV71 infection, the expression level of Staufen1 is higher than mock infection increased at the early stage, while its decreased expression phenomenon was detected at the late stage.
Materials and Methods:
We use the EV71 (TW/2231/98) to infect SF268 cell line neuroblasma. The virus was infected cell behind the overexpression or downregulation of Staufen1. The production of viral proteins was confirmed by Western blot. Staufen1 colocalized with VP that confirmed by Immunofluorescence or by immunoprecipitation. The overexpression or downregulation of Staufen1 compared between INF-β RNA level with Poly I:C transfection as a Positive control in SF268 cell.
Results:
Surprisingly, both overexpression and downregulation of Staufen1 result in the decrease of production in the EV71 viral protein, VPs and 3A. Colocalization of Staufen1 with EV71 viral protein, VPs, were observed using dual immunofluenscent staining upon viral infection, indicating this interaction might affect the viral replication.
Conclusion:
Our further studies will focus on the interaction between Staufen1 and viral protein or RNA at early stages of viral replication to form RNP, which facilitate its replication. Also, we will try to

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Study of S100P and G162R novel single point mutation of Carbonic anhydrase related protein 8 (CA8) in neuronal cell

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Backgrounds:

Carbonic anhydrase related protein VIII (CARPVIII/CA8), a member of carbonic anhydrase related proteins (CARPs), is an isozyme of α-carbonic anhydrases (α-CAs). Compared with CAs, CARPs are lack of a zinc ion in the active site, which catalyzes the reversible hydration of carbon dioxide to bicarbonate and a proton. CA8 is expressed in the Prukinje cells, as well as in peripheral tissues in both mouse and human. Previous studies showed that CA8 is a novel binding protein for inositol 1, 4, 5-trisphosphate receptor type and controls Ca2+ release from endoplasmic reticulum. Our previous results suggested that over-expression of wild-type CA8 promoted invasion and migration ability of mouse primary granule neurons. Moreover, we found that over-expression of wild-type CA8 increased cell proliferation, neurite out-growth and anti-apoptotic ability in SK-N-SH and Neuro-2a (N2a) cells. In the recent years, single point mutations of CA8, S100P and G162R, have been reported. These mutations are associated with novel syndromes including ataxia and mild cognitive impairment without quadrupedal gait. However, the mechanisms underlying the disease syndrome are unclear.

Materials and Methods:

Transient transfection and proteasome inhibitor (MG-132) treatment were used to detect CA8 protein degradation pathway in cytoplasmic hybrid cell line (D5-1). N2a-CA8-myc, N2a-S100P-myc and N2a-G162R-myc stable cell lines was established for cellular localization, neurite out-growth and cell proliferation analysis. Statistics T-Test was used and p value < 0.05 was considered statistically significant.

Results:

To study the expression of mutant CA8, cytoplasmic hybrid cell line (D5-1) was first used for transient expression of mutated CA8, transfected mutant CA8-S100P and CA8-G162R were accumulated under proteasome inhibitor MG-132 treatment, but wild-type CA8 did not respond to the same treatment. To further study the effects of CA8-S100P and CA8-G162R in neuronal cells, we established N2a-CA8-myc, N2a-S100P-myc and N2a-G162R-myc stable cell lines. Our results indicated that the expression levels of mutant CA8 were significantly lower than that of the wild-type CA8. In addition, cellular distributions of mutant CA8 were mainly localized in the cytoplasm, compared to both nuclear and cytoplasmic localization of the wild-type CA8. Furthermore, both of the mutant CA8 showed increased neurite out-growth and cell proliferation ability in N2a cells.

Conclusion:

Our analysis suggested the degradation pathway of mutant CA8 may be different from that of the wild-type CA8. Even though we observed differential effects of mutant CA8 on neurite out-growth and cell proliferation in neuronal cells, the mechanism underlying these observations is not clear. To obtain more solid evidence of the effects mutants CA8 in neural cells, we will use SK-N-SH and mouse primary granule neurons cells to confirm the effects observed in the N2a.

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Osteoblasts Survive the Arsenic Trioxide Treatment by Activation of ATM-Mediated Pathway

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Backgrounds:

Arsenic trioxide (ATO) is widely used in tumor treatment, but excessive arsenic exposure can have adverse effects. We recently found that, in primary osteoblasts, ATO produces oxidative stress and causes DNA tailing, but does not induce apoptosis. It is interesting to explore how osteoblasts can survive under the condition of ATO treatment.

Materials and Methods:

Primary rat calvarial osteoblasts were treated with 0.2, 2 or 6 μM ATO, then examined the cell survive by trypan blue exclusion, apoptosis by DNA fragmentation assay, DNA damage by comet assay, and cell cycle progression by flow cytometry analysis. In addition, the expressions of cell cycle- and DNA repair-related proteins were examined by western blotting.

Results:

We examined the signaling pathway by which osteoblasts survive ATO treatment. Results showed that primary osteoblasts arrested at G2/M phase of cell cycle at 30 h and overrode the G2/M boundary at 48 h of ATO treatment. There were increased Cdc2 phosphorylation and expression of Wee1, a Cdc2 kinase, and expression of the cell cycle inhibitor, $p21^{waf1/cip1}$, which interacts with Cdc2. Moreover, levels of the phosphatase Cdc25C, which activates Cdc2, were decreased, while the ratio of its phosphorylated/inactivated form to the total amount was increased. The phosphorylation/activation of the checkpoint kinases Chk1 and Chk2 and p53 levels were increased, as were levels of activated ATM. However, these effects were reduced by an ATM inhibitor.

Conclusion:

These findings suggest that G2/M phase arrest of osteoblasts is mediated by Chk1/Chk2 activation via an ATM-dependent pathway by which osteoblasts survive.

P371

Use of human primary leucocytes as the model to evaluate immune function - piceatannol as an example

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Backgrounds:

Human blood is the most accessible and relevant tissue for testing immune parameters in vitro. The purpose of this study is to explore leucocytes derived from peripheral blood as target cells for exogenous piceatannol.

Materials and Methods:

Human whole blood was separated to obtain PBMC (Peripheral blood mononuclear cell) and PMN, use PBMC and PMN for cell culture, then use LPS (Lipopolysaccharides) to stimulate the cells to release pro-inflammatory factors, and giving cells piceatannol, use cytokines' primer to run RT PCR amplifing product, and run the eelectrophoresis observe the product and observe the effect of piceatannol inhibit proinflammatory factors.

Results:

After stimulation with LPS, cells release more pro-inflammatory cytokine than without LPS stimulation. After stimulation with piceatannol, cells release less cytokine after stimulation with LPS.

Conclusion:

Our analysis indicated that after stimulation with piceatannol, cells release less pro-inflammatory factors. The higher concentration of piceatannol lead to pro-inflammatory factors gradually decreases. Inferred by the results that piceatannol can inhibit pro-inflammatory factor production, thus achieving the anti-inflammatory and boosting the immune effect. Piceatannol plays role in immunological improvement

P372

Effect of *Geloina eros* extract against apoptosis of hepa cancer cell Hep G2.

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Backgrounds:

Geloina eros , cultivated in Yulin country, is one of the most popular marine selfish in Taiwan. This study investigated the effect of *Geloina eros* extract(water, methanol, Ethyl acetate) for cytotoxicity and apoptosis of hepa cancer cell Hep G2.

Materials and Methods:

Geloina eros was homogenized and extracted with various solvents. The impact of *Geloina eros* extract and its downstream effectors on HepG2 cell growth were studied. The cytotoxicity were tested by the (MTT) colorimetric and trypan blue dye exclusion assay. PI stained cell flow cytometry measurement were introduced to observed the effect for cell cycle. Mechanism of apoptosis was investigated by Western blot analysis of P27, Bcl-2, Bcl-xL and Bax, and also caspases activity analysis.

Results:

Treatment with *Geloina eros* extract resulted in a dose-dependent decrease in cell viability of HepG2 cells. Cell cycle analysis indicated the increasing of G1 phase cells after treatment. *Geloina eros* extract enhanced the expression of P21(waf1), and reduced the expression of Bcl-2 and Bcl-xL proteins but induced the expression of Bax protein. *Geloina eros* extract induced apoptosis of DU145 cells by activation of the caspase-3 pathway.

Conclusion:

The results indicated *Geloina eros* extract induces apoptotic cell death in Hep G2 cell, by caspase-3 activation through activation of apoptotic proteins and down-regulation of antiapoptotic proteins.

P373
Clozapine Induced Autophagy and Apoptosis through Reactive Oxygen Species to Inhibit MCF-7 Human Breast Cancer Cell Growth

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Background:
Even though recent studies demonstrated antipsychotics have antiproliferative activity in cancer cell lines, the mechanisms are not fully elucidated.
Materials and Methods:
Cell proliferation was assessed using cell proliferation assay and clonogenic assay. Cell cycle arrest was monitored based on flow cytometry and western blot. Apoptosis was detected by annexin V-FITC/propidium iodide double staining assay, TUNEL assay and cleaved caspase-9. Autophagy was evaluated based on acridine orange activity and western blot detection of Atg 7, Atg 12/5 and LC3 II. All results were compared between those with and without clozapine treatment.
Results:
Clozapine has inhibitory effect on cell growth in MCF-7 cells by MTT and clonogenic assay. Flow cytometric analysis exhibited that the exposure of MCF-7 cells to clozapine led to the G0/G1 phase arrest. Further results revealed clozapine induced the arrest by decreasing CyclinD1, CDK4, CDK6 and increasing CDK inhibitor p21 and p27 protein levels. Exposure of MCF-7 cells to increasing dosage of clozapine during 72 h significantly increased the production of reactive oxygen species (ROS) accompanied by apoptosis and autophagy. Furthermore, vitamin E recoveried cell survival along with the reduction of ROS, apoptosis and autophagy. In addition, blocking autophagy by choloquine could enhance clozapine-induced apoptosis.
Conclusion:
Our study proved for the first time that clozapine inhibited MCF-7 cell growth by inducing G0/G1 cell cycle arrest, apoptosis and autophagy via a ROS dependent manner. Clozapine-induced autophagy prevented cancer cell apoptosis. These data suggested that combined clozapine and autophagy inhibitor will effectively kill MCF-7 cancer cells.

P374
Investigation of potential Chinese herbs in non-small cell lung carcinoma *in vitro* and *in vivo*

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Backgrounds:
Non-small cell lung carcinoma (NSCLC) is lung cancer with the highest incidence and mortality rate in the world. Chinese herb medicine (CHM) is an attractive therapy for many diseases due to its less side effect. Some CHMs have shown potential anti-cancer activity through reducing tumor recurrence, angiogenesis, metastasis, and drug-resistance.
Materials & Methods:
NSCLC cell lines H460, H520, and A549 were used as in vitro system to evaluate the effect of CHM. Male athymic nude mice (CAnN.Cg-Foxn1nu/CrlBltw, BALB/c nu/nu) subcutaneously injected A549 cells were used as in vivo system to validate the CHM effect. After the solid tumor volume has reached about 100 mm^3, each mouse received daily gravage of 400 μL Formula 3 (F3), 5 (F5), or 14 (F14) for 4 weeks.
Results:
Our studies have identified three of the fifteen Chinese herb formulae, F3, F5, and F14, showing potential anti-cancer activity in NSCLC cell lines. These formulae were found to have anti-proliferative activity through G1 arrest toward the A549 cells. We investigated the pharmaceutical effect of these formulae and active ingredients toward another two NSCLC cancer cell lines. Using MTT assay, we have determined the IC_{50} of F14 in NSCLC H460 and H520 to be 4.5% and 3.8%, respectively. Furthermore, F14 reduced the ability of colony formation, and induced G0/G1 arrest. In addition, we characterized their therapeutic potential *in vivo* using xenograft model. Furthermore, HC3 & HC4 have been identified as one common component from these three formulae. Our study identified that HC3 & HC4 reduced cancer stem cell population. The molecular mechanism of cancer stem cell inhibition was studied with epithelial mesenchymal transition (EMT) PCR array and western to identify specific genes affected by HC3 & HC4 treatments.
Conclusion:
CHM evaluated in this study show potential in cell cycle arrest and cancer stem cell suppression on NSCLC. These CHM might be considered to be an alternative approach toward lung cancer treatment.

P375
Analysis of Dry Eye Protection Effects of Chrysanthemum morifolium Flower Extract

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Backgrounds:
Short-term damages to the ocular surface caused by UV irradiation may be subtle and easily ignored. However, long term UV irradiation poses a critical threat to ocular surface health and may cause dry eye symptoms, which cannot be ignored. This study investigated the preventive effects of Chrysanthemum morifolium flower extracts (Cmfe) against UVB-induced ocular surface degeneration and dry eye.
Materials and Methods:
ICR mice were randomly divided into 14 groups: (1) blank control, (2) with UVB exposure but without Cmfe, (3-5) with UVB exposure and with 3 different doses of ethanol-extracted Cmfe from yellow flowers, (6-8) with UVB exposure and with 3 different doses of ethanol-extracted Cmfe from white flowers, (9-11) with UVB exposure and with 3 different doses of water-extracted Cmfe from yellow flowers, (12-14) with UVB exposure and with 3 different doses water-extracted Cmfe from white flowers. All groups were subjected to corneal surface evaluations, including smoothness, opacity, extent of staining, tear production, together with PAS staining, in order to determine the UVB-induced ocular surface injury, with or without various Cmfe treatments.
Results:
The results showed that chrysanthemum morifolium flower extracts were effective in the prevention of UVB-induced conjunctival damages in a dose-dependent manner. Regardless of extraction from yellow or white flowers and be it ethanol-extracted or water-extracted, Cmfe can effectively reduce UVB-induced ocular surface degeneration and dry eye from 100 mg/kg.
Conclusion:
Chrysanthemum morifolium flower extracts prevent UVB-induced conjunctival goblet cell degeneration and dry eye. Results of this study support that chrysanthemum morifolium flower extract may be used as a prophylactic agent prior to excessive UVB exposure.

P376
Runx1 Deficient Afferents Impair the Visceral Nociception and Exacerbate Chemical Induced Colitis

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Backgrounds:
There are progresses have been made in understanding visceral pain. However, the molecular mechanisms involved in the modulation of visceral pain remain to be clarified. Most of visceral pain is caused by inflammation in the internal organs. Researches have revealed that inflammation is also tightly controlled by nerves that reflexively monitors and adjusts the inflammatory response by inhibiting pro-inflammatory cytokine synthesis. Previously we demonstrated that Runx1, a Runt domain transcription factor, is restricted to nociceptors. In these neurons, Runx1 regulates the expression of many ion channels and receptors and controls the lamina-specific innervation pattern of nociceptive afferents in the spinal cord. Moreover, mice lacking Runx1 exhibit specific defects in thermal, neuropathic and inflammatory pain.
Materials and Methods:
To study the function of Runx1 in nociceptive neurons, we created double transgenic mice, Wnt-1-Cre: Runx1F/F, in which the expression of Runx1 was disrupted specifically in sensory neurons. To examine the role of runx1 in the visceral pain sensation, the Wnt-1-Cre: Runx1F/F mice and their littermates were treated with DSS to induce colitis. After DSS induction, ICP, EMG and von Frey were used to detect the hyperalgesia. The levels of cytokines in colon tissues were tested by using ELISA. The distribution of immune cells in mucosa layer was revealed by immunohistochemistry.
Results:
In this report, we found that disruption of Runx1 in sensory afferents resulted in: (1) the impairment of the visceral pain sensation in murine DSS-induced colitis, (2) more severe phenotypes in murine DSS-induced colitis, (3) differentially affected the production of proinflammatory cytokines in colon tissues isolated from DSS and TNBS -induced colitis, (4) the alteration of distribution of lymphocytes and mast cells in mucosa.
Conclusion:
Our study revealed that the function of Runx1 in sensory afferent is vital for the modulation of visceral pain and of neuro-immune axis.

P377
S100A4 Participates in the Pathogenesis of Areca Quid Related Oral Submucous Fibrosis

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Backgrounds:

S100A4, a member of calcium-binding proteins, is dramatically elevated involved in a variety of fibrotic diseases. Areca quid chewing is the most important etiological factor in the pathogenesis of oral submucous fibrosis (OSF). OSF has been considered as a pre-cancerous condition of oral mucosa. The aim of this study was to determine the critical role of S100A4 expression in the pathogenesis of OSF both in vitro and *in vivo*.

Materials and Methods:

Thirty OSF tissues from areca quid chewers and ten normal buccal mucosa samples without areca quid chewing were analyzed by using immunohistochemistry for S100A4 expression *in vivo*. Collagen gel contraction capability and expression of tissue inhibitor of metalloproteinases 1 (TIMP1)/ MMP9 in arecoline-stimulated BMFs with S100A4 knockdown was presented *in vitro*.

Results:

S100A4 expression was higher expression in areca quid chewing-associated OSF specimens than normal buccal mucosa specimens (p=0.001). Arecoline, a major areca nut alkaloid, led to dose- and time-dependent elevation of S100A4 expression in normal buccal mucosa fibroblasts BMFs (p<0.05). The additions of pharmacological agents rapamycin (mTOR inhibitor), PD98059 (ERK inhibitor), and Bay117082 (NF-kB inhibitor) were found to inhibit arecoline-induced S100A4 expression (p < 0.05) in BMFs. Down-regulation of S100A4 by lentiviral infection significantly reversed arecoline-induced collagen gel contraction and TIMP1/MMP9 expression.

Conclusion:

These results suggest that S100A4 expression is significantly up-regulated in OSF specimens. Arecoline-induced S100A4 expression was down-regulated by rapamycin, PD98059, and Bay117082. Targeting S100A4 might be a potential therapeutic target for OSF through TIMP1/MMP9 down-regulation.

P378
EAT-6, CSK-1 and KIN-2 calibrate extrinsic programmed cell death signaling in *C. elegans*

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Backgrounds:

Programmed cell death (apoptosis) functions in maintaining homeostasis and eliminating unwanted cells. Defects in programmed cell death has been implicated in cancerous growth and diseases. Over the past 30 years, about a dozen of genes, including the core cell death genes EGL-1 (a BH3-containing protein), CED-9 (Bcl-2), CED-4 (Apaf-1) and CED-3 (caspase), have been identified to function in the execution of programmed cell death in *C.elegans*. However, how the regulatory of extrinsic cell death process is still unclear.

Materials and Methods:

C.elegans as our animal model to study programmed cell death during embryogenesis. In genetics, we counted cell corpse numbers and used the microinjection to verify the relationship among interesting genes. *In vivo* analysis, we used yeast two hybridization system (Y2H) and bimolecular fluorescence complementation (BIFC) to analyze protein interaction.

Results:

Here, we demonstrated that EAT-6, a sodium-potassium ATPase alpha subunit, can mediate programmed cell death in *C.elegans*. A typical mammalian sodium-potassium ATPase is composed of two alpha subunits containing an ATPase activity and two beta subunits with/without one gama subunit, and functions to maintain membrane potential across plasma membrane. *C.elegans* has two subunits, *eat-6* and *catp-4*, and three subunits, nkb-1, *nkb-2* and *nkb-3*, but has no gama subunit. We found that loss of any beta subunit does not result in a detectable cell death defect during embryogenesis and, in contrast, loss of the alpha subunit *eat-6* significantly reduced cell death during embryogenesis. This result shows differential involvement of alpha and beta subunits in promoting cell death. In addition, overexpression of *eat-6* promotes cell death. Interestingly, the "pump dead" EAT-6 with a mutation disrupting the ATPase activity, can still promote programmed cell death, suggesting that *eat-6* has a function in promoting cell death independent of its pumping activity. In addition, EAT-6 can promote programmed cell death in the presence or absence of NKB-1 in a cell-specific manner. Our genetic data revealed that both CSK-1, an SRC family kinase member, and KIN-2, a cAMP-dependent protein kinase act downstream of *eat-6* to promote cell death. We observed a fluorescence signal of CSK-1::YN155 (N-terminal fragment of the YFP) with EAT-6::YC173 (C-terminal fragment of the YFP) fusion proteins on the surface of apoptotic cells using the bimolecular fluorescence complementation (BIFC) analysis and detected the interaction of CSK-1 with an EAT-6 intracellular region in a yeast two-hybrid assay. Moreover, the specific extracellular region of EAT-6 has distinguished in programmed cell death (apoptosis) and pumping activity. These results indicate that EAT-6 relays the cell-death promoting signal directly to CSK-1 by protein-protein interaction. We also explored the relationship of *eat-6* with respect to the core cell death genes by transcriptional overexpression.

Conclusions:

We found that the cell-killing function of EAT-6 depends on CED-3/Caspase 3, but not CED-4/ Apaf-1. We are currently doing more experiments to understand how EAT-6 promotes programmed cell death through CSK-1and KIN-2 depending on CED-3/Caspase 3.

P379
Induction of Mouse c-fos Gene Expression by Phorbol Ester Requires NF-κB Activation via p65 Homodimer Binding to Promoter

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Backgrounds:

c-Fos expression is mainly controlled by ERK and transcription factor Elk-1. In this study we unveiled how NF-κB's coordination with Elk/SRF regulates *c-fos* transcription.

Materials and Methods:

We are interested in testing the link between NF-κB and c-Fos. We used the Transcription Element Search System (TESS) and confirmed a previously characterized NF-κB binding site (TESS score: 10.7494) at the proximal region of mouse c-fos promoter. To prove its real transcriptional function in regulating mouse c-fos expression, we then designed oligonucleotides for EMSA assays. Next, to further verify the essential binding of p65 for PMA-induced c-fos promoter activation, we then performed ChIP assay using specific antibody against p65 to immunoprecipitate formaldehyde-fixed chromatin. To understand the involvement of MAPKs and downstream signals in induction of c-Fos expression, we introduced inhibitors of PKC and MAPKs. Finally, the possible signal interplay between IKK and ERK for c-Fos expression was assessed.

Results:

Our results revealed that PMA is a strong c-Fos inducer, while TNF-α is not. Compared to the prominent NF-κB activation by TNF-α, PMA is a weak activator. The c-Fos induction action of PMA in mouse fibroblasts is suppressed by the deficiency of IKKα, IKKβ, IKKγ or p65, and in the presence of ERK inhibitor. In contrast in human HEK293 cells this action is independent of p65. Consistently we identified a NF-κB binding site in mouse but not human *c-fos* promoter.

Conclusion:

In summary, this study provides evidence to link a species-dependent NF-κB activation to c-Fos induction. Besides the well identified ERK-Elk-1 pathway, p65 homodimer is indispensible for mouse *c-fos* expression.

P380
Lei Gong Teng induces autophagy in non-small cell lung carcinoma cells

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Backgrounds:

Lei Gong Teng (LGT), a Chinese herb from *Tripterygium wilfordii Hook. f.* was shown to inhibit the proliferation and reduce the growth of human tumor cells by caspase-dependent cell death. Autophagy is a cellular homeostatic mechanism. Previous studies indicate that increased autophagy can be found in dying cells, but the relationships between autophagy and programmed cell death remain unclear. In this study, we investigate LGT whether induced autophagy and elucidates the possible mechanism in non-small cell lung carcinoma cells A549.

Materials and Methods:

Cells were treated with LGT and viability was assessed by 3-[4,5-dimethylthiazol-2-

yl]-2,5-diphenyltetrazolium bromide (MTT). Cell cycle were measured by flow cytometry. Acridine orange and monodansylcadaverine (MDC) staining was used to monitor the vesicle formation. Autophagy related proteins expression were determined by Western blotting analysis.

Results:

Our results demonstrated that LGT inhibited the proliferation of A549 cells in a dose-time dependent manner. However, LGT treatment didn't caused apoptosis by flow cotymetry. Therefore, we confirmed LGT induced autophagy by acridine orange, monodansylcadaverine (MDC) staining of vesicle formation and the GFP-LC3 expression. Pretreatment with autophagy inhibitor 3-methyladenine (3-MA) suppressed the induction of acidic vesicular organelles and the autophagy related proteins in A549 cells treated with LGT.

Conclusion:

These results suggest that LGT induced autophagy in non-small cell lung carcinoma cells A549. On the other hand, Autophagy may play an important mechanism in LGT-induced cell death.

P381
Molecular mechanisms identification for Pb2+-induced IL-8 gene activation

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Background

The IL-8 is a proinflammatory cytokine that involves in several physiological functions. Our previous report has showed that Pb^{2+} induced cyclooxygenase-2 (COX-2) gene expression via epidermal growth factor receptor (EGFR) to incite inflammation responses. However the mechanism of how Pb^{2+} induced IL-8 expression is still unclear.

Materials and Methods

In this study, we used human epidermoid carcinoma cell line A431 as a model to clarify the mechanism by which Pb^{2+} induces such mediators of inflammation. RT-PCR and promoter assay were performed to analyze the Pb^{2+}-induced IL-8 gene expression. In addition, to identify whether EGFRs participates the signal pathway of Pb^{2+}-induced IL-8 expression, cells were treated with EGFR inhibitors (AG1478 and PD153035) during the exposure to Pb^{2+}.

Results

We found that IL-8 expression was induced by 0.1 μM Pb2+. Using the promoter deletion analysis approaches, our result suggested that the transcription factor AP1 was one of the necessary components in Pb^{2+}-induced IL-8 gene responses. Furthermore, phosphorylation of EGFR was involved in Pb^{2+}-induced signalling.

Conclusions

Our finding indicated the importance of EGFR and transcription factor AP1 in the Pb^{2+}-induced IL-8 gene expression.

P382
Detecting Electrical Signals on Interneuron Dendrites by Voltage Imaging

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Background:

How signals are initiated and transmitted along dendrites of oriens lacunosum-moleculare interneurons (O-LMs), a classical type of dendrite-targeting GABAergic neurons, is unclear. A previous study showed that strong current injection at dendrites can trigger direct AP initiation from dendrites because of high-density expression of dendritic voltage-gated Na+ and K+ channels. Results of electron microscopy also indicate that synaptic density of the distal dendrite is higher than that of the proximal dendrite in O-LMs. Here, we hypothesize that strong synaptic input may initiate ectopic APs on dendrites of O-LMs. Besides, pronounced 'sag' in response to somatic hyperpolarizing current injection suggests the expression of hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels and casts the question of how HCN channel affects EPSP along dendrites of O-LMs. The aim of this study is to set up voltage imaging system and to investigate electrical signals on dendrites of O-LMs.

Materials and Methods:

Acute hippocampal slices were obtained from mice or rats (P16-P22). A voltage-sensitive dye (JPW3028, 2 mg/ml) was loaded into neurons by whole-cell recording for 20-30 min. After recovery for ~2 hr, a whole-cell or cell-attached recording was made again on the same neuron. A laser light source (532 nm) was used to illuminate the neuron and a high-speed CCD camera (up to 10 kHz, RedShirt), which was controlled via Neuroplex (RedShirt), was used to detect the change of fluorescent signal on neurons. A stimulation electrode was placed on CA1 alveus to trigger synaptic inputs onto O-LMs' dendrites.

Results:

First, the optical signal can adequately re-capitulate the waveform of electrically recorded AP. Second, most of synaptically evoked APs were initiated in regions closer to somata and/or axons than farther dendrites. Third, with averaging multiple trials, subthreshold changes of membrane potential on dendrites can be detected.

Conclusion:

Using voltage imaging system, we can examine whether synaptically evoked APs can directly initiate from dendrites of O-LMs. Further, we will apply this technique to study how HCN channels influence dendritic signals of O-LMs. In sum, fast voltage imaging enables us to probe where digital and analogue signals are initiated and how are they transmitted along O-LMs' dendrites.

P383
Study on the anti-fibrosis effect of herb extract in human lung fibroblasts cells

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Backgrounds:

Idiopathic interstitial pneumonia, also known as idiopathic pulmonary fibrosis, is a type of diffuse parenchymal lung disease of unknown etiology.

Oleaceae plants (O.F.), referred to as sweet olives, O.F. possesses various functions, including neuroprotection, the scavenging of free radicals, and anti-oxidation. Transforming growth factor beta (TGF-β) is a vital growth factor that is essential for regulating cell growth, differentiation, immunoregulation, and extracellular matrices. This study investigates the mechanism O.F. on the regulation of pulmonary cellular fibrosis.

Materials and Methods:

The HFL-1 cells were cultured with 5 ng/mL of TGF-β1 for 24 h. After the induction of cell fibrosis, 200 μg/mL of O.F. was added and further cultured for 24 h. Subsequently, the enzyme-linked immunosorbent assay test was performed to quantify the extracellular fibronectins, and a Western blot analysis was conducted to examine the expression of the downstream TGF-β1 (i.e., the SMAD proteins).

Results:

The findings indicated that the presence of 200 μg/mL of O.F. reduced the expression of fibronectins and the TGF-β1 signaling protein, that is, the SMAD protein. Conclusion: O.F. treatment suppressed expression of fibronectin and TGF-β1-mediated signaling by Smads protein. O.F. apparently lessened the lung fibroblasts and decreased the expression of TGF-β1.

P384
Proinflammatory activity of type II interleukin 1 receptor, a decoy receptor

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We have previously demonstrated that type II interleukin 1 receptor (IL1R2), an IL-1 decoy receptor, plays a dual role on regulation of inflammatory signaling, i.e., IL1R2 inhibits the exogenous interleukin 1 (IL-1) signaling on the one hand but activates intracellular IL-1α and its downstream proinflammatory cytokines. To have a better understanding of the proinflammatory activity of IL1R2, we modulated the intracellular IL1R2 levels by ectopic expression or siRNA silencing techniques. In the present study, our results showed that ectopic expression of IL1R2 in HaCaT cells, an immortalized cell line of human keratinocyte, significantly enhanced the expression of IL-6, MMP-1, and VEGF-A. Since T4R2 cells, a tumorigenic cell line derived from HaCaT cells by long-term exposure to inorganic arsenic, expressed a significant amount of IL1R2, we therefore silenced the expression of IL1R2 in T4R2 cells by RNAi technique. Our results consistently showed that silencing of IL1R2 in T4R2 cells decreased expression of IL-6, MMP-1, and VEGF-A. Meanwhile, our results demonstrated that the intracellular levels of IL1R2 are closely associated with migration ability of HaCaT cells. Moreover, IL1R2 silencing reduced the growth rate of T4R2 cells in nude mice. The conditioned medium harvested from T4R2 cells was able to stimulate migration, proliferation, and tube formation of cultured endothelial cells, whereas the angiogenesis activity of conditioned medium harvested from IL1R2 silenced T4R2 cells was significantly suppressed. Furthermore, the enhanced migration ability of endothelial cells culturing in T4R2 conditioned medium was suppressed by antibodies against VEGF-A and IL-6. We also demonstrated that tumors derived from T4R2 cells contained higher levels of CD31 positive cells as compared to those derived from IL1R2 silenced T4R2 cells, indicating more blood vessels formed in T4R2 tumor tissues. Mechanistically, our present studies showed that IL1R2 may work together with cFos and bind to Ap-1 site of promoters of IL-6 and VEGF-A. Taken together, we revealed that intracellular IL1R2 acts as a proinflammatory factor and hence enhances the expression of several inflammatory cytokines involving in angiogenesis and tumor malignancy.

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Investigate the Role of AMPK in Regulating the Nuclear Localization and Tumor Suppressor Activity of the B56γ3 Regulatory Subunit of Protein Phosphatase 2A

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Backgrounds:

Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase which comprises three subunits, including a scaffolding A subunit, a catalytic C subunit, and a variable regulatory B subunit. The B subunits are involved in determining the activity, substrate specificity, and subcellular localization of PP2A. The AMP-activated protein kinase (AMPK) is involved in regulating metabolism and is activated during nutrient deprivation to arrest cell cycle at G1 phase. Similarly, B56γ3 is involved in regulating cell cycle at the G1/S transition. We have previously shown that a domain encompassing a.a 413 to a.a. 461 is critical in regulating nuclear localization of B56γ3, and sequence analysis predicted Ser440, residing within the nuclear localization regulating domain, as a potential phosphorylation site of AMPK. Our study mainly focuses on characterizing the role of AMPK in regulating the nuclear localization and activity of B56γ3, which has been reported to function as a tumor suppressor regulatory subunit of PP2A.

Materials and Methods:

We treated NIH3T3 cells stably expressing B56γ3 with pharmacological activator and inhibitor of AMPK to investigate the phosphorylation of Ser440. In parallel, siRNA knockdown of AMPKα1 was used to investigate the phosphorylation of Ser440 and nuclear localization of B56γ3. Immunofluorescence was used to investigate the subcellular localization of B56γ3. Co-immunoprecipitation analysis was used to investigate the interaction between AMPK and B56γ3.

Results:

We showed that treatment of AMPK inhibitor compound C reduced the phosphorylation levels of Ser440 and nuclear localization of B56γ3, whereas treatment of AMPK activator AICAR increased Ser440 phosphorylation and nuclear localization of B56γ3 in a dose-dependent manner. Further, siRNA knockdown of AMPKα1 reduced the phosphorylation levels of Ser440 and the nuclear localization of B56γ3. Co-immunoprecipitation analysis showed that B56γ3 interacted with the AMPKα1 and overexpression of the constitutively active form of AMPKα1 increased Ser440 phosphorylation of B56γ3.

Conclusion:

In summary, we demonstrate that AMPK may phosphorylate B56γ3 at Ser440 to regulate the nuclear localization of B56γ3, and we are currently investigating whether AMPK directly catalyzed phosphorylation at S440 to control B56γ3 nuclear localization and the role of AMPK in regulating B56γ3 tumor suppressor activity.

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Effects of Deep-Sea-Water on Liver Abnormalities in Type 1 Diabetes Rats

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Backgrounds:

Insulin-dependent diabetes mellitus, so-called type 1 diabetes mellitus (DM), accounts for three percent of all diabetic patients in Taiwan. Type 1 DM is an autoimmune disease, which frequently occurs in children and adolescents. Type 1 DM will increase lipid level in blood and leads to liver abnormality. However, little is known about the influence of type 1 DM on liver damage.

Materials and Methods:

Male SD rats were adopted as experimental animals to induce type 1 DM by administrating STZ. Different concentrations of Deep-Sea-Water (DSW) were used to treat STZ-induced DM rats. Immunoblots, gelatin zymography, TBARs, DPPH and GSH assays were performed to investigate the anti-oxidant activity and the expressions of apoptotic proteins.

Results:

Our results show that DSW not only increase the antioxidant activities but also reduce the inflammatory proteins. In addition, DSW also revels the alleviative effects on expressions of mitochondrial related apoptotic proteins.

Conclusion:

Herein we revealed the beneficial effects of DSW on type I DM rats and, therefore, reasonably conclude that DSW in the future may be useful to provide an alternative choice in treatment and prevention of type I DM.

P387

A Study Of Angiogenesis And Oral Cancer Stem-like Cells

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Backgrounds:

Some studies indicate that tumor sphere has more cancer stem-like (CSCs) cells. Angiogenesis is the propelling force for tumor growth and metastasis. Some cancer patients also accompany drug resistance during treatment, so they have poor prognosis after chemotherapy treatment. A portion of the patients can not be completely cured due to cancer metastasis. Therefore, many researchers highly suspect that cancer stem-like cells and angiogenesis may be the main reasons for this phenomenon.

Materials and Methods:

First, we compared human tongue squamous cell carcinoma (SAS) tumor sphere and monolayer for expression of stem cell genes and related proteins, including CD133, Nanog and ALDH1A1, using quantitative RT-PCR, Western blotting, and immunostaining to analyze these stem cell markers expression in tumor sphere. Next, we utilized angiogenesis PCR array to identify up-regulated angiogenic factors. We also confirmed the findings using real-time PCR on cell-sorted CD133+ cells to reconfirm the data.

Results:

The levels of CD133, Nanog and ALDH1A1 were up-regulated in tumor sphere compared with monolayer based on quantitative RT-PCR, Western blotting and immunostaining, We demonstrated tumor sphere have cancer stem-like cell characteristics. The data of angiogenesis PCR array identified up-regulation of several angiogenic factors, including CXCL10 (C-X-C motif chemokine 10), CXCL3 (C-X-C motif chemokine 3), IL8 (Interleukin 8) and TNF (Tumor nerosis factor). Most of them are chemotactic factors. Cell sorting for CD133+ cells also confirm CXCL3,IL8 and TNF were more expressed in CD133+ cells.

Conclusion:

Our analysis indicated that tumor sphere have cancer stem-like cell characteristics. We identified some angiogenic factors (chemotactic factors) in cancer stem-like cells. The results may help to develop new cancer therapies.

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Effect of Retinoic Acid, Galectin-1 and Gold Nanoparticles on Neuronal Cells

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Backgrounds:

Many investigations suggest that Retinoic acid (RA) plays an important role in neural development. RA is a metabolism activates retinol, which controls physiological processes including development, nervous system function, cell proliferation and differentiation, through the two of nuclear receptors, Retinoic acid receptors (RAR) and Retinoid X receptors (RXR). Galectin-1 (Gal-1) is one of galectin family, GAL-1 is a β-galactosyl binding lectin, which can binding with β-galactosides. GAL-1 can promote cell growth, differentiation , adhesion , and also participate in regulating cell proliferation. Gold nanoparticles (AuNPs) a novel agents, has good biocompatibility and been used in biomedical, investigations suggest AuNPs can promote cell differentiation, and intracellular can regulate cell function.

Materials and Methods:

In this study we used N2A cells (neuroblastoma cells), were divided into four groups with addition of control, RA (10 μM), GAL-1 (200 ng/ml) and AuNPs (10 ppm) separately. Experiments were divided into two parts. First, we use microscope and Micrometrics SE Premium to observe neurite outgrowth, take a photograph and measure.

Second, we use 3-(4,5-dimetnyl-thiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) and real-time quantitative polymerase chain reaction (QPCR) to analysis each groups cell Viability and gene expression.

Results:

Our results show that RA, Gal-1 or AuNPs promotes neurite outgrowth of N2A cells and significantly increases the neurite length. Some studies suggest retinoid X receptors (RXRs) and superoxide dismutase (SOD1) have associate with neurite outgrowth. Therefore, the transcript levels of RXRs and SOD1 in the N2A cells were effectively enhanced by RA, Gal-1 or AuNPs.

Conclusion:

The results show that N2A cell neurite outgrowth will better than control group with RA, Gal-1, AuNPs treat. Neurite outgrowth is the index of neuronal differentiation, Our results show that RA, Gal-1 or AuNPs maybe have neurite repair potential.

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Cycloheximide Stimulates Suppressor of Cytokine Signaling Gene Expression in 3T3-L1 Adipocytes via the Extracellular Signal-Regulated Kinase Pathway

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Backgrounds:

Suppressors of cytokine signaling (SOCSs) can act as regulators of energy metabolism and cytokine signaling in fat cells. They are regulated by hormones and toxicological factors. However, the action of cycloheximide on expression of adipocyte SOCS gene family is unknown.

Materials and Methods:

We identified whether the effects of cycloheximide on the expression of SOCSs gene in 3T3-L1 adipocytes via the MEK or JNK pathway by treatment with the MEK1 inhibitors, U126 and PD98059, or the JNK inhibitor, SP600125. We used real-time PCR and RT-PCR to detect the expression of SOCSs gene. Furthermore, we determined the phosphorylation of signal transduction proteins by western blot assay.

Results:

Using 3T3-L1 adipocytes, we found that cycloheximide up-regulated SOCS-3 mRNA expression in dose- and time-dependent manners. Treatment with actinomycin D prevented cycloheximide-stimulated SOCS-3 mRNA expression, suggesting that the effect of cycloheximide requires new mRNA synthesis. While cycloheximide was shown to increase activities of MEK1 and JNK, signaling was demonstrated to be inhibited by pretreatment with either MEK1 inhibitors U0126 and PD98059, or with the JNK inhibitor SP600125. U0126 and PD98059 respectively reduced cycloheximide-stimulated SOCS-3 mRNA expression, but SP600125 did not antagonize cycloheximide effect. Moreover, cycloheximide was found to up-regulate expression of other SOCS family members, such as SOCS-1, -2, -4, -5, -6, -7, and cytokine-inducible SH2-containing protein (CIS)-1 mRNAs. Such effects varied with the dosage and duration of cycloheximide treatment.

Conclusion:

These results imply the functional MEK1/ERK-mediated pathway is necessary for the cycloheximide stimulation of SOCS-3 gene expression.

P390

Interaction of BP180 and ADAM9 Enhances Cancer Cell Metastasis Potential

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Backgrounds:

Collagen XVII (BP180), a major component of the hemidesmosome is critical in maintenance cell adhesion. Clinical evidences indicated mutation or loss of BP180 leaded to subepidermal tissue separation. In addition, BP180 has showed to play important role of cancer migration and metastasis. However, the detail mechanism is still not clear. Hence, we aimed to investigate the mechanisms of BP180 in regulation of cancer migration and invasion.

Materials and Methods:

We first examine the expression levels of BP180 in different cell lines, including prostate, breast, renal and lung cancer cell lines. Immunoprecipitate ADAM9 followed by immunoblot analysis of BP180 expression was performed. Lentiviral knockdown of BP180 was constructed and cell-tracking assay was performed. Protein degradation of hemidesmosome components after knockdown of ADAM9 was determined by cycloheximide treated prostate cancer cells.

Results:

We noticed the differential expression pattern of BP180 across different cell lines, with strong expression in prostate cancer cells and lowest in lung cancer carcinoma. Inhibition of cell migration activities can be detected in prostate cancer cell line, PC3 after knockdown of BP180 expression. In addition, we also observed decreased of cell attachment, as well as haptotactic migration activities on matrices. Furthermore, we confirmed the interaction of ADAM9 and BP180 regulate hemidesmosome endocytosis and degradation. Knockdown ADAM9 expression decreased the degradation of hemidesmosome components, such as ITGB4. Immunoprecipitation studies confirmed the co-localization of ADAM9 and BP180.

Conclusion:

Our results indicated ADAM9 regulated hemidesmosome endocytosis and degradation by direct interaction with BP180. Knockdown of ADAM9 inhibited cell migration activities by upholding constitutive expression levels of hemidesmosome complex in prostate cancer cells.

Therefore, it is plausible to hypothesis the therapeutic strategy by blocking ADAM9-hemidesmosome interaction could inhibit cancer cell metastasis activities.

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In-vitro susceptibility test of tigecycline against multidrug-resistant *Klebsiella pneumoniae* in Taiwan

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Background:

Increasing emergence of multidrug-resistant Gram-negative pathogens is a major global challenge. *Klebsiella pneumoniae* is one important pathogen which can cause both community and nosocomial infections, and the isolates usually carry one or more antimicrobial resistance especially in the nosocomial infection. Tigecycline is a derivative of minocycline, which was approved by U.S. Food and Drug Administration (FDA) as a novel glycylcycline antimicrobial agent in 2005. Recent studies have showed that tigecycline has *in vitro* activity against multi-drug resistant (MDR) pathogens including *Klebsiella* spp. isolates.

Methods:

In this study, we examined the susceptibility to tigecycline of 80 MDR *Klebsiella pneummoniae* clinical isolates, which were collected nationwide in Taiwan during 1998 (Taiwan Surveillance of Antimicrobial Resistance; TSAR I) and 2006 (TSAR V).

Results :

Results demonstrated that the susceptibility to tigecycline of TSAR I MDR isolates is 79.4% by FDA criteria and 47.6% by EACUST, the TSAR V is 93.6% by FDA criteria and 66.0% by EACUST criteria respectively. The susceptibility of all MDR isolates is 86.4% (FDA criteria) and 63.0% (EACUST criteria), and rate is close to other studies.

Conclusion:

The resistant rate to tigecycline was not increased after the commercialized in Taiwan. It is suggested that tigecycline has the *in vitro* activity against MDR *Klebisella pneumoniae*. This may be helpful in treatment of MDR clinical isolates in Taiwan.

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In vitro MMP9 Inhibition Effectiveness Evaluations for Novel Wound Healing Biomaterials

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Backgrounds:

Matrix metalloproteinases (MMPs) are important in normal wound repair and have been shown to be abnormally expressed in diabetic wounds. Increased MMP-9 activity observed and related to the clinical severity of the ulcer. In this study, novel materials with MMP9 inhibition ability were designed and synthesized for chronic wound healing and the MMP9 inhibition evaluation method was established for the MMP9 inhibition effectiveness evaluation.

Materials and Methods:

The polymer-graft-poly (L-histidine) series materials with MMP inhibition capability were designed and synthesized for the application to chronic wound healing. Those polymers used here including polysaccharide, hyaluronan (HA) and synthetic polymers, poly(vinyl alcohol) (PVA) and ethylene vinyl alcohol (EVOH). Histidines or histidine derivatives were grafted to the polymer (HA, PVA or EVOH), which are hydroxyl group rich materials, and the grafted polymers were evaluated by in vitro MMP-9 inhibition effectiveness assay. The MMP-9 inhibition effectiveness evaluation method was incubated the grafted polymers with APMA-activated pro-MMP-9 at 37℃ , after 2 or 24 h, the residue solutions were harvested and the enzyme activity were determined using modified SensoLyte 520 MMP-9 assay kit.

Results:

MMP9 inhibition method was optimized for the effectiveness evaluations. Modified proMMP9 activation was used to access the wound dressing material evaluation and the concentration of the proMMP9 used here was 100 ng/mL. Our in vitro studies showed that both natural polymer and synthetic polymer-g-histidine significantly reduce MMP-9 activities and the MMP9 inhibition capability was raised by the histidine grafted ratios and polymer-g-histidine concentration. The synthesized grafted polymer can be powder or film type, the established MMP9 inhibition method was suitable to be used in both type materials. In addition, the materials were demonstrated their sustained MMP9 inhibition up to 24 hour.

Conclusion:

In vitro MMP9 inhibition effectiveness evaluation established here for novel wound dressing screening are useful to identify novel material or dressing with MMP inhibition ability.

These results show that histidine grafted materials with MMP9 inhibition properties which are potentially beneficial to wound healing dressing.

Those novel materials designed and synthesized here can be further combined with dressing or coating on the surface for chronic wound care management.

P393

Activation of Non-homologous End Joining (NHEJ) DNA Repair by Nuclear Translocation of RON in Cancer Cells under Hypoxia

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Backgrounds:

Cancer progression is accompanied, and is usually modulated, by alterations of micro-environment of tumor tissue, such as nutrient starvation, hypoxia and pH change. Hypoxia, for example, develops in the solid tumors where cancer cells outgrow the existing vasculature. To survive under such harsh condition, cancer cells must undergo a series of modifications to overcome the O_2- and/or nutrient-deprived stresses. Our laboratory is interested in identifying novel mechanisms of cancer cells in response to cellular stress. We recently reported that membranous RON receptor, in association with epidermal growth factor receptor, transports to the nucleus of cancer cells under serum deprivation. Nuclear RON could act as a transcriptional regulator in response to serum starvation. When cancer cells were exposed to hypoxia, nuclear translocation of RON was demonstrated in TSGH8301 bladder cancer cells. To identify its nuclear targets, nuclear fraction was co-immunoprecipitated with RON, fractionated by high-performance liquid chromatography, and then submitted for tandem mass analysis (Co-IP-HPLC-MS/MS). Among 80 candidate proteins, both Ku70 and DNA-PKs were chosen for further investigation.

Materials and Methods:

Human bladder cancer cell line-TSGH8301 is cultured in DMEM under hypoxia and analyzed by western blot and confocal microscopy. The interaction between RON and Ku70 or DNA-PKs was examined after co-immunoprecipitation. Using MTT assay, the importance of nuclear RON was investigated in the presence of DNA damaging anti-cancer drugs (Cisplatin, Etoposide, Doxorubicin, and Mitomycin).

Results:

Expression level of Ku70 and DNA-PKs remained unchanged; however we demonstrated a time-dependent increase of DNA sensor proteins, phospho-ATM and γ-H2AX, under hypoxia. The interaction between RON and Ku70 or DNA-PKs could be demonstrated after hypoxia for 3, 6, 12 and 24 hr, respectively, as well as by confocal microscopy. The survival rate of TSGH8301 cells with knock-down of RON is significantly lower than vector control. In the presence of hypoxia, knocking down of RON enhances the sensitivity of cancer cells to anti-cancer drugs with capability to induce DNA double strand break.

Conclusion:

RON can be translocated to nuclei of cancer cells in response to hypoxia, and interacts with Ku70 and DNA-PKs to activate DNA repair machinery of bladder cancer cells.

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Taurine Protects against Oxidized LDL-Induced Cytotoxicity in Human Proximal Tubular Cells

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Backgrounds:

Taurine (2-aminoethanesulfur acid, TAU), a semi-essential amino acid in humans, is important for the development of the nervous system, xenobiotic conjugation, osmoregulation, and other physiological processes. Recent studies suggested that TAU protected against renal damages induced by different models via decreasing production of oxidative stress and enhancing activities of antioxidant enzymes, yet the detailed mechanisms were not fully elucidated.

Materials and Methods:

Human proximal tubular cells (HK-2) treated with oxLDL (150 μg/ml) were used to explore the protective effects of TAU. Cytotoxicity of oxLDL on HK-2 cells was studied by measuring methylthiazol tetrazolium (MTT). Apoptotic cell death was characterized by TUNEL assay and stained with Annexin V. Production of reactive oxygen species (ROS) was also measured by using the fluorescnet probe 2', 7'-dichlorofluorescein acetoxymethyl ester (DCF-AM), to observe the activity of antioxidant enzymes. Furthermore, several apoptotic signaling pathway with alteration of mitochondrial membrane potential, cytochrome c release and activation of caspase 3 were also investigated.

Results:

Pretreatment with TAU (100 μM) significantly attenuated oxLDL-induced cytotoxicity, apoptotic features and generation of ROS of renal cells. OxLDL-induced mitochondria membrane potential collapse, cytochrome c release and activation of caspase 3 in HK-2 cells were also suppressed by TAU pretreatment.

Conclusion:

TAU was suggested to protect against oxLDL-induced dysfunction of renal cells, partly due to its anti-oxidant activity.

P395

Synergistic activity of EBNA1-derived polypeptide and 17-AAG suppress HER2/*neu* mediated cell transformation in HER2-overexpressing ovarian cancer cells

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Backgrounds:

Ovarian cancer is one of the most lethal malignancies in women. Currently studies considered the approximately 20–30% of human ovarian cancers arise from the amplification or overexpression of HER2/*neu* gene. It is also well known that the deregulation of HER2/*neu* oncogene is correlated with the progression of tumor metastasis and chemoresistance. Therefore, HER2/*neu* oncogene is a good target to design therapeutic agents against the HER2/neu-overexpressing human cancers. Previous studies demonstrated that EBNA1-derived polypeptide (EBNA1-ΔG/A) effectively suppress HER2/*neu* expression and inhibit cell growth in HER2-overexpressing ovarian cancer SKOV3 cells. However, HER2 is one of the most potent oncogenic client proteins of heat shock protein 90 (HSP 90). The rapid but transient HER2 degradation induced by HSP90 inhibition has been shown in several preclinical studies. 17-AAG, a potent HSP 90 inhibitor, downregulates the expression of many oncogenic proteins including the HER2. In this study, we investigated the effect of combination treatment with EBNA1-ΔG/A and 17-AAG on human ovarian cancer cells.

Materials and Methods:

SKOV3 and SKOV3- EBNA1-ΔG/A cell line has been constructed in our previous studies. Both cell lines were treated with the HSP90 inhibitor 17-AAG, respectively. Cell proliferation was assessed by Cell Titer 96® Aqueous One solution cell proliferation assay, and cell transformation was analyzed by soft agar assay. Analysis of the expression of HER2 and its downstream ERK/AKT signaling pathways was performed by Western blot.

Results:

Our results revealed that the combination treatment with EBNA1-ΔG/A plus 17-AAG was more effective in reducing the HER2 protein level than 17-AAG alone. In addition, the combination of EBNA1-ΔG/A and 17-AAG had synergistic inhibitory effects on cell proliferation and transformation through the inhibition of the AKT and ERK signaling pathways.

Conclusion:

These data together suggest that targeting HSP90, alone or in combination with EBNA1-derived polypeptide may be a promising therapeutic approach in the treatment of HER2/*neu*-overexpressing ovarian cancer

P396

Functional analysis of zebrafish NF-YC (nuclear factor Y subunit C) during early embryonic development

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Backgrounds:

Nuclear factor-Y (NF-Y) is a CCAAT-box-binding transcription factor which is composed of three subunits (NF-YA, NF-YB, and NF-YC). In this study, we used zebrafish as an animal model to study their roles during early developmental stage.

Materials and Methods:

We observed the expression of *nfyc* in zebrafish embryos using whole-mount in situ hybridization. While endogenous *nfyc* was knocked down by antisense morpholino of *nfyc* (*nfyc*-MO), a reduction in eye size was observed in *nfyc*-MO-injected embryos.

Results:

nfyc mRNA expression was found in brain, eyes and neural tube. Immunostaining with neuron-specific antibodies revealed that *nfyc*-MO affected nfyc expression in ganglion cell layer (GCL), inner plexiform layer (IPL) and outer nuclear layer (OPL), suggesting that *nfyc* is associated with the development of retinal neurons. TUNEL assay results revealed the apoptosis in head and eyes of *nfyc*-MO-injected embryos. Our in situ hybridization data showed significant change in eye developmental markers (pax6a, six3b, *mitfa, crx, rx1*,and *vsx2*) , suggesting an impaired cell differentiation in *nfyc*-MO-injected embryos.

Conclusion:

Our results suggested that zebrafish nfyc may affect the development of retinal neurons, and further affect zebrafish eye development.

P397
The functional role of a novel RNA binding protein in the innate immune response

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Background:

The innate immunity is the first line of defense in the body. This system is vital as it provides immediate protective functions when the body is under infection and thus contributes to the clearance of pathogens and the regulation of the adaptive immunity. Toll-like receptors (TLRs) plays an critical role in the innate immunity. They recognize pathogen-associated molecular patterns (PAMPs) upon infection and then initiate downstream pro-inflammatory and antiviral responses. However, dysregulation of inflammatory response has been associated with a variety of diseases, such as cancer, metabolic diseases, inflammatory bowel disease. Thus, the TLR-mediated responses must be tightly controlled. In the previous study, our lab identified an RNA-binding protein (RNABPX) that is upregulated in murine macrophages upon LPS stimulation. Here we would like to address the role of RNABPX in regulating the TLR4-mediated innate immune response.

Materials and Methods:

RNABPX were knocked down in RAW 264.7 cells (murine macrophage cell line) using lentiviruses containing shRNA. The control and shRNABPX RAW 264.7 cells were treated with TLR agonists and the cytokine expression was determined by quantitative PCR. One of the cytokines which was affected was interleukin-10 (IL-10). The cells were induced by LPS then treated with actinomycin D and total RNA was collected to determine the stability of IL-10 in the control and the shRNABPX cells. Furthermore, transcriptional control of IL-10 was determined by Luciferase assay in the control and shRNABP RAW 264.7 cells.

Results:

After knocking down RNABPX in RAW 264.7 cells and treating them with TLR agonists including LPS, Pam3CSK4 and Poly(I:C), IL-10 was found to be upregulated, while IL-6 was downregulated. We focused on the regulation of IL-10 upon LPS treatment and found that the mRNA stability of IL-10 was similar in control and shRNABPX cells, with half-lives of 45 and 50 minutes, respectively. Furthermore, the shRNABPX cells had increased IL-10 promoter activity upon LPS treatment using the luciferase reporter assay.

Conclusion:

Our results showed that RNABPX is responsible for the regulation of the IL-6 and IL-10 cytokines after numerous TLRs stimulation, including TLR4, TLR3 and TLR1/2. Furthermore, we found that RNABPX regulated IL-10 mRNA upon LPS stimulation, and that this regulation occurred at the transcriptional level and not at the mRNA stability level.

P398
從缺

P399
Hypoxia-Induced Retinal Neovascularization in Zebrafish Embryos: a Possible Model of Retinopathy of Prematurity

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Backgrounds:

To establish a zebrafish model of retinopathy of prematurity (ROP).

Materials and Methods:

We established a ROP model using a green fluorescent blood vessel zebrafish transgenic line (*fli*-EGFP) treated with cobalt chloride (CoCl2, a hypoxia-inducing agent) and followed by GS4012 (a vascular endothelial growth factor inducer) since 24 hpf, and we found that numbers of vascular branches and sproutings were significant increased in the central retinal vascular trunks in 3-5 days.

Results:

By a special angiography using tetramethyl rhodamine-dextran which displayed severe vascular leakage through the vessel wall into the surrounding retinal tissue. Furthermore, Real-time quantitative PCR revealed expression of *hif1*α and *vegfaa* to increase by 1.68 and 1.56 folds in comparison with the corresponding control group, indicating increased VEGF signaling in hypoxia condition. Specifically, according to the effect of SU5416 (VEGF receptor tyrosine kinase inhibitor), we demonstrated hypoxia-induced angiogenesis in retina also needs the the effects from VEGF.

Conclusion:

Our findings also provide simple and highly reproducible and clinically relevant ROP model using zebrafish embryos, which may be served as a platform for understanding the mechanisms of ROP development and progression, and provide an efficient way to screen candidate drugs in the future.

P400
Synergistic Activity of Antrodia Camphorata Mycelia Purified Components on Targeting Oral Cancer Stem Cell Characteristics

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Background:

Cancer stem cells (CSCs), also termed as Cancer initiating cells (CICs), representing a rare subpopulation of cancer cells responsible for tumor growth. CICs such as oral cancer -cancer initiating cells (OC-CICs), are often resistant to either radio- or chemotherapy. Therefore, screening and development of new drug candidates that specifically targeting OC-CICs is crucial for future oral cancer therapy.

Methods:

In this study, we used the in vitro cell-based ALDH activity assay system, which has been demonstrated as a CICs marker, to screen for the active purified components from Antrodia Camphorata Mycelia extract (ACME), which can target OC-CICs.

Results:

Herein, we first discovered that ACME purified compounds (YMGKI-1 and YMGKI-2) significantly down regulate ALDH activity of oral cancer cells or OC-CICs, and reduce the self-renewal ability, tumorigenic properties and stemness properties of OC-CICs in vitro. Treatment of YMGKI-1 and YMGKI-2 significantly induced cell death and enhanced the differentiation capability of OC-CICs, respectively. Furthermore, treatment of YMGKI-1 and YMGKI-2 effectively suppressed tumor growth of nude mice bearing tumor xenografts. We found that treatment of YMGKI-1 resulted in down-regulation of mTOR/PI3K/HER2 molecular mechanisms, which play important roles in CICs. Whereas, treatment of YMGKI-2 inhibited the activation of Src, leading to a decrease of EMT and an induction of differentiation of CICs. Finally, combined treatment of YMGKI-1 and YMGKI-2 drastically induced the cell death of OC-CICs through blocking the dual-pathway.

Conclusion:

Our studies reveal the combination of ACME purified components specifically target on oral cancer stem cell characteristics. Eventually, we can further discover different future drug candidates from ACME purified components for alternative oral cancer treatment.

P401
Effects of macrophage on lipid droplets formation and lipolysis of adipocytes

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Obesity is a chronic and low grade inflammation which can cause insulin resistance and affect the functions of adipocytes. Different types of macrophages (M1 or M2) may be involved in the metabolism of adipose tissue. In our previous studies, the conditioned medium from anti-inflammatory macrophages (M2) can affect the 3T3-L1 adipocytes differentiation and decrease the size and quantity of lipid droplets inside of adipocytes. The adipocyte differentiation marker (PPAR-γ, AP-2) had no significant change. Then we focused on Fat-specific protein 27 (FSP27) and Perilipin, both are lipid-droplet coating proteins and correlated with oil droplet formation and lipolysis. Our data showed that the RNA and protein levels of perilipin are significant decrease in the M2 conditioned group. It is suggested the M2 conditioned medium contained certain secreted factors to affect the lipid droplets formation and enhance lipolysis.

P402
Investigation of The Regulation of *Irxl1* Gene in Cartilage Development

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Backgrounds:

Iroquois homeobox-like 1 (Irxl1) is a novel member of the TALE superfamily of homeobox genes that is most-closely related to the Iroquois class. During zebrafish development, *Irxl1* is expressed mainly in the brain and the first two pharyngeal arches. Antisense morpholino knockdown of *Irxl1* results in defective pharyngeal cartilage in zebrafish larvae. Analysis of *Irxl1* promoter reveals four putative Sox5 binding sites in the -1342/+249 region. Since the Sox trio genes (Sox5,Sox6,Sox9) play a key role in regulating chondrocyte differentiation, we investigate whether and how *Irxl1* is involved in Sox5-mediated cartilage development.

Materials and Methods:

Whole-mount *in situ* hybridization was performed to compare the expression of *Irxl1* and *Sox5* during zebrafish embryogenesis. Antisense MO was injected to knockdown *Sox5* and alcian blue staining was used to reveal pharyngeal cartilage morphogenesis. Transcriptional control of Sox genes on *Irxl1* promoter was assayed using luciferase reporter coinjected with Sox5 or Sox5 MO into embryos. In addition, lentiviral-based shRNA knockdown of *Irxl1* was performed in mouse ATDC5 cells to study the function of Irxl1 on chondrocyte differentiation. The morphologic features and gene expression patterns of chondrocyte differentiation were investigated.

Results:

Irxl1 and Sox5 were co-localized at the pharyngeal arch of zebrafish embryos at 48hpf. At 120 hpf, Sox5 morphants displayed defective pharyngeal cartilage formation similar as in Irxl1 morphants. Overexpression of Sox5 repressed *Irxl1* promoter activity, and knockdown of Sox5 enhanced it. The Sox trio genes together can activate Irxl1 promoter activity whereas Sox5/6 act the opposite way. Irxl1 knockdown results in delayed expression of the late phase differentiation markers of ATDC5 cells.

Conclusion:

Both Sox5 and Irxl1 play a role in pharyngeal cartilage development in zebrafish. Irxl1 is transcriptionally regulated by Sox5 and may participate in chondrocyte differentiation in the Sox- mediated pathway.

P403
Ceramide decreases insulin signaling through activating mTORC1 pathway

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contribute equally.

Backgrounds:

Insulin resistance caused by obesity is related to chronic accumulated free fatty acids in plasma. In cells, ceramide converted from fatty acids is known to negatively regulate insulin actions. In this study, we intend to investigate the detail molecular mechanisms of ceramides on insulin signaling.

Materials and methods:

C2C12 myotubes were treated with ceramides at different dosage and timing. The insulin signaling molecules such as insulin receptor, Akt, S6K, and their phosphorylated status were analyzed by Western blots. RNAi of signaling molecules such as Rheb was also adopted to verify the relationship. C2C12 cells were infected with Rheb shRNA and followed by treatment of ceramide to assess various signaling molecules. In addition, cells were fractionated into cytosol and membrane to detect the distribution of signaling molecules involved in insulin pathway.

Results:

We confirmed that 100 μM C2-ceramide reduced Akt Ser473 phosphorylation stimulated by insulin without affecting upstream signaling molecules, such as IRS1 and PI3K. Simultaneously, elevated phosphorylation of S6K Thr389 and a decrease in Akt Ser473 phosphorylation was observed under such a condition. The phenomenon was reversed by mTORC1 inhibitor, rapamycin. Rheb RNAi also prevented the decrease of Akt Ser473 phosphorylation caused by C2-ceramide plus insulin treatment. Moreover, membrane-bound TSC2 translocated from the cytosol after C2-ceramide plus insulin incubation implied that TSC2 could also be involved.

Conclusion:

Taken together, we explored a novel pathway of C2-ceramide-impaired insulin signaling through activation of mTORC1 cascade. Downstream of PI3K or upstream of TSC2 may be possible molecules affected by ceramides.

P404
Identification and Characterization of An Interacting Protein of Metastasis-Associated NDP Kinase A

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Background:

Metastasis remains as a major cause of death in cancer patients. Nucleotide diphosphate kinase A (abbreviated as NDP kinase A or NDPK-A) is the first metastasis-associated protein identified in humans, and encoded by the *nm23-H1* gene. NDPK-A was originally identified as a metastasis suppressor in melanoma, and then in breast carcinoma. In contrast, our lab found that NDPK-A functions as a metastasis promoter in neuroblastoma. In addition to overexpression, our lab has detected the S120G mutation of NDPK-A in patients with advanced stages of neuroblastoma. These NDPK-A alterations not only abrogate neuronal differentiation and increase invasiveness of neuroblastoma cells, but also promote tumor metastasis in mice. To date, the molecular mechanism by which NDPK-A participates in metastasis still remains largely unknown. In this study, we aim at identifying interactive protein partners of NDPK-A in order to improve our understanding of the molecular network of tumor metastasis.

Materials and Methods:

Yeast two-hybrid system was used to identify interacting proteins of NDPK-A. One of the interacting proteins, termed Protein Y, was cloned into mammalian pIRES-DsRed or lentiviral pLKO-AS2 expression vectors to generate stable transfectants and transducents respectively in human neuroblastoma NB69 and SH-SY5Y cells. Antibodies specific for Protein Y or a tagged HA epitope were used to perform immuno-cytochemistry, Western blot analysis and immuno-precipitation.

Results:

We have identified Protein Y as one of novel interacting proteins of wild type NDPK-A, but not NDPK-A^{S120G} mutant in the yeast two-hybrid system. Interestingly, Protein Y is also overexpressed in advanced stages of human neuroblastoma based on public microarray data. The mRNA of Protein Y was differentially expressed in various human tissues based on our Northern blot analysis. Immuno-cytochemistry shows that Protein Y was co-localized with NDPK-A to cell nuclei, even though NDPK-A was also found in cytosol. Nuclear localization signal(s) of Protein Y appear to reside in both N- and C-termini based on the mutation experiment. Studies are undergoing to dissect the domain(s) required for the interaction of NDPK-A with Protein Y by immuno-precipitation. Effects of Protein Y on cell proliferation and migration are being examined.

Conclusion:

Identification and characterization of novel Protein Y as a NDPK-A interacting protein would improve our understanding of the molecular mechanism by which NDPK-A is involved in neuroblastoma metastasis.

P405
Proteomic analysis of CYP26B1 associated proteins in betel quid induced oral cancer patients

許正傑

There are about 600 million betel quid chewers in the world. In 2004, the International Agency for Research on Cancer (IARC) had declared betel quid (BQ) chewing without tobacco to be carcinogenic to humans, and areca nut (AN) itself is carcinogenic to humans in carcinogen. The practice of BQ chewing is widespread in Taiwan, with approximately three million habitual users,about 13% of Taiwan population. Previous study indicated the quantity of habitual BQ use (quids/per day) is positive correlated significantly with blood concentration of arecoline and arecaidine, the two major alkaloids in AN. A genome-wide microarray chip analysis detected 12 genes may provide susceptibility to betel quid chewers who arecoline damage biomarkers, and a candidate gene CYP26B1 which is significant to betel nut caused cancer and cancer of the process was selected. From human paired tissues. preliminary data confirmed *CYP26B1* gene expression was consistently higher in cancerous tissues compared to non-cancerous tissues. Additionally, information of BQ chewing on *CYP26B1* expression with oral cancer, CYP26B1 gene expression in oral cancer may be highly related with substance use (especially the use of betel nut). This preliminary study results suggested that CYP26B1 gene associate with the metabolic mechanism of betel nut. Furthermore. Arecoline treated oral cancer cell line Ca922 and HGF shows CYP26B1 level was increased. We also study the proteomics of CYP26B1 of BQ-induced oral cancer. LC-MS/MS method was used to annotate proteins with high CYP26B1 expression in oral cancer. Taking together, CYP26B1 was confirmed to be overexpressed in BQ-induced oral cancer, and proteomic studies was used to investigate the underlying mechanisms.

P406
Anticancer Molecular Mechanism of *Ganoderma* Triterpene T-612

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Backgrounds:

Ganoderma (Lingzhi) has been a notable Chinese traditional medicinal herb for centuries. T-612, a triterpenoid from *Ganoderma tsugae*, was previously found to possess anticancer activity, but the mechanism remains unclear. Breast cancer is the most life threatening malignant disease in women worldwide. In this study, we studied anticancer activity of T-612 in breast cancer cell line and characterized its inhibitory activity on topoisomerase II (Topo II), an enzyme essential for DNA replication and transcription, and crucial for cancer cell proliferation. Whether T-612 is able to enhance the efficacy of breast chemotherapeutic drug doxorubicin was also studied.

Materials and Methods:

Topo II inhibition and Topo II-DNA complex formation abilities of T-612 were investigated *in vitro* by DNA relaxation assay. MDA-MB-231, a human breast carcinoma cell line, is used to analyze the effects of T-612 *in vivo*. Molecular mechanism and cellular response towards drug were explored by real-time PCR, cell cycle analysis and apoptosis assays.

Results:

We found that T-612 irreversibly inhibited Topo II activity with an IC_{50} of 1.2 μM. Both in vitro and *in vivo* data showed that T-612 caused accumulation of Topo II-DNA cleavable complex. In MDA-MB-231 cells, T-612 treatment caused the increases in DNA breaks and DNA damage-related gene expression, G2/M cell cycle arrest, and apoptosis. These responses suggest that T-612 is a Topo II poison. We found that T-612 is able to enhance the efficacy of doxorubicin (another Topo II inhibitor). The combination of T-612 and doxorubicin enhanced DNA-Topo II complex formation in MDA-MB-231 cells. The combined treatment displayed synergistic cytotoxicity through higher level of G2/M arrest and apoptosis compared to doxorubicin alone.

Conclusion:

Topo II poisoning could be a mechanism contributing to the anticancer activity of T-612. In clinical, the breast cancer cells which overexpress estrogen receptor, progesterone receptor, or HER-2 genes are satisfactorily treated by targeted therapy. However, these targeted therapeutic drugs are not effective for triple negative breast cancer (TNBC), which should be treated by chemotherapy. The synergistic effect of T-612 and doxorubicin in TNBC cell MDA-MB-231 indicates that T-612 is a valuable anticancer drug/adjuvant for TNBC.

P407
The enzyme activity of the branched-chain alpha-ketoacid dehydrogenase complex using high-performance liquid chromatography

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Backgrounds:

Maple syrup urine disease (MSUD) is an autosomal recessive inborn error disorder caused by a congenital defect of the mitochondrial branch-chain α-ketoacid dehydrogenase complex (BCKDH). It is the second most common amino acid metabolic disorders identified in newborn screening in Taiwan. Although molecular analysis is a technically easy way for early confirmation in some inherited metabolic disorders, it can be time-consuming as the disease-causing mutations for MSUD can be found in any of the four genes encoding the subunits of BCKDH. We therefore develop a rapid and simple enzymatic assay for early confirmation using high performance liquid chromatography (HPLC).

Materials and Methods:

Human lymphocytes were isolated from heparinized or EDTA venous blood and cultured with the stimulation of phytohaemagglutin (PHA) for 5 days. According to a modification of a previously reported method (Tajima et al., 2004), a crude enzyme solution was incubated with 50mM Tris-HCl (pH 10.0), 0.2mM EDTA, 7.5mM 2-ketoisocaproic acid, 7.5mM Coenzyme A trilithium salt, 35mM MgCl2, 1mM TPP, 4mM NAD+ and 2% FBS at 37oC for 60 min. The reaction was terminated by adding 0.3N HClO4. Denatured protein was removed by centrifugation, and a 20μl aliquot of the supernatant was analyzed by HPLC. The substrates and the product were separated using a reverse-phase C-18 column of Finepak STR ODS-II (150mm x 4.6mm). The mobile phase was composed of 0.1M NaH2PO4 (pH 4.0) and 15% v/v acetonitrile, at a flow rate of 1.5ml/min. The production of isovaleryl-CoA was quantified by an external standard method.

Results:

BCKDH was found to be unstable, particularly in disrupted lymphocytes, gentle operation was strongly recommended. Ten normal controls and one MSUD patient were recruited in this study. BCKDH activity in normal samples was ranged from 2.2~5.6 pmol/min/106 lymphocytes (mean ± SD = 3.5± 0.9), whereas in samples from patient was 0.4 pmol/min/106 lymphocytes. The relative residual activity in the MSUD patient was calculated to be 11.7% of the average in normal subjects.

Conclusion:

The enzyme assay by HPLC is a simple and sensitive assay for confirmatory diagnosis of MSUD detected by newborn screening. This is the only study performing the BCKDH enzyme assay and reported a reliable reference range for the enzyme in Taiwan.

P408
Helicobacter pylori CagA Expression Pattern Correlates with Disease Outcome

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Backgrounds:

Gastric cancer is the second most common cancer worldwide. The occurrence is especially high in East Asia. *Helicobacter pylori (H.pylori)* has been closely linked to chronic gastritis, peptic ulcer, gastric cancer and MALT-lymphoma. Previous study indicated that in Western countries, *cag*A is present in about 50 to 60% of H. *pylori*. Among these, 60% are EPIYA-ABC, 20% are EPIYA-ABCC, and the rest of the 15% are EPIYA-ABCCC and others. *H. pylori* containing *cag*PAI or carrying more EPIYA C repeats is associated with gastric cancer, but not all infections could find a relationship between *cag*A genotype and disease outcome, such as in East Asia. The aim of our study was to investigate cagA genotype and *CagA* biological function among *H. pylori* strains from clinical patients.

Materials and Methods:

In this study, we used PCR and western blot to analyze *cagA* genotype and its protein expression pattern in different *H. pylori* strains isolated from 99 patients with chronic gastritis, gastric ulcer, duodenal ulcer and gastric cancer.

Results:

There was no significant correlation between diseases and cagA EPIYA types. The majority of the *cagA* EPIYA type is ABD type (95.5%) followed by AABD type (2.9%), BD type (1%) and ABXD type(0.7%). From the western blot analysis of CagA protein from 99 *H.pylori* strains, we found more than one *CagA* protein bands in some *H.pylori*, including $p135^{CagA}$, $p110^{CagA}$ and $p100^{CagA}$. The bands can be distinguished into four types, and the band patterns correlate with the disease outcome.

Conclusion:

In our result, there is a significant correlation between disease outcome and CagA protein expression patterns.

P409

Ganoderma Triterpenoid GC538 Enhanced Anti-cancer Activity of Gefitinib in Drug-resistant Cell Line.

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Backgrounds:

Non–small cell lung carcinoma (NSCLC) accounting for 80-85% of lung cancer incidence often harbors epidermal growth factor receptor (EGFR) mutations. Gefitinib is an EGFR tyrosine kinase inhibitor especially effective for NSCLC patients with EGFR mutation, but the development of drug resistance is a challenge in clinical practice. Therefore, therapeutic strategies enhancing efficacy of gefitinib in the drug resistant cells would be valuable. Ganoderma is a well-known traditional Chinese medicinal herb been used for centuries in Asia. In this study, a novel triterpenoid was isolated from *G. colossum* and its efficacy in gefitinib-resistant cells was studied.

Materials and Methods:

Human lung adenocarcinoma cell line PC9 and gefitinib-resistant cell line PC9/gef were the experimental models of this study. Triterpenoid GC538 was isolated from G. colossum through ethanol extraction, ethyl acetate partition, silica gel chromatography and high performance liquid chromatography. Drug efficacy was evaluated by cell viability measurement and apoptosis assays.

Results:

PC9/gef cells were less sensitive to gefitinib than PC9 cells with the half maximal inhibitory concentration (IC_{50}) at 26 μM and 13 nM, respectively. Gefitinib treatment inhibited the phosphorylation of EGFR, AKT and ERK in both cell lines. This result indicated that the gefitinib-resistance of PC9/gef is not through the EGFR pathway. In PC9/gef cells, the combined treatment of gefitinib with GC538 displayed synergistic cytotoxicity (combination index<1), and enhanced cell apoptosis as shown by the increases in the cell populations with lowered mitochondrial membrane potential (6%), with fragmented DNA (19%), with caspase-9 and caspase-3 activation (13% and 14%, respectively).

Conclusion:

Novel triterpenoid GC538 was isolated from G. colossum. The compound has the potential to be an adjuvant to enhance efficacy of gefitinib in gefitinib-resistant cells.

P410

Evaluation of the Genebuster Rapid TB Diagnostic Kit for Detection of Mycobacterium tuberculosis Complex in Clinical Specimens

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Backgrounds:

Control of the global Tuberculosis spread has been slowed down by the lack of a simple and cost-effective diagnostic test that can be performed in poor and resource-limited countries. We have developed a rapid and low-cost Taqman qPCR base TB diagnostic test, Genebuster Rapid TB Diagnostic Test, which includes a single-tube DNA extraction step prior to direct nucleic acid analysis by a fast Taqman qPCR. Each diagnosis from specimen extraction can be finished in 90 min and the cost can go down to $15 per diagnosis.

Materials and Methods:

From June 2011 to September 2012, a total of 213 clinical specimens, including 69 acid-fast smear-positive and 144 acid-fast smear-negative specimens, were collected at Sijhih Cathay General Hospital, Taipei and investigated. Of those samples, 203 were sputum, 1 was pus, 3 were lung tissues, 3 were synovial fluid, 2 were pleural effusion, and 1 was bronchial washings. Diagnostic cultures performed with 7H11 agar were used as the reference method. Specimens were digested and decontaminated by the *N*-acetyl-L-cysteine-NaOH method. After wash with the phosphate buffer (67 mM, pH 6.8) and centrifugation, pellets were extracted with the Genebuster Extract buffer containing internal control then Taqman qPCR was performed with 2 ul of the extracts. The qPCR conditions were as follow: 95°C for 3 min, then 45 cycles of 95°C for 3 seconds and 65°C for 20 seconds. Fluorescence signals for the amplifications of the internal control (VIC) and TB target gene Rv3618 (FAM) were simultaneously recorded for the further data analysis. Sensitivity and specificity were calculated to evaluate staining and the Genebuster Rapid TB Diagnostic Kit in comparison with culture as a gold standard. The Ct value of the internal control had to be within 25±1 as a successful test and the cut-off Ct value for TB positive sample was set at 42.

Results:

Of 213 specimens, four acid fast-positive and 10 acid fast-negative were culture positive for nontuberculous mycobacterial (NTM) species. These specimens were all tested negative by the Genebuster Rapid TB Diagnostic Kit. The sensitivity and specificity of the Genebuster Rapid TB Diagnostic Kit compared with those of culture were 90% and 95%, respectively. Whereas, the sensitivity and specificity of the traditional Acid Fast smear method compared with those of culture were 76% and 98%, respectively.

Conclusion:

In this pre-clinical trial, we demonstrated that the Genebuster TB Diagnostic Kit can be used to detect *Mycobacterium* tuberculosis in not only sputum, but also lung tissues and bronchial washings. This kit allows detection of TB in approximately 90 min and has potential to provide quick and accurate diagnosis. This work was support in part by research grants (EG-31-08-10) from Southern Taiwan Science Park, the National Science Council, Taiwan.

P411

Relationship Between Monoamine Oxidase A Gene And Smoking Behavior In Young Adults

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Backgrounds:

Monoamine oxidase A (MAOA) may be associated with smoking behavior.

Materials and Methods:

In this study, 378 males aged 20~25 years were enrolled. A self-report questionnaire regarding behavior of smoking was requested and polymorphisms of MAOA *EcoRV* and MAOA Variable Number Tandem Repeat (VNTR) were determined by the methods of PCR-restriction fragment length polymorphism and PCR, respectively. For MAOA *EcoRV* polymorphisms, restriction enzyme *EcoRV* yielded 260- base pair (bp) and 297-bp fragments for homozygous wild-type subjects (1460T/O) and only a 557-bp band for those carrying homozygous variation (1460C/O). For MAOA VNTR polymorphisms, the primers used yielded 321- and 351-bp fragments corresponding to the 3- and 4- repeat alleles, respectively.

Results:

Analysis of the questionnaire revealed that there were 16 quitters, 190 current-smokers and 172 never-smokers. Since number was too small, the 16 quitters were neglected for further investigation. Odds ratios (OR) of smoking were 0.961 (P = 0.866) and 1.220 (P = 0.391) in the subjects carrying MAOA *EcoRV* 1460 T/O compared with the variant-carriers (1460 C/O) and in those possessing MAOA VNTR 3 compared with the variant-carriers (MAOA VNTR 4), respectively. When the 190 current-smokers were divided into moderate/heavy smokers and light smokers, the ORs of moderate/heavy smoking were not statistically significant between MAOA *EcoRV* 1460 T/O and MAOA *EcoRV* 1460 C/O carriers and between MAOA VNTR 3 and MAOA VNTR 4 carriers (OR = 0.551, P = 0.080 and OR = 0.665, P = 0.242, respectively).

Conclusion:

In conclusion, variation status of MAOA *EcoRV* and MAOA VNTR are not related to smoking behavior in male Taiwanese adults.

P412

Exploring Brain Monoaminergic System in ADHD Animal Model Using Nuclear Medicine Imaging

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Backgrounds:

Attension-deficit/hyperactivity disorder (ADHD) is a kind of behavioral disorder. Patients' symptoms include difficulty in sustaining attention, impulsivity, and hyperactivity. ADHD may affect on children and the symptoms can be continually persisted into adulthood. Even though ADHD is yet to be completely established based on pathopsysiology; in addition, according to the previous studies, they showed that the mainly affected systems seem to be involved within the dopamine and serotonin systems. Moreover, the mechanisms of ADHD therapeutic drugs, such as methylphenidate (MPH), were not completely to be understood.

Materials and Methods:

We will use two radiopharmaceuticals, [^{123}I]ADAM (targeting to serotonin transporters) and [^{99m} Tc]TRODAT-1 (targeting to dopamine transporters) coupled with single photon emission computed tomography (SPECT) to imaging the ADHD rat models to address following issues: 1) the possible mechanism of the pathophysiology and validity of these imaging techniques. 2) Evaluating the correlation between imaging changes and therapeutic effects after MPH treatments in the ADHD animal models in order to understand the therapeutic mechanism of MPH.

Results:

The preliminary data shows the two ADHD rat models revealed the ADHD behavior; they both showed hyperactivity than normal rats. In SHR rat (spontaneous ADHD model), it shows higher [^{123}I]-ADAM uptakes in various brain regions than those of WKY rat (control group). In contrast, the [^{123}I]-ADAM uptakes in brain regions of neonatal 6-OHDA lesion SD rat were less than those of normal SD rat.

Conclusion:

With this study, the roles of monoaminergic system in ADHD may be well understood. Hence, provide useful information in developing the new drugs, and develop better methods to monitor their therapeutic responses.

P413

Comparison of [^{18}F]BF_4 and [99mTc]TcO4 Uptake/ Dynamics in Thyroid Tissues with Various NIS Expression or Under Different Pharmacological Treatment

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Backgrounds:

The thyroid gland with Na+/I- symporter (NIS) expression can uptake radioiodine and tetra-hedral complex such as TcO4 and, thus, allows its function assessed by these radionuclides. Recently [18F] BF4 (TFB), another NIS substrate, is investigated for the potential use as thyroid imaging in PET. We propose to compare TFB, TcO4 and iodine uptake and their dynamic change in thyroid tissues with various NIS expression.

Materials & Methods:

Male SD rats (6~9 wks, 250~300 g) were used throughout the experiments. Intervening reagents including ClO4 (3 mg/kg,i.v), methimazole (5mg/day x 10days,i.p) and thyrotropin (1IU x 4 days, i.p) were used to alter NIS expression. After [99mTc] TcO4 and [18F]TFB 1mCi, i.p., PET and gamma camera were utilized for animal imaging and ex vivo study was conducted by tissue counts by gamma counter normalized by tissue weight.

Results:

By animal study, we noted similar time-activity curves with a stable thyroid/background ratio 40 min post injection (TcO4 vs. TFB, 2.41: 2.14) and similar bio-distribution (accumulated in stomach and thyroid while excretion from genitourinary route). In normal thyroid functional status, TFB PET and TcO4 scintigrapghy demonstrated fairly equivalent contrast (neck muscle as background). Based on imaging analysis, the ClO4 treatment lead to reduced thyroid uptake with 26% of decrement for TcO4 vs. 74% for TFB. After TSH treatment, the enhanced tracer uptake in thyroid appeared to be more intense on TFB PET than on Tc99m scan.

Conclusion:

The preliminary results demonstrate that, in modulation of NIS function with ClO4 and TSH, respectively, TFB PET shows more pronounced change than TcO4 imaging to detect NIS alteration. It suggests that TFB PET might be more sensitive to detect the subtle change of NIS function. As PET can offer better quantitative data, TFB might be an alternative to measure thyroid functional status in conjunction with the use of PET. It warrants for further clinical correlation.

P414

The TGFBRI-regulated microRNA is a critical mediator in the mobility of trophoblast through epigenetic modification

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Backgrounds:

There are many similarities between cancer cell invasion and trophoblast invasion. Recently, the researcher also postulated that the invasive growth of tumor and blastocyst implantation may share the similar mechanisms of epigenetic regulation. However, there are few studies concerning the invasion pathway of trophoblast. MicroRNAs (miRNAs), a novel class of gene regulator that are involved in many biological processes. The purpose of our study is to identify the TGFBRI regulated miRNA, which participates in TGFBRI mediated trophoblast mobility.

Materials and Methods:

To investigate the role of TGFBRI level in the regulation of trophoblast cell invasion in trophoblast-like 3A-sub-E, JAR and JEG-3 choriocarcinoma cells was performed. In order to identify TGFBRI-mediated mobility in trophoblast-like cell and choriocarcinoma. Subsequently, ectopic expression or knockdown of TGFBRI and its downstream signaling affects mobility properties of trophoblast cell. In this study, we used a miRNA microarray strategy to identify the expression level of thousands miRNA and confirmed by qPCR and western blot. After confirming by functional assays, these miRNAs will be compared with GEO database in invasive trophoblast group and analysed by Gene set enrichment analysis (GSEA).

Results:

We used lentiviral based shRNA strategy to knock down TGFBRI in trophoblast cells. To search for miRNAs regulated by TGFBRI in trophoblast cells, we further applied TGFBRI-silenced cells and TGFBRI-overexpressed cells to miRNA microarray study. Furthermore, we found that TGFBRI downregulation promoted cell migration and invasion in transwell assay. By using TGFBRI inhibitor SB231542, we found that increased cell migration and invasion in 3A-sub-E cell.

Conclusion:

Trophoblast invasion involved in TGFβ signaling through non-canonical pathway. To detect critical TGFBRI-relative miRNA that may provide early diagnostic marker for preeclampsia. The results of this study may apply to explore the new blood or serum early detection and treatment strategy for preeclampsia.

P415

Study of Differences in Differentiation between Mouse Embryonic Stem Cells and Induced Pluripotent Stem Cells

林煒哲

Pluripotent stem cells possess the powerful ability to replicate indefinitely and can differentiate into various cell types derived from three germ layers. It has been reported that induced pluripotent stem cells (iPSCs) reprogrammed from somatic fibroblasts have been generated by transfecting four transcription factors including *Oct4, Sox2, Klf4* and *c-Myc*. After the reprogram, iPSCs has provided great advantages in many applications, such as developmental studies, drug screening, and autologous cell transplantation.

In this study, we compared the neural differentiation ability between two pluripotent stem cells, mouse embryonic stem cells (mESCs) and mouse induced pluripotent stem cells (miPSCs) with the neural inducer “retinoic acid (RA)” treatment. After RA-induction, both mESC- and miPSC-derived cells exhibited neuron-like processes. The potency of neurodifferentiation was different between mESCs and miPSCs after RA-induction. In order to clarify this phenomenon, we further characterized the mESC- and miPSC-derived cells by reverse transcription polymerase chain reaction (RT-PCR)/quantitative polymerase chain reaction (qPCR) and immunocytochemical approaches. More mesodermal lineage cells could be found from miPSCs after RA-induction.

Protein levels of retinoic acid receptors (RARs) involved in RA signaling pathway were also examined in both stem cells. The protein level of RARα in mESCs was higher than that in miPSCs. Using the RARα antagonist to attenuate RARα activity resulted in down-regulation of RARα and β-tubulin III in both stem cells. On the other hand, the level of RARβ was higher in miPSCs before RA treatment, but was dramatically down-regulated after RA-induction in comparison with mESCs. Our data indicated that the propensity of neuroectodermal differentiation could be correlated with the different distributions of RARα and RARβ induced by RA treatment between mESCs and miPSCs.

We suggested that the cell memory of miPSCs could be one of the key factors triggered the mesodermal differentiation. The neuroectodermal differentiation could be easily induced by RA treatment via RAR signal pathway in the pluripotent mESCs.

P416

Effects of HYS-32 on Microtubule and Microtubule-Associated Protein EB1 in Rat Astrocytes

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HYS-32 [4-(3,4-dimethoxyphenyl)-3-(naphthalen-2-yl)-2(5H)-furanone)] is a novel analogue of combretastatin A-4 (CA-4) containing a cis-stilbene moiety. We have previously demonstrated that treatment of rat primary astrocytes with HYS-32 (5 μM) for 24 h caused a random-coiled staining pattern of microtubule (MT). In the present study, immunofluorescence and confocal microcopy showed that HYS-32 induces MT catastrophes in a dose- and time-dependent manner, whereas the protein levels of β-tubulin remain unchanged as demonstrated by Western blot analysis. In control astrocytes, MTs projected toward the cell periphery and MT plus ends reached the cell edge. In HYS-32-treated astrocytes, MT catastrophes occurred at the cell periphery and the numbers of cell cortex-bound MTs were greatly reduced. However, removal of HYS-32 from culture medium rescued the HYS-32-induced MT catastrophes in astrocytes. Double immunofluorescence microscopy further revealed that EB1, a MT-associated protein, accumulate at growing MT plus ends, where they appear as bright comet-like structures. Treatment of HYS-32 depleted EB1 accumulation at MT plus ends and resulted in a ubiquitous distribution of EB1 along the MTs. Furthermore, HYS-32 induced GSK3β activation in astrocytes without changing the levels of EB1. Pre-treatment of astrocytes with GSK3β inhibitor SB415286 attenuated the HYS-32-induced MT catastrophes. Taken together, our results suggest that HYS-32 causes MT catastrophes by affecting MT−EB1 interactions via a GSK3β-mediated signaling pathway.

P417

Wharton's Jelly Mesenchymal Stem Cell Ameliorate Diabetes by Inducing Insulin-Producing Cell Differentiatinn and Suppressing T Cell Mediated Autoimmunity in NOD Mice

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Backgrounds:

Type 1 diabetes mellitus (T1DM) is caused by T cell mediated autoimmune destruction of pancreatic β-cells. Recently stem cell therapeutic approach has been implicated for the treatment of T1DM. Systemic administration of mesenchymal stem cells (MSCs) have been shown to be incorporated into a variety of tissues and have immunosuppressive effects, results in the regeneration of pancreatic islets. We showed that pancreatic endocrine precursors generated from human umbilical cord (Wharton's Jelly) MSCs are potential cell sources to treat diabetic animals. However, the underlying mechanisms remained to be elucidated.

Materials and Methods:

The purpose of this study was to discern whether the transplanted MSCs are able to differentiate into insulin-producing cells in pancreas and modify immunological responses in NOD mice. Wharton's Jelly MSCs were transduced with green fluorescent protein (MSC-GFP) by lentiviral vector and then injected into retro-orbital venous sinus in NOD mice. Fluorescent islet-like cell clusters in pancreas of NOD mice could be tracked 7 days post MSC-GFP transplantation.

Results:

Significant decreases of blood glucose and higher survival rate were found in MSC-GFP treated NOD mice as compared with PBS control. Colocalization of human C-peptide and GFP could be found in pancreas of NOD mice 23 days post transplantation of MSC-GFP. Additionally, significant higher human C-peptide and mouse insulin, more intact islets, and less severe insulitis could also be detected in these mice. Systemic and local reduction in the abundance of auto aggressive and IL-17 producing T cells together with an increase in regulatory Tcells were found in these mice. Furthermore,in the pancreas of NOD, we observed an increase of antinflammatory cytokines and decrease of dendritic cells.

Conclusion:

Wharton's Jelly MSCs can differentiate into insulin-producing cells and have immunosuppressive effect in NOD mice.

P418

Effect of Hyperbaric Oxygen and Hyperbaric Air on Stem Cell-based Tissue Engineering

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Backgrounds:

Cartilage defects are accompanied by persistent pain and functional limitations of the joint and are therefore considered a severe medical and therapeutic problem. Cartilage has a poor intrinsic healing response, and neither the innate healing response nor current clinical treatments can restore its function. Therefore, articular cartilage tissue engineering is a promising approach for the regeneration of damaged tissue. Because cartilage is exposed to mechanical forces during joint loading, many tissue engineering strategies use exogenous stimuli to enhance the biochemical or biomechanical properties of the engineered tissue. Hyperbaric oxygen(HBO) is commonly used gas pressure in clinical. Literatures suggests that treatment of full-thickness osteochondral defects with hyperbaric oxygen resulted in a clear improvement in cartilage repair.

Materials and Methods:

Human adipose derived stem cells (hADSCs) were seeded on the gelatin/polycaprolactone biocomposites. Totally 6 groups were included in the study: negative control, 1 absolute atmosphere(ATA) air, 2 ATA oxygen/air and 2.5 ATA oxygen/air groups. The hyperbaric oxygen/air therapy was then applied for 1 hour in a consecutive 5 days period of time. The hADSC/gelatin/PCL scaffolds implant to articular cartilage defects rabbit and sacrifice at 2 weeks and 1 month after implant the scaffold. The series of exam including the behavior analysis, immunofluorescence stain, Histochemistry stain were tested to evaluate the production of GAGs, collagen type II and SOX9. We also build an NaF treated PET model to evaluate and compair the osteogenesis of lesioned cartilage at 2 and 4 weeks.

Results:

The preliminary results of behavior test at 1 week and 2 weeks showed that after 2 and 2.5ATA oxygen/air treated, the cell-based tissue engineering cartilage affect the articular cartilage defect rabbits become more beneficial characteristics in movement and weight barrier.

Conclusion:

After finish all of the test, we expect the hyperbaric oxygen and air treatment can provide a high pressure environment to help the cartilage matrix synthesis and improve the quality of cartilage tissue engineering.

P419

The Role of Sclerostin in Therapeutic Mechanism of Calcitonin in Ovariectomy-Induced Osteoporotic Rats

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Backgrounds:

Osteoporosis is a common disease characterized by a systemic impairment of bone mass and microarchitecture that result in fragility fractures. Sclerostin is an osteocyte-expressed negative regulator of bone formation. Previous research revealed estrogen deficiency inhibits osteocyte-derived sclerostin synthesis. Other paper showed calcitonin increases the expression of sclerostin in osteocyte. In this study, we evaluate the role of sclerostin in therapeutic mechanism of calcitonin in ovariectomy (OVX)-induced osteoporotic Rats.

Materials & methods:

Using aged (6-month-old) OVX Sprague-Dawley rats (n=18), the following experiments were performed: 1) To examine the effect of calcitonin and alendronate on the expression of sclerostin in osteocyte by immunohistochemistry (IHC) of tibia. 2) To examine trabecular number and bone volume, and bone formation rate by microCT and bone histomorphometry. Sham operated group (n=6), OVX rats (n=6) and alendronate-treated OVX rats (n=6) were used as control.

Results:

Both calcitonin and alendronate increased bone volume, trabecular number, trabecular thickness and reduce trabecular separation in OVX rats. In IHC stain, the expression of sclerostin in osteocyte was increased in cortical bone of sham and alendronate-treated groups as compared to those in calcitonin-treated and OVX group. Increased bone formation was observed in OVX rats as compared to sham group. Further increase of bone formation was observed in the calcitonin-treated OVX rats but not in the alendronate-treated rats.

Conclusion:

Both calcitonin and alendronate increased bone volume in OVX rats. The increase of bone volumne in calcitonin-treated OVX rats may partly due to the decrease expression of sclerostin.

P420

Inhibitory Effects of Cordyceps Sinensis on B16 Melanoma Cells

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Backgrounds:

The potential of the health food supplement Cordyceps sinensis may inhibit proliferation of cancer cells. Melanoma is a cancer that starts in a certain type of skin cell. This research aimed at the exploration of Cordyceps sinensis extract on the proliferation effect and possible related mechanisms on B16 melanoma cells.

Materials and Methods:

In this study, the dried powder of Cordyceps sinensis was extracted with water in a water bath of 90°C for 2 h. The water extract of Cordyceps sinensis (WECS) was used in this study. B16 melanoma cells were treated with WECS (0, 20, 50, 75, and 100 mg/mL), and evaluated the survival and proliferation rate using MTT assay. The tyrosinase and melanin in cells treated with WECS were measured, and the effects of WECS on apoptosis and cell cycle were analyzed by flow cytometry.

Results:

The results indicated that B16 melanoma cells treated with WECS can significantly inhibit the proliferation effect than the control groups. The synthesis of tyrosinase and melanin were diminished by the addition of WECS, which effects could be contributed to the suppression proliferation of B16 melanoma cells. The results of flow cytometry found that WECS can dose-dependently increase B16 melanoma cells to apoptosis. Percentages of cells in the G0/G1, S, and G2M phases of the cell cycle determined by flow cytometry were similar to control groups. In summary, this study demonstrates that WECS is a potential remedy to prevent the proliferation of melanoma cancer cells.

Conclusion:

Our results suggest that WECS might inhibit the proliferation of B16 melanoma cells and decrease tyrosinase production possibly via increasing cells to apoptosis. WECS might be beneficial in the prevention of cancer cells proliferation as an adjuvant agent in cancer therapy.

P421
Roles of miR-22 in tumorigenesis of gastric cancer

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Gastric carcinoma is one of the most common cancers and lethal malignancies worldwide. Up to date, the regulatory mechanism has not been understood clearly. MicroRNAs (miRNAs) are increasingly implicated in regulating the malignant progression of gastric cancer. In this study, we overexpressed miR-22 by recombinant adenovirus-expressing system. Our preliminary results showed that colony forming, migration, and invasion abilities were suppressed by miR-22 in SC-M1 gastric cancer cells. These results suggest that miR-22 inhibits may act as a tumor suppressor in gastric cancer progression.

P422
Maternal poly I:C exposure affects the hippocampal structure and function of the offspring.

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Backgrounds:
Maternal immune activation during pregnancy has been linked with the some developmental psychiatric disorders, such as schizophrenia. However, the pathogenic mechanisms are largely unclear.
Materials and Methods:
In this study, we administered a viral mimic, polyriboinosinic-polyribocytidilic acid (poly I:C, 20 mg/Kg, ip) to pregnant C57BL/6J mice at gestation day 9 to simulate prenatal immune challenge. Normal saline treatment was used as control. First, we evaluated the emotion related behaviors (elevated plus maze test and forced swimming test) as well as hippocampus-related cognitive function (Morris water maze) in adult male offspring. After behavioral tests, animals were perfused with 4% paraformaldehyde. Brains were then impregnated in Golgi solution for 18 days at room temperature, and cut at a thickness of 150 μm with a vibratome. Reconstruction and quantitative analysis of granule cells in dorsal- and ventral-blade of the dentate gyrus were performed using Stereo Investigator and Neurolucida system.
Results:
In the elevated plus maze test and forced swimming test, no signs of depression or anxiety were noticed in poly I:C-offspring compared to controls. However, poly I:C-offspring exhibited impaired spatial memory in Morris water maze. Morphologically, the dendritic architecture of the granule cells in the dentate gyrus (DG) was altered in poly I:C-offspring. In both dorsal- and ventral-blades, the DG granule cells had reduced dendritic complexity and shorter dendritic length compared to control mice. In addition, the density of dendritic spine in DG granule cells was also decreased in poly I:C-offspring.
Conclusion:
Our data showed that the structure and function of hippocampus are affected by prenatal PolyI:C exposure. Early adverse environmental factors could thus influence the developmental processes and ascribe to the susceptibility to mental disorders.

P423
Upregulation of GAP-43 is linked to the cartilage repair by microarray analysis

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Background:
Better quality of cartilage repair in developing skeleton is recognized. The associated repair factors may be important in osteoarthritis and those factors would be the targets for the management of osteoarthritis. Microarray analysis of cartilage repair in rat knee joint was therefore carried out. Surgical injury on the femoral cartilage of the right patello-femoral joint (PFJ) in the 3-week-old and 8-week-old rats for two weeks was first made. The left side of joint cartilage was used as the sham control.
Materials and Methods:
The results showed that cellular proliferation over the surgical injured cartilage in the 3-week-old rats was identified by histology, whereas not in the sham control side and 8-week-old joint cartilage. Primary cultures from the joint cartilage with 1x105 cells to observe cell proliferation between sham control and injury groups were also performed. Fibroblastic morphology with increased growth rate in injured groups was seen. Then, the gene expression level in the sham control and injury groups by microarray analysis demonstrated some novel genes involvement in this process.
Results:
The top 5 upregulated genes were asporin (log2 ratio 4.49), growth associated protein 43 (GAP-43) (log2 ratio 4.43), tenascin N (log2 ratio 4.36), C1q and tumor necrosis factor related protein 3 (log2 ratio 4.06), and ADAM metallopeptidase (log2 ratio 3.94).
Both asporin and GAP-43 upregulation were confirmed by real time polymerase chain reaction. Further functional verification by cartilage frozen sections in different time courses including 1, 2, 3, 4 weeks was carried out, especially GAP-43. GAP-43 has been known as a nerve growth associated protein which involves on neurite outgrowth with transducing intra- and extracellular signals to regulate cytoskeletal organization in the nerve ending. Here, we novelly identified that GAP-43 was expressed strongly on 2 weeks cartilage repair period by immunofluorescence. The GAP-43 expression was correlated with the cyclooxygenase 2 (COX-2) expression during the repair process.
Conclusion:
On present data, the upregulation of GAP-43 is novelly linked to the cartilage repair process. The target of GAP-43 in osteoarthritis pathogenesis may be value of further investigation.

P424
Applying biopolymers dressings to promote wound healing on rats

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Backgrounds:
Alginate is a natural substance that can be found in brown algae. Its abilities to heal wound by binding with water soluble molecules to form a gel that keeps moisture over the wound bed to speed up healing and to provide glycosaminoglycans (GAGs) are widely known. The process of wound healing can be briefly divided into stages. After bleeding, fibroblasts are transported to the wound site within 72 hours, which is also known as inflammatory stage. Next, collagen that were secretes by fibroblast at the wound bed would serve as a scaffold for other cells to attach and rebuild. It is also the major critical cell during the proliferation stage till scar formation. In this report, collagen alone, collagen plus alginate, alginate alone and carboxymethycellulose (CMC) were compared for the efficacy on wound healing. Four materials produced different result in various healing stages.
Materials and Methods:
There were 12 rats to be divided into four groups (n=3), CMC, collagen, collagen plus alginate, and alginate. After weighted, rats were given different amount of anesthetics based on their weight recorded. After shave, we made an artificial square wound with sides of 3 cm on the back of each rat. The tested wound dressing was placed on top of the wound and wrapped after covering with a piece of PU film. The wounds were observed and their sizes were measured and recorded in the first, second, fourth week.
Results:
The observation showed that alginate alone significantly minimized the wound size in the first week and continued to the second week but its healing slowed down in the third and fourth week. For collagen alone, the wound did not heal as fast as alginate in the first week. However, the wound healing took off drastically in the second week and the size of wound reduced at the end. Next, collagen plus alginate was able to heal the wound at an above-average and steady speed. Within two weeks, the original 9cm2 wound bed was almost healed. Lastly, CMC showed a constant but slower rate of healing the wound in the fourth week.
Conclusion:
Our study proved alginate has the best healing effect during the first two week. Moving into the third and fourth week, collagen overtook others to be the best dressing. Of all four materials, CMC was the most ineffective dressing. Even though rats that applied CMC, wound did heal. It took longer to heal than the other three. In conclusion, collagen plus alginate pad combines the two strong points of alginate and collagen and smoothest rate of healing of all four tested materials.

P425

The efficacy of adsorbent ingestion in treating hepatic encephalopathy in a rat model

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Backgrounds:

Hepatic encephalopathy (HE) is a complication of the central nervous system in liver failure. It could cause brain edema, intracranial hypertension and a number of neuropsychiatric disturbances including somnolence, confusion, impairments of sensory-motor integration, cognitive performance, attention and memory, or even coma. High ammonia level is believed to be the cause for neuropsychiatric disturbances. Ingestion of adsorbent has been a way to treat diarrhea and renal failure. Adsorbent in the alimentary canal is believed to function by indirectly adsorbing redundant ions in the blood and digestive tract. This leads to the intriguing question of whether it can be used to lower blood ammonia to prevent the development of HE syndromes in case of liver failure.

Materials and Methods:

The absorbing abilities of the adsorbents zeolite, activated carbon and kaolin were assessed first with an in vitro immersion method. Three concentrations of the adsorbents, 5%, 10% and 30%, were mixed with 10-4M NH4Cl solution for an hour and then measured the remaining ammonia concentration using an Ammonia Assay Kit. In animal experiments, male Sprague-Dawley (SD) rats that were fed with saline, zeolite, activated carbon or kaolin (1g/kg/day) for 2 weeks and then intravenously infused with NH4Cl (0.5 g/kg) in normal saline for an hour. Blood samples collected from these animals afterward were assessed for blood ion (Na+ and Ca2+) and ammonia levels. HE was induced in Male SD rats (250-350 g) with bile duct ligation (BDL) and then intraperitoneal infusion of 5 M NH4Cl (143 μl/day) via a subcutaneously implanted osmotic mini-pump (HA) for 2 weeks. Sham control, BDLHA+saline, BDLHA+zeolite, BDLHA+activated carbon and BDLHA+kaolin were examined. Rats were fed with adsorbent or saline daily after mini-pump implantation. Normal cage activity, rota-rod and Morris Water Maze tests were used to assess animal behaviors before BDL and at the end of the experiment before been sacrificed. To sacrifice, rats were perfused with paraformaldehyde and the removed brain was cryoprotected, cut into 25-μm-thick sections, and processed for nNOS, Iba1 (microglial marker) and GFAP (astrocyte marker), for morphological assessment.

Results:

Adsorbents effectively decreased ammonia level in vitro ammonia adsorptive test, intravenous ammonia infusion test and HE rats. The adsorptive ability of zeolite was better than activated carbon and kaolin. These adsorbents did not affect blood ion levels, including Na+ and Ca2+. HE rats were found to have poorer normal cage activity, sensory-motor integration ability and space learning and memory than control. At the same time, their cortex were filled with activated microglia and swollen astrocytes. Oral adsorbent treatment rectified these behavioral deficits of HE rats and ameliorated their microglial and astrocytic reactions as well.

Conclusion:

In our HE rat model, ingestion of adsorbent effectively reduced blood ammonia level without affecting the liver abnormalities caused by diseases. Oral adsorbent at the same time attenuated central glia reactions and eased the behavioral impairments associated with HE. Thus adsorbent ingestion is an effective measure to prevent hyperammonemia-induced central neuronal damages and behavior impairments while seeking medical treatment of the abnormalities that cause liver malfunction.

P426

Therapeutic Effects of Ganoderma in the Cardiac Injury Induced by Ractopamine

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Backgrounds:

Ganoderma tsugae (GT), one of the ganoderma (Lingzhi in Chinese) species, has been used as a medicinal mushroom in traditional Chinese medicine. GT contains polysaccharides, nucleotides, triterpenoids that are responsible for its bioactivities. The triterpenoids fraction from GT consists of a mixture of nine structurally related ganoderic acids (GAs). Our previous study shows that oral *Ganoderma tsuga* (GT) or subcutaneous injection of ganoderic acids (GAs) both protect the mice from isoproterenol (ISO) induced cardiac injury and cell death.β1-adrenoceptor (β1-AR) generally predominates on the cardiomyocytes. ISO is a β1-adrenoceptor agonist used in treating heart block or bradycardia. However, long-term stimulation by ISO may induce myocardial injury in mice. Although, the numbers of β2-adrenoceptors (β2-AR) are less than of β1-AR in cardiomyocytes, but both overexpression β2-AR and stimulation of β2-AR agonist induce cardiac hypertrophy and apoptosis. There are several β2-AR agonists such as ractopamine, clenbuterol and salbutamol. Although it has been suggested that ractopamine induced myocardial toxicity in greyhounds, the physiological role of ractopamine remains unclear. We intended to examine the effects of ractopamine in mice and to test the therapeutic potential of GT against ractopamine.

Methods and Results:

We tested the effects of ractopamine through different delivery channels (subcutaneous, intraperitoneal or osmotic pump) with various doses, frequency on different strains of mice (C57BL/6 or BALB/c). The cardiac tissue were analyzed by H&E and trichrome staining. We found that BALB/c mice were more sensitive than C57BL/6. BALB/c mice consistently displayed myocardial damage and fibrosis 4 days after receiving a subcutaneous injection 300 mg/kg of ractopamine.

Conclusion:

β2-AR stimulation by ractopamine possesses cardiotoxicity in mice. The protective effect of GT or GAs against the ractopamine cardiotoxicity is currently under investigation.

P427

Inhibition of the glycolytic enzyme hexokinase isozyme HK1 but not HK2 accelerates tumor malignancy via deregulation of energy metabolism

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Backgrounds:

Malignant tumors often display an aberrant energy metabolism that uses predominantly glycolysis for ATP generation, the so-called Warburg effect. Hexokinase (HK) catalyzes the first reaction in glycolysis and also attenuates mitochondrion-mediated apoptosis. Thus, cancer cells with overexpression of HK isozyme HK1 or HK2 are generally more resistance to antitumor therapy. However, the roles of both HK1 and HK2 in cancer progression are not fully elucidated yet particularly in their deficiencies.

Material & Methods:

To investigate whether attenuated glycolytic activity modulates tumor progression, the first and rate-limiting glycolytic enzyme HK isozyme HK1 and HK2 were examined, silenced, and the effects analyzed.

Results:

Human cancer cells exhibited a strong inverse correlation between HK1 and HK2 expressions. In human cervical carcinoma cells, RNAi-mediated HK1 but not HK2 knockdown induced phenotypic change characteristic of the epithelial-mesenchymal transition (EMT). This switch greatly accelerated cancer cell metastasis and growth in both *in vitro* assays and *in vivo* tumor xenograft models. Notably, HK1 silenced cells displayed severe dysfunction in respiratory pathway and great increase in glycolytic activity, but no effect on ATP production. These metabolic alterations associated with strong increases of HK2 and lactate dehydrogenase 1 (LDH1) but a marked decrease of citrate synthase (CS). In particular, this HK1 knockdown induced aberrant energy metabolism was recapitulated by either HK2 overexpression or CS silencing. Moreover, the HK1 knockdown cells exhibited strong glucose-dependent growth and great 2-deoxyglucose (2-DG)-induced inhibition.

Conclusion:

These results demonstrate that inhibition of the HK1, but not HK2, alters energetic metabolism and induces EMT phenotype, resulting in accelerating tumor malignancy.

P428

The Effect of *Ganoderma Tsugae* Extracts on Adipogenesis and Lipid Metabolism in the Murine 3T3-L1 Cell

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Backgrounds:

Lingzhi is a medicinal fungus and has long been used for promoting health and longevity in Asian countries. *Ganoderma tsugae* (GT), a popular species of Lingzhi cultivated in Taiwan, is found to have potential in affecting adipocyte differentiation and other metabolic functions. It is known that adipocyte differentiation is a key aspect of obesity development when homeostasis is unbalanced. Additionally, obesity is a major culprit of many metabolic disorders. However, some contradictory results on adipocyte differentiation have been reported. To further investigate the real role of GT on adipogenesis and lipid metabolism, we performed this study.

Materials and Methods:

GT was obtained from the Luo-Kuei-Ying Fungi Agricultural Farm, Taoyuan, Taiwan. The ethanolic extract of GT (GTEE), was used to treat the pre-adipocyte 3T3-L1 cells. After treatment of 3T3-L1 cells with GTEE, the cell viability and toxicity was determined by MTT assay, the lipid accumulation and lipid droplets size was observed by Oil red-O staining, and the changes of adipogenic markers and the genes associated with lipid metabolism were identified by Q-RT-PCR.

Results:

GTEE had no effect on cell viability of 3T3-L1 cells but accelerated adipocyte differentiation. Furthermore, GTEE altered the lipid droplet size and adipogenic marker genes, including PPARγ2, C/EBPα and adiponectin. Therefore, we infer that GTEE may affect the expression of BAT-related genes.

Conclusion:

We demonstrate that GTEE accelerates adipocyte differentiation, alters the lipid droplet size and regulates the genes related to adipogenesis and lipid metabolism. In conclusion, GTEE may promote health by modulating adipocyte differentiation.

P429

Characterization of *Methanohalophilus portucalensis* FDF1^T S-Adenosyl Homocysteine Hydrolase SAHH1 and SAHH2

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Backgrounds:

S-Adenosylhomocysteine (SAH) is a competitive inhibitor of the SAM-dependent osmolyte glycine betaine synthesizing process in halophilic methanogen *Methanohalophilus portucalensis* FDF1^T. In order to accumulate high level of betaine to overcome hyperosmotic stress, SAH must be removed efficiently through SAH hydrolase (SAHH) to form adenosine and homocysteine. Both bacterial/eukaryotic type SAHH1 and archaeal type SAHH2 are co-exist in *M. portucalensis*. High transcriptional levels of SAHH1gene under salt up-shock suggested it plays the major role in SAH removal.

Materials and Methods:

Both *E. coli* heterologous over-expressed *Mp*SAHH1 and *Mp*SAHH2 were purified by Ni Sepharose 6 Fast Flow column. The activity was measured by the TNB formation at 412 nm using spectrophotometer.

Results:

After one step purification, 11.96 g of *Mp*SAHH1 and 0.59 g of *Mp*SAHH2 were each obtained from one liter of their *E. coli* host cultures. The purification fold and specific activities of *Mp*SAHH1 and *Mp*SAHH2 were 11.99, 0.013 μmol/min/mg and 5.23, 0.006 μmol/min/mg respectively. The catalytic efficiency of recombinant *Mp*SAHH1 is 0.0002 $min^{-1}\cdot\mu mol^{-1}$, which is much lower than SAHH from other organisms (0.0024 to 0.303 $min^{-1}\cdot\mu mol^{-1}$).

Conclusion:

The primitive kinetic study showed low catalytic efficiency of recombinant *Mp*SAHH1 which indicate it may not be capable to hydrolysis SAH efficiently while cell encounter salt stress. Although this may not be similar for the origin SAHH1 in methanoarchaea, high transcription level of *sahh1* and low accumulated level of SAH in *M. portucalensis* indicate efficient SAHH hydrolysis may be contributed by immediate increased high amount of SAHH1 instead the high catalytic activity.

P430

Studies of the active site of human 4-hydroxyl-phenyl-pyruvate dioxygenase by site-direct mutagenesis

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Backgrounds:

4-hydroxyl-phenyl-pyruvate dioxygenase(HPPD) catalyzes the second step in the pathway of tyrosine catabolism, to convert the substrate 4-hydroxyl-phenyl-pyruvate(HPP) to Homogentisate(HG). 4-HPPD belongs to the alpha-keto acid-dependent non-heme dioxygenase. The active site of enzymes were located in the C-domain in which the metal ion binds with a 2-His-1-carboxylate facial triad.

Materials and Methods:

WT and mutant enzyme were purified through ion-exchanger, hydrophobic and gel filtration chromatography. The enzyme activities were determined by HPLC, oxygraph assay. ITC assay was used to determine the thermodynamics for metal ion binding.

Results:

The kcat and Km value of HPP for wild type were 3.276±0.4046 s-1 and 0.2161±0.0529 mM. The Kd value determined from ITC was 1μM. No activity was observed for Q251E mutant and about 95% activity was lost for Q265E mutant. The kcat ,Km and kcat /Km for Q265E were decreased about 20, 4, and 100-fold, respectively. The activity of Q334N was decreased about 96%. The Km value for substrate was similar, but increased about 3-fold for Iron ion. The activities for H183A,H266A,E349Q and E349G were decreased by about 95%, 90%, 95% and 95% respectively. No HG product was detectible for H266A mutant by HPLC assay. However HPA intermediate was produced indicating the uncouple reaction.

Conclusion:

Q251 and Q265 that interact with the 4-hydroxyl group of HPP might play an important role on substrate binding and catalysis. Q334 affects iron binding which might be related to catalysis. Any mutation would result in decrease of activity, and increase the possible uncouple reaction in metal binding residues H183, H266 and E349.

P431

TMPRSS2 is involved in tight junction formation of Caco-2 cells

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Backgrounds:

Pericellular serine proteases have an important role in tissue homeostasis and tight junction formation. Recently, type II transmembrane serine protease (TTSP) TMPRSS2 has received increasing attention because its expression level is correlated with prostate cancer progression. Several tissues such as prostate and colon also selectively express this protease. However, the biological function and substrate(s) of TMPRSS2 are still unclear. Our preliminary data show that TMPRSS2 can induce the activation of matriptase, another member of TTSP, in prostate cancer cells. Since recent studies have shown that matriptase can regulate intestinal tight junction formation and permeability, we then hypothesize that TMPRSS2 exhibits a role in the tight junction formation of colorectal cells and is crucial for barrier function and permeability.

Materials and Methods:

Caco-2 cells were used as a cell model that exhibited a differentiation program with tight junction formation. TEER (transepithelial electrical resistance) was used to monitor the formation of tight junction in TMPRSS2-knockdown or -overexpressed Caco-2 cells.

Results:

The TEER value reached to the plateau on Day 7 in the differentiation program of Caco-2 cells. TMPRSS2 knockdown delay the TEER value to reach the plateau of Caco-2 cells, indicating that TMPRSS2 plays a role in tight junction formation. Consistently, TMPRSS2 overexpression accelerated Caco-2 cell differentiation, indicated by the fact that the TEER value in TMPRSS2-overexpressed Caco-2 cells reached the plateau two days earlier than control cells.

Conclusion:

Taken together, the results indicate that TMPRSS2 may play a role in tight junction formation in Caco-2 cells. We will further investigate if matriptase is involved in the TMPRSS2-regulating tight junction formation and delineate the role of the proteolytic cascade from TMPRSS2 to matriptase in barrier formation and function.

P432

Prostaglandin E2 enhance Hepatocellular Carcinoma HA22T cells Survival and Metastasis effects via EP2/EP4 signaling pathways

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Backgrounds:

Earlier studies have shown that, Prostaglandin E2 (PGE2) enhances the growth of various cancers (breast, stomach, pancreas, lung and prostate) by activating various proteins that are involved in controlling proliferation and survival pathway. However, the mechanisms of PGE2 regulate HA22T hepatocellular carcinoma cell survival and metastasis is still unknown, roles of EP receptor and related signaling pathways are waiting for further investigation.

Materials and Methods:

Firstly, we investigated the Prostaglandin E Receptor 2(EP2) and Prostaglandin E Receptor 4(EP4) protein level in liver cancer tissues via Immunoblotting. Then, we used HA22T cells to investigate PGE2 induced EP2/EP4 receptor expression and also β-catenin, pEGFR, PI3K, Akt and their downstream anti-apoptotic protein levels by western blot analysis. Moreover, the role of PGE2 in regulating HA22T cell migration was investigated by Boyden chamber.

Results:

High levels of EP2 and EP4 proteins were expressed in human hepatocellular carcinoma than normal liver tissue. Using HA22T cell line we observed PGE2 induced survival pathway by either activating EP2/EP4 expression or EGFR expression. Furthermore, PGE2 induced HA22T cell migration by up-regulating GSK3-β and β-catenin expression in a dose dependent manner.

Conclusion:

EP2 and EP4 could be used as novel prognostic markers in hepatocellular carcinoma and activation of these two receptors by PGE2 enhanced HA22T cells survival and migration abilities.

P433

Explore Functions of The Mouse Double Homeobox gene, *Duxbl*, in myoblasts, satellite cells and zebrafish

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Backgrounds:

Homeobox genes encode transcription factors that regulate embryonic development programs including organogenesis, axis formation and limb development. Previously, we identified and cloned a mouse double homeobox gene, *Duxbl*. We found that *Duxbl* expressed in embryonic myocytes and promote C2C12 myoblast proliferation but inhibit differentiation. The amino acid sequences of Duxbl homeodomains are most similar to those of human DUX4 protein, associated with facioscapulohumeral muscular dystrophy (FSHD). The mechanisms causing FSHD and functions of double homeobox genes remain unclear. In this study, we analyze the molecular mechanism(s) of Duxbl promoting proliferation and inhibiting differentiation in C2C12 cells. Satellite cells and zebrafish were also used to decipher the function(s) of Duxbl in muscle regeneration and myogenesis.

Materials and Methods:

To investigate the molecular mechanism(s) of Duxbl in myogenesis, Duxbl were overexpressed in C2C12 cells and then cultured in growing medium or induced to differentiation. Total proteins and RNAs were extracted for Western blot and RT-PCR analyses. To study the function(s) of Duxbl in muscle regeneration, myofibers or satellite cells were isolated from EDL muscles then activated or induced to differentiation. Transgenic zebrafish with striated muscle-specific Duxbl expressions were generated, and then myogenesis of zebrafish were analyzed by H&E staining.

Results:

Duxbl overexpression in C2C12 cells promoted cell proliferation by activating cyclin D1 and reducing P21. The ratios of S and G2/M cell cycles are higher in Duxbl-overexpressed cells than those in vector-only cells. Duxbl activated MyoD expression slightly in growing medium. Although the expression of MyoD, master gene of myognesis, was not influenced in differentiating medium, but the myocyte enhancer factors (MEF2A) were inhibited in Duxbl-overexpressed C2C12 cells. Since MEF2 and MyoD protein cooperately activate muscle-specific gene expressions, the expressions of early differentiated markers or their downstream gene products including MyoG, P21, cyclin D3 were all inhibited by Duxbl overexpression. The fusion index was decreased by Duxbl overexpression in differentiating medium. However, the expressions of the cell cycle regulators, cyclinD1 and CDK4/6, were increased by Duxbl overexpression. From these results, we suggest that Duxbl overexpression increase cyclinD1 and CDK4/6 expressions, then inhibit MyoD and MEF2 activities in differentiating condition. Furthermore, results of co-immunoflorescence analysis showed that Duxbl was expressed in activated satellite cells but not in quiescent ones. Finally, the Duxbl overexpression caused the loose of somite muscles and the production of muscles with no obvious nucleus in zebrafish.

Conclusion:

In addition to embryogenesis, Duxbl is also expressed in satellite cells after birth. We suggest that Duxbl play a role in muscle repair and regeneration. Duxbl overexpression promotes C2C12 cell proliferation by increasing cyclin D1 and inhibiting P21 expressions in growing medium. In differentiating medium, Duxbl activated cyclinD1-Cdk4/6 complexes block MyoD or MEF2A, and then inhibit myoblast differentiation. Ectopic Duxbl expressions cause the muscle loose and production of muscles with no obvious nucleus in zebrafish.

P434

Nanogold Particles Combined with Glucose Regulated Protein 78 Binding Peptides for In Situ Colorectal Tumor Detection in Mice

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Backgrounds:

In epidemiological studies showed that the incidences of colorectal cancer have increased over the past several decades. Therefore, an early, accurate diagnosis of colorectal tumor could offer the best chance to increase therapeutic effects and survival rate. The enhanced permeability and retention (EPR) effect is the property by which certain sizes of nanoparticles tend to accumulate in tumor tissue much more than in normal tissues. Furthermore, a lot of research has been indicated that glucose-regulated protein (GRP78) was over-expressed in colorectal tumor tissue. Therefore, the goal of this study is to utilize the nanogold particles (NGPs) combined with GRP78 binding peptide for detecting the in situ colorectal tumor in carcinogen induced-animals.

Materials and Methods:

The NGPs were synthesized by HAuCl¬4 and NaBH4. The chemical reaction was performed at room for 1 h. After dialysis, the synthesized NGPs were connected with GRP78 binding peptide to form a tumor detector (NGPs-GRP78bp). The particles size of NGPs-GRP78bp was detected by nano-particle analyzer. Then, the toxicity of tumor detector was measured in normal liver and kidney cells. In addition, the in situ colorectal tumor was induced by treating dimethyl hydrazine (DMH, with oral administration, 20mg/kg, twice a week) for 6 months in C57BL/6 mice (n=20). The liver sections of DMH treated-animals were observed by HE stain. The tumor induced-animals were intravenous injected the NGPs-GRP78bp and analyzed the distribution of tumor by in vivo imaging system (IVIS).

Results:

The size of NGPs-GRP78bp was about 170 nm which was achieved the EPR effects for tumor accumulation. For cytotoxicity assay, the tumor detector showed low toxicity in normal cells and liver tissue. Therefore, we suggested that the materials have slight side effect in animals. The colorectal tumor was confirmed by a postmortem examination and the rate of tumor induction was more than 90% in all treated group. After IVIS assay, the tumor was significant expression in colorectal region of DMH treated group.

Conclusion:

Our analysis indicated that the NGPs combined with GRP78 binding peptide could provide tumor detection in vivo. The fairly low side effect and tumor diagnostic function of NGPs-GRP78bp maybe provide for clinical practice.

P435

Anti-inflammatory Activity of Peroxyauraptenol Extracted from Cnidium monnieri Cuss

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Backgrounds:

Cnidium monnieri Cuss. is not only a traditional Chinese herb, but also an economically important agricultural product via artificial planting. Five compounds were isolated from the ethanol extract of seeds of C.monnieri including osthole, imperatorin, xanthotoxin, isopimpinellin, and peroxyauraptenol. The structure between osthole and peroxyauraptenol are similar, and osthole has been reported to exhibit various biological activities, but the function of peroxyauraptenol has not been reported, and hence, in this study we investigated the effect of peroxyauraptenol on lipopolysaccharide (LPS)-induced inflammation in macrophages.

Materials and Methods:

Cytokine secretion and protein expression in LPS-activated macrophages were measured by ELISA and western blot respectively. NLRP3 inflammasome activation in LPS- and ATP-activated macrophage was monitored by detection of IL-1β secretion and caspase-1 activation. The caspase-11 mRNA expression in LPS- and IFN-γ-activated macrophage was quantified by qPCR.

Results:

Peroxyauraptenol reduced NO generation, iNOS expression, and IL-6 secretion through inhibiting the phosphorylation levels of mitogen-activated protein kinases and protein kinase C-α/δ, as well as decreased reactive oxygen species production. Peroxyauraptenol also decreased NLRP3 inflammasome activation, whereas did not affect caspase-11 mRNA expression.

Conclusion:

Peroxyaurpentol has anti-inflammatory activities, and has the potential to be developed as an anti-inflammatory in the future.

P436

The effect of noni juice on mice inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a chronic relapsing and remitting immune disorder that can be explained as exuberant immune activity causing a dysregulated immune profile in the intestinal mucosa. IBD have two main forms are Crohn's disease (CD) and ulcerative colitis (UC). The cause of IBD is unknown, but involves interplay between genetic predisposition, defective mucosal immune regulation and environmental factors. In the research, *Morinda critifolia* (noni) has many physiological activity, such as anti-inflammatory, anti-oxidation, anti-tumor. However, Western-style diet let inflammatory bowel disease incidence gradually increased, so in this mainly ulcerative enteropathy, selection of Tahitian Noni juice to explore the effect of inflammatory bowel disease. In the animal experiments, divided into a control, negtive control, positive control and the group of fed noni juice 1X, 2X, 4X , n=10. Use DSS-induced inflammatory bowel disease produce. The experimental test blood of lipid oxidation, antioxidant enzymes SOD, GPx activity, NO content and cytokines of TNF-α, IL-6 production. Result show that NO level decreased in noni group compared with negative control. Antioxidant enzyme levels also improved compared with the negative control. In noni group the cytokine of TNF-α and IL-6 level decreased compared with negative control. In particular IL-6 decreased significantly, the group of 4X Noni decreased 80% compared with the negative control. The findings suggest that noni juice are effective in symptom by IBD.

Keyword:

Inflammatory bowel disease, *Morinda critifolia*

P437
Functional Analysis of Drosophila UBPY in Autophagic Regulation

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To maintain homeostasis, cells mainly depend on ubiquitin-proteasome system (UPS) and autophagy to eliminate unnecessary materials. Ubiquitin plays a dominant role in these two pathways. Besides marking materials to be degraded, it is also able to regulate enzyme activity and alter the cellular location of key molecules. It is much well known about the ubiquitination process; however, the reverse reaction, deubiquitination, and its connection with autophagy remains largely to be explored. Among these deubiquitinating enzymes, one especially highlighted: UBPY (ubiquitin isopeptidase Y), which loss of function activates autophagy under normal condition, whereas UBPY overexpression inhibits starvation-induced autophagy. UBPY had been shown to be involved in the regulation of ESCRT (endosomal sorting complex required for transport) complex whose malfunction impairs autophagy. We further found that overexpression of the active-site mutation of UBPY fails to inhibit autophagy, so UBPY may regulate autophagy by its deubiquitinating activity.

P438
Study the function of N-terminal domain in human 4-hydroxyphenylpyruvate dioxygenase by terminal truncation.

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Background:
4-Hydroxyphenylpyruvate dioxygenase (HPPD) catalyzes the second step of tyrosine catabolism which converts substrate 4-hydroxyphenylpyruvate(HPP) to homogentisate(HG). This enzyme belongs to the member of the alpha-keto acid-dependent non-heme iron(II) dioxygenase. Human HPPD is a dimer protein. Each subunit composed of two structural similar domain , the N-terminal and C-terminal domain. The active site of human HPPD is located in the C-terminal domain and the ferrous ion is bound through 2-His 1-carboxylate facial triad.
Material and method:
WT and mutant enzyme were purified through (ion-exchanger , hydrophobic and gel filtration)chromatography. The enzyme activity were determined by HPLC and Oxygragh assay.
Results:
The specific activities(SA) for wild type and mutants of H89A, S5, A9, R13, H18 and T23 were 1.35±0.24, 1.29±0.23, 0.94±0.26, 1.09±0.18, 0.25±0.03, 0.85±0.16 and 0.96±0.15 nmole/min/ug , respectively. Compared to wild type , only the kcat and kcat/km values of R13 mutant were decreased. The thermostability of mutant enzymes were less stable than wild type.
Conclusion:
The result indicates that residues of K10 , P11 and E12 plays important role in catalysis. In contrast , the highly conserved H89 in domain interface is not related to enzyme activity , but might maintain the stability of the protein.

P439
Role of Tissue Transglutaminase 2 on the Invadopodia Formation in Highly Invasive A431-III Tumor Cells

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Backgrounds:
Cancer progression is closely linked to the epithelial-mesenchymal transition (EMT) process. Invasive cancer cells increase tissue tranglutaminase (TG2) expression. Our laboratory obtained a highly invasive tumor cell subline (A431-III) from parental A431 tumor cells (A431-P) using a Boyden chamber system with matrigel-coated membrane support. Previous studies have shown that the A431-III cells display increased expression of TG2. The upregulation of TG2, also correlated with Snail and MMP-9 expression and resulted in greater cell motility. Invadopodia are believed to be the crucial structures that allow cancer cells to penetrate across extracellular matrix (ECM) by recruiting matrix metalloproteinases (MMPs). In this study, we investigate TG2 on invadopodia formation.
Materials and Methods:
A413 human epidermal cancer cell line was purchase from American Type Culture Collection (ATCC, Manassas, VA, USA). The highly invasive A431-III cells were isolated in our laboratory from the parental A431 tumor cells (A431-P) by three successive passages through Boyden chamber (Kao et al., 2008).
Results:
We found A431-III cells exerted greater ability to form invadopodia degrade ECM and increase expression of TG2. Treating A431 cells with TG2 siRNA resulted in inhibition of invadopodia formation and decreased ECM degradation. Src and phosphorylation of Src have been reported as main regulator of invadopodia formation and function. The result showed that invadopodia formation was dramatically reduced by TG2 siRNA treatment, resulted from inhibition of Src and phosphorylation of Src expression.
Conclusion:
We found A431-III cells exerted greater ability to form invadopodia degrade ECM and increase expression of TG2. The elevation of TG2 played important roles in invadopodia formation, by regulating Src kinase activity in A431-III. The enhancement of Snail expression by TG2 induces the acquisition of the mesenchymal-like phenotype, promotes the secretion of MMP-9, enhance invadopodia formation and metastatic potential in A431-III cells. We conclude that TG2 could affect the formation of invadopodia, MMPs secretion and metastasis potential.

P440
The Anti-Cancer Effects of Clioquinol on Human Oral Squamous Cell Carcinoma

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Backgrounds:
Clioquinol (CQ) was used clinically as an anti-amoebic drug, an antibiotic for the treatment of diarrhea and skin infection. More recently, CQ is a newly discovered anticancer agent. In this study, we focus on the anti-cancer effects of CQ on human oral squamous cell carcinoma (OSCC).
Materials and Methods:
Cell viability was determined via MTT assay. Caspase-3 activation, mitochondria membrane potential change and ROS production were analyzed by flow cytometer and analyzed by WinMDI analysis software. Protein expression was detected by western blot. The localizations of copper were measured by Olympus FluoView 1000 confocal laser scanning microscope.
Results:
We found that CQ with copper could enhance the cytotoxicity of CQ in oral cancer cells. Further, CQ with copper would decrease mitochondria membrane potential, increase ROS production, and induce cell apoptosis in OSCC cells. Moreover, we found that copper was transported into mitochondria upon CQ treatment. The effect of CQ with copper caused aberrant expression of apoptosis-related protein in intrinsic pathway.
Conclusion:
CQ could transport copper into mitochondria and induce mitochondria disruption. CQ with copper could also induce ROS production, and trigger cell apoptosis via intrinsic apoptosis pathway in OSCC cells. Our results supported that CQ would act as a potential selective anti-cancer drug due to the accumulation of copper in tumor part but not normal tissue.

P441
OO7 Inhibits Human Hepatocellular Carcinoma Cell Growth

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Backgrounds:

Hepatocellular carcinoma (HCC) is the sixth most common newly diagnosed cancer and the third most common cause of cancer mortality worldwide. At present, the sorafenib treatment of advanced HCC widely used in clinical practice. However, the survival benefit was only a few months. A high-throughput screen of the cytotoxic activity of 300 molecules from a commercial library in four human hepatocellular carcinoma cell lines identified the OO7 to be a HCC active drug.

Materials and Methods:

The OO7 inhibits the growth of HCC cell lines in a dose-dependent manner. Additionally, OO7 induced apoptosis of HCC cell lines by western blot, and was confirmed by flow cytometry analysis. In animal model, nude mice that received subcutaneously Mablavu cell xenografts were administered the vehicle or 2mg/Kg of OO7 by daily intraperitoneal injection. Treatment was initiated 10 days after implantation and continued for 35 days.

Results:

OO7 suppressed the growth of HCC cells in a dose-dependent manner and the IC50 was lower than sorafenib for 10 fold. In addition, OO7 caused apoptosis in sub-G1 phase by flow cytometry analysis and by increasing the expression of Bak and cleaved-caspase-3. In animal testing, tumors were palpable in vehicle treated mice by day 10 and grew to approximately 2000 mm3 by the end of the experiment, whereas mice treated with OO7 experienced tumor growth to approximately 500 mm3, indicating that OO7 has the potential to inhibit tumorigenesis.

Conclusion:

Together, the strategy used seems promising for identification of new diagnosis-specific cancer drugs.

P442
SOCS6, a Tumor Suppressor, Promotes Mitochondrial Fission and Apoptosis

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Mitochondria are highly motile organelles that constantly undergo fission and fusion. Impairment of mitochondrial dynamics is associated with mitochondrial dysfunction and is frequently linked to the pathogenesis of neurodegenerative diseases and cancer. We have previously shown that biallelic inactivation of the *suppressor of cytokine signaling 6 (SOCS6)* gene is a frequent event in human gastric cancer. In this study, we recapitulated the event of *SOCS6* loss using a Lentivirus-based knockdown approach, and demonstrated the linkage between SOCS6 depletion and the suppression of programmed cell death. SOCS6 promotes intrinsic apoptosis, with increased Bax conformational change, mitochondrial targeting, and oligomerization. Most importantly, SOCS6 is targeted to mitochondria and induces mitochondrial fragmentation mediated through an increase in DRP1 fission activity. Here, we show that SOCS6 forms complex with DRP1 and the mitochondrial phosphatase PGAM5, attenuates DRP1 phosphorylation, and promotes DRP1 mitochondrial translocation. Based on mutation analyses, SOCS6-mediated apoptosis is tightly coupled to its ability to induce mitochondrial fission. This study demonstrates an important role for SOCS6 in modulating mitochondrial dynamics and apoptosis.

P443
Identification of a monomeric K315A mutant δ-crystallin in the unfolding pathway by urea

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Backgrounds:

δ-crystallin is a taxon specific protein in eye lens of birds and reptilia. It shares 70% identity in amino acid sequence with argininosuccinate lyase. δ-crystallin is a homotetramer, each monomer has 3 highly conserved regions. Our previous study found out that residues in double dimer interface are important for structural stability. K315A mutant presents as a stable intermediate in the presence of urea.

Materials and Methods:

Goose wild-type and mutant δ-crystallin were expressed in E.coli. Proteins were purified through anion exchange, hydrophobic and gel filtration chromatography. δ-Crystallin was equilibrium unfolded and refolded in different urea or GdnHCl concentration. Protein structure were determined by using circular dichroism, fluorescence spectroscopy and analytical ultracentrifugation.

Result:

In the presence of 2~5M urea, the K315A mutant existed a as stable conformation as determined by tryptophan fluorescence. The state has highly exposed hydrophobic regions in 2M urea concentration as measured by ANS fluorescence. Analytical ultracentrifugation analysis identified that this state is a dissociated monomeric form. After dilution, the monomers could refold to tetramers. In the presence of granidine hydrochloride, monomeric K315A mutant was reversible unfolding. However, partial unfolded monomeric K315A mutant in 1M GdnHCl concentration exposed most of the hydrophobic region. Dilution of unfolded monomeric K315A mutant resulted in a conformation with increased hydrophobic regions and is prone to aggregate.

Conclusion:

δ-Crystallin K315A reversibly dissociates to a stable monomer in the presence of urea. The stable monomers partially unfold to a highly exposed hydrophobic intermediate and is prone to aggregate.

P444
Anti-Tumor Effects of OO7 Against Non-Small Cell Lung Cancer Cells

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Backgrounds:

Using high content screening (HCS) (96 well) of the cytotoxic activity of 300 small molecule compounds from a commercial library and seven distinct structural isomers in two non-small cell lung cancer cells identified OO7 as a human Non-Small Cell Lung Cancer Cells (NSCLC) active drug.

Materials and Methods:

The OO7 Lead to cell changes (morphology) and induced the process of programmed cell death simultaneously in dose-dependent manner in human NSCLC cell lines. Furthermore, the OO7 caused cell growth arrest in G2/M phase in A549 cell line by flow cytometry analysis. In vivo testing, nude mice bearing A549 lung cancer cell line by i.v. injection. Treatment of nude mice was started in 10th day and continued for 35 days with OO7 drug (2mg/kg) by daily i.p. injection.

Results:

Result of the mice treated with OO7 group compared with the vehicle-treated control, has distinctly decrease tumor size and tumor spots in lung surface. Likewise, we also found the fibrosis levels remarkably induced in lung slice.

Conclusion:

we demonstrates that OO7 treatment of mice bearing established tumors resulted in growth arrest, providing another anti-tumour strategy by using OO7 to against NSCLC cell lines.

P445

Investigating The Factor of N-Methyl-N'-Nitro-Nitrosoguanidine (NTG) Treated Zymomonas mobilis Mutant Responding to Acetate

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Backgrounds:

Recent high oil prices, concerns over energy security, and the negative impact of fossil fuels on the environmental have reawakened interest in finding renewable fuel alternatives. One of the most common renewable fuel today is bio-ethanol produced from cheap crops and lignocellulosic materials. *Zymomonas mobilis* is the most efficient ethanol producer, which have the potential to replace yeast for ethanol production. The major problem on lignocellulosic caused its needed pretreatment to increase accessibility to produce ethanol efficiently. However, pretreatment created some inhibitor compounds like acetic acid as an important inhibitor that reduced ethanol production. Therefore, improvement the acetate tolerance of *Z. mobilis* is necessary.

Materials and Methods:

The acetate tolerant mutant *Z. mobilis* SAT212 was generated from *Z. mobilis* ATCC31823 by NTG. Chromosome DNA of *Z. mobilis* SAT212 and ATCC31823 were isolated to investigate the differences between SAT212 and ZM4/AcR. Samples were collected for measuring ethanol and glucose concentration by High Performance Liquid Chromatography. Protein extraction were prepared from *Z.mobilis* SAT212 and ATCC31823 grown to the stationary phases in the presence and absence 195 mM NaOAc without pH control and were analyzed by two-dimensional gel electrophoresis (2-DE). Then, the protein identification was performed by MALDI-TOF.

Results:

Based sequencing result, *nhaA* region in SAT212 was existed in the full length and indicated that there were no deletion in *nhaA* region. SAT212 was able to tolerate NaOAc up to 250 mM and ATCC31823 was significantly inhibited at 195 mM NaOAc without pH control. Under the same condition without pH control, the glucose was completely consumed in 24 h by SAT212 and 57 g/l ethanol was produced with the ethanol yield of 0.58 g/g (Yp/s). 2-DE analysis founded ten proteins that were apparently alerted acetate tolerance. Then, a protein, which was named AHP, was related to the acetate tolerant ability of SAT212.

Conclusion:

Our analysis indicated that the acetate tolerant mechanism of SAT212 is different from ZM4/AcR. Meanwhile, we found that AHP was increased the acetate tolerance of *Z. mobilis*.

P446

Association of Fish and Long Chain n-3 Fatty Acid Consumption With Renal Function in Diabetic Nephropathy

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Background:

N-3 PUFA are increasingly being studied for their clinical benefits in a variety of medical conditions. The purpose of this research is to clarify the long-chain n-3 fatty acids and fish consume to affect subjects.

Material and Method:

Subjects were DM patients with proteinuria >30mg/dlCr more than 3 months in Taoyuan Armed Forces General Hospital , in 2012, 36 subjects were recruited.

We used food frequency questionnaire to measurement fish and long-chain n-3 fatty acids consumed in diabetic nephropathy and to detect subjects with urine protein > 30μg/mgCr, observation fish and long-chain n-3 fatty acids intake changes the subjects involved in experimental urine protein excretion and glomerular filtration rate (eGFR).

Result:

At the end follow-up, pearson correlation used to assess the validity of FFQ. Average correlation FFQs are in the range of r = 0.4~0.7 . EPA (C20:5) intake DM group was significantly higher than DN group (1.133 ±1.841vs. 0.377 ±0.693; p=0.019). DHA (C22:6) intake DM group was significantly higher than DN group (2.639 ±5.281vs. 0.269 ±0.368; p <0.0001).

Conclusion:

The present study shows that an increased dietary intake of long-chain n-3 PUFA and fish reduces the prevalence of diabetes nephropathy. Hence, a diet rich in n-3 PUFA could have a role in maintaining healthy kidney function.

P447

Betanodavirus non-structural protein B1 plays a new role on oxidative stress regulation in fish cells

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Background:

As we known, some fish species are infected by betanodavirus such as grouper, which results in severe mortality and significant economic losses on the aquaculture industry. In previous studies, RGNNV can induce necrotic cell death via mitochondrial membrane (MMP) loss in grouper cells. Then, RGNNV infection can induce reactive oxygen species (ROS), but it how to induce this oxidative stress is still uncover. On the other hand, a non-structural protein B1 from RGNNV plays a crucial role on anti-apoptotic function. In the present, we try to understand the other functions on ROS production.

Materials and Methods:

The grouper fin cell line, GF1, was obtained from Dr. Chi (Institute of Zoology and Development of life Science) Taipei, Taiwan. ROC. Naturally-infected red grouper larvae were collected in 2002 in the Tainan prefecture and were the source of the RGNNV Tainan No. 1 (RGNNV TN1) used to infect GF-1 cells.

A B1 coding sequence from RGNNV B1 were cloned into pcDNA3.1 vector (Clontech Laboratories, Palo Alto, CA), p3XFLAG-myc-CMV-26 vector (Sigma).

Generation of reactive oxygen species was determined using fluorescence microscopy or flow cytometry after live cells were stained with MitoSOX or Carboxy-H2DCFDA (Molecular Probes). Cellular hydrogen peroxide (H_2O_2) was assayed using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Molecular Probes).

Western blot were incubated with polyclonal antibodies to mouse Cu/Mn SOD, catalase or actin (1:15000; Upstate, Charlottesville, VA, USA), and then with peroxidase-labeled goat anti-rabbit conjugate (1:7500; Amersham Biosciences, Piscataway, NJ).

Results:

We found that B1–producing stable cells can tolerance the H_2O_2- induced cell damage. Then, very interesting, the B1 can induce superoxide anion (O_2-) production at 24 h and increased H_2O_2 level at 48 h after sub-cultured in fish cells, which may trigger a oxidative stress via up-regulating some anti-oxidant enzymes such as Cu/Zn SOD and Mn SOD.

Conclusion:

Taken our results, the B1 protein may play a new role on regulate the metabolic superoxide anion via triggering oxidative stress for up-regulating antioxidant enzymes. This finding may provide an insight into the molecular pathogenesis of betanodavirus infection and treatment.

P448

The Roles Of 5-HT2A And 5-HT2B In 5-HT-induced Thermal And Mechanical Hyperalgesia

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Backgrounds:

Following tissue damage, peripheral tissues release inflammatory mediators that activate and sensitize the nociceptors, inducing inflammatory pain. Serotonin (5-HT) is, one of the important inflammatory mediators which is released from platelets and mast cells, involved in pain and hyperalgesia. Previous studies have found that 5-HT2A antagonist and 5-HT2B antagonist inhibits 5-HT-induced thermal hyperalgesia in rats and mechanical hyperalgesia in mice, recepectively. Moreover, 5-HT potentiates TRPV1 functions in rat DRG neurons. TRPV1 is important for heat and mechanical sensitivity because PKC-evoked nocifensive behaviour and the heat injury-induced thermal and mechanical hyperalgesia are attenuated in TRPV1 KO mice. However, it remains unclear whether TRPV1 and PKCε are involved in 5-HT2A-mediated thermal hyperalgesia and 5-HT2B-mediates mechanical hyperalgesia.

Materials and Methods:

In this study, Von Frey filaments and Hargreave's apparatus were used to assess pain-like behaviors in mice injected with agonists or antagonists. I also used in vitro cell-based assays to examine the specificity of agonists and antagonists.

Results:

5-HT2A antagonist reduced 5-HT-induced thermal hyperalgesia. 5-HT2B antagonist, phospholipase C inhibitor, Protein kinase Cε inhibitor, but not adenylyl cyclase and protein kinase A and Gi protein inhibitors, significant inhibited 5-HT-induced mechanical hyperalgesia (p=0.001). 5-HT2B antagonist partially inhibited CFA-induced mechanical hyperalgesia. TRPV1 inhibitor significantly inhibited 5HT-induced mechanical hyperalgesia (p=0.001).

Conclusion:

5-HT2A mediates 5-HT-induced thermal hyperalgesia. 5-HT2B mediates 5-HT-induced mechanical hyperalgesia through Gq-PKCε pathway and TRPV1.

P449
Selective Cytotoxicity of Novel Host Defense Peptides against Colorectal Cancer Cell Lines

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Backgrounds:

Due to its malignancy, the development of effective therapeutic strategies for colorectal cancer is of urgent needs. Natural antimicrobial peptides (AMPs), also known as host defense peptides (HDPs), not only act as direct antimicrobial agents, but also represent important regulators of the innate immune system. It has been reported that cationic HDPs may exhibit cancer-selective toxicity.

Materials & Methods:

We have designed a series of novel HDPs with potent antimicrobial activity against a broad spectrum of bacterial pathogens. In the current study, we evaluate the antitumor potency of these HDPs toward colorectal cancer cell lines WiDr and HCT116. MTT assay was performed to measure cell viability upon HDP treatment. Flow cytometry was used for cell cycle analysis. Western blot was applied to analyze cell death mechanisms. Confocal microscopy was used to monitor cellular localization of FITC-labeled HDPs. Xenograft experiment will be done to evaluate in vivo efficacy of selected HDPs.

Results:

Selected HDPs inhibit the viability of colorectal cancer cells in a dose-dependent fashion, while the normal 3T3 cells were significantly less susceptible to these HDPs. GW-Q4-a showed potent anticancer activity with IC_{50} of less than 10 μM against both colorectal cancer cell lines. Flow cytometry, western blot analysis, confocal microscopy and xenograft experiments are now under investigation.

Conclusion:

In this study, we seek to find out whether HDP GW-Q4-a induce selective cell death of colorectal cancer cell lines through apoptosis, autophagy, or simply membranolysis. These findings would provide support for future application of novel cationic HDPs as potential therapeutic agents for colorectal cancer treatment.

P450
Using RNA-sequencing data of a non-familial cancer to screen causative single-nucleo tide variants (SNVs)

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Backgrounds:

Finding causative genes of cancers is challenging. It has been complicated by the high abundance of variations that could be found in cancer genomes. To date, in only a few types of cancers have mutated genes been implicated in carcinogenesis. The genetic drivers of cancers remain largely unknown.

In this study, we attempt to create a pipeline to look for protein-coding genes likely impaired in functions in a subtype of malignancies that share distinctive phenotype. Our input is RNA-seq data, which technology is transcriptome sequencing method capable to capture expressed genes and their variations simultaneously.

Materials and Methods:

The data that our pipeline starts with is the pre-called variations derived from RNA-seq, and the possible consequences of the variations already predicted by using the Ensembl variation effect predictor.

Our pipeline integrates all the information to find genes that recurrently carry potentially damaging variations in multiple cancer samples. To facilitate the further prioritization of the candidate driver genes, our pipeline creates a report that tabulate the mapping quality, alternative-allele quality, and sequencing depth for each potentially damaging variation detected within these genes. Finally, the remaining multi-hit loci across different samples are mapped to the known cases of RNA editing in the human genome.

Results:

Even though the data sources and formats to be integrated by our pipeline is complex, our pipeline is very efficient. It can complete the analysis of 6 samples, each composed of 500~800 million reads and 400~700 thousand called variations, in less than 5 minutes, consuming less than 10G memory.

Conclusion:

Our pipeline can prove effective screening causative variants and prioritization of the candidate driver genes from the RNA-seq data of non-familial malignancies case.

P451
Glycoproteomic Identification of Potential Biomarkers from Plasma of Patients with Asthma

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Backgrounds:

Asthma is a common chronic respiratory disease worldwide, with estimated about 300 million affected individuals, and the prevalence is increasing year by year. Asthma is a lifelong disease, an increasing number of patients with asthma caused personal and family distress, and more medical costs. In Taiwan, some 20% of severe asthma patients do not know their own morbidity and never receive appropriate treatment.

Materials & Methods:

In the current study, we used glycoproteomic approaches for the identification of potential disease biomarkers from plasma of patients with asthma. We purified glycoproteins in plasma obtained from asthma and non-asthma patients based on concanavalin A (Con A)-affinity method. With this treatment, a large number of high-abundance non-glycosylated proteins could be removed. Glycoprotein samples were then separated on two-dimensional gel electrophoresis (2-DE). Protein spots significantly altered as revealed by SameSpots image analysis software were excised and subjected to in-gel trypsin digestion. Tryptic peptide mixtures were then subjected to LC-ESI-Q-TOF MS/MS analysis and protein identification was performed using Mascot program.

Results:

2-DE were performed as triplicates in three independent experiments. Image analysis revealed 15 protein spots with significantly altered expression levels between asthma and non-asthma patients. These protein spots will be in-gel digested and subject to mass spectrometry. Western blot analysis will also be performed to verify the proteomics data.

Conclusion:

According to our findings in the current study, we might be able to discover asthma-associated plasma proteins. These potential biomarkers may be developed as promising diagnostic indicators for the early detection of the disease.

P452
To Evaluate the Effect of thromboxane A_2 synthase inhibitor on the Bone Mass of Senile Mice

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Background:

Ghosal hematodiaphyseal dysplasia syndrome (GHDD) is a disorder with higher bone density. Genetic studies of patients with this disorder had identified mutations in the gene encoding thromboxane A_2 synthase (TXAS), which is the key enzyme for producing thromboxane A_2 (TXA_2). TXA_2 are involved in the maintenance of bone homeostasis. Via ovariectomized (OVX) mice model, the effect of TXA_2 on the activities of osteoblasts/osteoclasts and BMD was evaluated by treating with the inhibitors of TXAS. It is very clear that inhibitor of TXAS effectively increase the bone mass in OVX female mice through the TXA_2-TP signaling pathway. Thus, through blocking the signaling of TXA_2-TP pathway, TXAS inhibitor may also be benefit for senile male osteoporotic people or female osteoporotic people with low or no response to estrogen. To prove this, senile osteoporotic mouse model (aged male mice) was established.

Marterials and Method:

Fifteen month-old senile male *Balb/c* mice were separated into two groups: control and inhibitor of TXAS (5mg/kg/day) for 16 weeks. In the end of this experiment, long bone was fixed, decalcified, and sections were collected for H&E stain. Micro-CT scan was used for BMD analysis and trabecular parameters measurement. The mRNA level of bone marrow cells flushed from the femur were determined by quantitative RT-PCR.

Result:

The preliminary results of this study show that the femur BMD was decreased in the inhibitor group. And the body weight is proportional to BMD in both groups. Although the level of cPGES, IL-1β, TNFα mRNA is lower in the inhibitor group, it did not show significantly difference when compare to the control group.

Conclusion:

Our results demonstrated that the supply of TXAS inhibitor may not result in the increase of bone mass in senile male mice. It is probable that the fifteen month-old senile male *Balb/c* mice were too old to express the genes for bone formation and bone resorption. Thus, we will try to use the younger mice, for example, 10-month-old mice to prove if TXAS inhibitor plays any roles in the decrease of bone loss.

P453
Deciphering the role of DNA binding activity of Mer2 in meiotic DNA double-strand breaks

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Meiotic recombination is 100-1,000 fold more frequent than mitotic recombination because of widespread DNA double-strand breaks (DSBs) that are introduced in a programmed fashion early on in meiosis. The programmed DNA DSBs are essential for proper chromosome segregation in the first meiotic division. Genetic studies in yeast, *Saccharomyces cerevisiae*, have identified at least 10 genes, including Mer2, involved in the formation of DSBs. The knock out phenotypes of Mer2 exhibited defective in sporulation and spore viability. Moreover, no DSBs are formed in mer2 null cells. Recent study from Franz's research group suggested that Mer2 binds to chromatin and then the phosphorylation of Mer2 can recruit its associated protein components to generate DNA breaks. Although Mer2-mediated DSBs are established, the detail mechanism still remains largely unknown. We will characterize the biochemical properties of Mer2 and decipher their biochemical properties in contributing the formation of DSBs. To address this issue, we have purified near homogeneous recombinant Mer2 proteins and our initial results documented that Mer2 can directly bind double-strand DNA. Our recent progress towards understanding the role of DNA binding activity of Mer2 in meiosis will be presented.

P454
The investigation of oxidative damage in liver of ayu with aging

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Backgrounds:
Oxidative stress is due to the imbalance between the in vivo activity of reactive oxygen species (ROS) and anti-reactive oxygen species, which caused oxidative damage ,resulting in the change of cell and organ function, triggering age-related disorder syndromes such as cognitive and Parkinson's disease. To most animals, aging is a progressive process characterized by accumulated damages of organs functions.
Materials and Methods:
Taiwan Yilan is the largest Ayu aquaculture County, the lifespan of ayu is short and will be sexual maturity in one year, then the aging rate of the various organs suddenly rise, they will die after spawning. The present study indicated that ayu produced ROS higher than other species and suggested that high levels ROS might be related to their short lifespan.
The expression of anti-oxidant enzymes (Ex. SOD and GPx) in ayu after spawing were significantly different from ROS induced aging. In this study, we divided ayu into four periods by calculated gonadal somatic index: young, before spawing, during spawing, and after spawing.
Results:
We found the levels of superoxide dismutase, glutathione peroxidase, and malondialdehyde increased in ayu's liver after spawing. Furthermore, it was revealed that the expression of phosphor-p53, cleavage caspase-3, and cleavage caspase-9 proteins were much higher in ayu's liver whereas heat shock protein 70, the aging-related protein, significantly decreased after spawing. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) scavenged the superoxide anion and H2O2 to prevent damage induced by reactive-oxygen-species- (ROS-).
Conclusion:
These results suggest that ayu can be an special animal model to reproductive aging research.

P455
Effect of Four-Agents-Decoction and Its Single Herb Extracts on the Cell Motility of Endometrial Carcinoma

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Backgrounds:
Endometrial carcinoma (EC) is a common gynecological cancer in Taiwan. The cases of patients with EC waere increased in recent years. Traditional Chinese medicine (TCM) has been used for medicinal purposes for centuries in Asian countries. Four-Agents-Decoction (FAD), also known as Si-Wu-Tang, is a TCM and composed of four herbs, dang gui, chuan xiong, bai shao and shou di huang. FAD has been used in regulating menstrual cycle and gynecologic diseases in ancient China for a long time. Our previous studies have found that the water extract of FAD (FADE) may affect genes relevant to cell adhesion and epithelial-mesenchymal transition (EMT) in ovarian and breast cancers. In this study, we investigate whether FADE has effect on cell motility of EC.
Materials and Methods:
Three EC cell lines, HEC-1-A, KLE and AN3CA were used in this study. EC cells were treated with FADE, and MTT assay was used to compare the anti-proliferation activity of FADE and its single herb extracts. Cell motility was analyzed by using wound healing assay and transwell migration assay. Invasion assay was used to further study cell invasion.
Results:
Our results suggest that FADE and its single herb extracts inhibit cell proliferation of EC cells. FADE also inhibits EC cell migration in a dose-dependent manner. Especially bai shao extract (BSE) has more significant inhibiting effect on EC cell motility and invasion than FADE and other single herb extracts.
Conclusion:
These data demonstrate that FADE inhibits cell migration, and its single herb extract BSE has even more inhibiting effect on EC cells.

P456
Characterization of Ligand Binding Properties of A Novel Cell Penetrating Peptide Employing Quartz Crystal Microbalance

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Background:
Protein-protein and protein-carbohydrate interactions play central roles in cell signal transduction as well as host-pathogen recognition. Both qualitative and quantitative assays are required to precisely characterize molecular interactions between these biomolecules. Quartz crystal microbalance (QCM) is an ultrasensitive mass sensor, though conventionally used to monitor the formation of thin films in vacuum systems; its application to aqueous solutions has brought a new research insight in the area of biosensors and impacted the field of drug discovery. Being integrated with a flow injection analysis system, QCM is a convenient tool for real-time monitoring and thermodynamic parameter measurement, which has been successfully applied to analyses of a variety of proteins, DNA, and small organic molecules.
Materials and Methods:
Trinitrobenzenesulfonic acid (TNBS) and radioactive labeling methods were used to determine functional amino groups on QCM *N*-link sensor chip prior to characterization of protein concentration coated on the chip as well as ligand binding activity. Besides, a novel cell penetrating peptide derived from eosinophil cationic protein (CPP*ecp*) reported to interact with cell surface glycosaminoglycans (GAGs) was immobilized on QCM *N*-link sensor chip through Schiff base formation and reductive amination, and binding affinities between CPP*ecp* and heparin molecules of different sizes were measured. Subsequently, heparin derivatives lacking of sulfo groups at specific positions were tested to demonstrate that CPP*ecp* binding strength correlated with sulfation degrees. Moreover, highly sulfated high molecular weight heparin (HMWH), moderately sulfated chondroitin sulfate C (CSC) and dermatan sulfate (DS), and non-sulfated hyaluronic acid (HA) were also tested.
Results:
Both HMWH and low molecular weight heparin (LMWH) interacted with CPP*ecp* and the equilibrium constants (K_d) fell into micro molar level. Besides, *N*- and O-sulfo groups on heparin were highly involved in molecular interaction with CPP*ecp*. Furthermore, sugar moiety might involve in CPP*ecp*-GAG interaction since the CPP*ecp* binding affinity of CSC was 2 fold weaker than that of DS.
Conclusion:
Our CPPecp preferred to bind to sulfated GAGs, marking feasible application of QCM N-link sensor chip for determination of protein-glycan analysis.

P457
Development of HBV surface antigen specific antibodies and their application on HBV detection

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Backgrounds:
There are about 350 million chronic carriers in the world. Furthermore, Taiwan is also a highly endemic area. There is approximately 90% of the population over the age of 30 have been infected with hepatitis B and 15-20% chronic carrier in adults. The infection of HBV may cause acute or Chronic hepatitis. Moreover, Sustained liver inflammation will lead to the liver fibrosis, cirrhosis and HCC. Therefore, detect the infection of HBV is important to prevent the HBV-related liver disease. There're many target can use for diagnosis. One of them is HBV DNA. Most of HBV DNA assay are based on PCR. The advantages of PCR are few sample used and sensitivity. But cost high is the disadvantage. cccDNA is another useful marker for detection. But it needs an invasive procedure. HBsAg is a common target for detection. The advantage of use the HBsAg as target is easy and cheaper. Therefore, in our research we choose the HBsAg to be the detect target.
Materials and Methods:
The processes of our research start from PCR to get the target gene, including LHB, MHB, SHB ,preS and HBV_a. Then, clone it to suit express vector, such as pET28a. After expression and purification, we get the proteins that use for immunization of mouse. With the specific antibodies, I development a diagnosis system for detection of HBV surface antigen rapid and sensitivity that based on ELISA.
Results:
We have the entire five target gene. And express the recombinant proteins of preS and HBV_a in BL21 strain. After immunization we scarified the mouse for hybridoma. And tried to get the specific antibodies.
Conclusion:
Hepatitis B is a global healthcare problem. It can cause many liver diseases. And the majority of the transition is from the mother to the fetus. Therefore, to prevent the HBV-related diseases a sensitivity and rapid diagnosis method is required.

P458
Mitochondrial Dysfunction-Induced VLDLR Overexpression and Fatty Acid Synthesis in Human Hepatoma HepG2 Cells

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Backgrounds:
One of our previous studies revealed that mitochondrial dysfunction-induced cell migration and chemo-resistance in human hepatoma cells. Meanwhile, we found that mitochondrial dysfunction upregulated VLDLR1 and VLDLR2 overexpression in HepG2 cells. To our knowledge, VLDLR majorly function in lipid metabolism and transport. However, the evidence of VLDLR in cancer malignance is limited. Therefore, this study aims to investigate the mechanism of mitochondrial dysfunction-regulated VLDLR expression and lipid metabolism and its responsible to cancer progression in hepatoma cells.
Materials and Methods:
By using shRNA of mitochondrial transcription factor A (shTFAM) and inhibitor of mitochondrial ATPase (oligomycin), mitochondrial dysfunction were induced in human hepatoma HepG2 cells. Besides, the oxygen consumption rate was analyzed as an indicator of mitochondrial oxidative phosphorylation (OXPHOS). The expression levels of VLDLR were quantified by real-time PCR and immunobloting, simultaneously. By using Oil Red O staining and BODIPY fluorescence staining, the intracellular neutral lipid content was determined. The activity of fatty acid synthase (FAS) was measured by spectrophotometrically monitoring oxidation of NADPH at 340 nm.
Results:
Pretreatment with shTFAM and oligomycin impaired OXPHOS in HepG2 cells, respectively. Subsequently, overexpression of VLDLR1 and VLDLR2 were observed in a time-dependent manner. Moreover, mitochondrial dysfunction-induced VLDLR proteins were located in intracellular fraction but not cell membrane. Furthermore, mitochondrial dysfunction caused increasing of FAS activity and lipid accumulation in HepG2 cells.
Conclusion:
These results suggested that mitochondrial dysfunction upregulated intracellular VLDLR overexpression as well as improved fatty acid synthesis in HepG2 cells. Additionally, the roles of VLDLR induction and FAS activation in mitochondrial dysfunction-induced cell migration and chemoresistance are under investigation.

P459
Effects of Diallyl Disulfide on the Human Oral Cancer Cells (OC2)

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Backgrounds:
Diallyl disulfide (DADS), one of the major organosulfur compounds (OSCs) of garlic, is a potential chemopreventive compound that can induce oxidative stress in carcinoma cells and cause apoptotic cell death. However, the anticancer's potential of DADS on human oral cancer remains unexplored. This study was performed to assess the effects of DADS against oral cancer cell to investigate the underlying mechanisms.
Materials and Methods:
The anti-proliferative effect of DADS was initially evaluated on OC2 human oral cancer cells using the MTT assay. The suppressive effects of DADS on cell cycle and Ca^{2+} signal were further determined.
Results:
From MTT assay, DADS could decrease cell viability in a dose-dependent manner. Similar result was observed by fluorescence microscopy. Treatment of OC2 cells with DADS (500 μM) resulted in a massive accumulation of the cells in the G2/M phase of the cell cycle. Using fura-2 as a fluorescent Ca^{2+} indicator, DADS induced a significant and prolonged $[Ca^{2+}]i$ increase in OC2 cells.
Conclusion:
Our analysis indicated DADS could affect human oral cancer cells in anti-proliferation, cell cycles, and Ca^{2+} signaling. This class of naturally occurring organosulfur compounds thus have potential for clinical use as anticancer's treatments.

P460
Thrombin Induces COX-2 Expression Mediated through PKCs/c-Src/EGFR/AP-1 Cascade in Human Tracheal Smooth Muscle Cells

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Backgrounds:
The thrombin/proteinase-activated receptors (PARs) have been shown to regulate smooth muscle cell proliferation, migration, and vascular maturation. Thrombin upregulates expression of several proteins in tracheal smooth muscle cells (TSMCs) which may contribute to airway inflammatory diseases. Here, we investigated the mechanisms underlying thrombin-induced cyclooxygenase-2 (COX-2) expression and its effect on human TSMCs (HTSMCs).
Materials and Methods:
HTSMCs were used in the study. The mechanisms of thrombin-induced COX-2 expression were investigated by Western blot, RT-PCR, real time-PCR coupled to using pharmacological inhibitors of PI3K (LY294002), MEK1/2 (U0126), JNK1/2 (SP600125), p38 MAPK (SB202190), and AP-1 (Tanshinone II A), transfection with respective siRNAs, and promoter assay. Finally, PGE2 production was detected by an ELISA kit.
Results:
We demonstrated that thrombin induced COX-2 protein and mRNA expression, promoter activity, and PGE2 production, which were attenuated by pretreatment with the inhibitors of PAR-1 (PPACK), PKC-α (Gö6976), PKCδ (rottlerin), c-Src (PP1), EGFR (AG1478), PI3K (LY294002), MEK1/2 (U0126), p38 MAPK (SB202190), JNK1/2 (SP600125) and AP-1 (Tanshinone II A). Thrombin-stimulated phosphorylation of c-Src, EGFR, Akt, Erk1/2, p38, JNK1/2 and cJun were attenuated by pretreatment with respective inhibitors. Moreover, transfection with respective siRNAs also inhibited thrombin-induced COX-2 expression. These results suggested that thrombin induces COX-2 expression and PGE2 production through the PAR-1-dependent PKCs/c-Src/EGFR/PI3K/Akt/MAPKs signaling pathway linking to activation of AP-1.
Conclusion:
We demonstrated that thrombin-induced COX-2 expression and PGE2 production is mediated through PAR-1/PKCs/c-Src-dependent EGFR transactivation and then linking to PI3K/Akt/MAPKs cascade, which leads to AP-1 activation in HTSMCs.

P461

Quantitative Proteomic Analysis Reveals the Mechanism of a Potential Anticancer Drug Tanshinone IIA on the Growth Inhibition of Gastric Cancer Cells

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Backgrounds:

Gastric cancer is the second common cause of cancer-related death in the world. Discovering effective drugs against gastric cancer is necessary. Tanshinone IIA (TIIA) is a diterpene quinone extracted from traditional Chinese herbal medicine, Danshen (Salvia miltiorrhiza). It has been reported to have anti-tumor potentials against several cancers including gastric cancer, breast cancer, prostate cancer, colorectal cancer, lung cancer, liver cancer and leukemia. However, the mechanism of gastric cancer cells induced by TIIA is still unclear. Here, we perform quantitative proteomic analysis to reveal the effect of TIIA in gastric cancer cells.

Materials and Methods:

Human gastric cancer cell line AGS was treated with TIIA dissolved in DMSO by a concentration of IC50 for 48 hours. We compared its protein expression profile with cells treated with DMSO only. The differentially expressed proteins were identified and quantified by isobaric tags for relative and absolute quantification (iTRAQ)-coupled LC-MS/MS system.

Results:

TIIA significantly inhibited cell growth of AGS in a dosage-dependent manner (P <0.0001, Wilcoxon Signed-Rank test), and the IC50 after 48 hours of treatment is 5.3 μM, which was calculated by a series of growth curves resulted from four different TIIA concentrations (0.1μM, 1μM, 5μM, 10μM). We identified 102 differentially-expressed proteins by using iTRAQ. Among them, three proteins including ATPase family AAA domain-containing protein 5 (ATAD5), 40S ribosomal protein S10 (RS10), and proteasome subunit beta type-3 (PSB3) were significantly up-regulated, and three proteins including 40S ribosomal protein S2 (RS2), glucose-6-phosphate isomerase (G6PI) and 60S ribosomal protein L7 (RL7) were significantly down-regulated by TIIA treatment. In previous studies, ATAD5 and PSB3 were reported to be involved in DNA damage response, and PSB3 also contributes to cell cycle arrest. G6PI is well known for its role in glycolysis and gluconeogenesis, and have a negative regulation toward caspase activity. RS10, RS2, and RL7 are essential to protein translation. ATAD5, G6PI, RS2, and RL7 were shown to be involved in carcinogenesis.

Conclusion:

TIIA can significantly inhibit gastric cancer cell proliferation in a dosage-dependent manner, and might serve as a potential anticancer drug for gastric cancer.

P462

A Quick Method to Detect Splicing Pattern Change by Using RNA Sequencing

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Backgrounds:

Alternative splicing is a way to increase the diversity of gene expression in normal cells. However, perturbation of either the cis- or trans-acting elements of the splicing apparatus may change the ratio of different splicing form or even yield novel splicing patterns. Existing tools for analyzing the RNA sequencing data, such as Cufflinks, are not satisfactory. Thus, we have developed a pipeline to select and to rank the results derived from existing tools.

Materials and Methods:

The level of gene expression is usually measured by the fragments per kilobase of transcript per million fragments mapped (FPKM). However, the key point in detecting a splicing pattern change is the over- or under-expression of a few exons. Thus, it is more sensible to define the expression per kilobase of exon per millions fragments mapped (EPKM) than FPKM. In this case, the expression level of individual exons can be compared between two different states, such as case and control. If the splicing pattern of two states are identical, the expression level of each exon will not be identical due to experimental errors. We assume that the difference of expression level of all exons of a gene will form a distribution, which is similar to normal distribution. When there is a splicing pattern change between two states, the expression of some exons will be altered. Those alternations, which is above a threshold, will be picked for further examination. This method was named as DIfferential Splicing Pattern detected by ExoN-Selective Expression (DISPENSE).

Results:

Cufflinks is the most popular software to detect the changes of splicing patterns, because this program does not count on previous gene annotation. This program is quite successful in detecting the splicing pattern change for novel but simple genes. Unfortunately, Cufflinks' algorithm to assign reads to isoforms is based on probability. If the depth of RNA sequencing is not large enough, it is difficult to get enough samples of different patterns. In other words, the level of gene expression for complex genes may not be correctly estimated. This issue is particularly serious for complex genes, i.e. a gene with multiple transcripts. We have used Cufflinks and DISPENSE to analyze the same set of RNA sequencing data. Most of the top candidates of Cufflinks can be hit by DISPENSE, but Cufflinks run at least 100 times slower than DISPENSE.

Conclusion:

Although DISPENSE needs previous gene annotation, this method is more sensitive in detecting splicing pattern changes of complex genes. Moreover, this method may drastically decrease the computing time, which is needed to detecting the pattern change.

P463

Investigate the association of high triglyceride, Interleukin-6 and high blood sugar in Hepatitis B Patients

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Backgrounds:

Several studies have pointed out the performances of the hypertriglyceridemia, are correlated with the occurrence of high inflammatory hormones, Interleukin-6 (IL-6) and hyperinsulinemia in Hepatitis B patients. The study also pointed out that hypertriglyceridemia is the important features of inflammatory in hepatitis B patients, and the pro-inflammatory hormone Interleukin-6 is the important cause of the occurrence of hyperglycemia.

Materials and methods:

Patients are collected and confirmed by the gastroenterologist and endocrinologist with the informed consent. 43 type 2 diabetic patients, 68 patients without hepatitis B and type 2 diabetes are control group, and 68 patients with hepatitis B.Fasting blood glucose, HbA1c, Triglyceride and IL-6 are analyzed by Roche C501,Perkin Elmer 1470 instrument and Thermo Multiskan Spectrum. Thedata were compared and determined the difference in degree with t test.

Result:

The standardized level of Triglyceride, HbA1c, fasting blood glucose and in 68 patients with hepatitis B(1.006±0.573, 1.081±0.221, 1.184±0.454,12.699±25.277 %), 43 type 2 diabetic patients(1.093±0.573, 1.242±0.194, 1.399±0.366, 1.749±11.598 %) are higher than those patients without hepatitis B and type 2 diabetes, and showing highly significant differences of(***P<0.001).

Conclusion:

According to our study and the association of Triglyceride, IL-6 and fasting blood sugar, in hepatitis B patients, the occurrence of high and blood sugar may be due to the expression of triglyceride and pro-inflammatory factorIL-6.

P464

Investigate the CRP with High Blood Sugar of Hepatitis B Patients

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Backgrounds:

The literature pointed out that, Performance of the inflammatory factor with high blood sugar of the derived diabetes. In order to understand the correlation of hepatitis B patients with inflammatory CRP expression and high blood sugar, In this study, patients with hepatitis, Discussion on correlation, In order to provide a reference to the medical personnel to do follow-up medical services for patients with hepatitis B.

Materials and methods:

Collected the blood by the gastroenterologist and Endocrinologist to confirm, To attract the informed consent letter of 43 diabetic patients, 68 patients without hepatitis and diabetes were randomly, and 68 patients with hepatitis, Analysis of glucose, HbA1c and CRP by Roche C501, Inspection data to the t test to compare and determine a difference in degree.

Result:

The standardized level of CRP, HbA1c and fasting blood glucose in 68 patients with hepatitis B(1.457±2.130, 1.081±0.221, 1.184±0.454 %), 43 type 2 diabetic patients(2.740±4.507, 1.242±0.194, 1.399±0.366 %) are higher than those patients without hepatitis B and type 2 diabetes, and showing highly significant differences of control group(***P<0.001). Although CRP is no obvious significant difference between hepatitis B and control group, but the level of CRP in hepatitis B with type 2 diabetes (1.857±2.778 %) was higher than those patients of hepatitis B without type 2 diabetes(1.290±1.930 %), and showing nearly significant differences of control group(P=0.054).

Conclusion:

According to our study and the association of CRP and fasting blood sugar, in hepatitis B patients. Through the performance with CRP and fasting blood sugar studies in HBV infection can help us understand more detail in hepatitis B.

P465
Silencing of Signaling Pathways in Host Cells Affects Japanese Encephalitis Virus Replication

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Background:
It has been reported that viruses interact with many host cellular signaling pathways. We investigate the effects of PI3K/Akt, JAK/STAT3, and MEK/ERK signaling pathways on replication of JEV in this study.
Materials and Methods:
Human embryonic kidney cells (HEK239T) were cultured in DMEM medium (GIBCO) supplemented with 10% fetal bovine serum (FBS) (GIBCO), 2 mM L-glutamine, 3.75 g/l sodium bicarbonate, 100 U/ml penicillin, and 100 U/ml streptomycin under standard conditions (5% CO2, 37oC). shRNA double-stranded oligonucleotides cloned into lentiviral pLKO.1 vector were purchased from National RNAi Core Facility, Academia Sinica, Taiwan.
Results:
PI3K/Akt, JAK/STAT3, and MEK/ERK signaling pathways were blocked with shRNAs targeting Akt, STAT3, and ERK respectively. We found that blocking of PI3K/Akt, JAK/STAT3, MEK/ERK signaling pathway individually reduced the expression of JEV E and NS5 proteins. It was observed that JEV infection caused the detachment of various signaling molecules knocked-down HEK293T cells. This was particularly evident when ERK expression was knocked down. Furthermore, JEV infection caused more apoptosis in ERK-knockdown HEK293T cells than in control cells.
Conclusion:
Blocking of PI3K/Akt, JAK/STAT3, MEK/ERK signaling pathway individually reduced the replication of JEV. And JEV infection caused more apoptosis in ERK-knockdown HEK293T cells than in control cells. The mechanism of JEV interacting with MEK/ERK signaling pathway needs further investigations.

P466
Analysis of the Active Components of Pathogenic Leptospira Outer Membrane Protein LipL32 that Activate TLR2 Signal Transduction Pathway

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Backgrounds:
Leptospirosis is the most widespread zoonosis caused by pathogenic *Leptospira* worldwide. In mammalians, proximal tubule renal cells of kidney are the major target for pathogenic Leptospira colonization and invasion, resulting in inflammation and severe tubular injuries. Protein extract from outer membrane of this pathogenic strain contains a major component of a 32-kD lipoprotein (LipL32), lacking in the counter membrane of nonpathogenic strains. Previous studies indicated that LipL32 protein interacts with Toll-like receptor 2 (TLR2) on human kidney cell (HK2 cell) surface and succeeding elicits inflammatory responses.
Materials & Methods:
In this study, the active components of LipL32 were identified by individually truncation of distinct fragments. The binding abilities of separated truncated fragments of LipL32 were determined by using atomic force microscopy (AFM) and Enzyme-linked immunosobent assay (ELISA), respectively. In addition, the inflammatory responses of hIL-8, hTNF-α, and hMCP-1 stimulated by the different LipL32 fragments were simultaneously measured by real time PCR. Moreover, the synthesized peptides relative to LipL32 were subsequently proved to associate with TLR2.
Results:
The binding ability of truncated N and C termini of LipL32 decreased to 54% and 42% as compared to full-length LipL32, respectively. Meanwhile, the double truncated N and C termini of LipL32 decreased to only 13% as compared to full-length LipL32. In the inflammatory responses, the full-length LipL32 stimulated the expression of hIL-8 and hMCP-1 to 5.1 and 9.0 folds as compared to buffer control, respectively. The truncated N terminus decrease the stimulation of hIL-8 and hMCP-1 to 1.2 and 1.4 folds, while the truncated C terminus decrease the stimulation of hIL-8 and hMCP-1 to 3.6 and 3.2 folds. Interestingly, the double truncated N and C termini lessened the stimulation folds of hIL-8 and hMCP-1 to 1.7 and 1.5 folds. The full-length LipL32, with higher affinity to TLR2, could stimulate strong inflammatory responses while the N and C termini truncated LipL32, with low affinity to TLR2, could not stimulate the inflammatory responses.
Conclusion:
In summary, LipL32 is a novel ligand from pathogenic *Leptospira* that interact with TLR2. Both the N and C termini of LipL32 play critical roles in pathogen recognition involved in TLR2 interaction. This finding is crucial to understand how pathogenic *Leptospira* could interact with innate immunity TLR through active components of the outer membrane protein.

P467
Expression of mitochondrion-encodend Nad2 and Nad5 subunits of bamboo *Phyllostachys edulis* mitochondrial NADH:ubiquinone oxidoreductase in yeast *Saccharomyces cerevisiae*

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Backgrounds:
NADH:ubiquinone oxidoreductase (Complex I) is the first complex of mitochondrial electron transfer chain. Complex I catalyzes electron transfer from NADH to ubiquinone and pumps protons from matrix to intermembrane space so that proton concentration gradient is generated to drive ATP synthesis. *Nad2* and *Nad5*, encoded by mitochondrial nad2 and nad5 genes, are subunits on the membrane arm of complex I and may be involved in proton pumping or ion translocation.
Materials & Methods:
The cDNAs of *nad2*, 1.5 kb, and *nad5*, 2 kb, from bamboo mitochondria were cloned and constructed into a galactose inducible pYES2-*mt*-egfp plasmid. The mt (mitochondrial targeting peptide gene) and *egfp* (enhanced *gfp gene*) were fused at 5'-terminal and 3'-terminal of *nad2* and *nad5*, respectively. The constructed pYES2-mt-nad2-egfp and pYES2-mt-nad5-egfp plasmids were transformed into yeast *Saccharomyces cerevisiae*. Nad2 and Nad5 yeast transformants were confirmed by PCR and restriction enzyme digestion, and the expected 2.3 kb of *mt-nad2-egfp* and 2.8 kb of *mt-nad5-egfp* fragments were observed.
Results:
The location of Nad2-GFP and Nad5-GFP fusion proteins at yeast mitochondria were observed by fluorescence microscope. The Nad2-GFP and Nad5-GFP fusion proteins of 84 kDa and 103 kDa were also observed in yeast membrane protein extracts by western blot analysis while the molecular weight of Nad2 and Nad5 subunits are 55 kDa and 74 kDa. To analyze the activity of ion translocation of Nad2 and Nad5 subunits, the Nad2 and Nad5 yeast transformants were cultured in two high ion concentration media of 600 mM NaCl and 800 mM KCl. The results showed that the mutants could survive under high salts. Moreover, the mutant cells with expressed Nad2 and Nad5 grew faster than the control cells. Both bamboo Nad2 and Nad5 subunits seemed to function independently to translocate Na^+ and K^+ ions in yeast mutants.
Conclusion:
The bamboo *P. edulis* Nad2 and Nad5, encoded by mitochondrial genome, could be expressed in yeast mitochondria. It showed that Nad2 and Nad5 subunits may have the activities of ion translocation.

P468
Function Analysis of GPCR56 in Chemical Induced K562 Human Leukemia Cell

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Backgrounds:
The expression level of GPR56, a member of the adhesion G protein-coupled receptor (GPCR) family, was 4 times down regulated in leukemia model cells, K562 cell, as compared with clinical samples, also the expression was correlated to white blood cell counts at diagnosis from children with acute lymphoblastic leukemia. K562 cell is used as model to study the blood cell differentiation by chemicals induction. In this report, the GPR56 was expressed in K562 cell to character the function of GPR56 in blood cells.
Materials and Methods:
K562 cells were treated with hemin and huangqi and HMBA. Then, the expression level of GPCR56 was RT-PCR examined in chemicals induced K562 cells and equal loading was normalized with GAPDH. The recombinant GPR56 was expressed by trasnfection CMV based GPR56 expression plasmid into K562 cells and several cell linage makers were studied.
Results:
The GPCR56 was up-regulated in hemin and huangqi treated K562 cell while in HMBA treated cell the receptor was down regulated. The growth of K562 cells was not affected by the expression of GPCR56, however, promegakaryocytic phenotype were increased. To further investigation the function of GPCR56, the expression of hemoglobins and cluster of differentiation makers (CD markers) of erythroblast (β-globin, γ-globin), myeloid lineage (CD13, CD33), monocyte (CD14, CD68) and megakaryocyte (CD41, CD61) were detected by RT-PCR. Among these, γ-globing, CD13, CD33, and CD61 were up-regulated, β-globing and CD41were not affected, CD14 and CD68 were down-regulated.
Conclusion:
GPR56 was induced in hemin and huangqi treated cells and the expression of GPR56 increased CD13, CD33, CD61 and γ-globin presentation in K562 cells. We proposed that the expression of GPCR56 expanded myeloid cell numbers and the GPR56 signals inhibit monocyte differention. These expansion numbers of myeloid cells and inhibition of monocyte patways result in the increasing megakaryocyte and erythrocyte population in hemin and huangqi treted K562 cells.

P469
RNA-seq Profiling Reveals the Role of miR-148a in Gastric Cancer AGS Cells

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Backgrounds:

MicroRNAs play an important role in various biological processes by post-transcriptionally regulating gene expression. To investigate the role of miR-148a in gastric cancer, the next generation RNA sequencing (RNA-seq) was applied to reveal the miR-148a-regulated gene expression profiles in gastric cancer cells.

Materials and Methods:

To uncover the role of miR-148a in gastric cancer, miR-148a was overexpressed in gastric cancer cell line AGS and followed by RNA-seq analysis. Using RNA-seq analysis, gene expression levels were estimated to identify differentially expressed genes in miR-148a-overexpressed cells. The biological processes and functional networks associated with the regulated genes were further analyzed by Ingenuity Pathway analysis (IPA). To validate the finding from RNA-seq, the expression level of the interested genes was measured by real-time reverse transcription PCR (qRT-PCR), the cell growth was monitored in real-time using the xCELLigence system, and the cell cycle analysis was examined by flow cytometry.

Results:

We identified a total of 16,254 genes by comparing RNA-seq profiles of control (NTC) and miR-148a-overexpressed AGS cells. Among them, 63 up-regulated and 48 down-regulated genes were differentially expressed in miR-148a-overexpressed cells. The IPA results showed that these miR-148a-regulated genes were significantly involved in the biological pathways including cell death and cell cycle. We demonstrated overexpression of miR-148a reduced the cell growth and induced G2/M cell cycle arrest. Additionally, we found miR-148a promoted the expression levels of ATM (ataxia telangiectasia mutated), ATR (ataxiatelangiectasia and Rad3-related), TP53 (tumor protein p53) and GADD45B (growth arrest and DNA-damage inducible, beta).

Conclusion:

This study illustrates the new role of miR-148a in controlling cell cycle and provides information in gastric cancer therapy.

P470
Structural Folding, Purification and Functional Characterization of the Presumed Ring Contraction Enzyme for Indolocarbazole Glycoside Biosynthesis

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Backgrounds:

Indolocarbazole natural products are an emerging class of bioactive compounds holding potential therapeutic applications in the treatment of cancer and neurodegerative disorders. K-252a, for example, exhibits potent neuroprotective and cytotoxic activities against numerous cancer cells, and is a structurally unique indolocarbazole alkaloid containing a rare furanose moiety. We have recently cloned the entire biosynthetic gene cluster for K-252a, where the key enzyme presumably responsible for the conversion of pyranose to furanose has been identified. This key enzyme for the hexose ring contraction is valuable in that little is known in the enzyme of this class in terms of molecular mechanism and structure-and-activity relationship in natural product biosynthesis. Availability of functional form of this enzyme would greatly facilitate the synthesis of furanose-containing natural products for drug development, which has attracted us to carry out heterologous expression, purification and functional characterization of this enzyme. Therefore, our study has been to get access to large quantity of this enzyme and subsequently to investigate its catalytic function and mechanism.

Materials and Methods:

The enzyme has been cloned for expression as a His-tagged recombinant protein in Escherichia coli. For functional characterization and future X-ray structural determination, we aimed to obtain a large quantity of soluble protein by protein refolding techniques. Upon optimization of expression condition, the expression was induced to give substantial amount of protein, which was further treated with high concentration of denaturants (such as urea and guanidinium HCl). The protein refolding was carried out by dialysis against various buffers to allow a transformation of the enzyme from unfolded state to the folded state.

Results:

Throughout examinations with a series of expression and refolding conditions, a sufficient amount of soluble protein has been obtained for functional characterization. The refolded enzyme was further examined for enzymatic activity on several hexose nucleotide diphosphates as potential substrates. The hexose nucleotide diphosphates were synthesized by a combinatorial biosynthetic approach developed in our lab using a combination of various NDP-hexose biosynthetic enzymes. As a result, the enzyme has successfully displayed catalytic activity as analyzed by high performance liquid chromatography, showing a time-dependent consumption of NADPH cofactor. Interestingly, further enzymatic coupling reaction of the refolded form of the enzyme with the NDP-sugar epimerase from the K-252a biosynthetic pathway also generates unanticipated products.

Conclusion:

In summary, this study has for the first time developed a method to gain soluble form of the key enzyme presumed for ring contraction in indolocarbazole glycoside biosynthesis. Unprecedentedly, the enzyme has also been revealed to possess substrate promiscuity on the given sugar substrates. The information gained here has provided critical insights into molecular mechanism of this class of catalyst, as well as materials useful to carry out combinatorial biosynthesis of unusual NDP-sugars and indolocarbazole glycosides for development of potential therapeutic agents.

P471
Transcription Factor Elf3 Mediates Vasopressin-Regulated Aquaporin-2 Expression in mpkCCD Cells

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Backgrounds:

Vasopressin (AVP) is a peptide hormone that plays a key role in whole body water balance in mammals. When the osmoreceptors in the brain detect increased osmotic pressure in the blood, AVP is released from the pituitary and circulates to the kidneys where it increases water permeability of the collecting ducts thereby reducing water excretion. AVP increases collecting duct water permeability by two mechanisms. In the short-term mechanism, AVP triggers redistribution of aquaporin-2 (AQP2) water channel protein from intracellular vesicles to the apical plasma membrane to increase water transport. In the long-term mechanism, AVP increases transcription of AQP2 gene, resulting in increases in AQP2 mRNA and protein levels. Dysregulation of AQP2 protein abundance via the long-term regulation of AVP is associated with many water balance diseases. Despite potential clinical relevance of the latter mechanism, vasopressin-mediated regulation of AQP2 gene transcription is poorly understood. Several recent microarray and proteomic studies have alluded to potential roles of the ETS-like transcription factor Elf3 in vasopressin-mediated AQP2 transcription. The goal of the current study was to investigate whether the transcription factor Elf3 is involved in the regulation of vasopressin-mediated AQP2 gene expression in a mouse collecting duct cell model (mpkCCD).

Materials and Methods:

The mpkCCD cells were polarized on permeable membrane supports (Transwell®) before experiments. shRNA-based gene knockdown was conducted to examine functions of Elf3 in vasopressin analog dDAVP-induced AQP2 mRNA and protein expression using quantitative RT-PCR and immunoblotting. The functions of Elf3 on AQP2 promoter was further examined using luciferase based promoter activity assay.

Results:

In the mpkCCD cells, the vasopressin analog dDAVP induced AQP2 mRNA and protein expression in a time-dependent manner. Stable Elf3 knockdown significantly reduced dDAVP-induced AQP2 mRNA and protein expression. Overexpression of Elf3 isoform 1 or isoform 2 in the mpkCCD cells increased AQP2 promoter activity in the absence and presence of dDAVP.

Conclusion:

The transcription factor Elf3 mediates basal and vasopressin-induced AQP2 gene expression in the mpkCCD cells.

P472
Effect of Acarbose on *p*-Cresylsulphate-Induced Vascular Inflammation in Human Umbilical Vein Endothelial Cells

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Backgrounds:

Recruitment of specific leukocyte subpopulations at the site of inflammation requires a series of cell adhesion molecules (CAMs)-mediated interactions. The major CAMs, viz., intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin are expressed on endothelium in response to various cytokines. Acarbose, a glucosidase inhibitor, has been shown to reduce cardiovascular diseases. We investigated the effect of acarbose on the expression of CAMs by human umbilical vein endothelial cells (HUVECs) stimulated with *p*-cresylsulphate (PCS).

Materials and Methods:

Human umbilical vein endothelial cells (HUVEC) were incubated with PCS and acarbose. Thereafter, the expression of adhesion molecules was evaluated by Western blot.

Results:

Time course of the effect of PCS (10ug) on VCAM-1 expression in HUVEC, VCAM-1 expression significantly increased within 9hrs and significantly decreased within 24hrs ($P < 0.05$). Effects of acarbose on VCAM-1 expression on HUVEC stimulated with PCS for 9hrs, acarbose (10mmol/L) significantly decreased the VCAM-1 expression. Furthermore, effects of acarbose on VCAM-1 expression on HUVEC stimulated with PCS for 24hrs, acarbose (1mmol/L and 10mmol/L) significantly decreased the VCAM-1 expression.

Conclusion:

Due to acarbose significantly decreased the VCAM-1 expression; our study may indicate that acarbose may play a role in the reduction of cardiovascular diseases by inhibiting endothelial adhesion molecule expression.

P473
Stably Transfection of ROGDI Sensitizes Human Brain Glioma Cell Lines to Cisplatin-induced Apoptosis

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Backgrounds:

ROGDI is a novel gene which locates on human chromosome 16p13.3 and has unknown functions. According to GenBank database, ROGDI coding region is 864 bp which encodes 287 amino acids and contains leucine zipper domain. Earlier studies in our laboratory showed that overexpression of ROGDI induces p53 and p27 mRNA levels in human glioma cell line T98G and U251, besides , ROGDI gene suppresses growth in Hep G2 and Hep 3B cell hepatocellular carcinoma cell lines by apoptosis and induce sensitization to anticancer drugs.

Materials & Methods:

This study was purposed to explore the mechanism of augmenting apoptosis-inducing effects of cisplatin on human glioma cell by stable transfection of ROGDI gene.

The expression level of ROGDI of U87 cells stably transfected with ROGDI (U87/ROGDI) and pcDNA3.1/Neo(+) empty vector stably transfected cells (U87/Neo) was examined by western blot. The effects of cisplatin in combination with ROGDI on the proliferation and apoptosis of human glioma cells were measured by using WST-1 analysis, and western blot, respectively.

Results:

The results showed that ROGDI levels of U87/ROGDI were higher than U87 and U87/Neo, respectively. Overexpression of ROGDI gene reduce the proliferation percentage of U87/ROGDI cells induced by cisplatin, as compared U87 and U87/Neo. In addition, the ROGDI induction of p53, p21, c-caspase, Bax upregulation and Bcl-2 downregulation of U87/ROGDI cells induced by cisplatin, as compared U87 and U87/Neo.

Conclusion:

It is concluded that stable transfection of ROGDI gene can notably improve apoptosis-inducing effects of cisplatin on human glioma cells, which is a novel strategy to better chemotherapeutic effects on human brain tumor.

P474
Aqueous Extracts of *Pluchea Indica* (L.) Less. Induce Apoptosis in Human Carcinoma Cell Lines

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Backgrounds:

Pluchea indica is a perennial shrub plant indigenous to many Asian Countries. Anti-inflammatory properties were found in the methanolic fraction of the chloroform extract of *P. indica root.* In addition, significant anti-ulcer and anti-tuberculosis activities were found in the methanol fraction of *P. indica* root and leaf extracts, respectively. Recently studies in our laboratory showed that crude aqueous extracts of *P. indica* leaf and root suppressed proliferation, viability, and migration of GBM8401 and HeLa cells.A number of natural herbs and herbal extracts can induce apoptosis in cancer cells, but review about herbal related with molecules on cancer-cell-apoptosis was seldom. This study is going to evaluate the effect and mechanism of *P. indica* in cancer cell lines .

Materials & Methods:

Human malignant glioma cells U87, human NPC cells NPC-TW04, human colon adenocarcinoma cells SW480 were used. These cells were treated with low concentrations of *P. indica* root aqueous extracts 20, 50, 100μg/mL for up to 48 hours, respectively. Cell proliferation was determined using WST-1 assay. Apoptosis assay was performed to determine the effects of *P. indica* root aqueous extracts in U87, NPC-TW04 and SW480 by western blotting.

Results:

The results showed that *P. indica* root aqueous extracts reduce the proliferation percentage and induce p53, c-caspase, cytC, Bax upregulation and p-AKT downregulation in U87. But, low concentrations of *P. indica* root aqueous extracts have no effect in NPC-TW04 and SW480.

Conclusion:

The study indicated that *P. indica* root aqueous extracts may trigger U87 cells apoptosis, that it has sufficient potential to warrant further examination and development as a new anticancer agent.

P475
A Study of P-cresol Induce ER Stress and Apoptosis in Human Umbilical Vein Endothelial Cells (HUVEC)

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Backgrounds:

Cardiovascular disease (CVD) is highly prevalent in patients with chronic kidney disease (CKD). Endothelial dysfunction plays an important role in the development of cardiovascular diseases , which are the leading cause of mortality in CKD patients. Several uraemic retention of compounds have been associated with cardiovascular damage. A major prototypic representative of the group of protein-bound uremic retention solutes is *p*-cresol (pC; 4-methylphenol). It has been demonstrated that the *p*-cresol is related to endothelial dysfunction in patients with CKD. However, the relationship between *p*-cresol and endothelial dysfunction have not been elucidated.

Materials & Methods:

This study was purposed to explore the mechanism of augmenting apoptosis-inducing effects of *p*-cresol on Human umbilical vein endothelial cells (HUVEC). HUVEC were treated with physiological concentrations of *p*-cresol 1, 10, 100μg/mL for up to 1, 3, 6, 9, 12, 24, 24, 48 hours, respectively. Cell proliferation was determined using WST-1 assay. Western blotting was also performed to determine the effects of *p*-cresol on endoplasmic reticulum stress (ER stress) and apoptosis in HUVEC.

Results:

The results showed that *p*-cresol induce activation of ER stress-regulated proteins BiP, CHOP, and upregulation of apoptosis-associated proteins p53, p21 and cytochrome c (cytC) . In addition, *p*-cresol reduce the proliferation percentage of HUVEC.

Conclusion:

Endoplasmic reticulum stress is known to promote cell apoptosis, thus, this study revealed that *p*-cresol induces cell apoptosis by endoplasmic reticulum stress.

P476
Anti-NLRP3 Inflammasome Activity of Osthole

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Backgrounds:

IL-1β release is controlled by caspase-1-containing multi-protein complexes called inflammasomes, the most thoroughly characterized of which is the NLRP3 inflammasome, which controls caspase-1 activity and IL-1β release in the innate immune system and controls inflammatory responses caused by bacterial infection, viral infection, fungal infection, obesity, cholesterol crystals, silica crystals, amyloid-beta, and uric acid crystals. Osthole, 7-methoxy-8-(3-methyl-2-butenyl) coumarin, is an ingredient of the fruit of *Cnidium monnieri (L.)* showing various biological activities including anti-inflammation, anti-tumor, and anti-apoptosis; however, the effect of osthole on NLRP3 inflammasome activation is unclear.

Materials and Methods:

Effect of osthole on NLRP3 inflammasome activation in LPS- and ATP-activated macrophages was monitored by detecting IL-1β secretion and caspase-1 activation using ELISA and western blot respectively. LPS- and ATP-mediated signaling associated with NLRP3 inflammasome activation were measured by western blot.

Results:

Osthole reduced IL-1β secretion and caspase-1 activation by inhibiting priming signal, but not activation signal of NLRP3 inflammasome in LPS- and ATP-activated macrophages. The osthole also reduced LPS-induced protein expression levels of NLRP3 and IL-1β precursor. The underlying mechanisms for the anti-NLRP3 inflammasome activity were demonstrated as reducing ATP-induced ROS production and phosphorylation of PKC-α. Interestingly, osthole increased the activation of NLRP1and NLRC4.

Conclusion:

These results demonstrate that osthole inhibited NLRP3 inflammasome activation and has the potential to be developed as preventing/therapeutic agent for NLRP3 inflammasome-related disease.

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Biocompatibility and biological function of mesenchymal stem cells and MC3T3 cells on poly (ethylene glycol)-nanogold nanocomposites

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Backgrounds:

Vascularization and osteogenesis play a critical role for bone tissue regeneration. The main goal of this proposal was intended to design a kind of ideal biomaterial for bone tissue engineering application. A simple surface modification method, comprising of a thin coating with gold nanoparticles (AuNPs) and poly (ethylene glycol) (PEG) was developed to improve the biocompatibility required for bone tissue regeneration.

Materials and Methods:

The surface morphology of PEG-AuNPs was characterized by the UV-Vis spectrophotometry (UV-Vis), and Fourier Transform Infrared spectroscopy (FTIR). The biocompatibility effect and biological performance of the PEG-AuNPs was evaluated by in vitro study.

Results:

The behavior of human umbilical cord-derived mesenchymal stem cells (MSCs) and osteoblast (MC3T3) on PEG-AuNPs was further investigated. Cells on PEG-AuNPs particularly that containing 43.5 ppm of AuNPs showed had better cell proliferation, low ROS generation, less monocyte activation, as well as increases in the protein expression levels of matrix metalloproteinase-9 (MMP-9), which may account for the enhanced cell migration on the PEG-AuNPs.

Conclusion:

These results suggest that the PEG-AuNPs nanocomposite thin film coating may serve as a potential and simple solution for the surface modification of bone-contacting devices.

P478

Activated microglia promotes glioma growth in mice with deficiency of tumor necrosis factor receptor type II

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Microglia play a critical role in the immune response of the central nervous system (CNS). Similar to other neurodegenerative disorders, microglia accumulation is observed in glioma. The recent findings have indicated that activated microglia can enhance glioma progression. It has been known that systemic inflammation increase the production of proinflammatory cytokines, including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). These cytokines not only control local inflammation in peripheral tissues, but also trigger neuroinflammation in the CNS. The multiple actions of TNFα are resulted from the complexity of TNF receptor type I (TNFRI) and type II (TNFRII)-triggered signaling pathways. Our recent findings showed that intraperitoneal (ip) injection with lipopolysaccharide (LPS) injections into TNFRII$^{-/-}$ mice (0.5 mg/kg/day) significantly increased CD11b^{+} microglia/macrophages in the cortex. In addition, progressive C6-glioma cell formed tumor was observed in the implanted site of TNFRII$^{-/-}$ mice brain when compared to that observed in TNFRII$^{+/-}$ mice. We found that no significant survival rate of TNFRII$^{-/-}$ mice treated with peripheral LPS injection before C6 glioma cell implantation was observed when compared to that of WT and TNFRII$^{+/-}$ mice. However, pre-treatment with LPS increased tumor volume in TNFRII$^{-/-}$ mice bearing C6 glioma implantation. Alternatively, the lower survival of TNFRII$^{-/-}$ mice receiving peripheral LPS injection after the implantation of C6 glioma cells was observed when compared to that detected in wild type (WT) and TNFRII$^{+/-}$ mice. Yet, there was no difference in the tumor size between surviving WT, TNFRII$^{+/-}$, and TNFRII-/- mice with formed glioma. The low surviving rate of TNFRII$^{-/-}$ mice receiving post LPS injection might be due to systemic immune response. Together, the results suggest that TNFRII deficiency and microglia activation could play the role in enhancing glioma growth.

P479

Developments of chemical probes that label protein tyrosine phosphatases *in vivo*

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Backgrounds:

The goal of the research is to establish a method for the analysis of protein tyrosine phosphatase activities *in vivo*. Here we report the design, synthesize, and characterization of a series of new chemical probes that can be used for *in vivo* labeling of cellular PTPs.

Materials and Methods:

Chemical probes **LCL08037, LCL09012, LCL08021, LCL09011**, and **LCL10005** were designed and synthesized. These chemical probes were tested for their labeling specificity and efficacy toward PTPs *in vitro* and *in vivo*. Purified recombinant PTPs were used to evaluate the labeling properties of these probes *in vitro*. Cell based system was use to evaluate the labeling efficiency of these probes *in vivo*.

Results:

Chemical probes **LCL08037** was first designed and characterized that had a 1,4-fluorine reactive group to be recognized and labeled classical PTPs. However, this probe cannot enter cells to label phosphatases directly. We then applied multiple approaches including two-step labeling system, addition of acyloyxmethyl to neutralize the charge of the phosphotriesters, and adopted a non-polar BODIPY group as the reporter to facilitate the entering of these probes into cells. These approaches proved to be useful that effectively labeled PTPs when incubated with cells *in vivo*.

Conclusion:

Our newly designed probes could be used as a tool in detecting and monitoring the cellular activities of PTPs *in vivo*. These newly designed PTP probes can analyze PTPs active in diseases or identify unknown PTPs in cells.

P480

Identification and Characterization of *Bacillus* strains Isolated from honey

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A total of 86 bacterial strains were isolated from 29 honey samples collected from different countries. According to VITEK AutoMicrobic system tests and partial 16S rDNA sequences analysis, the isolates were belonging to several common species of the genus *Bacillus.* The *Bacillus* species identified included *B. subtilis, B. pumilus, B. megaterium, B. gibsonii, B. indicus,* and species of the *B. cereus* group, whereas a number of our isolates could not be classified. Among these strains, five strains were selected and subjected to analysis by API-ZYM commercial kit system based on presence of 19 different enzymes. The results showed that esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, β-galactosidase, α-glucosidase, and β-glucosidase were produced by all tested strains. However, the properties of their enzymes varied from strain to strain. In addition, all isolated 86 strains harboring riboflavin (*rib*) genes were detected by PCR amplification of the *rib* gene. As observed, 31 out of 86 strains yielded positive PCR result bearing *rib* gene. These results are encouraging as *Bacillus* strains bearing some useful enzymes are found in different country sources of honey. The present study is an approach initiated from diversity screening of *Bacillus* species to further potential enzyme production and application from the specified *Bacillus* strains.

P481
The generation of proinflammatory mediators on macrophages by Okadaic acid

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Backgrounds

Okadaic acid is the protein phosphatase 1/2A inhibitor and one of main diarrhetic shellfish poisoning toxins. However, the mechanism of okadaic acid induced proinflammatory generation in activated microglia is unclear. At present, we found okadaic acid had no cytotoxic effect on macrophage RAW264.7 cells.

Materials and Methods

Cytotoxicity was measured by tetrazolium bromide reduction assay. interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)α was analysed by enzyme-linked immunosorbent assay. Generation of nitric oxide (NO) were investigated by Griess assay. Statistical analyses were performed using anova followed by the Bonferroni's t-test for multigroup comparisons.

Results:

Okadaic acid induced generation of proinflammatory mediators, such as nitric oxide (NO), interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)α, in a concentration-dependent manner.

Conclusion:

The mechanism of okadaic acid induced macrophages activation near further studies. These results indicated that okadaic acid induced proinflammatory mediators generation and cytotoxicity on macrophages.

P482
Silibinin could modulate PI-3 kinase/Akt/mTOR pathway to inhibit hypoxia-induced VEGF secretion and aged-macular degeneration

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Background:

Hypoxia-mediated neovascularization plays an important role in age-related macular degeneration (AMD). There are few animal models or effective treatments for AMD. Here, we investigated the effects of the flavonoid silibinin on hypoxia-induced angiogenesis in a rat AMD model.

Materials and Methods:

Retinal pigmented epithelial (RPE) cells were subjected to hypoxia in vitro and the effects of silibinin on activation of key hypoxia-induced pathways were examined by elucidating the hypoxia-inducible factor-1 alpha (HIF-1α) protein level by western blot. A rat model of AMD was developed by intravitreal injection of vascular endothelial growth factor (VEGF) in Brown Norway rats, with or without concomitant exposure of animals to hypoxia. Animals were treated with oral silibinin starting at day 7 post-VEGF injection and AMD changes were followed by fluorescein angiography on days 14 and 28 post-injection.

Results:

Silibinin pretreatment of RPE cells increased proline hydroxylase-2 expression, inhibited HIF-1α subunit accumulation, and inhibited VEGF secretion. Silibinin-induced HIF-1α and VEGF downregulation required suppression of hypoxia-induced phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (mTOR) pathway. In the rat model of AMD, silibinin administration prevented VEGF- and VEGF plus hypoxia-induced retinal edema and neovascularization.

Conclusion:

The effects of silibinin, both in vitro and in vivo, support its potential as a therapeutic for the prevention of neovascular AMD.

P483
The Inhibitory Effect of Hibiscus Sabdariffa Leaf Extract on α-MSH-Induced Melanogenesis in Murine Melanoma B16F10 Cells

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Background:

Melanogenesis has many important physiological functions, including photo-protection of human skin from UV irradiation. Melanin synthesis is stimulated by α-melanocyte stimulating hormone (α-MSH) and cyclic AMP (cAMP) elevating agents. However, abnormal melanogenesis causes hyperpigmentation in the skin, which results in serious aesthetic issues and increases the risk of skin cancer. Previous studies have indicated Hibiscus sabdariffa L. leaf extract (HLE) has antioxidant and anticancer activities. Therefore, in this study we examined the effect of HLE on α-MSH-induced melanogenesis and signaling pathways in melanoma B16F10 cells.

Materials and Methods:

The inhibitory effect of HLE on α-MSH-induced melanin synthesis and tyrosinase expression was evaluated. The expressions of TRP-1, 2 (tyrosinase-related protein 1, 2), MITF (the transcription factor of tyrosinase) and CREB (the transcription factor of MITF) were also examined to explore the anti-melanogenic mechanisms of HLE in the B16F10 cells pretreated with α-MSH. Additionally, intracellular cAMP content was analyzed by ELISA assay.

Results:

Non-cytotoxic doses of HLE reduced α-MSH-induced both tyrosinase activity and melanin production. Western blotting data showed HLE inhibited the expression of tyrosinase and TRP-1, cooperative with intracellular cAMP content. Further, HLE also suppressed the nuclear levels of CREB causing the consequent disturbed activation of MITF in α-MSH-stimulated B16F10 cells. The HLE-inhibited α-MSH-induced tyrosinase expression appeared be a consequence of MITF inactivation, because its DNA binding activity was suppressed by HLE.

Conclusion:

Our data showed HLE inhibited α-MSH-induced melanogenesis in B16F10 cells. Thus, HLE might be used as a potential natural whitening agent.

P484
Cell Survival Effects of Scopoletin on Lung Cancer Cell Line A549

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Backgrounds:

Cancer has been the leading cause of death in Taiwan. Lung cancer accounted for the most deaths followed by the liver, breast, and colorectal cancer. Inflammatory responses play roles at different stages of tumor development, including initiation, invasion, and metastasis. Scopoletin belongs to the groups of coumarin. Because of its anti-inflammatory effect, the aim of this study is to explore the biological effects of scopoletin on human lung adenocarcinoma epithelial cell line (A549).

Materials and Methods:

Human lung adenocarcinoma epithelial cell line A549 treated with scopoletin for 24-72 hours incubation period. Morphological change was detected by direct observation. Cell viability and cell cycle analysis was assayed by MTT and flow cytometry. .

Results:

The cell morphology was change obviously while treated with scopoletin after 48 hours incubation. The cell viability was analyzed by MTT assay. We found that the cell viability decreased for more than 30% by scopoletin at the dosage of 0.05-0.4 μg/μL for 48-72 hours incubation. The sub G1 phase was detected by flow cytometry analysis at the dosage of 0.2-0.4 μg/μL for 72 hours.

Conclusion:

Our study indicated that scopoletin induced A549 cells inhibition in a concentration-dependent manner probably through the apoptosis mechanism. The exact apoptosis pathway is under investigation in our laboratory.

P485

Hibiscus Sabdariffa Leaf Polyphenolic Extract Induces Autophagy and Apoptosis in Human Melanoma Cells

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Background:

Hibiscus sabdariffa leaf, the edible part of *H. sabdariffa* Linne, is usually ignored and discarded. Previous studies have indicated that *H. sabdariffa* L. leaf extract (HLE), rich in polyphenols, has been demonstrated to hypoglycemic, hypolipidemic, antioxidant and anticancer effects. In this study investigations were conducted to examine the anticancer activity of *H. sabdariffa* leaf polyphenolic extract (HLP) *in vitro*.

Materials and Methods:

We utilized trypan blue assay to analyze the effect of HLP on human melanoma A375 cell viability. Using a set of cell death detection assays, including Flow cytometric analysis, DAPI assay, and AVO stain, the effects of these agents on the cell cycle distribution, apoptosis, and autophagy were defined *in vitro*. The expressions of molecular proteins were measured by Western blotting.

Results:

HLP could inhibit A375 cell growth in a dose-dependent manner. Our results also revealed the cells presented AVO-positive morphology, and had an increase in autophagosomes with double-membrane structure after a 24-h treatment with HLP. This effect of HLP in A375 cells might be mediated via regulation of the autophagy-related proteins, including ATG5, Beclin-1, P62 and LC3. In addition, HLP could induce apoptosis and the expression of caspase-3/8/9 in A375 cells.

Conclusion:

Our findings indicated HLP induced A375 cell death through autophagy and apoptotic signaling. These results suggested that HLP potentially could be developed as an anti-cancer agent, and may open interesting perspectives to the strategy in human skin cancer treatment.

P486

Effects of Butachlor, Carbosulfan, And Fipronil on Early Embryo Development of Zebrafish (*Denio rerio*)

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Backgrounds:

Recently, fish embryo toxicity test was proposed to be an alternative method on aquatic safety assessment.

Materials and Methods:

In this study, three pesticides including butachlor, carbosulfan, and fipronil were used to evaluate the aquatic environment safety with the acute zebrafish embryo toxicity and the extent of fish embryo abnormal development.

Results:

In process, the 48-h acute zebrafish embryo toxicity test was performed and the resulting median lethal concentration (LC_{50}) or median effect concentration (EC_{50}) for these three pesticides was 2.764 (LC_{50}), 0.035 (EC_{50}) and 6.284(LC_{50}) mg/L, respectively. Based on the EC_{50}/LC_{50} values, four treatment levels of each pesticide were used to evaluate the developmental toxicity on zebrafish embryo. The results showed butachlor and carbosulfan caused significant early-hatching effect at 1.0 and 0.1 mg/L on 50 hours-post-fertilization embryos. On the observation of morphological defects, fipronil significantly caused shortened body length at 0.07 mg/L and curved body axis of larval fish at 0.60 mg/L. Moreover, butachlor and fipronil affected the embryo development by decreasing the melanin accumulation on the surface of zebrafish trunk.

Conclusion:

In conclusion, the data revealed that these pesticides may cause developmental toxicity on zebrafish embryo after short and continuous exposure.

P487

Benzo(a)pyrene Inhibits Skeletal Myogenic Differentiation and Myotube Formation in Human Skeletal Muscle Progenitor Cells

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Backgrounds:

Recent epidemiologic researches show the evidence that transplacental exposure to airborne polycyclic aromatic hydrocarbons (PAHs) may compromise fetal development, including low birth weight and length. Benzo[a]pyrene (BaP), a member of PAHs, is known as a risk associated with growth restriction. However, the action and mechanism of BaP on the skeletal myogenic differentiation and muscle fiber development still remain unclear. Here, we investigate that low-dose BaP and the main metabolite influence the myogenic differentiation and myotube formation in human skeletal muscle progenitor cells (HSMPCs).

Materials and Methods:

HSMPCs were isolated from skeletal muscle biopsies of patients undergoing knee surgery. HSMPCs were exposed to low dose (0.25 and 0.5 μM) BaP and the main metabolite, benzo(a)pyrene-7,8-dihydrodiol-9,10-expoxide (BPDE), during muscle differentiation. After 4 days of treatment, myoblast differentiation was determined morphologically by analysis of multinucleated myotube formation and by hematoxylin and eosin staining. The cell with or without treatment in GM was counted by trypan blue staining. The associated-protein expressions during myoblast differentiation were analyzed by Western blotting.

Results:

The results demonstrated that low dose BaP and BPDE (0.25 and 0.5 μM) significantly inhibited the myotube formation after 4 days of differentiation in C2C12 myoblasts, as well as in HSMPCs. C2C12 myoblasts were dramatically decreased until 10-day BaP pretreatment, indicating BaP could disrupt C2C12 myoblast growth resulting in the poor myotube formation. Estrogen receptors (ER) antagonist ICI182780 effectively enhanced the inhibitory effect of BaP and BPDE on the differentiation-associated protein expressions of MHC, myoD. Furthermore, BaP and BPDE were capable of inhibiting skeletal myogenic differentiation by Akt suppression and NF-kB activation in HSMPCs.

Conclusion:

This study provides the evidence that exposure to BaP intervenes in skeletal muscle differentiation and myotube formation via an antagonism on ER.

P488

Assessing cytotoxic effects of PAEs on mesenchymal stem cells isolated from umbilical cord

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Backgrounds:

The phthalate esters (PAEs) are a kind of environmental hormone ubiquitously present in the environment and in human tissues. They bind to nuclear hormone receptors and affect cellular and developmental processes. The goal of this study is to monitor and evaluate dose-dependent responses of human umbilical cord mesenchymal stem cells (UCMSCs) exposed to PAEs. (DEHP, MEHP, BBzP, & MBzP)

Materials and Methods:

Electric Cell-Substrate Impedance Sensing(ECIS): Approximately 2 days after MSCs (3×104) were cultured onto ECIS electrode wells, cells were treated with different types and concentrations of PAEs. The time course impedance values were measured at multiple frequencies for 20 hours and dose-dependent changes of the impedance drop were generally observed. For detection of cell micromotion, rapid time collection of the impedance data for each well was taken every 0.2 second with exquisite sensitivity until 4500 points were collected and then another well was measured. To quantify the changes of cell morphology in response to PAEs, impedances of the cell-covered electrode wells were measured as a function of frequency. By comparing the experimental data with the calculated values obtained from cell-electrode model, morphological parameters such as the junctional resistance between cells (Rb) and the average cell-substrate separation (h) can be determined.

CyQUANT® Cell Proliferation Assays: Approximately 1 day after MSCs (3×103) were cultured onto the 96-well plates, cells were treated with different types and concentrations of PAEs for 2 days. Cell proliferation was then measured by CyQUANT NF Cell Proliferation Assay Kit (Invitrogen). The fluorescence intensity of each sample was measured using a fluorescence microplate reader with excitation at 485 nm and emission detection at 530 nm.

Results:

The data were first presented as the measured resistance normalized to its value at the start of each run. The resistance drop due to the addition of DEHP, MEHP or MBzP was not evident. However, a drastic drop of resistance was observed if high concentration of BBzP was added. The frequency scan measurement showed that high concentration of BBzP caused a decrease of the junctional resistance between cells. In addition, the rapid time collection measurement showed that the addition of DEHP, MEHP and BBzP caused the reduction of cell micromotion even though the overall resistance value was not much changed.

Conclusion:

We have applied various ECIS assays to follow the activities of UCMSCs in response to different concentrations of PAEs. Our results provide information about the potential risk and minimum toxic concentration of PAEs-containing solution on both morphology and motility of the UCMSCs.

P489

Iron Oxide Nanoparticles Attenuated Phagocytic and Antigen-presenting Functions of Murine Microglia

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Backgrounds:

Superparamagnetic iron oxide nanoparticles have been used as magnetic resonance imaging (MRI) contrast agents for a variety of diagnostic applications, including the imaging of the central nervous system (CNS). As the major resident immune cells in the CNS, microglia participate in both innate and acquired immune responses. The objective of this study was to investigate the effect of iron oxide nanoparticles on the phagocytic and antigen-presenting functions of microglia.

Materials & Methods:

Primary murine microglial cells were exposed to carboxydextran-coated iron oxide nanoparticles (0.1-50 μg Fe/mL) for 24 h. The activation of microglia was evaluated by the expression of ionized calcium-binding adaptor molecule-1 (Iba-1). The phagocytosis, antigen uptake and processing were determined by flow cytometry using pHrodo E. coli, ovalbumin (OVA)-Alexa fluor 647 and DQ-OVA, respectively. The antigen presenting ability of microglia was evaluated by the expression of the costimulatory molecules CD40 and CD86, following stimulation with lipopolysaccharide and interferon-γ.

Results:

Exposure to iron oxide nanoparticles attenuated the expression of Iba-1, the phagocytosis of pHrodo E. coli, and the uptake and processing of OVA by murine microglial cells. In addition, the expression of CD40 and CD86 by activated microglia was suppressed by iron oxide nanoparticles.

Conclusion:

These results indicate that exposure to iron oxide nanoparticles may attenuate the phagocytic and antigen-presenting functions of microglia. The impact of iron oxide nanoparticles on acquired immune responses mediated by microglia warrants further investigation.

P490

Free radical scavenging and inhibitory effects of lipid peroxidation stimulated by linoliec acid hydroperoxide on rat lung mitochondria of selected spices herbs

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Backgrounds:

Oxidative stress is closely related to lung diseases. The spices are used as flavor and Chinese medicine for lung diseases. In this study, the bioactivity of ten spices on antioxidant, free radical scavenging and ROS damage protection on rat lung mitochondria were investigated.

Materials and Methods:

The ethyl acetate extracts of 10 spices as follow, *Illicium verum, Foeniculum vulgare, Ramulus Cinnamomi (Cinnamomum cassia), Cortices Citrus (Citrus reticulata), Amomum tsao-ko, Crevost et Lemaire, Kaempferia galangal, Amomum cardamomum, Piper nigrum, Syzygium aromaticum,* and *Perilla frutescens* were prepared. Lipid peroxidative inhibitory assay was induced by linoleic acid hydroperoxide as reactive oxygen species (ROS) inducer on rat lung mitochondria. Moreover, free radical scavenging activities of each extract were displayed by DPPH, superoxide anion scavenging, hydrogen peroxide scavenging, xanthine oxidase inhibitory, and ferrous ion chelating assay.

Results:

The extracts of *Illicium verum, Kaempferia galangal* and *Syzygium aromaticum* showed more potential LPO inhibitory effect (IC_{50}: 36.83±2.67, 23.01±2.75 and 8.43±1.70μg/ml).

Syzygium aromaticum extract has highest DPPH scavenging capability (EC_{50}9.58±0.23μg/ml) and phenolic content (329.31±2.74 μg equivalent gallic acid /mg).

Kaempferia galangal exerts remarkable xanthine oxidase inhibitory effect (IC_{50} 34.29±0.22μg/ml). *Crevost et Lemaire* shows highest flavonol content with 165.55±4.88μg/mg equivalent EGCG.

Conclusion:

Overview of above evidence suggests that 10 spices are potential antioxidants and LPO inhibitors to develop a natural lung damage protector, and *Syzygium aromaticum* and *Kaempferia galangal* are most potential.

P491

Immune Response and Allergic Contact Dermatitis of Chromium (VI) with Nanoparticle on Dendritic Cells Activation and Function.

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Backgrounds:

The purpose of this study is to determine whether Cr^{+6} combined with nanoparticles enhances the effects of allergic contact dermatitis (ACD) and/or immunotoxicity compared with treatment alone by using DCs and animal model.

Materials and Methods:

In *in vitro* study, DCs were treated with Cr^{+6}, titanium dioxide nanoparticles (nano-TiO_2) alone and in combination. Then, cytotoxicity was analyzed by MTS assay to determine the optimal doses. The percentage of mouse bone marrow-derived DCs and the expression levels of maturation were determined by flow cytometry. Cytokine production was measured by ELISA.

Results:

Our results suggested that about 70 % of dendritic cells could be derived from mouse bone marrow cells. The percentage of DCs viability was determined after treatment of different doses of Cr^{+6}, nano-TiO_2 and combined treatment for 24 hrs. The results indicated that DCs survival rate was above 80 % at doses less than 2 μM Cr^{+6} and 50 μg/ml nano-TiO_2. In addition, the expression levels of MHC class II and co-stimulatory molecules CD86 were higher in Cr^{+6}, nano-TiO_2 alone and in combination (Cr^{+6}: 15 nM / nano-TiO_2: 25 μg/ml) than in control. Furthermore, the production of proinflammatory cytokins, including TNF-α and IFN-γ were significantly increased in combined treatment group.

Conclusion:

These results show that hexavalent chromium combined with nanoparticles could have the potential to induce more dendritic cells activation and related immune response.

P492

Advanced Glycation End Products (AGEs) Increase Proliferation in Human Fibroblast-Like Synovial Cells via Receptor for AGEs and Mitogen-Activated Protein Kinases.

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Backgrounds:

Aged people have higher risk of osteoarthritis (OA) which is characterized synovial hyperplasia. Advanced glycation end products (AGEs) are highly accumulated in aged and diabetes patients, especially in knee joint. The influence of AGEs on fibroblast-like synovial cells (FLS) still remains unclear. This study is investigating that AGEs potentially influence on FLS proliferation whether regulated by mitogen-activated protein kinase (MAPKs) and receptor for AGEs (RAGE) pathway.

Materials & Methods:

Synovial tissue was obtained from OA patients and digested to isolate FLS. FLS is treated with 10, 20, 50, 100 and 200 μg/ml AGEs to evaluate cell viability by MTT assay. The protein expressions of pERK, pJNK, pP38, pP65, RAGE and NF-κB ligand (RANKL) was measured by western blot assay. Furthermore, FLS was treated with 5μg/ml anti-RAGE antibody and 10 μM the ERK1/2 inhibitor U0126 to determine represented cellular signaling which regulates cell proliferation by MTT assay.

Results:

The MTT assay showed that AGEs increase FLS proliferation in a dose-dependent manner, and the negative regulation of AGEs on FLS proliferation resulted from suppression of ERK1/2 signaling and was restored by RAGE neutralizing antibody. AGEs mediated that FLS expresses increased protein level of MAPK including pERK, pJNK and pP38, and activates pP65 and RAGE. Besides, AGEs could increase the expression of RANKL in FLS.

Conclusion:

The present study shows that AGEs increase FLS proliferation, and activate NF-κB might induce inflammation response. Overall, these results suggest that AGEs enhance FLS proliferation through MAPK and RAGE pathway would involve in osteoarthritis progression. On the other hand, AGEs induce the expression of RANKL that may be potential to result in osteoclastogenesis.

P493
Effects of Perfluorinated chemicals on Embryonic Development in Medaka (Oryzias latipes)

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Backgrounds:

The perfluorinated chemicals (PFCs) are used as surfactants and surface protectors in paper, food containers, and other applications. Due to the resistance to hydrolysis, photolysis, and biodegradation, they can accumulate in tissues through the food chains. Thus, the chemicals have been detected in the environment, wildlife, and humans all over the world.

In this study, we investigated whether PFCs had any effects on gonadotropin-releasing hormone (GnRH) neuronal and embryonic development, using a transgenic medaka (Oryzias latipes) in which the green fluorescent protein (GFP) was placed under the control of the gnrh3 promoter. GnRH3 is one of the three forms of medaka GnRH, and is involved in the reproductive function. As the medaka embryos are transparent, the GnRH neurons expressing GFP can be monitored in vivo during embryonic development.

Materials and Methods:

The gnrh3-GFP medaka embryos were collected within 5 h post-fertilization, and each placed individually in a 24-well plate. They were then randomly selected and treated with 10-100 ppm of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorononanoic acid (PFNA) (n = 12–15/group), and maintained at 27 ℃ . All solutions were replaced every 24 h. The embryos were exposed until hatch, and their images were recorded. Along with the fluorescent intensity of the GnRH/GFP neurons, several endpoints of embryonic development, such as the eye development, time to hatch, hatchability, larval body length and weight, and swimming ability were also observed and analyzed.

Results:

The PFCs significantly altered the GFP fluorescence intensity, eye development, heart rate, and larval weight and/or length, caused spinal deformities, affected swimming ability, lowered the hatchability, and shortened the time to hatch.

Conclusion:

We have demonstrated that PFCs affect embryonic development at several levels, including the expression of the GnRH neurons, the eye and the heart development, the hatchability of the embryos, and the hatchings' swimming behavior. We will explore the underlying gene regulation during the PFC exposures in further studies.

P494
Effect of Penconazole, Propiconazole and Triadimefon on Estrogen Receptors with Whole Rat Embryo Culture

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Backgrounds:

Penconazole, propiconazole, and triadimfefon were most common triazole pesticides in Taiwan. They were developed to inhibit the ß-tubulin assembly in mitosis, it is likely to disturb the synthesis of steroid hormone in mammals. A few reports showed that triazole chemicals antagonized the aromatase, which transfer testosterone into 17ß-estradiol in mammals.

Materials and Methods:

This study aimed to investigate the effect of these three pesticides on estrogen receptor (ERα, ERß) activity in whole rat embryo culture (WREC) on gestation day (GD) 10.5. The concentrations of WREC were pencoazole, 0.004, 0.008, 0.016 ppm; propiconazole, 0.005, 0.01, 0.02 ppm; triadimefon, 0.005, 0.009, 0.019 ppm. The culture period was 48 hours. After evaluation of embryo development it was fixed in formalin or kept in HBSS for immunohistochemistry (IHC) and Western blot (WB), respectively.

Results:

The positive control of 17ß-estradiol (ERα, ERß) showed positive effect for its receptor expression. Results showed that these three triazoles induced expressions of ERα and ERß in WREC. This result basically meets the principle that triazoles were designed to disrupt the synthesis of steroid hormone. We need to study the antagonistic effects by adding the antagonists for estrogen receptors. The quantification of IHC and WB will be the important endpoints in addition to the images.

Conclusion:

Penconazole, propiconazole, and triadimfefon significantly induced the estrogen receptor expressions. It seems that WREC can be used as a robust method of endocrine disrupting screening for estrogen receptors.

P495
FIP-fve, an Immunomodulatory Protein from Flammulina Velutipes, Inhibits Inflammation Induced by Respiratory Syncytial Virus

張語琦

Backgrounds:

Respiratory syncytial virus (RSV) causes bronchiolitis in children followed by inflammation and asthma-like symptoms. To date, the development of a vaccine for this virus has been met with many problems. Fungal immunomodulatory proteins (FIPs) exhibit anti-inflammatory function. FIP-fve is an immunomodulatory protein isolated from Flammulina velutipes. To determine whether FIP-fve affects the infection or consequence of immunity of RSV, we investigated viral titers of RSV and inflammatory cytokine (IL-6) levels in vitro and in vivo.

Materials and Methods:

RSV replication and IL-6 level in RSV-infected HEp-2 cells were compared with those following FIP-fve treatment. Female BALB/c mice were administered 200 μg FIP-fve orally once a day from 2 days before RSV infection and intranasally administered w/o 2 × 105 plaque-forming units (PFU) of purified RSV.

Results:

We demonstrated that FIP-fve inhibits viral titers on plaque assay and Western blot, as well as the RSV-stimulated expression of IL-6 on ELISA and RT-PCR. On Western blot, FIP-fve decreased both phosphorylated IκBα and NF-κB expressions and inhibited NF-κB translocation into nucleus of A549 cells in a confocal manner. Moreover, oral FIP-fve decreased RSV-induced airway hyperresponsiveness (AHR), airway inflammation, and IL-6 expression in BALF of BALB/c mice.

Conclusion:

The results of this study suggested that FIP-fve decreases RSV infection, RSV-induced inflammation and respiratory pathogenesis. FIP-fve is a natural compound from Flammulina velutipes that is widely used, and may be a safe agent for viral prevention and even therapy.

P496
Growth Inhibitory Effect of Ethanol Extract of Water Caltrop Shell in Human Breast Cancer cells

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Background:

Water caltrop is one of the most popular vegetables in Taiwan due to its special taste and medical functions. The water caltrop shell are commonly discarded as fertilizer. However, there are many evidences showing that high fiber content and polyphenols of shell attracted more attention on antioxidants and health related properties, which play an important role in the prevention of cancer and cardiovascular disease. In this study, we investigated cytotoxic effect and apoptosis mechanism of ethanol extract of water caltrop (EEW) on human breast cancer cell lines.

Materials and Methods:

Cell viability was assayed by trypan blue exclusion and MTT assay. Apoptotic effects were evaluated by DNA ladder assay and flow cytometry. ROS was measured with DCFDA using flow cytometry.

Results:

The viabilities of breast and cervical cancer cells decreased in time course and dose dependent manner after treatment with the EEW. Apoptosis analysis revealed that the percentage of MDA-MB231 cells in the SubG1 phase (a marker of apoptosis) was increased upon EEW treatments, indicating that DNA fragmentation existed concomitantly with cellular death. The EEW decreased reactive oxygen species (ROS) production in MDA-MB231 cells.

Conclusion:

The EEW induced apoptosis in MDA-MB231 cells partially through ROS reduction. Therefore, the ethanol extract of water caltrop could be considered a source of new anti-cancer healthy food.

P497

To Develop Gold Nanoparticles Conjugated with Conventional Chemotherapy Drug to Achieve Anti-Oral Squamous Cell Carcinoma Synergism

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Backgrounds:

Chemotherapy is the standard treatment for oral squamous cell carcinoma (OSCC). However, chemotherapy drugs, such as 5-fluorouracil (5FU) and methotrexate (MTX) often indicate significant toxicity to healthy cells. Gold nanoparticles (AuNPs) have gained considerable attention due to potential use in biomedical applications. They show tumor-selective uptake with multiple targeting functional groups. In this study, we aim to investigate whether AuNPs conjugated with chemotherapy drugs exhibit selective cytotoxicity on cancer or normal cells.

Materials and Methods:

Synthesized serial 5FU-AuNP and MTX-AuNP nanodrugs by different conditions was characterized their physical properties by UV-vis absorption spectroscopy, energy dispersive X-ray fluorescence spectrometry, Fourier transform infrared spectroscopy, and transmission electron microscopy. all synthesized 5FU-AuNP and MTX-AuNP were tested the cytotoxicity effects of cancer cells and healthy human oral mucosa fibroblasts by MTT assay. In mechanism level, LC3 expression was tested by western blot.

Results:

At the end of follow-up, we found that 5FU-AuNP-dep#3, 8 and 9 displayed lower IC50 value compared to parental drug, and the size and shape of these candidates were all spherical figure and approximately 20 nm diameter. However, there were no significant improve of drug efficacy in MTX-AuNPs group. Furthermore, 5FU-AuNP-dep#9 displayed extremely low cytotoxicity in normal cells. In mechanism level, 5FU-AuNP-dep#9 induced lower LC3 expression, the essential element of autophagosome formation, than parental drug, which indicated that 5FU promoted autophagy to escape apoptosis, but not 5FU-AuNP-dep#9.

Conclusion:

5FU-AuNP-dep#9 demonstrated a stronger cytotoxicity effect in OSCC than parental 5FU drug, and showed less off-target effects in human healthy cells

P498

Antioxidant activity analysis of *Hedychium coronarium* extract

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Backgrounds:

Hedychium coronarium belongs to Zingiberaceae family and is widely distributed throughout tropical and subtropical regions. The aim of the present study was designed to investigate the antioxidant properties of Hedychium coronarium.

Materials and Methods:

The freeze drying leaf and stem of *Hedychium coronarium* were extracted by hot water for 180 mins. The antioxidant activities were examined by DPPH, reducing power and ferrous chelating ability. Additionally, we also measured the amount of total flavonoids and total polyphenolic in the *Hedychium coronarium*.

Results:

The results showed that DPPH radical scavenging activity, ferrous ion chelating activity, reducing power all increased with increasing concentrations of leaf and stem extracts of *Hedychium coronarium*. Additionally, the total phenolic contents of leaf and stem extracts were 66 μg/ml and 12 μg/ml, respectively. The total flavonoids contents of leaf and stem extracts were 88 μg/ml and 22 μg/ml, respectively.

Conclusion:

Therefore, the total flavonoids and total phenolic content of *Hedychium coronarium* are reflected by its in vitro antioxidant properties. Based on the assays presented here, it can be concluded that *Hedychium coronarium* is an accessible source of natural antioxidants that provides the expected health benefits.

P499

Mechanisms of Sphingosine 1-Phosphate Induced Intercellular Adhesion Molecule-1 Expression in Human Pulmonary Alveolar Epithelial Cells

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Backgrounds:

Sphingosine-1-phosphate (S1P) has been shown to regulate expression of several inflammatory genes and contributes to inflammatory responses. Several studies demonstrate that expression of adhesive molecules on the cell surface of epithelial cells plays a critical role in inflammatory responses. Here we investigated the mechanisms regulating intracellular cell adhesion molecule (ICAM)-1 expression by S1P in human pulmonary alveolar epithelial cells (HPAEpiCs)

Materials and Methods:

To investigate whether S1P induces ICAM-1 expression, Western blot, RT-PCR, and real-time PCR were performed. Moreover, the monocyte adhesion was detected by adhesion assay. To study the mechanisms of S1P-induced ICAM-1 expression, the pharmacological inhibitors or specific siRNAs were used.

Results:

We found that HPAEpiCs express S1P receptor subtypes including S1PR1, S1PR2, and S1PR3, analyzed by RT-PCR. Time-course incubation of S1P resulted in an increased in ICAM-1 mRNA, protein expression, the promoter activity, and monocyte adhesion. Moreover, we investigated the signaling pathways of S1P-induced ICAM-1 expression in HPAEpiCs. The data showed that S1P-induced ICAM-1 expression and THP-1 adhesion were attenuated by pretreatment with the inhibitor of S1PR1/3 (W123, CAY10444), Gi protein (GP2A), c-Src (PP1), EGFR (AG1478), PDGFR (AG1296), PI3K (LY294002), MEK1/2 (U0126), p38 MAPK (SB202190), AP-1 (Tanshinone IIA), or NF-κB (Bay11-7082) and transfection with respective siRNAs, revealed by Western blot. These results sugegsted that c-Src, RTKs, PKCs, MAPKs, PI3K/AKT, AP-1, and NF-κB were involved in S1P-induced ICAM-1 expression in HPAEpiCs.

Conclusion:

We demonstrated that S1P-induced ICAM-1-dependent monocyte adhesion was mediated through activation of the c-Src, EGFR, PDGFR, ERK1/2, p38 MAPK, PI3K/Akt, AP-1, and NF-κB pathways in HPAEpiCs.

P500

Phthalate -Induced Cardiac Toxicity *in vitro* and *in vivo*.

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Backgrounds:

Di(2-ethylhexyl) phthalate (DEHP), as a plasticizer, is a well- known endocrine-disrupting chemical and became infamous caused by a serious food safety issues in 2011 in Taiwan. In mammals, DEHP is rapidly hydrolyzed to its monoester, mono-(2-ethylhexyl) phthalate (MEHP) by esterase. MEHP is thought to be 10-fold more potent in its reproductive toxicitycompared to DEHP. The level of DEHP and its metabolite MEHP in the blood can be measured before and after cardiopulmonary bypass (CPB). DEHP in the general population is in the range of 3 to 30μg/kg of body weight per day. Although the developmental and reproductive toxicities of DEHP are well recognized, little is known about the potential adverse effects of phthalates on the heart.

Materials and Methods:

The primary cultured mice neonatal cardiomyocytes and rat neonatal cardiomyoblasts (H9C2 cells) were incubated with DEHP and MEHP (10 to 100 μM) for 72hr. The cellular morphological changes and cardiotoxicity markers were determined via immuno-fluorescent, real-time RT-PCR analyses, and Western blotting. The C57BL/6 mice with chronic exposure of DEHP (100mg/kg.bw or 200mg/kg.bw) by oral gavage for 16 weeks and the pathohistological changes of heart were examined via hematoxyllin/eosin staining and staining of collagen with a Masson's Trichrome (MT) Kit assessed myocardial fibrosis. Transthoracic echocardiography was examined at 8 weeks and 16 weeks after DEHP exposure during the treatment phase.

Results:

We found that treatment of neonatal cardiomyocytes with 10 to 100 μM DEHP and MEHP for 72hr decrease the cell viability , and decrease the activity of sarcoplasmic/endoplasmic reticulum Ca2+-ATPase2 (SERCA2), as well as SERCA2 at the mRNA and protein level in H9C2 cells. The chronic exposure of DEHP in mice can significant increase in heart rate as compared to control group (control vs. DEHP-treated mice, $p<0.05$). Transthoracic echocardiography showed that the fractional shortening and wall thickness was decreased in DEHP-treated group versus control group. An increasing in heart weight was noted after 16 weeks of DEHP treatment. MT staining was performed to show the significant myocardial fibrosis can be seen in the hearts from DEHP-treated animals.

Conclusion:

Exposure in clinical-related dosage of DEHP can lead to cardiac adverse effects and myocardiol fibrosis *in vivo*; the potential mechanisms may due to the cytotoxicity to cardiomyocyte and cause the decrease of the SERCA2 expression, which may lead to the dysfunction of heart. This study strongly suggest that using tubing free from DEHP during cardiopulmonary bypass, which may minimize exposure to phthalate and reduce the potential risk of cardiotoxicity.

P501
Liuwei Dihuang Wang Protects NSC34 Cells against SMN Deficiency

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Backgrounds:
Liuwei dihuang wang (LDW) is a widely used traditional Chinese medicine for diabetics, renal disorders, and neurosis. LDW has been revealed its benefits in kidney lesion and diabetic mellitus via anti-oxidative, anti-inflammatory, and anti-apoptotic properties. Survival motor neuron (S¬¬mn) protein deficiency is a major cause of motor neuronal death. In this study, we aimed to examine the neuroprotective effects of LDW on motor neuronal NSC34 cells under Smn deficiency.

Materials and Methods:
Inducible *Smn*-knockdown NSC34 cells mimicked SMN deficiency condition were used. Cell viability and cytotoxicity were measured by MTT assay and LDH assay, respectively. Protein expressions were determined by western blot analysis. Cell morphology and neurite length were observed by inverted microscope and measured by the Image J software.

Results:
Smn-knockdown activated apoptotic pathway and increased motor neuronal death. However, in inducible *Smn*-knockdown NSC34 cells, LDW (0.01-10 μg/mL) increased cell viability and Smn expression. Moreover, LDW upregulated anti-apoptotic protein Bcl-2, reduced cytosol level of cytochrome *c* and decreased the cleavage of caspase-3. Furthermore, LDW could attenuate Smn deficiency-induced neurite damage.

Conclusion:
The present results reveal the novel protective effect of LDW against Smn deficiency in NSC34 motor neuronal cells, which suggest that LDW might be used as an alternative and complementary medicine for neuroprotection.

P502
The Mechanism of Dengue Virus Core Protein Alter the Dynamic Balance of Positive-Transcription elongation factor b(P-TEFb)

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Background:
Dengue virus (DENV), a member of Flavivirus, can causes dengue fever (DF) and dengue hemorrhagic fever (DHF). In the past 50 years the WHO reported cases of DENV infection have increased noticeably. Therefore, the effective medical treatment or prevention of DENV infection becomes more and more urgent in need. Several studies have noted that DENV triggers the infected cells releasing large amounts of cytokines. Previous study indicates that DENV core protein induces infected cell IL-8 gene expression through associating with Positive-transcription elongation factor b (P-TEFb), composed of cyclin T1 and cyclin-dependent kinase 9 (CDK9), that is responsible for stimulating transition of transcription initiation step into elongation step. Although the total protein level of P-TEFb did not change in DENV core expressed cells, but it notes a possibility that DENV core protein may affects the activity of P-TEFb. The amount of active P-TEFb is regulated by a dynamic balance between Brd4-P-TEFb active complex and 7SK snRNA-HEXIM1 (hexamethylene bisacetamide-induced protein 1)-P-TEFb inactive complex. The purpose of this study was to investigate the how the dengue core protein alters the dynamic balance of P-TEFb.

Materials and Methods:
Flag tagged-full length and C-terminal 28 amino acids-truncated dengue core protein were used in this study. The C-terminal truncated dengue core is a negative control because of lacking nuclear localization signals (NLSs). In DENV core protein over-expression HeLa cells, whole cell lysate was immunoprecipitated by anti-P-TEFb (anti-CDK9 and anti-cyclin T1) or anti-Flag and then proceeded to immunoblotting for Flag-core, Brd4 and HEXIM1, furthermore, RNA will be extracted from immunoprecipitates followed by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) to examine the amounts of 7SK snRNA.

Results:
The immunoblotting result of flag-immunoprecipitates shows that flag tagged DENV core protein is not associated with Brd4 and HEXIM1. The immunoblotting result of CDK9-immunoprecipitates shows that the HEXIM1 relative binding level of P-TEFb has no significant change. However, the HEXIM1 relative binding level of P-TEFb was decreased in cyclin T1-immunoprecipitates. This result was strongly suggests that DENV core may direct interacts with cyclin T1 and interrupts the binding between cyclin T1 and anti-cyclin T1.

Conclusion:
Overall, our result indicates that DENV core protein is not associates with Brd4 and HEXIM1 and reveals the relationship between DENV core protein and P-TEFb. The accomplishment of this work may provide a novel target for the development of anti-DENV drugs.

P503
Leptin increases VEGF expression in human chondrosarcoma cells through the MEK, ERK, and c-Jun signaling pathways

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Background:
Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. Leptin, the product of the obese gene that plays an important role in the regulation of body weight that induces neuroprotection, neurogenesis, and angiogenesis. However, the effect of leptin on VEGF expression in human chondrosarcoma cells is mostly unknown. The aim of study is try to examine the effect of leptin in VEGF expression in human chondrosarcoma cells

Materials & Methods:
The qPCR was used to examine the mRNA expression of VEGF. The MEK, ERK, c-Jun phosphorylation was examined by using Western blot method. A transient transfection protocol was used to examine AP-1 activity.

Results:
We found that leptin increased the VEGF expression in human chondrosarcoma cells. Leptin-mediated VEGF up-regulation was attenuated by MEK inhibitors (PD98059 and U0126) and AP-1 inhibitors (Curcumin and Tanshinone). Incubation of cells with leptin increased MEK, ERK, and c-Jun phosphorylation as well as AP-1 luciferase activity.

Conclusion:
Our results indicate that leptin enhances the VEGF expression in chondrosarcoma cells. One of the mechanisms underlying leptin-directed VEGF expression was through the MEK, ERK, and c-Jun signal transduction pathway.

P504
KMUP-1 Ameliorates Neuropathic Pain via Inhibition of ERK and NF-κB Pathways in the Dorsal Horn of Spinal Cord

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Backgrounds:
Many reports have been shown that peripheral nerve injury could induce inflammatory states, resulting in the genesis and maintenance of neuropathic pain. We aimed to investigate whether KMUP-1 could attenuate pain hypersensitivity and inflammatory mediators, and to explore its possible mechanisms in the dorsal horn of spinal cord of rats after chronic constriction injury induced neuropathic pain.

Materials and Methods:
Chronic constriction injury (CCI) of sciatic nerve induced painful neuropathy is a widely employed model for induction of neuropathic pain in experimental animals. Sprague–Dawley rats were randomly divided into four groups including sham, sham with KMUP-1, right-sided CCI, and right-sided CCI with KMUP-1. KMUP-1 (5 mg/kg) was administrated intraperitoneally once daily starting at day 1 after CCI surgery. Mechanical allodynia and thermal hyperalgesia were assessed before surgery and at day 3, 7, 14 after sciatic nerve injury. The dorsal horn of spinal cord was divided into ipsilateral and contralateral parts for western blots and enzyme-linked immunosorbent assay to analyze the levels of inflammatory proteins and cytokines, respectively.

Results:
In this study, CCI rats markedly increased the proinflammatory and inflammatory proteins. The expression of p-ERK, p-PKA, p-PKC, COX2 and iNOS proteins and proinflammatory mediators (IL-1β, TNF-α) induced by CCI were significantly decreased in KMUP-1-treated group. The activation of ERK pathways contributes to neuropathic pain in CCI rats, and the function of pERK might partly be accomplished via gene expression to induce inflammatory responses.

KMUP-1 decreased phosphorylation of ERK in the spinal dorsal horn is mediated by multiple kinase pathways. Both pain markers of BDNF and CGRP activate ERK protein by way of the PKA and PKC pathways. Additionally, KMUP-1 also inhibited CCI-induced IκBα phosphorylation and NF-κB translocation to nuclei.

Conclusion:
In conclusion, the results suggest that KMUP-1 has anti-inflammatory and anti-pain hypersensitivity properties in CCI-induced neuropathic pain through inhibition of ERK and NF-κB. Therefore, KMUP-1 could be a potential pharmacotherapeutic agent for the treatment of neuropathic pain.

P505

Regulation of Hepatic RhoA/Rho Kinase by cyclic guanosine monophosphate (cGMP) Pathway and Sensitized peroxisome proliferator activated receptor (PPAR) for Improving Hyperlipidemia

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Purpose and background:

Xanthine-based caffeine and theophylline have been used for lowering body-weight. This study investigated whether xanthine-based KMUP-1 improves high-fat-diet (HFD)-induced dyslipidemia and body-weight gain of mice, and if so, compared the mechanism of actions of KMUP-1 for associated pleiotropic effects via both endothelium nitric-oxide synthase (eNOS)/cGMP-dependent pathway and also caffeine-like hormone-sensitive lipase (HSL) activation activity.

Methods:

KMUP-1 (1, 2.5, 5.0 mg/kg, p.o.) was administered in C57BL/6J mice treated with HFD for 8 or 12 weeks. Body-weight changes, serum triglyceride/total cholesterol and lipoprotein levels were measured for monitoring hyperlipidemia; western blotting was used to measure the protein expression during long-term administration of KMUP-1 and HFD.

Results:

KMUP-1 reduced HFD-treated mice serum triglyceride, total cholesterol and sGOT/sGPT. KMUP-1 restored eNOS and peroxisome proliferator activated receptor γ (PPARγ) expression, inactivated RhoA/Rho kinase II (ROCKII) and reduced body-weight gain of mice treated with HFD. Treatment of HepG2 cells with C3 exoenzyme and Y27632 also inactivated ROCKII, which is associated with restoration of PPARg. Pretreatment with cGMP-antagonist reversed KMUP-1- induced ROCKII inactivation. Like caffeine, KMUP-1 indicated that activation of HSL can induce lipolysis in adipocytes.

Conclusion and implication:

KMUP-1 increases PPARγ via eNOS/cGMP through a modulator ROCK, creating co-localized signaling of pleiotropic effects, useful for lowering body-weight and hyperlipidemia

P506

DV128 enhances the cytotoxic effects of DNA-damaging agents against human breast cancer cells

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Backgrounds

Breast cancer is the cancer with the highest prevalence among women, and the major cause of death worldwide. Despite their serious side effects, DNA-damaging agents are still among the most effective cancer therapies. Drug combination therapy is a key strategy to improve treatment efficacy and to reduce adverse effects of DNA-damaging agents. Compound DV128 is a synthetic derivative of the flavonoid protoapigenone, which has been shown as a potent anticancer agent. In this study, we evaluated the cytotoxic effect of DV-128, alone and in combination of DNA-damaging agents (mitomycin C and cisplatin) against two human breast cancer cell lines. The mechanism underlying the interaction between these agents was also investigated.

Materials & Methods

The human breast cancer cell lines MCF-7 (estrogen receptor-positive) and MDA-MB-231 (estrogen receptor-negative) were used in this study. We studied the effect of DV128, mytomycin C, and cisplatin on cellular viability, clonogenic survival assays, and apoptosis. Cellular viability was measured by the 3-[4,5-dimethylthiazole-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. Levels of total and phosphorylated checkpoint kinase1/2 and the stress-activated protein kinases p38, JNK, the apoptotic marker poly (ADP-ribose) polymerase (PARP), and pro-caspase were determined by Western blot.

Results

Although DV128 alone had only little effect on cell viability of human breast cancer cells, it significantly enhanced the effects of mitomycin C and cisplatin on inhibiting proliferation, clonogenic survival, and inducing apoptosis. DV128 did not affect mitomycin C-induced activation of Chk1 and Chk2, which are mediators of DNA damage checkpoints. In contrast, the activation of stress-activated protein kinases JNK and p38 was enhanced when mitomycin C was used in combination with DV128. Because sustained activation of JNK and p38 has been shown to have a pivotal role in stress-induced apoptosis, our results suggest that the enhancement of DNA-damaging agent-induced cytotoxicity by DV128 may be resulted from stimulation of JNK and p38.

Conclusion

In the present study, we have demonstrated that DV128 potentiates DNA-damaging agent-induced cytotoxicity and apoptosis in human breast cancer cells. Enhanced activation of JNK and p38 by DV128 may play a crucial role in the combination effect.

P507

The Effects of *C*-phycocyanin on Skin Regenerative Activity

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Backgrounds:

Burn injuries are among the most devastating of all injuries and accompanied by both local and distant effects leading to intense inflammation, tissue damage, infection, and even a significant incidence of death. Today, most of the drugs used on burn injuries are mainly focused on anti-microbial activity. However, there are still no useful agents that effectively improve the progression of burn injuries. As a consequence, it's urgent to find a new drug that performs great wound-healing effect on skin wound cares. In present study, we would investigate the skin regenerative activity of *C*-phycocyanin on both *in vitro* and *in vivo*.

Materials and methods:

We used wound-healing scratch assay as described by Rodriguez et al. (2005) to test *C*-phycocyanin on EA.hy926 fibroblasts migration activity. Furthermore, we also performed burn injuries that modified from Schaffer et al. (1997) on both normal rats and diabetic rats. And also, we produced excision wounds that modified from Huang and Yang (2008) on normal rats and diabetic rats respectively.

Results:

The *C*-phycocyanin did not enhance the migration of EA.hy926 fibroblasts in the scratch assay. However, in the burn injury animal study, *C*-phycocyanin significantly enhanced the wound healing rate in the early phase of the experiment and promoted the dead skin detached from wounds in the middle phase on normal rats, but did not show significant wound-recovering effects on diabetic rats. As in the excision wound animal study, *C*-phycocyanin showed no effects on both normal rats and diabetic rats in the late phase of the experiment. But, in the early phase of excision wound, *C*-phycocyanin showed significant effects in wound area recovery on diabetic rats.

Conclusion:

C-phycocyanin enhanced the wound healing rate on burn injuries wound but not excision wounds in normal rats, and did not enhance burn or excision wounds healing in diabetic rats. This result suggests that *C*-phycocyanin might involve in different mechanisms of skin regeneration on normal rats and diabetic rats respectively.

P508

Therapeutic Benefits of Low-Dose Dextromethorphan Plus Valproic Acid in Bipolar Disorder

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Background The over activation of inflammatory cytokines and dysfunction of the neurotrophic system might be related to developing bipolar disorder. We evaluated whether inflammatory or neurotrophic factors or both are related to bipolar disorder and investigated whether treating bipolar disorder with low-dose dextromethorphan plus valproic acid is more effective than valproic acid only, and whether dextromethorphan affects plasma cytokine and brain-derived neurotrophic factor levels.

Methods In a 12-week, randomized, double-blind study, 209 patients were randomly assigned to groups: valproic acid plus either placebo or dextromethorphan. The Young Mania Rating Scale and Hamilton Depression Rating Scale were used to evaluate symptom severity, and ELISA to analyze cytokine and brain-derived neurotrophic factor levels.

Results Before treatment, patients had significantly higher plasma interleukin-1β and interleukin-8 levels, and significantly lower brain-derived neurotrophic factor levels than did healthy controls. Brain-derived neurotrophic factor levels were significantly negatively correlated with depression but not mania scores. After 12 weeks of treatment, these scores significantly improved in both groups. However, Changes in depression scores were significantly lower in the dextromethorphan group than the placebo group at week 12. Plasma cytokine and brain-derived neurotrophic factor levels were significantly improved only in the dextromethorphan group but not the placebo group.

Conclusions Plasma brain-derived neurotrophic factor levels may indicate the severity of depression. Valproic acid plus low-dose dextromethorphan treatment provided patients with bipolar disorder significantly more anti-inflammation and neurotrophic benefit and ameliorated depressive symptoms more than did valproic acid treatment alone.

P509
Suppression of Gefitinib-resistant Human Lung Cancer Cells by Indolizino[6,7-b]indole Derivatives

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Backgrounds

According to the most prevalent, poor prognosis, and lowest 5 year survival rate, patients with non-small cell lung cancer (NSCLC) are one of the most common issues in global health problem. Although tyrosine receptor inhibitors (TKI), cetuximab, erlotinib, and gefitinib, which target epidermal growth factor receptor (EGFR), are only effective in certain subpopulations, TKI resistance is developed after a median of 10 to 14 months via acquisition of second mutation as well as several other mechanisms. Developing therapeutic agent against TKI resistance is of critical and essential.

Materials & Methods

The derivatives of 1,2-bis-hydroxy- indolizino[6,7-b]indiole diols were examined their therapeutic effects on suppressing NSCLC cell lines including PC9 (exon 19 deletion), PC9/gefb4 (exon 19 deletion and resistant to gefitinib), CL100 (exon 19 deletion), and CL97 (exon 19 deletion and T790M) by the alamarBlue® assay *in vitro* and the xenograft tumor model with intravenous injections *in vivo*. In order to investigate whether the derivatives are capable of causing DNA double-strand crosslink, disrupting cell-cycle progression, and inducing cell apoptotic death, alkaline gel shift assay, modified comet assay, flow cytometric assay were performed.

Results

In this study, we demonstrated that the derivatives of 1,2-bis-hydroxy- indolizino[6,7-b] indiole diols, a novel class of DNA crosslinking agent, effectively kill NSCLC cell lines including PC9 (exon 19 deletion), PC9/gefb4 (exon 19 deletion and resistant to gefitinib), CL100 (exon 19 deletion), and CL97 (exon 19 deletion and T790M). Our results also showed that BO-1922, one of derivatives, was almost equally effective in suppression the growth of various H1299 cell lines which ectopically expressed wild type or a variety of mutant EGFR (such as including L858R, exon 19 deletion, L858R/T790M, and exon 19 deletion/T790M, respectively). The selected derivatives, BO-1922 and BO-1978, were shown to induce DNA double-strand crosslink by modified comet assay and modulate the expression of DNA repair proteins. By aid of flow cytometric assay, we found that exposure of PC9 and PC9/gefb4 cells to BO-1922 resulted in accumulation of cells at the G2/M phase, while BO-1978 causes cell cycle delay at the G1 and S phase. Furthermore, BO-1922 and BO-1978 given at 25 mg/kg significantly suppressed the growth of PC9/gefb4 xenograft tumors.

Conclusion:

Our present results revealed that BO-1922 and BO-1978 may have potential against NSCLC with EGFR mutations and gefitinib resistance.

P510
Study on Pure Compounds Extracted from *S. arisanensis* against Interleukin-6-Induced Hepatic Stellate Cell Activation

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Backgrounds:

Activation of hepatic stellate cells (HSCs) plays an important role in the pathogenesis of liver fibrosis. The cytokine Interleukin-6 (IL-6) has been reported to stimulate HSC activation. *Schisandra arisanensis* is an indigenous medicinal plant in Taiwan for the treatment of inflammatory illnesses. In this study, we aimed to examine if pure compounds extracted from *S. arisanensis* could inhibit IL-6-stimulated HSC activation.

Materials and Methods:

A cell line of rat hepatic stellate cells (HSC-T6) was stimulated with IL-6 (1 ng/ml) for 24 hours. A1-A24, 24 pure compounds from *S. arisanensis* were tested for their inhibitory activities in HSC migration and signal transducers and activators of transcription 3 (STAT3) phosphorylation. Cytotoxicity was assessed by MTT assay.

Results:

The results showed that among the 24 compounds, A3, A4, A5, A6, A8, A9-A15, and A17-A23 (0.1 mg/ml) inhibited IL-6-stimulated HSC migration without cytotoxicity. Furthermore, A8 also attenuated IL-6-stimulated STAT3 phosphorylation in HSCs.

Conclusion:

In conclusion, some compounds extracted from *Schisandra arisanensis* could inhibit HSC migration and STAT3 phophorylation.

P511
Neuroprotection by Intrathecal NERV-1 in Experimental Spinal Cord Injury

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Backgrounds:

The NERV-1 was originally isolated from wild type soft corals, but now it can obtained from established cultivation systems to help perform pre-clinical studies in the future. Our preliminary results demonstrate that NERV-1 significantly decreases the expression of pro-inflammatory proteins. Moreover, it attenuates neuronal cytotoxicity and apoptosis. The aim of the present study was to determine whether NERV-1 serves as an anti-neuroinflammatory agent by attenuating the progression of spinal cord injury (SCI)-associated neurodegeneration.

Materials and Methods:

NERV-1 was administered by intrathecal injection after thoracic spinal cord injury in rats. The expressions and localizations of pro-inflammatory and survival-signaling proteins were examined by immunoblot and immunofluorescence analyses. The expression of glial cell specific marker and neuroprotective factors were examined by immunofluorescence. Spared white matter was compared after eriochrome cyanine R staining. The Basso-Beattie-Bresnahan (BBB) scale was used to evaluate locomotor function after NERV-1 administration.

Results:

We found that NERV-1 treatment improved locomotor function and reduced the lesion size after a SCI event. It also attenuated the SCI-induced upregulation of pro-inflammatory proteins and provided neuroprotection by increasing the expression of Bcl-2 through Akt-dependent CREB activation. Moreover, the NERV-1 also inhibited SCI-induced microglia activation and enhances GDNF and VEGF expression in normal and injured spinal cord.

Conclusion:

In summary, we conclude that NERV-1 is a potential candidate for the treatment of SCI because of its dual anti-apoptotic and anti-inflammatory mechanism of action.

P512
The role of NMDA and GABA receptors in acute alcohol intoxication-related cardiovascular effects in conscious SD rats

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Backgrounds:

Intake of ethanol (alcohol) affects cardiovascular system. Acute ethanol intoxication-related cardiovascular effects include tachycardia, peripheral vasodilation and hypotension. The precise mechanisms underlying ethanol regulation of cardiovascular function remains unclear. Alteration of sympathetic activity in the central nervous system (CNS) has been suggested to participate in ethanol-induced changes in blood pressure. Emerging evidence indicated that glutamate and GABA system may play a crucial role in the regulation of several neurobiological effects of ethanol. The present study was undertaken to examine the hypothesis that NMDA and GABA receptors in the CNS may participate in alcohol-induced cardiovascular actions.

Materials and Methods:

The blood pressure and heart rate in freely moving SD rats (300~350g) were measured for 24 hr by radio-telemetry methods. Alcohol was applied by intraperitoneal injection (IP). The antagonists of NMDA receptors or GABA receptors were applied by intracerebroventricular injection (ICV).

Results:

IP high doses of alcohol (3.2g/kg) caused a significant decrease in blood pressure; the maximal depressor responses were reached at about 1 hr following the injection and the responses lasted for over 3 hr. A significant increase in heart rate was observed at about 3 hr following administration of alcohol and the tachycardia lasted for over 10 hr. The alcohol-induced depressor effects and tachycardia were diminished by ICV post-treatment with higher dose of NMDA receptor antagonists (memantine, ketamine) or a GABA receptor antagonist (bicuculline), which was applied 5 min after administration of alcohol. Lower dose of the antagonists of NMDA receptors or GABA receptors decreased the tachycardia caused by acute alcohol administration, but had little effects on alcohol-induced depressor effects.

Conclusion:

The results indicated that IP administration of high dose of alcohol caused a decrease in blood pressure and an increase in heart rate, NMDA receptors and GABA receptors in the CNS may play an important role in mediating acute alcohol intoxication-related cardiovascular effects.

P513

Influence of Folate Deficiency with High Glucose and Hypoxia in Hep G2 Cells

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Backgrounds:

Folate deficiency (FD), hyperglycemia, and hypoxia were proved that increase oxidative stress, inflammation and angiogenesis. The aim of this study is to estimate the effects of folate ameliorating the inflammatory response of FD in Human hepatoma cell (Hep G2) with or without high glucose (HG) and hypoxia.

Materials and Methods:

The Hep G2 cell was cultured in FD with or without HG for 7 days, and followed culturing in hypoxia for 24 hours. On the other hands, treated with 25mM folate in above condition to observe the effects of folate.

Results:

The cell growths were slowed down in FD combined with or without HG and hypoxia. After culturing in FD for 7 days followed with hypoxia for 24 hours, the protein expressions of iNOS, COX-2 and mitogen-activated protein kinases (MAPKs: ERK1, JNK1, p38 and p65) and its' phosphorylated form of Hep G2 cell were increased. On the other hand, we found Hep G2 cell cultured in FD, HG for 7 days followed with hypoxia for 24 hours that the iNOS, COX-2 and MAPKs protein expressions had more enhancement. Treated 25mM folate in FD combined with or without HG and hypoxia, the cell growth, iNOS, COX-2 and MAPKs protein expressions were ameliorated obviously.

Conclusion:

According to the results, we proved that FD, HG and hypoxia increased inflammation. Moreover, additional folate could improve the inflammation through decreasing MAPKs.

P514

Mechanisms of Electronegative LDL-Induced Cardiac Senescence and Dysfunction

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Backgrounds:

Growing evidence has shown that the dyslipidemia, especially electronegative low-density lipoprotein is one of the main reasons leading to cardiovascular diseases. In our previous studies, we found the most negatively charged LDL isolated from human plasma could induce inflammation of vascular endothelium and apoptosis of cardiomyocytes. However, whether L5 would cause cardiomyocyte senescence followed by cardiac dysfunction is still unclear. To unravel the pathological effects of L5 on heart and the underlying mechanisms is an important issue in prophylaxis, diagnosis, and treatment of dyslipidemia-induced cardiac dysfunction.

Materials and Methods:

Adult male C57B6/J and L5 receptor (LOX-1) knockouot mice were intravenously injected with human LDL (L1, L5, or vehicle) thourgh the tail vein for four weeks. PV-loop analysis was performed by inserting a micro-tip pressure-volume catheter through right carotid artery into left ventricle, and preload was changed by depressing the inferior vena cava to obtain the preload-independent parameters. The ventricular myocytes were enzymatically isolated by Langendorff perfusion method to measure myocyte shortening and intracellular calcium transient simultaneously by using the Myocytes Calcium and Contractility Recording System. Telomerase activity measured by TRAPEZE-RT telomerase detection kit and β-galactosidase staining (X-gal) were used to determine the senescence of cardiomyocytes. Mitochondria were also isolated to analyzethe function and biogenesis, and cardiac fibrosis was performed with Masson's trichrome staninig and related protein markers.

Results:

Both end-systolic pressure-volume relationship (ESPVR) and end-diastolic pressure-volume relationship (EDPVR) in PV-loop analasis were altered after 4 weeks of L5 injection. Intracellular calcium transient also showed abnormality in calcium handling, which is essential for cardiac contraction and relaxation. Both cardiomyocytes and fibroblasts isolated from L5-injected mice showed senescence, characterized by positive X-gal staining and decrease of telomerase activity. . L5 could increase fibrosis in heart, enhance γ-H2AX and Galectin-3, but suppressed SCN5A, Connexin 43, PGC-1α protein levels, telomerase activity, and quantity of mitochondria .

Conclusion:

Cardiac senescence and dysfunction induced by L5 is correlated with the LOX-1 pathway, which then increased ROS and inhibited PGC-1α.Furthermore, L5 could also interfere the connection between myocytes including affecting SCN5A and Connexin 43 protein expression. Combination of all these signaling pathways could cause reduction of mitochondria biogenesis, fibrosis, senescence, and following cardiac dysfunction with abnormal calcium handling.

P515

Amphiregulin increases α6β1 integrin expression and cell migration through Ras, Raf, MEK, ERK, c-Jun in human chondrosarcoma cells

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Background:

Amphiregulin is a member of epidermal growth factor (EGF)-like family. It is associated with the disease status and outcomes of cancers. However, the effect of amphiregulin on migration activity in human chondrosarcoma cells is mostly unknown.

Materials & Methods:

Cancer cells migration activity was examined using the Transwell assay. The RaS, RaF, MEK, ERK, and c-Jun phosphorylation was examined by using Western blot method. The qPCR was used to examine the mRNA expression of integrins.

Results:

Our results show that amphiregulin increased the expression of α6β1 integrin and the migration ability in human chondrosarcoma cells. Amphiregulin-induced migration activities and the expression of α6β1 integrin were inhibited by the specific inhibitors and mutants of Ras, Raf, MEK, ERK and c-Jun cascades. Activation of the Ras, Raf, MEK, ERK, and c-Jun signaling pathway after amphiregulin treatment was demonstrated.

Conclusion:

Our results found that amphiregulin play a critical role in cancer migration and metastasis. Therefore, amphiregulin enhanced the migration of chondrosarcoma cells and the expression α6β1 integrin through the Ras, Raf, MEK, ERK, and c-Jun signal transduction pathway.

P516

Interaction of Immobilized Plasminogen Activators with Plasma Proteins

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Backgrounds:

Urokinase-type plasminogen activator (uPA) has been used to induce thrombolysis in thromboembolic diseases. We have demonstrated that target delivery of plasminogen activators with magnetic nanoparticles (MNP) may increase local retention of the drug and reduce the dose required for its pharmacological efficacy in rat. To optimize the system *in vivo*, we asked how plasma components may alter the characteristics of MNP and the enzyme activity of immobilized uPA.

Materials and Methods:

Platelet-poor plasma (PPP) was obtained by centrifugation of the blood from rats. MNP with a hydradynamic diameter of 264 nm in water was produced with an alkaline precipitation method. A tunable pore system (qNano), equivalent to a nanoscale flowcytometer, was used to determine the diameter and surface charge of chitosan-coated MNP subjected to sonication prior to mixing with phosphate buffer (PB; 0.1 M, pH 7.0) or PPP. After uPA was immobilized to MNP using a glutaraldehyde method, the fibrinolysis efficacy of MNP-uPA was determined by thromboelastometry.

Results:

The diameters of MNP (0.1 mg/ml) in PB vs. PPP measured with tunable pore were 914 ± 85 *vs.* 1250 ± 83 nm (n=5-6; p<0.05), respectively, suggesting that both salt and plasma proteins may cause increase in particle size. In addition, the duration of particles going through the tunable pore was 3.2 ± 0.9 *vs.* 10 ± 2 ms (n=5-6; p<0.05), respectively. The results suggest that interaction with plasma protein may reduce surface charge of MNP and prolong the time going through the pore. With thromboelastogram, $CaCl_2$-induced fibrin clot formation in PPP was measured with free vs. immobilized uPA. Free form of uPA (100~1000 IU/ml) induced a concnetration-dependent fibrinolysis and maximal clot formation (MCF). After 60 min of incubation, 300 *vs.* 1000 IU/ml of uPA induced fibrinolysis with a lysis index (LI_{60}) of 53 ± 4 % *vs.* 9 ± 9 %, and MCF of 9.7 ± 2.3 *vs.* 2.7 ± 1.3 mm, respectively (n=3). With an enzyme activity equivalent to 300 IU/ml of free uPA, MNP-uPA induced fibrinolysis with LI_{60} of 40 ± 17 % (n=3); whereas MNP *per se* did not induce significant fibrinolysis.

Conclusion:

Nanoparticle carriers such as chitosan-coated MNP may increase in size and decrease in surface charge when encountered with salts and proteins in plasma. Nevertheless, immobilized vs. free plasminogen activator exerted similar fibrinolytic activity in plasma. MNP-uPA may be used in target therapeutics for treatment of thromboembolic diseases.

P517
Hepatitis B virus-encoded X protein regulates the EGFR expression via microRNA-7 in hepatocellular carcinoma cells.

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Background & Aims:

Hepatocellular carcinoma (HCC) is a complicated disease. To date, there is no efficient therapy for HCC. It is known that hepatitis B virus (HBV) infection accounts for over half of HCC and its regulatory protein, HBV-encoded X (HBx) plays critical roles in HBV-associated hepatocarcinogenesis. Interestingly, the poor prognosis is particularly observed in HBV-infected HCC patients with epidermal growth factor receptor (EGFR) expression. Therefore, the study is to investigate whether there is an association between HBx and EGFR

Methods:

EGFR protein expression was examined in HCC cells and their derivatives with HBx expression. The expressions of EGFR mRNA and microRNA-7 (miR-7) were analyzed by reverse-transcription quantitative polymerase chain reaction (RT-qPCR). The 3'un-translated region (3'UTR) activity of EGFR was determined by reporter gene assay. The growth rates of HCC cells were analyzed by MTT assay, cell counting and crystal violet staining assay.

Results:

HBx up-regulates miR-7 expression to target at 3'UTR of EGFR mRNA, which in turn results in the reduction of EGFR protein expression in HCC cells. HBx renders HCC cells present a slow-growth pattern. Deprivation of HBx or miR-7 expression or restoration of EGFR expression without miR-7-targeting sites reverses the slow-growth rate in HCC cells.

Conclusion:

This is the first study not only addressing that HBx is able to regulate the EGFR protein level via miRNA expression in HCC, but also indicating that miR-7 expression is up-regulated by HBx. The regulatory trait of HBx-miR-7-EGFR is critical for control the growth rate of HCC cells.

P518
The Effect of N-acetylcystein on Rat Model of Valproate-induced Autism

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Backgrounds:

Autistic spectrum disorder (ASD) has been recognized as a neurodevelopmental disorder characterized by impairment in social interaction, deficit in communication, as well as stereotypic/repetitive behaviors. However, causes of autism remain elusive. Recent studies had implicated that the excitatory/inhibitory imbalance caused by disturbed glutamatergic transmission might contribute to the pathophysiology of ASD. Moreover, *N*-acetylcysteine (NAC) as a glutamatergic modulator has showed its possible therapeutic effect in a clinical trial. Here we try to investigate the effect of NAC and its underlying mechanism by using the valproate (VPA)-induced rat model of ASD.

Materials and Methods:

The rats were tested for the open field test and the elevated plus maze test, as well as the social interaction test.

Results:

The results showed the 3 weeks VPA offspring exhibited significantly social interaction impairment. Besides, in the open field test and in the elevated plus maze test, we found the VPA offspring showed anxiety was increased. After 7 days administration of NAC, the social interaction duration and frequency of the VPA offspring increased significantly (n=16 of the VPA offspring treated with NAC, $p<0.001$ vs. the VPA offspring treated with saline). Moreover, the VPA offspring with NAC administration exhibited increasing time spent in center zone in the open field test and time spent in the open arms in the elevated plus maze test (n=16 of the VPA offspring treated with NAC, $p<0.05$ vs. the VPA offspring treated with saline).

Conclusion:

The results suggested NAC administration might rescue the social interaction impairment and also decrease anxiety. The results further supported the role of disturbed glutamatergic transmission and amygdala excitatory/inhibitory imbalance in the pathophysiology of ASD.

P519
The Regulatory Effect of Hypericum Sampsonii on High-Fructose and High-Fat Diet Induced Nonalcoholic Fatty Liver Disease On The Development Of Metabolic Disorders In Murine Model.

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Backgrounds:

Non-alcoholic fatty liver disease has become the most common cause of liver disease in the world. Non-alcoholic fatty liver disease may be deterioration to form non-alcoholic steatohepatitis, leading to end-stage liver disease. The pathogenesis of non-alcoholic fatty liver disease is not entirely understood, but we know that insulin resistance is a major pathogenetic key. Insulin resistance is often preceded by metabolic syndrome (MS) and may develop in normal adults ingesting a high fructose diet.

Materials and Methods:

Our objective is fed mice 30% fructose in their water and high fat diets to induce MS, and observed feeding the mice with *Hypericum sampsonii* (HS) whether the improved insulin resistance. C57BL/6 mice were divided into four groups. The control group were administered 30% fructose in their water and high fat diets. The treatment groups were administered 30% fructose in their water and high fat diets, and fed the HS (150, 750 mg/kg/d). The naive group were administered water and normal diet. At the end of administration, serum alanine aminotransferase (ALT), triglyceride and cholesterol determination in serum or liver and oral glucose tolerance test (OGTT), 4-hydroxynonenal (4-HNE), Glucose transporter type 4 (GLUT4), and lipid accumulation in liver tissue sections by immunohistochemical, Oil Red O staining were determinate.

Results:

HS reduced ALT and triglycerides levels in serum, and also decreased accumulation of triglycerides in liver. We provide information on the distribution of inflammatory factors in the liver and/or adipose organ, where their aberrant expression in NAFLD. GLUT4 play a central role in the regulatory glucose transport in progressions of NAFLD.

Conclusion:

These data suggest that HS enhanced glucose sensitivity, reduced triglyceride level and prevent hepatic steatosis. HS prevent high-fructose and high-fat diet induced hepatic steatohepatitis in mice through involvement in down-regulated GLUT4, and further hepatoprevention.

P520
Polylactic acid scaffold with Cuscuta chinensis extracts modulated macrophage infiltration in bone healing

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Backgrounds:

Macrophages have been demonstrated to the phenotypes which led to activated status in classical (M1) and alternative (M2) macrophages. M1 are activated by pro-inflammatory signals, IL-6 and TNF-α including Th1 related cytokines, IFN-γ and interleukin (IL)-12, and M2 are activated by pro-healing signals including Th2 related cytokines, IL-4, IL-5, IL-10 and IL-13. Epithelial-derived IL-33 and thymic stromal lymphopoietin (TSLP) are critical regulators with Th2 cytokine-mediated immune response. IL-33 and TSLP are expressed not only in epithelial cells but also fibroblasts, endothelial cells, and smooth muscle cells.

Materials and Methods:

Mouse bone marrow (BM) cells were cultured on polylactic acid scaffolds, with or without added herb extracts from *Cuscuta chinensis*. In animal model, femoral drilling for 1 mm. In diameter, cover with PLA plus various dose of herb extract for 24, 48, 1wk to 2 wks in BALB/c mice. Histological stains were carried out for hematoxylin and eosin (H&E), alkaline phpsphatase (ALP), tartrate-resistant acid phosphatase (TRAP). Cell proliferation and differentiation were studied using cytokines ELISA and alkaline phosphatase.

Results:

Pro-inflammatory cytokine, IL-6, the naïve group was lower than the other groups. Th1 related cytokines, IFN-γ and IL-12, except naïve the others decrease in 1 wk and 2 wks. Th2 related cytokines IL-10 increase in the seconded week. IL-33 and TSLP are higher then the control group. In BM cells, herb extracts increased cell. In histomorphological analysis, herb extracts groups present faster bone healing then control group after 2 wks treatment.

Conclusion:

Herb extracts from *Cuscuta chinensis* increased bone marrow differentiation and bone healing. Th2 related cytokines played an important role in increase polylactic acid scaffold with *Cuscuta chinensis* extracts modulated macrophage into M2 status.

P521

Medical Management of Gastroesophageal Reflux Disease in Taiwan: Analysis of 2006-2011 Nationwide Health Insurance Database

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Backgrounds:
Gastroesophageal reflux disease (GERD) was one of the most common gastrointestinal disorders in Taiwan. Current BNHI reimbursement guidance on PPI once daily treatment for patients with GERD with erosive esophagitis. We aimed to study the treatment duration and efficacy in PPI once daily for GERD control in Taiwan.
Materials and methods:
GERD patients were selected by International Classification of Disease, Ninth Revision, Clinical Modification (ICD-9-CM) diagnostic criteria (ICD-9 code 530.81). PPI and H2 blockers identification was according to the Anatomical Therapeutic Chemical classifications A02BC and A02BA. Prevalence of GERD/PPI usage and comedication were studied.
Results:
The prevalence of GERD was 0.21-0.66%, and among these GERD patients 41.88-37.14% were prescribed PPI as their medication in year 2006 to 2011. The average treatment period was 69.15-74.27 days, and the average dose was 1.02-1.03 tablet per day in 2006 to 2011. However, nocturnal gastricacid breakthrough (NAB) occurs in many patients, and adding an H2-blocker at bedtime may decreases NAB. The prevalence of H2 blockers comedication in PPI treated patients was 27.14-35.37%, and treatment cost per cycle was NT 3,712-2,298 in year 2006 to 2011. The study suggests proton pump inhibitor once daily may not enough to control gastric pH at bedtime, H2 blockers may be needed to NAB control.
Conclusion:
In patients receive PPI once daily therapy, bedtime H2-blocker administration should be considered in patients who require continued NAB control.

P522

Antitumor potentials of Palmitic acid methyl ester on melanoma in vitro and in vivo

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Backgrounds:
This study investigates the anti-tumor effects of palmitic acid methyl ester (PAME) in a highly metastatic murine melanoma cell line (B16F10) *in vitro* and *in vivo*.
Materials & Methods:
Cell viability was assessed using the MTT colorimetric assay. Metastatic capabilities including migration of B16F10 melanoma cells were examined by Boyden Chamber migration, scratch assays, respectively. To investigate the possible mechanisms involved in the metastasis inhibitory effect of PAME, the phosphorylation of Akt, ERK and IκB was analysis by western blot. To investigate the *in vivo* anti-tumor effect and evaluate the combination therapy efficacy, PAME was administration alone or treated with gemcitabine in melanoma xenograft mouse model.
Results:
We first demonstrated that PAME did not affect the cell viability as compared to vehicle-treated and control cell line. In non-toxic concentration, PAME does-dependently inhibited the migration of B16F10 cells *in vitro*. PAME time-dependently affected the Akt, ERK and IκB phosphorylation in B16F10 cells. The antitumor effect of PAME was demonstrated in the melanoma model of B6 mice that was known as the most difficult tumor model to cure. PAME administration alone or treated with gemcitabine was able to inhibit tumor value significantly in melanoma xenograft mouse model.
Conclusion:
Taken together, these findings suggested that PAME could reduce the growth and metastasis of melanoma cells, thereby constituting an adjuvant treatment for melanoma skin cancer control.

P523

Role of pro-inflammatory cytokine in ischemia-induced dysfunction of astrocytic glutamate transporter

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Backgrounds:
Glutamate is a excitatory neurotransmitter and play a role in central nervous system (CNS). Sodium-dependent glutamate/aspartate transporter (GLAST) is the critical manner to clean the glutamate, because excess of glutamate would be damage the CNS and lead to neuronal death. In recent studies, brain ischemia can down-regulate the expression of GLAST on astrocytes and lead to the accumulation of glutamate. Moreover, ischemia can also induce the inflammation and make the inflammatory cell release TNFα and HMGB1, which are inflammatory mediators.
Materials and Methods:
Rat primary cultured astrocytes are exposed to oxygen-glucose-deprivation (OGD) as a model of ischemia. This model can result in the GLAST dysfunction and down-regulation. We tested with numerous concentration of recombinant TNFα and HMGB1 to investigate the effect of astrocytic glutamate transporter
Results:
We found that in the OGD duration, cultured astrocytes were synthesis TNFα and HMGB1, and the mitogen-activated protein kinase(MAPK) family were involved in the formation of TNFα and HMGB1. We also used the cyclosporine A and FK506, which can inhibit the calcium induced signaling pathway, can inhibit the TNFα and HMGB1 synthesis. Moreover, BAPTA-AM can chelate the calcium in the intracellular space, which also reduced the formation of TNFα and HMGB1.
Conclusion:
Recombinant rat TNFα can down-regulate the expression of GLAST on cultured astrccyte. Improve the relation about the OGD induced inflammation and GLAST down-regulation.

P524

KMUP-1 Lessens Neuropathic Pain via inhibition of PKA and PKC Pathways in the Dorsal Root Ganglion

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Backgrounds:
Chronic neuropathic pain is a refractory pain characterized by its complex mechanisms and diverse clinical manifestations. Traditional therapies usually bring about many side effects and limited success. In the study, we attempt to investigate whether KMUP-1 could reduce hyperalgesia and inflammatory mediators, and to reveal its underlying mechanisms in the dorsal root ganglion (DRG) following chronic constriction injury (CCI)-induced neuropathic pain.
Materials and Methods:
Sprague–Dawley rats were randomly divided into four groups: sham, sham with KMUP-1 (5 mg/kg, i.p), CCI and CCI with KMUP-1 (5 mg/kg, i.p). KMUP-1 (5 mg/kg) was administrated intraperitoneally once daily starting at day 1 after CCI surgery. Each group of rats (n=5) were sacrificed and L4-L6 DRGs removed quickly at day 3, 7 and 14 after CCI. Behavior tests were assessed before surgery and at those scheduled time-points after CCI.
Results:
KMUP-1 decreased mechanical allodynia at day 3, 7 and 14, and thermal hyperalgesia at day 7 and 14 after CCI ipsilateral side, but not CCI contralateral side. KMUP-1-treated group significantly inhibited CCI-induced inflammatory mediators (iNOS, COX2) and proinflammatory mediators (TNF-α, IL-1β). Activation of PKA, PKC and ERK in the DRG contributes to the initiation of CCI-induced pain hypersensitivity. KMUP-1 also inhibited the PKA, PKC and ERK activations that could attribute, at least in part, to its possible mechanisms in CCI-induced neuropathic pain.
Conclusion:
Based on our results, KMUP-1 has anti-inflammatory and anti-hyperalgesia properties in CCI-induced neuropathic pain via inhibition of PKA, PKC and ERK. We suggest that KMUP-1 might be a potential agent for the control of neuropathic pain.

P525
IL-1β-induced MMP-9 Expression is Mediated through Receptor Tyrosine Kinases and NF-κB Pathways in Corneal Epithelial Cells

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Backgrounds:
Increasing evidences show that the dry eye induces inflammation on the ocular surface through increases of pro-inflammatory mediators and catalytic enzymes. Matrix metalloproteinases (MMPs), MMP-9 especially, have been demonstrated to play a key role in the pathogenesis of inflammation and tissue wound healing in cornea. Many studies have shown that MMP-9 can be induced by several stimuli such as IL-1β, which may contribute to collagen degradation and tissue remodeling in the inflammatory responses of cornea. However, the mechanisms underlying IL-1β-induced MMP-9 expression in cornea remain unknown

Materials and Methods:
Here we applied the Statens Seruminstitut Rabbit Corneal Cells (SIRCs) to investigate the mechanisms of IL-1β-induced MMP-9 expression. Data obtained with Western blot, RT-PCR, co-immunoprecipitation, cell fraction isolation, and promoter luciferase activity analyses coupled to using pharmacological inhibitors of various signaling molecules, including c-Src (PP1), EGFR (AG1478), PDGFR (AG1296), PI3K (LY294002), and NF-κB (Bay11-7082).

Results:
In this study, we demonstrated that IL-1β-up-regulated MMP-9 protein, mRNA, and promoter activity, which were attenuated by PP1, AG1478, AG1296, LY294002, or Bay11-7082. Moreover, IL-1β can stimulate Akt phosphorylation which was attenuated by pretreatment with PP1, AG1478, AG1296, or LY294002. These signalings lead to both IκBα degradation and NF-κB p65 translocation in SIRCs. Interestingly, we found that IL-1β stimulates c-Src, EGFR, and PDGFR complex formation resulting in up-regulation of MMP-9.

Conclusion:
These results revealed that IL-1β-induced MMP-9 expression is mediated through c-Src-dependent transactivation of EGFR and PI3K/Akt cascade linking to NF-κB activation in SIRCs.

P526
Study on The Mechanisms of PPE8-induced Apoptosis Through Endoplasmic Reticulum Stress.

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Backgrounds:
To investigate the mechanisms of cell death induced by the new synthetic compound of naphthoquinone treatment in human non-small lung carcinoma cell line H1299.

Materials and Methods:
The effect of PPE8 on H1299 cells viability was obtained by MTS assay. Mophological changes of ER observed by immunofluorescence microscopy. Expression levels of ER-related protein were determined to investigate their role in PPE8-induced cell death by western blotting assay. The effect of PPE8-induced ER stress and cell viability of H1299 cells were investigated by siRNA knockdown of IRE1.

Results:
PPE8-induced cell death was on dose-dependent manner. PPE8-induced ER morphological changes. PPE8-induced ER stress was evidenced by increased expression of p-IRE1 and p-JNK in H1299 cells. Knockdown of IRE1 expression by siRNA reduced PPE8-induced JNK phosphorylation and cell death in H1299 cells.

Conclusion:
Our data demonstrated that PPE8 can induce cell death through ER stress in human non-small lung carcinoma cell line H1299. Thus, PPE8 may serve as an anticancer agent by inducing ER stress in human non-small cell lung cancer.

P527
The Mechanism of Endomitosis Inhibited by PKA Isoforms

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Backgrounds:
The phenomenon of polyploidy, also known as endomitosis, is composed of interruption of cytokinesis and re-synthesis of DNA. The deregulation of cell cycle may cause physiological abnormality. It's reported that endomitosis can be inhibited by cAMP-PKA signaling axis. However, the relationship of cell cycle factors and endomitosis is still not clear. In this study, we investigate the mechanism in inhibition of endomitosis by PKA isoforms in human erythroleukemia (HEL) cells.

Materials and Methods:
Human erythroleukemia (HEL) cells were cultured in RPMI 1640 containing 2% FBS, 1 mM sodium pyruvate, 2 ml glutamine and 100 IU/ml streptomycin/penicillin in the humidified incubator at 37℃ with 5% CO2. Cells were collected every 24 hours for a 4-day period after drug treatment. PMA (25nM), a common agent used for induction of polyploidization in HEL cells, and forskolin (FSK, 50μM), adenylyl cyclase activator, is used for raising cAMP level. The cell cycle factors were detected with immunolabeling and Acurri C6 flow cytometry, and analyzed with Flowjo software.

Results:
In our results, the raising of p21 activity started at second day after PMA treatment and FSK can reverse PMA-induced effect. The levels of cyclin B1 and cdc2Y15 were not significantly altered between PMA treatment and PMA/FSK co-treatment. Also, the level of cyclin D3 was indeed increased in PMA-treated cell.

Conclusion:
P21, known as cell cycle inhibitor, may has its specific function in the process of endomitosis. Furthermore, cyclin D3, involved in G1 phase, is indeed play an important role in endomitosis. To investigate the effect of cAMP on cell cycle related factors, we will continually study the alternation of cell cycle-related factors under PMA treatment and PMA/FSK co-treatment. Next, we will determine which PKA isoforms is the downstream of cAMP and clarify the inhibitory mechanism of cAMP-PKA signaling pathway.

P528
The effects of marine-derived compound, WSS-10 on 6-hydroxydopamine model of Parkinson's disease

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Backgrounds:
Parkinson's disease (PD), an important neurodegenerative disorder, is characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra results in motor defects. However, current treatments for PD are limited and drug of PD is needed urgently. Our previous studies had found that marine-derived compound, WSS-10 provides neuroprotection against 6-hydroxydopamine (6-OHDA)-induced neurotoxicity by anti-apoptotic and anti-inflammatory actions. The present study, we would further examine the cellular mechanisms of neuroptrotetive effect of WSS-10 in Parkinson's animal models.

Material and methods:
Zebrafish larvae were treated with 6-OHDA in the absence or in the presence of WSS-10. Motor activities (total distance and velocity) were monitored by animal behavior system (SINGA). In Parkinson's rat model was induced by lesion of middle forebrain bundle (MFB). After lesion, amphetamine-induced rotation behavior was evaluated every week. One month after lesion, rats were sacrificed after reperfusion and the section of brains were performed in immunohistochemical analysis.

Results:
WSS-10 markedly rescued the deficit of loco-motor activity in 6-OHDA-treated zebrafish. In addition, WSS-10 also attenuated the number of amphetamine-induced rotation behavior and DA neuronal death in 6-OHDA rat model of PD. After knock down of DJ-1 protein expression by siRNA could decrease the protective effects of WSS-10 on 6-OHDA-induced cytotoxicity in neuron cells.

Conclusion:
We confirmed the therapeutic efficacy of WSS-10 in zebrafish and rat PD model. Moreover, we strong proposed that WSS-10 is a promising candidate for the treatment of Parkinson's disease through DJ-1 mediated cascade.

P529

The Antinociceptive Mechanisms of Transforming Growth Factor-β1 on Neuropathic Rats: Association With Attenuation of Neuroinflammation and Downregulation of Glutamate Transporters at the Spinal Level

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Backgrounds:

The molecular and cellular mechanisms involved in the development and maintenance of neuropathic pain are unclear, and there is an urgent need for further research in this area. Previous studies have reported that intrathecal (i.t.) transforming growth factor-β1 (TGF-β1) could prevent and reverse nerve injury-induced neuropathic pain. However, only limited information is available regarding the possible role and effects of spinal TGF-β1 in neuropathic pain.

Materials and Methods:

In the present study, we investigated the time course of effects of i.t. TGF-β1 on glutamate transporters and chronic constriction injury (CCI)-induced spinal neuroinflammation in neuropathic rats.

Results:

Prior to exploring the possible effects of exogenous TGF-β1 on neuropathic pain, we demonstrated that sciatic nerve injury could cause downregulation of endogenous TGF-β1 on the ipsilateral side of the lumbar spinal dorsal gray matter at 7 and 14 days after CCI surgery. In CCI rats at 14 days after surgery, we found that a single i.t. administration of TGF-β1 (0.01–10 ng) significantly attenuated CCI-induced thermal hyperalgesia in a dose-dependent manner. The antithermal hyperalgesia effects of 5 ng of TGF-β1 reached the maximum value at 30 min after i.t. injection, then decreased progressively with time, and lasted for at least 18 h. We then focused on the periods of 30 min, 3 h, and 6 h after i.t. administration of 5 ng of TGF-β1, to examine the possible effects of i.t. TGF-β1 on neuroinflammation and glutamate transporters at the spinal level in neuropathic rats. Immunohistofluorescence analyses showed that i.t. TGF-β1 (5 ng) significantly inhibited CCI-induced neuroinflammation, microglial and astrocytic activation, and the upregulation of tumor necrosis factor-α (TNF- α) in the ipsilateral dorsal gray matter of the lumbar spinal cord. In addition, confocal double-immunostaining images further revealed that astrocytes are the primary sources of TNF-α production, rather than neuronal or microglial cells, at 14 days after CCI surgery. I.t. TGF-β1 (5 ng) also significantly attenuated CCI-induced downregulation of 3 glutamate transporters (GTs), Glu transporter-1 (GLT-1), glial transporter Glu–Asp transporter (GLAST), and excitatory amino acid carrier 1 (EAAC1), on the ipsilateral side of the lumbar spinal dorsal gray matter. Moreover, i.t. TGF-β1 (5 ng) significantly upregulated GLT-1 and EAAC1 on both the ipsilateral and contralateral side of the lumbar spinal dorsal gray matter in neuropathic rats. Furthermore, i.t. TGF-β1 (5 ng) significantly decreased the concentration of 2 excitatory amino acids (EAAs), aspartate and glutamate, in the spinal dialysates in neuropathic rats.

Conclusion:

In summary, the antinociceptive effects of i.t. TGF-β1 on neuropathic pain are associated with both attenuation of neuroinflammation and downregulation of glutamate transporters at the spinal level. Thus, our present findings indicate that spinal TGF-β1 may be a potential therapeutic target for neuropathic pain.

P530

A prodeath role of autophagy in MPP+-induced neurotoxicity in vivo: the involvement of α-synuclein and mitochondria

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Autophagy, also known as an intracellular self-eating mechanism, is essential for the cellular homeostasis between biosynthesis and catabolism. However, the role of autophagy in the central nervous system (CNS) neurodegenerative diseases remains undetermined. In the present study, a Parkinsonian animal model was employed using an intranigral infusion of 1-methyl-4-phenylpyridinium (MPP+, 3μg/injection site), a neurotoxin commonly used in Parkinsonian studies. Our in vivo data showed that MPP+ infusion elevated lipid peroxidation and decreased tyrosine hydroxylase levels (a rate-limiting enzyme of dopamine biosynthesis) in the infused substantia nigra (SN). At the same time, dopamine content was reduced in the striatum ipsilateral to MPP+-infused SN, indicating MPP+-induced neurodegeneration of the nigrostriatal dopaminergic system of rat brain. The molecular mechanisms underlying MPP+-induced neurotoxicity were investigated. Intranigral infusion of MPP+ increased heme oxygenase-1 (HO-1) level (a redox-regulated protein) and α-synuclein aggregation (a pathological hallmark of Parkinson's disease) as well as attenuated cytochrome c oxidase levels (a biomarker of mitochondria mass). Our Western assay showed that MPP+ significantly increased LC3-II (a hallmark protein of autophagy) and cathepsin B (a lysosomal cysteine proteinase) levels in the infused SN, indicating MPP+ is capable of inducing autophagy. In vivo siATG7 transfection was performed 3 days prior to MPP+ infusion. Western blot assay showed that siATG 7 transfection attenuated MPP+-induced elevation in LC3-II and active caspase 9 as well as reduction in TH levels in the infected SN, indicating that autophagy is pro-death. Furthermore, MPP+-induced elevation in α-synuclein aggregation and reduction in cytochrome c oxidase was diminished in the siATG7-transfected SN. Immunostaining study showed co-localization of α-synuclein with LC3 and cathepsin B, indicating that autolysosomes may engulf α-synuclein. Moreover, colocalization of LC3 and succinate ubiquinone oxidoreductase (mitochondria specific protein) was observed, indicating that autolysosomes may engulf mitochondria. The pro-inflammatory mechanism of MPP+ was demonstrated by reduction in procaspase 1 level as well as elevation in active caspase 1 level and IL-1β levels (biomarkers of inflammasome) in the infused SN. In conclusion, our data show that autophagy and inflammasome may contribute to the pathophysiology of Parkinsonism.

P531

The Effects of Estrogen on Lipopolysaccharide-Induced Rhabdomyolysis in Ovariectomized Rats

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Backgrounds:

Infection is one of the causes of Rhabdomyolysis. Sepsis patients complicated with rhabdomyolysis led to a high mortality. Animal studies have demonstrated that estrogen may potentially provide protection from exercise-induced muscle damage by acting both as an antioxidant and a membrane stabilizer. In this study, the protective effect of estrogen on the lipopolysaccharide (LPS)-induced rhabdomyolysis in ovariectomized (OVX) rats was evaluated.

Materials and Methods:

The female Wistar rats were assigned into four groups: (1) sham + saline: the rats were sham-operated; (2) sham + LPS: the rats were sham-operated. After 14 weeks, LPS was administrated i.v. with a dosage of 30 mg/kg in 9 mL saline for 4 hours; (3) OVX + LPS: the rats were OVX bilaterally. After 14 weeks, LPS was administrated i.v. with a dosage of 30 mg/kg in 9 mL saline for 4 hours; (4) OVX + E_2 + LPS: OVX rats were given E_2 (50 mg/kg, 3 times per week, s.c.) for 14 weeks beginning at 1 week after OVX. Fourteen weeks after OVX, LPS was administrated i.v. with a dosage of 30 mg/kg in 9 mL saline for 4 hours. Plasma levels of lactic dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine (Cre), glutamic pyruvic transaminase (GPT), and creatine phosphokinase (CPK) were measured at 0 hour, 4 hour, 6 hours after LPS administration. Plasma levels of nitric oxide (NO) metabolites, estrogen (E_2) and myoglobin (MYO) were measured at 6 hours. Rats were euthanasia at 6 hours after LPS administration, the skeletal muscle from gastrocnemius were removed immediately for determining superoxide anions production and mitochondria oxygen consumption.

Results:

The mean arterial pressure (MAP) of rats in the OVX+LPS group significantly reduced at 6 h after LPS initiation when compared with the sham+LPS group. The MAP in the OVX+E2+LPS group was significantly higher than the OVX+LPS group at 6 h. Plasma levels of CPK and MYO significantly elevated in all LPS-treated groups. The CPK and MYO levels in sham+LPS and OVX+E2+LPS groups was also significantly lower than that of OVX+LPS group. The superoxide anions production in sham+LPS and OVX+E2+LPS groups was also significantly lower than that of OVX+LPS group. There was no difference in CPK, MYO and superoxide anion levels between sham+LPS and OVX+E2+LPS groups.

Conclusion:

Both endogenous and chronic of E2 treatment prevent circulatory failure and attenuate the damage of skeletal muscle in sepsis. The underlying mechanism needs to be f

P532

Secondary Metabolites From The Leaves of Cinnamomum macrostemon Hayata

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Backgrounds:

Cinnamomum macrostemon Hayata is a medium-sized evergreen tree, and it's endemic in Taiwan, distributed at medium altitudes throughout the island .

Materials and Methods:

The air-dried leaves of *C. macrostemon* (3.5 kg) were extracted with MeOH (10 L×5) at room temperature and a MeOH extract (112.6 g) was obtained upon concentration under reduced pressure. The residue was placed on a silica gel column and eluted with CHCl3 gradually enriched with MeOH to afford 4 fractions. The compounds were characterized by comparison of their physical and spectral data with values obtained in the literature.

Results:

The MeOH extract of its leaves was subjected to solvent partitioning and chromatographic separation to afford 12 pure substances.

Conclusion:

Fraction 1 (4.62 g): coumarin (1), isoscopoletin (2), and scopoletin (3). Fraction 2 (14.38 g) : β-sitostenone (4), β-sitosterol (5), cinnamic acid (6), and eugenol (7). Fraction 3 (7.43 g) : (+)-yangambin (8) and (+)-syringaresinol (9). Fraction 4 (15.23 g) : tenuifolin (10), reticuol (11), and subamol (12). In addition to 3-7 and 9, all of these compounds were found for the first time from this plant .

P533
Autophagy Inhibition Enhances Celecoxib-induced Apoptosis in Human Urothelial Carcinoma Cells

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Backgrounds:
Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, has been reported to elicit anti-tumor effects in various malignancies. In this study, we attempted to clarify the role of autophagy in celecoxib-induced cytotoxicity in human urothelial carcinoma (UC) cells.

Materials and Methods:
Cell viability and apoptosis of UC cells were determined by MTT assay and flow cytometry. The formation of autophagy was examined by the staining of immunofluorescence and LysoTracker and immunoblotting analysis for microtubule-associated protein 1A/1B-light chain 3 (LC3).

Results:
The results showed that celecoxib induced cell death and apoptosis in human UC cells. In addition, increased cellular stress-related molecules such as endoplasmic reticulum (ER) stress-related molecules, phsophorylated SAPK/JNK, phsophorylated c-Jun, phsophorylated AMPKα, and LC3 could be detected in celecoxib-treated UC cells. The presence of autophagy could be proved by the increases of LC3 puncta in cells and LysoTracker-positive cells. Co-treatment with 3-methyladenine (3-MA), an autophagy inhibitor, enhanced the apoptotic effect of celecoxib in UC cells. The enhancement of autophagy by rapamycin, an inhibitor of mTOR, could also alleviate celecoxib-induced apoptosis. Consistently, up-regulation of autophagy by GFP-LC3-transfection decreased celecoxib-induced cytotoxicity in human UC cells.

Conclusion:
Taken together, these results indicate that the inhibition of autophagy enhances celecoxib induced-apoptosis, suggesting a novel therapeutic strategy against UC.

P534
The Regulation of the STAT3 in AEA Induced Hepatoma Cell Apoptosis

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Backgrounds:
Arachidonoyl ethanolamide (Anandamide, AEA), the main endogenous agonist were discovered class of lipid mediators term endocannabinoids. AEA is implicated in a variety of pathological contents (inflammation, eating disorder, analgesia and cancers). Recently, anti-tumor activity of AEA have been reported in various cancer (breast, prostate, colorectal cancers).

Materials and Methods:
HepG2 cells was growth in modified Eagles medium (MEM) supplemented with 10% fetal bovine serum. Briefly, Cell were treated with various concentrations of AEA (0, 1, 10, 20, 30, 50 μM) treatment for 72 hrs, The anti-proliferative activity of the AEA was assessed by XTT and methylene blue assay. Immunofluorescence (IF) assay cell were cultured in 12-well plates, then cells were fixed with 4% paraformaldehyde before stained with the antibody to STAT3, then cell nucleus were counterstained with DAPI. Pictures were captured by (Axioskop 2 plus, ZEISS, NA, USA). EMSA assay was detected the nuclear translocation of STAT3. Total cell lysate were subjected to SDS-PAGE. Monoclonal antibodies against Janus- activated kinase 2(Jak2) , p-STAT3 (Y705, S727) were used.

Results:
The signal transducer and activators of transcription (STAT) family protein, especially STAT3 play a consistent activation role in tumorgenesis, In AEA induced human hepatoma cell apoptosis, we observed the inhibition of STAT3(Y705, S727) phenomenon in Western blotting analysis and electrophoretic mobility shift assay (EMSA),From both cytoplasma and nuclear separation we found the same results

Conclusion:
AEA caused hepatoma cell growth inhibition and apoptotic induction that may be through the regulation of transcription factors STAT3.

P535
The Role of Hsp70 in the Protective effect of Hsp90 Inhibitor 17-DMAG on the Heat Stroke Rats.

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Backgrounds:
Heat stroke (HS) is a serious medical emergency. Despite through rapid cooling the body temperature and aggressive support treatment, heat stroke is often fatal. Thus, it is important to find a new drug to protect the function of organ and to prolong the survival time of heat stroke rats. In recently studies showed that inhibition of Hsp90 can activate heats hock factor-1 (HSF-1) to following by the induction of production of Hsp70, as well as of other chaperones. Several studies have been published showing that Hsp70 can protect against numerous stresses, including heat shock, oxidative stress, apoptotic stimuli, and ischemia. 17-dimethylamino-ethylamino-17-demethoxygeldanamycin (17-DMAG), is a water soluble analog of geldanamycin and has excellent bioavailability and tissue distribution in animals. The aim of the present study was to evaluate the role of Hsp70 in the protective effect of Hsp90 inhibitor 17-DMAG on heat stroke rats and it possible mechanism.

Materials and Methods:
The right femoral artery and vein of adult, male Sprague-Dawley rats weighing 280-350 g, under urethane (1.4 g/kg, IP) anesthesia, were cannulated with polyethylene tubing (PE 50). Four groups of animals were studied. (1) Normothermic control (NT) group : the Tco was maintained at about 36 ℃ with a heating chamber at a room temperature of 24 ℃ , through out the entire experiments. (2) Vehicle-treated heat stroke group (HS) : heat stroke was induced by putting the rats into a chamber at 42℃ . When mean arterial blood pressure (MAP) dropped to a value of 25 mmHg from the peak level and Tco was elevated to about 42℃ . (3) 17-DMAG (5 mg/kg, IP) pretreatment with heat stroke (17-DMAG + HS) group. (4) Quercetin (Hsp inhibitor) and 17-DMAG pretreatment with heat stroke (Q +17-DMAG + HS) group. The following were measured in these four groups of animals: (1) rectal temperature (Tco), MAP, heart rate; (2) survival time; (3) serum biochemical and cytokines assay; and (4) to analysis the Hsp70 expression in liver, kidney and hypothalamic.

Results :
The value of MAP, heart rate and Tco of untreated heat stroke (HS) group were all significantly lower than that of normothermal (NT) group. 17-DMAG pretreatment with heat stroke (17-DMAG + HS) group significantly reduced heat stroke induced hyperthermia, arterial hypotension and heart rate, and prolong the survival time. The expression of Hsp70 in liver and renal of 17-DMAG + HS group was significantly higher than that of HS group.

Conclusion :
Results of this study demonstrated that 17-DMAG could improve survival rate, hemodynamic and organ function of heat stroke rats. This beneficial effect of 17-DMAG may mediate by the increasing of Hsp70 ovrexpression.

P536
The Role of PKCμ during Macrophage Differentiation

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Backgrounds:
Recent years, researchers have found that cell differentiation usually accomplishes with death, but this branch issue has not well studied. PKC isoform has reported that regulates cell migration, differentiation, proliferation, membrane trafficking and immunoreactions. In this study, we investigate the role of PKC isoform in macrophage differentiation.

Material and Methods:
THP-1 is often used as a model of macrophage differentiation that stimulated with PMA. After PMA treatment within different times, collect cells by centrifugation and aspirate supernatant. Add lysotracker and DAPI to observe the morphology of lysosome/autophagosome. And determined death percentage by flow cytometer. On the other hand, lyse cells with RIPA and measure protein expression level by Western blot.

Result:
We observed that CID755673 can increase differentiation cell proportion in PMA-induced THP-1 cells. Moreover, the phosphorylation of PKCμ (S744/S748) increases after PMA treatment, and returns to normal level after 4 hours. Previous studies have shown that autophagy is observed during differentiation. We used lysotracker to define the distribution and morphology of the lysosome/autophagosome. We found that PMA increases the number of lysosomes that fuse into huge vacuoles after 2 days. Furthermore, CID755673 can reduce superoxide production. This data represent that PKCμ participates in microphage differentiation.

Conclusion:
Our results indicate that PMA activates expression of PKCμ and may induce autophagy during macrophage differentiation.

P537

BMP-7 promotes αvβ3 integrin expression and cell motility through c-Src, PI3K/Akt, and NF-κB signaling pathway in human chondrosarcoma

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Background:

Bone morphogenetic protein 7 (BMP-7) encoded in a member of the TGF-β superfamily. Like other members of the BMP family of proteins, it plays a key role in the transformation of mesenchymal cells into bone and cartilage. However, the effect of BMP-7 on migration activity in human chondrosarcoma cells is still unknown.

Materials & Methods:

Cancer cells migration activity was examined using the Transwell assay. The c-Src, PI3K/AKT, and NF-κB phosphorylation was examined by using Western blot method. The qPCR was used to examine the mRNA expression of integrins. A transient transfection protocol was used to examine the NF-κB activity.

Results:

Here we found that BMP-7 directed chondrosarcoma cells migration involves αvβ3 integrin up-regulation. BMP-7 mediated migration and αvβ3 integrin up-regulation were attenuated by the inhibitors and the mutant of c-Src, PI3K/Akt, and NF-κB cascades. It activated the c-Src, PI3K/Akt and NF-κB signaling pathway after BMP-7 treatment was demonstrated.

Conclusion:

Taken together, our results indicated that BMP-7 enhances the migration of chondrosarcoma cells by increasing αvβ3 expression through c-Src, PI3K/Akt, and NF-κB signal transduction pathway.

P538

Effects of glucose on NHE activity and cellular functions in human vascular smooth muscle cells

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Backgrounds:

Na+/H+ exchanger (NHE) is involved in regulation of intracellular pH (pHi) homeostasis and affects cell contraction, proliferation, growth, migration, and apoptosis. Diabetes mellitus (DM) has been one of the top ten causes of death for a long time in Taiwan. DM is a major cause of vascular morbidity and mortality. The aims of the study are, in the human vascular smooth cells (HVSMCs), to investigate effects of (1) glucose on contractile force, cell proliferation and growth; (2) Andrographolide (Andro), a Chinese traditional herbal medicine, on glucose-induced changes on parameters of cell contractile force, proliferation and growth.

Materials and Methods:

HUASMCs were isolated/cultured from human arteries/veins obtained from patients with the approval of the institutional review committee and with prior informed consent. Contractile force was detected by force transducer. Cell growth and proliferation were evaluated using electric cell-substrate impedance (ECIS) assay.

Results:

In study of contracture experiment, human saphenous vein contraction was stimulated by norepinephrine. The change of different glucose concentration (2.8 mM, Emax=1.0g; 15 mM, Emax=1.78g; 20 mM. Emax=2.0g) affects vein contraction in a concentration-dependent manner (-32%, +20%, +35% respectfully, n=5, in comparison with control group (5.5 mM, Emax=1.48g). In study of ECIS assay, human umbilical artery smooth muscle cells (HUASMCs) have been successfully isolated and cultured in different concentration of glucose. Andrographolide (10~30uM) significantly reduced the high-glucose (20~25 mM) induced impedance and resistance changes.

Conclusion:

Glucose affects cellular contraction and growth concentration-dependently in human smooth muscle cells. Andrographolide improve the high-glucose induced cellular functions.

P539

Effect of Intermittent Hypoxia on Airway Hyperresponsiveness in Brown Norway Rats

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Backgrounds:

Obstructive sleep apnea (OSA), manifested by intermittent hypoxia (IH), is associated with airway hyperresponsiveness (AHR). AHR, a fundamental component of the airway inflammatory process, is associated with hyperreactive airway diseases causing airway narrowing on exposure to a bronchoconstrictor stimulus, which in turn causes patients to experience symptoms of breathlessness and chest tightness. Although the pathophysiological mechanism of OSA-associated AHR remains unclear, airway inflammation is likely to play a critical role. In this study, we investigated 1) whether long-term exposure to IH causes AHR, and if so, 2) whether inflammatory mediators, such as reactive oxygen species (ROS) and cyclooxygenase products, are involved in the response.

Materials and Methods:

Male Brown Norway rats were exposed to repetitive 1.25-min cycles (30 s of N2 + 45 s of 21% O2) of IH for 6 h/day for 14 consecutive days and matching control animals (receiving room air exposure). On day 15, lung resistance (RL) and dynamic lung compliance (Cdyn) to right atrial injection of methacholine were measured as an index for AHR.

Results:

The baseline RL and Cdyn were not significantly different between the two groups; however, the methacholine injection induced a significantly more intense bronchoconstriction in IH-exposed rats. Furthermore, pretreatment with N-acetyl-L-cysteine (an antioxidant, 25 mg/kg) or ibuprofen (a cyclooxygenase inhibitor, 15 mg/kg) inhibited the enhanced bronchomotor responses to methacholine injection.

Conclusion:

These results suggest that IH exposure induces AHR, and that the endogenous ROS and cyclooxygenase products are responsible for the response.

P540

Murine Resistin Gene Promoter Contains Multiple FOXO3-binding Regions.

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Backgrounds:

Resistin is an adipocyte-specific secretory hormone that was discovered to cause insulin resistance. It can be regulated by transcriptional factors, but the possible role of the forkhead transcription factor FOXO3 in regulating resistin gene expression is still unknown.

Materials and Methods:

We used electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) assay for searching the FOXO3-binding site in the resistin gene promoter *in vitro* and *in vivo*. Additionally, we used PCR to assess changes in levels of resistin and FOXO3 mRNAs during differentiation of C3H10T1/2 and 3T3-L1 preadipocytes to adipocytes.

Results:

In vitro, EMSA showed that GST-FOXO3 fusion protein could directly bind several nucleotide regions of the resistin promoter, at least including -3439~-3205, -2789~-2615, -2561~-2397, -2074~-1895, -1894~-1715, -1504~-1285 and -954~-759 bp. The binding specificity was confirmed by an EMSA competition experiment since the added competitive probe blocked the binding of GST-FOXO3 to each individual resistin promoter region. *In vivo*, ChIP assay indicated that the fourth day and the sixth day of differentiating C3H10T1/2 adipocytes, but not preadipocytes or differentiated adipocytes, exhibited the strong binding of the endogenous FOXO3 protein to the following nucleotide regions of resistin promoter: -3444~-3155, -2799~-2615, -2571~-2380, -2074~-1895, -1894~-1705, -1558~-1385, -1404~-1285, and -954~-724 bp. Additionally, levels of both resistin and FOXO3 mRNAs increased during differentiation of preadipocytes to adipocytes.

Conclusion:

These data indicate the existence of the multiple FOXO3-binding regions in the adipocyte resistin promoter, and the binding of FOXO3 to resistin promoter varies with the developmental status of fat cells.

P541
Functional Roles of Protein Degradation Pathways in Central Nervous System and Peripheral Tissues Before the Onset of Huntington's Disease

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Backgrounds:
Huntington's disease (HD) is an autosomal dominant disorder caused by a mutantion of CAG repeat expansion in *huntingtin* (HTT) gene, resulting an abnormally long polyglutamine tract at the N terminus of HTT. The *HTT* gene is widely expressed in human tissues, and mutant HTT protein forms aggregates in central nervous system (CNS) and several peripheral tissues, such as heart and liver, leading to widespread pathology. In order to prevent pathological phenotypes, clearance of mutant HTT, mediated by both the ubiquitin–proteasome system (UPS) and autophagy, is one of potential strategies. Previous studies showed that impairment of UPS was important for the accumulation of N-terminal mutant HTT in the brain of HD models; however, clearance pathway of mutant HTT in peripheral tissues before the onset has not been determined. Therefore, the aim of this study was to compare these two degradation pathways in CNS and peripheral tissues before the onset of HD *in vivo.*
Materials and Methods:
HD transgenic mice carrying GFP fused with mutant HTT exon 1 were used in this study. According to our previous studies, these HD transgenic mice showed the onset of motor deficit after 8.5 months of age. In order to determine UPS and autophagy before the onset of HD, we collected tissues of these HD transgenic mice at three different age points (2, 5 and 8 months) to compare expression profiles of ubiquitin, an UPS marker, and LC3-II, an autophagy marker, by western blot analyses.
Results:
The data showed that the expression of ubiquitin in cortex, striatum and liver significantly increased in 5 and 8 months old HD mice compared to those of 2 months old HD mice. Then, the expression of LC3-II in cortex and liver significantly decreased in 8 months old HD mice compared to those of 2 months old HD mice. However, both ubiquitin and LC3-II expression were unchanged in hearts of HD mice at three different age points.
Conclusion:
Our findings indicated that UPS was more important than autophagy in cortex, striatum and liver, but not in heart, before the onset in our HD mouse model. With the achievement of this study, it would provide an insight on potential therapies for HD.

P542
Establishment of a Rat Model for Studying Cardiac Arrhythmias: the Role of Tyrosine Phosphorylation in the Regulation of Cardiac Rhythm

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Yen-chang Lin

Background:
To investigate the effects of a small molecule, sodium orthovanadate (Na3VO4), on ventricular arrhythmias induction to develop a rat model with controllable occurrence of ventricular arrhythmias without cardiac structural remodeling.
Materials and Methods:
2% isoflurane mixed with oxygen at the flow rate of 2 liter/min inhaled by adult SD rats was used for anesthetization in accordance with Animal Care and Use Committee guidelines for implantation of catheters. Different doses of $Na3VO_4$ were applied for the induction of ventricular arrhythmias. At the beginning, 0.8-1.2 mg/rat of Na3VO4 was administrated via the catheter injection which catheter has been placed in jugular vein. An increased dose of Na3VO4, 1.2-1.6 mg/rat, was then applied to induce arrhythmias. For more severe arrhythmias induction, a relatively higher dose of $Na3VO_4$ (1.6-2.4 mg/rat) was applied. All the results are repeated by at least 10-12 times.
Results:
We have reproducibly detected several major ventricular arrhythmias by using three different doses of sodium orthovanadate. At low dose (0.8-1.2 mg/rat), premature ventricular contraction was detected. Increasing the dose to 1.2-1.6 mg/rat induced more frequent PVCs leading to ventricular tachycardia (VT). Fragmented QRS, a strong marker of abnormal conduction in the ventricle, also appeared. At the higher dose (1.6-2.4 mg/rat), which is still within the pharmacological concentration range in the study of isolated myocytes, frequent VT and ventricular fibrillation occurred.
Conclusion:
We established a valid small-molecule inducible rat model to study the mechanisms of ventricular arrhythmias. This model will help us understand the molecular mechanisms and neural causes of arrhythmia-associated ventricular contraction and blood pressure.

P543
For Better Senior Sex – insights from Drosophila

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Backgrounds
Better senior sex has long been an important objective in healthy aging research. This is a big question about the declination of sexual activity and sexual desire along with age has been proven in various clinical studies. Dopamine has been proven to participate in the sexual desire control of mammals. The involved cellular and molecular mechanisms of sexual problem in senior have not been clarified.
Materials and Methods
The GAL4/*UAS* gene expression system is a precise means of targeted gene expression employed to study sexual activity in *Drosophila*. *Drosophila* also provides a research advantage of large-scale analyses that the analyses and quantification of courtship behavior have been clearly confirmed. In this study, we using tissue-specific GAL4 drivers resulted in tyrosine hydroxylase (TH) expression that was restricted, and expected dopamine level will be boost to those specific tissues for large amount screening dopamine-mediate sexual activity circuits.
Results:
More compelling evidence was shown when increasing dopamine level in dopaminergic neurons, the declination of courtship strength of *Drosophila* along with ageing could be slowed down. It increases the DA level in different types of dopaminergic neurons. Interestingly, we demonstrate the existence of critical circuit; in VPL neurons which innervate the calyx of mushroom bodies, through DA, may boost the male courtship strength.
Conclusion:
VPL neurons are necessary and sufficient, through dopamine, enhance male sexuality; and age-related declines in sexual activity also leads to alterations of dopamine in VPL neurons. As such a complicated behavior could be controlled by this simply neural circuitry, it is expected that the outcome would become an effective platform for relevant research on sexual activity.

P544
Human Gingival Fibroblasts Cultured On Chitosan Film Crosslinked By Glutaraldehyde Could Inhibit Bacterial Invasion And IL-8 Relative Inflammatory Responses.

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Chitosan, which is a nature polymer, has been fabricated into various forms such as antibiotic additives in bone cements, micro/nano particles or reservoir devices for drug delivery and membranes or matrices for tissue engineering. However, there is no research indicating chitosan anti-bacterial abilities after cell culture. In this study, we have fabricated chitosan solutions, partices and films by glutaraldehyde crosslinking reaction to study their effect on bacterial killing and the prevention of bacterial invasion on human gingival fibroblasts (HGF). The results show that all chitosan forms have anti-bacterial abilities on E. coli, Staphylococcus Epidermidis and Streptococcus mutans. In comparison with chitosan particles, chitosan films and solutions demonstrated higher antibacterial ability and no bacterial invasion into HGF. Interestingly, chitosan films crosslinked by glutaraldehyde present hydrophobic surface and which could not support cell adhesion. Therefore, various proteins were added into chitosan films for modifying their hydrophobicities. After bacterial invasion, HGF cultured on chitosan films could inhibit bacterial invasion and gene expressions of inflammation markers such as IL-8, ICAM and VCAM but not COX-2 gene. Based on these results, we conclude that chitosan films could not suppress inflammation responses on COX-2 pathway but could prevent cell adhesion and gathering in immune system through the inhibition of IL-8, ICAM and VCAM expressions. Chitosan films crosslinked by glutaraldehyde showed good cell attachment and proliferation and can inhibit bacterial invasion and further immune responses. Therefore, chitosan films crosslinked by glutaraldehyde could be potential in tooth and bone biomaterial applications.

P545

Smap1 and ccdc75, genes downstream of NRF-1, have differential function in axonal and dendritic growth in rat hippocampal neurons

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Backgrounds:

Outgrowth of axon and dendrites is important for neuronal differentiation and nerve regeneration. We have identified that SMAP1 and CCDC75 are two genes downstream of nuclear respiratory factor 1 (NRF-1) and mediate its functions in neurtie outgrowth in human neuroblastoma cells. SMAP1 encodes the GTPase activating protein of Arf GTPase and CCDC75 is predicted to encode a nuclear protein with the potential of DNA binding. It remains unknown whether these genes play roles in axonal and dendritic growth in neurons. Here, we use the development of cultured rat hippocampal neurons as a model to investigate whether these two genes have differential function in axonal and dendritic growth.

Materials and Methods:

Rat hippocampal neurons were cultured from embryonic day 18. The morphologies of hippocampal neurons at each developmental stage were observed under light microscope. Total RNAs of hippocampal neurons were extracted and subjected to semi-quantitative RT-PCR analysis. Full-length cDNA rat Smap1 and Ccdc75 was constructed separately into the pCMS-EGFP vector and then transfected into hippocampal neurons 1 day after cell plating. Twelve hours after transfection, neurons were grown for another 3 days to stage 5. Neurons were then fixed and stained with anti-GFP for morphological observation. With the help of ImageJ program, the number of axons and axonal collaterals, dendritic branches, axonal length and total dendritic length were determined.

Results:

The mRNA levels of rat Smap1 and Ccdc75 increased gradually from early to later stages, suggesting that expression of these two genes play important roles in later stages of axonal and dendritic growth. Overexpression of Ccdc75 in rat hippocampal neurons increased the number of axonal collaterals and the number of primary, secondary, and tertiary dendrites but has no effects on the length of axons and dendrites at stage 5. In contrast, overexpresion of Smap1 decreased the length of axons and dendrites at the stage but has no effects on the number of axonal collaterals and dendritic branches.

Conclusion:

These results suggest that ccdc75 and smap1, two genes downstream of NRF-1, have differential functions in axonal and dendritic growth in cultured hippocampal neurons. In the future, effects of these two genes on axonal and dendritic growth in vivo will be investigated. These results will not only increase our knowledge on the molecular network underlying axonal and dendritic growth, but also provide potential therapeutic target for neuronal regeneration after injury.

P546

Involvement of BDNF in the thalamic hypersensitivity in CPSP

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Backgrounds:

After stroke, about 7~10% patient will develop chronic pain syndrome after 6 month later. This chronic pain condition is called central post stroke pain syndrome (CPSP). Lenz supported a hypothesis CPSP is due to the unbalanced oscillation of thalamocortical circuit. Recent research results also indicated that abnormal increased secretion of brain derived neurotrophy factor (BDNF) in spinal cord tissue after spinal cord or peripheral neural injury. Expression of NKCC1 and NCC2, Cl- related channel, were influenced by the over expression of BDNF that the balance and functional role of Cl- in mature neuron were also re-modified. CPSP animal model of brain hemorrhage in ventral-posterior area of thalamus (VP) was well established but the pathological mechanism of CPSP is not well studied.

Materials and Methods:

In the present study, 0.125U/0.5μl type 4 collagense was injected into SD rats' VP area and nociceptive responses were tested with Von Frey and Plantar test. After 4 weeks of injection, in light isoflurane anesthesia situation, rat brain neuronal response was recorded with multi-channel system and analyzed with homemade Matlab programs. Animal scarified with PBS/paraformadehyde perfusion, rat brain was removed, sliced to 30~40μm with frozen section and stained with 1' anti body, Neu-N/Gila or OX42/Glia, and 2' 488/594nm fluoresce antibody. Expression of BDNF, TrkB receptor, GABA receptor, KCC2 channel and NKCC1 channel in MD area were be measured by Western Blotting method.

Results:

After 4 weeks of injection, the number of neuron was decreased and the cell number of astroglia, microglia and the mRNA level of BDNF were increased in lesion brain area. The decreasing of neuron was positively correlated with the degree of pain hypersensitivity of CPSP. In the electrophysiology recording, activity of medial-dorsal thalamus nucleus (MD) was enhanced after repeated noxious stimuli in CPSP animal and this enhancement could be blocked by acute TrKB-FC (an extracellular scavenger of BDNF) injection. Instead of inhibition by GABA system in normal rat, MD multiunit activity was enhanced after microinjection of muscimol in CPSP animal. After CPSP, expression of BDNF was enhanced in MD area tissue but the expressions of GABAa channel and KCC2 channel were decreased in the same area.

Conclusion:

The change of cell composition in VP area was a key factor of CPSP syndrome and over expression of BDNF probably induced by the proliferation of glia or microglia cell. MD neurons plasticity changes after stroke may be due to the composition changes of Cl- related channels that caused by BDNF re-modulation.

P547

Effect of Cistanche tubulosa on Male Reproductive Function in Streptozotocin-Nicotinamide Induced Diabetic Rats

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Backgrounds:

Hypogonadism and oxidative stress occurs commonly in men with diabetes associated male infertility. *Cistanche tubulosa* is the Chinese herbal medicine have traditionally been used for treatment of impotence, female sterility, and cold sensation in the loins and knees. The aim of this study was to investigate the capability of anti-inflammatory and anti-oxidative on *Cistanche* tubulosa extract, and to evaluate the protective effects on male reproduction of *Cistanche tubulosa* extract against diabetic rats.

Materials and Methods:

In vivo, the model diabetic rat was induced by nicotinamide (230 mg/kg), streptozotocin (65 mg/kg) and 45% hight fat diet. *Cistanche tubulosa* extract was tested in three doses (80, 160 and 320 mg/kg, p.o daily) for 6 weeks. Besides, rosiglitazone (RSG) administration (0.571 mg/kg) as positive control.

Results:

Cistanche tubulosa extract administration for 6 weeks improve hyperglycemia and insulin resistance, lipid peroxidation, superoxide dismutase (SOD), and sperm abnormal numbers, motivity increased significantly. But only 320 mg/kg of *Cistanche tubulosa* extract could significantly restore hypertriglyceridemia. Besides, it has restored KiSS1, GPR54 and SOCS-3 mRNA expression in the hypothalamus, and recover LH and testosterone level. This study clearly indicates that *Cistanche tubulosa* extract impaired markedly this animal model mediated insulin resistance, and *Cistanche tubulosa* extract treatment increased the activities of testicular antioxidant enzymes and restored sperm characteristics.

Conclusion:

In conclusion, not only the antioxidant and anti-inflammatory property but also steroidogenesis effect of *Cistanche tubulosa* extract might have contributed for its ability to decrease this animal model mediated insulin resistance, and protect male reproduction.

P548

Effects of Single Nucleotide Polymorphisms of MAPK8IP1 and MTERFD3 Genes on Mood Disorders

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Backgrounds:

Affective disorders, mainly depressive (MDD) and bipolar disorders (BPD, including BP I and BP II), are common in the general population. However, little is known between genes and their clinical features. The aim of this study is to correlate SNPs of MAPK8IP1 and MTERFD3 genes with clinical features.

Materials and Methods:

There were 1242 participants in this study, including 464 probands, 522 relatives and 256 controls from six clinics, regional hospitals or medical centers in the southern and northern Taiwan from 2008 to 2012. Participants were interviewed by well-trained interviewers using Composite International Diagnostic Interview (CIDI) to collect data on demographic and clinical features. The data were collected from patients with depression or bipolar disorder, their families and other members. We also cloned the promoters of MAPK8IP1, and MTERFD3 genes to study the correlation of SNPs and clinical features and compared the promoter activities between the promoters, which contain different variations. These genes are associated with mental diseases or respond to environmental factors. We also studied the correlation of these variations with performance of patients suffered by mental diseases under high pressure.

Results:

At the Pittburgh Sleep Quality Index, PSQI0, used to assess the sleep situation of subjects in the past month, There were significant differences among probands, relatives and controls. The items includ quality ($p<0.001$), latency ($p<0.001$), efficiency ($p<0.001$), disturbances ($p<0.001$), use of medications ($p<0.001$) and day-life dysfunction ($p<0.001$). However, There was no significant difference among groups in sleep duration ($p=0.274$). Mood disorder was not associated with SNPs of MAPK8IP1 (rs1554338, $p=0.887$) and MTERFD3 (rs2287161, $p=0.439$).

Conclusion:

The results from this study may provide us more information for development of diagnosis and medical resource utilization for preventing mental diseases in the future.

P549
Impedance Monitoring of Mesenchymal Stem Cell After In Situ Electroporation by ECIS

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Backgrounds:
The genetic transformation of mesenchymal stem cells (MSCs) using genes that enhance their homing ability, as well as their proliferation and survival capacities when transplanted to sites of injury, is an important alternative to improve MSC function, especially for tissue regeneration. Electroporation has been considered one of the most efficient non-viral based methods to deliver genes, but high cell mortality has been frequently observed. Understanding the mechanism underlying electroporation and improving the effectiveness of this technique is therefore important, and the motivation of this study.
Materials and Methods:
In this study we used electric cell-substrate impedance sensing (ECIS) technique to optimize the delivery of Lucifer Yellow into MSCs derived from human umbilical cord of Wharton's jelly. In this instrumental method, small gold electrodes serve as substrata for mammalian cells, and the impedance of these electrodes to an AC signal is followed with time. Due to the small electrode size and the close association of the anchored cells with the substrata, only a few volts will be required to achieve electroporation fields. Electroporation was performed by using AC sinusoidal voltage pulses of varying frequency, amplitude, and duration. Permeabilization and re-closure of the plasma membrane were evaluated by the uptake of the fluorescence probe, Lucifer Yellow, from the extracellular fluid.
Results:
We observed that high-frequency AC voltage pulses of hundreds millisecond duration are most suited to electroporate cells cultured on gold-film electrodes. For the MSCs, we achieved the most efficient but still reversible permeabilization of the plasma membranes and the corresponding probe uptake when 40 kHz AC pulses of 1.8 mA current amplitude and 100~300 ms duration were applied. Under these conditions, we successfully introduced low molecular weight probes, Lucifer Yellow (457 g/mole), into the cell interior. In addition, the cells recovered from these electroporation pulses in 60-90 min.
Conclusion:
In this work we systematically established optimized pulsing parameters for Lucifer Yellow delivery into human MSCs using ECIS electroporation technique. The use of computer controlled impedance measurement allows rapid feedback coupling, so that "intelligent" electroporation systems which would enable a fully automated optimization of the cell-type-specific electroporation conditions are technically feasible. This in turn should greatly facilitate rational design of electroporation protocols for rare and valuable human adult stem cells. The method reported here may also be useful for generation of induced pluripotent cells where high cell recoveries and high gene expression are crucial.

P550
The Expressions of PKMζ and KIBRA are Involved in the Prefrontal Cortex-Mediated Working Memory in Rats

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Backgrounds:
Working memory is a temporary memory store that is held briefly until the appropriate behavior is produced. It has been demonstrated that the formation and maintenance of working memory is closely related to the prefrontal cortex (PFC), however, the molecular mechanisms underlying PFC-mediated working memory are still unclear. Recent evidence has revealed that the persistent activity of protein kinase M zeta (PKMζ), a brain-specific variant of protein kinase C zeta (PKCζ), is required for memory formation and maintenance. In addition, kidney and brain expressed protein (KIBRA), a memory-related protein, is suggested to be a substrate of PKCζ. Whether the expressions of PKMζ and KIBRA are related to the performance of PFC-mediated working memory in normal adult rat provoke our interests.
Materials and Methods:
Sprague-Dawley male rats aged 12 weeks were used. All animals were habituated in a T-maze and then were trained to perform the delayed nonmatch-to-sample (DNMS) task in which working memory is involved. At the end of task, the levels of PKMζ and KIBRA in rat's PFC were examined by Western blot analysis. In addition, brain-derived neurotrophic factor (BDNF) has been shown to enhance the expression and activity of PKMζ. In order to confirm whether KIBRA is a substrate of PKMζ, BDNF treatment in the presence or absence of PKMζ inhibitor ZIP was given to primary cultured hippocampal neurons, and then the levels of PKMζ and KIBRA were examined by Western blot analysis.
Results:
In the T-maze task, the percentage of correct choices was calculated to evaluate the behavioral performance. Well-trained animals had a significantly increased percentage. After training successfully, animals performed the DNMS task, in which a delay of 10 sec (D10) or 30 sec (D30) was given to allow the execution of working memory. Compared with the pre-trained animals, the percentage was only significantly higher in the D10 group, indicating that the working memory was functioning in the D10 but not the D30 group. After finishing the DNMS task, the expressions of PKMζ and KIBRA in the PFC were significantly increased in the D10 group. In addition, pretreating cultured neurons with ZIP prevented BDNF-enhanced expressions of PKMζ and KIBRA.
Conclusion:
This study reveals that, in adult rats, performing PFC-mediated working memory enhances the expressions of PKMζ and KIBRA in the PFC. Furthermore, in primary cultured neurons, PKMζ inhibitor, ZIP, prevents the enhanced effect of BDNF on the expressions of PKMζ and KIBRA. These results indicate that the expressions of PKMζ and KIBRA are involved in the performance of working memory and that PKMζ plays a role in the expression of KIBRA.

P551
Exercise training attenuates aging-induced cell apoptosis in SD rat heart

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Backgrounds:
Healthy problem is the major concern of aging population in present society. Exercise is considered one of the necessary methods to promote healthy in aged population. The effects and mechanisms against aging and heart protections in long-term exercise training are our main purpose in this research.
Materials and Methods:
We use intraperitoneal injection D-galactose (150mL/kgBW, 8 weeks) as an acute aging model and combined with or without the exercise training. Our exercise training prescription is swimming in warm water 60 min per day and five times per week. H & E staining assay was applied in tissue analysis. DAPI / TUNEL staining assay was used to evaluate the apoptosis cells ratio in tissue slides. Proteins extracts were analyzed by western blotting.
Results:
Although the macroscopic phenomenon results showed the animal heart weight has no significantly change in the four groups. However the heart cell composition was disordered in intraperitoneal injection D-galactose as acute aging model then normal SD rats. Long-term exercise training had improved the disordered. Besides, the cardiac cell apoptotic ratio were significantly lower on long-term exercise training group by DAPI /TUNEL assay. Moreover, the proteins analysis observed higher expression of SIRT1-PGC1α-AMPK anti-aging signaling pathway in long-term exercise training animals.
Conclusion:
Our research results determined the situation of heart tissue senescence was similar between intraperitoneal injection D-galactose as an acute aging model and normal aging model. Long-term exercise training can enhance the SIRT1 anti-aging signaling pathways to protect heart tissue against aging. Our research results suggest that exercise can promote healthy and heart function in this aged population.

P552
The Role of XRCC6 T-991C Genetic Polymorphism in Taiwan Renal Cell Carcinoma.

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Background:
The DNA non-homologous end-joining repair gene *XRCC6* (Ku70) plays a key role in both the DNA double-strand break (DSB) repair and cell cycle arrest. Defects in DSB repair capacity can lead to genomic instability. We hypothesized that a variant in the *XRCC6* gene was associated with susceptibility to renal cell carcinoma (RCC).
Materials and Methods:
In a hospital-based case–control study of 92 patients with RCC and 580 cancer-free controls frequency matched by age and sex, the associations of *XRCC6* promoter T-991C (rs5751129), promoter G-57C (rs2267437), promoter A-31G (rs132770), and intron 3 (rs132774) polymorphisms with RCC risk in a Taiwanese population were investigated. At the same time, 30 adjacent renal tissue samples were tested to estimate the *XRCC6* mRNA expression by real-time quantitative reverse transcription.
Results:
Compared with the TT genotype, the TC genotype had a significantly increased risk of RCC [adjusted odds ratio=2.24, 95% confidence interval=1.25-4.08, p=0.0175]. The *in vivo* mRNA expression in renal tissues revealed a statistically significantly lower *XRCC6* mRNA expression in samples with TC/CC genotypes compared with those with TT genotype (p=0.0039).
Conclusion:
The evidence suggests that the *XRCC6* T-991C genotype together with its mRNA expression are involved in the etiology of RCC and may be a marker for susceptibility to RCC in the Taiwan population.

P553

The Role of the BDNF-TrkB Pathway in Treadmill Exercise-Induced Facilitation of Long-Term Potentiation in the Rat Hippocampal Dentate Gyrus

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Backgrounds:

Many studies have indicated that chronic exercise training enhances learning and memory as well as long-term potentiation (LTP), the best-described neurobiological substrate of learning and memory to date. Previous studies have shown that treadmill exercise enhances passive avoidance (PA) memory by down-regulating the serotonin type 1A ($5\text{-}HT_{1A}$) receptor system and up-regulating levels of brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin-related kinase B (TrkB) in the rodent hippocampus and amygdala, brain areas highly associated with PA memory. Our recent studies have indicated that treadmill exercise potentiates high-frequency stimulation (HFS)-induced LTP in the rat hippocampal dentate gyrus (DG) and lateral amygdala (LA) via reducing $5\text{-}HT_{1A}$ receptor activation. In addition, we have further demonstrated the involvement of the BDNF-TrkB pathway in treadmill exercise-induced enhancement of rat LA-LTP. However, it is unclear whether the BDNF-TrkB pathway is also involved in treadmill exercise-induced enhancement of rat DG-LTP.

Materials and Methods:

We used the extracellular electrophysiological recording technique and pharmacological methods to determine the role of the BDNF-TrkB pathway in four-week treadmill exercise-induced facilitation of DG-LTP in male rats.

Results:

The mean magnitude of DG-LTP (116 ± 2.1% of baseline, n = 8) in exercise rat slices measured 40 min after HFS was significantly greater than that (97 ± 3.5% of baseline, n = 12) observed in sedentary rat slices ($p < 0.001$, unpaired Student's *t* test), confirming our previous findings that treadmill exercise facilitated DG-LTP in male rats. Moreover, bath perfusion of the protein kinase C (PKC) inhibitor Bis I abolished exercise-induced facilitation of DG-LTP, suggesting that exercise acted through PKC to facilitate DG-LTP.

Conclusion:

Our results demonstrated that four-week treadmill exercise facilitated DG-LTP in male rats and that PKC was involved in this effect of exercise. The mechanisms underlying exercise-induced facilitation of DG-LTP are currently under investigation.

P554

The Short-Term Effects of Ping-Shuai Gong on Human Health Evaluated by Ryodoraku.

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Backgrounds:

Ping-Shuai Gong is a simple Qigong and easy to implement in public. It's generally considered to enhance human body health and improve disease state, but only very few studies mentioned the effect of this qigong to human body status. In this study, we aim to investigate the short-term effects of Ping-Shuai gong on human body health determined by Ryodoraku.

Materials and Methods:

Subjects 24~30 years old was participated in this study. The Meridian Electroacupuncture Analysis Device (Ryodoraku) was used to measure the subject's body status, including body energy, spirit activity state (spirit ratio), musculoskeletal ratio, metabolic functions, and autonomic nerve balance. Subjects practiced Ping-Shuai Gong for 20 minutes about 3:30 pm each day continued for 1 week. Parameters were determined just before and after the first qigong practice (acute effect) and repeated measurements after a week (short-term effect).

Results:

In acute effect, the body energy before and after practice tended to decrease and most significant in male subjects (mean ± SE, 56.2 ± 5.1 vs. 48.5 ± 6.0 in all subjects and 64.0 ± 3.4 vs. 52.1 ± 3.8, $p<0.05$ in male subjects). Other parameters had no significant difference in the acute state. In short-term study, the body energy was elevated after 1 week qigong performance (49.4 ± 5.3 vs. 62.6 ± 4.2, $p<0.05$) but there showed no significant difference in spirit activity, musculoskeletal balance, basic metabolic rate and autonomic nerve balance.

Conclusion:

Our preliminary data indicated that short-term performance of Ping-Shuai Gong may reduce stress in acute stage and elevate human body energy after 1 week practice. The effects of Ping-Shuai Gong on body functions of spirit activity, musculoskeletal balance, metabolic rate and autonomic nerve balance are remain unclear and need to be further investigated.

P555

Interactions Between Nicotinamide and Resveratrol in Aortic Relaxation

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Backgrounds:

Both resveratrol (RSV) and caloric restriction (CR) exert protective effects in cardiovascular tissues and may involve the activation of sirtuins. It was, however, unclear whether sirtuin is directly responsible for beneficial vascular functions, e.g., endothelium-dependent relaxation (EDR), often observed in RSV- or CR- treated rodents.

Materials and Methods:

We tested the effects of nicotinamide (NIC, 12h pre-trentment), a noncompetitive inhibitor of sirtuins, on EDR in rats with or with out RSV-pretreatment (2 wks). We also determined plasma level of nitric oxide (NO) and vascular ROS (reactive oxygen species).

Results:

NIC (10 and 20 mM) inhibited Ach-induced EDR in control aortic rings, however, only high-dose of NIC inhibited EDR in rings derived from RSV-treated rats. Endothelium-independent relaxation induced by sodium nitroprusside was not different in any groups. Further, RSV rats exhibited higher plasma NO level than that of control and NIC (20 mM only) reduced endothelium-derived NO in RSV rings. We have also determined the NADPH-sensitive ROS production of aortic rings and found no significant effects in control but stimulation by NIC in RSV rings.

Conclusion:

These data suggest that NIC, a sirtuin inhibitor, reduced EDR possibly involving both NO and ROS. It thus provides a tool to compare the potential sirtuin activator such as RSV and CR. (Supported by NSC101-2320-B-255-001 to YT Lau)

P556

Association of DNA double strand break gene *Ku70* Genotypes and lung cancer in Taiwan.

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Background:

The DNA repair gene *Ku70 (XRCC6)* is thought to play an important role in the repairing of DNA double strand breaks. It is known that defective in double strand break repair capacity can lead to irreversible genomic instability. However, the polymorphic variants of *XRCC6*, has never been reported about their association with lung cancer susceptibility. In this hospital-based case-control study, the association of *Ku70* promoter T-991C (rs5751129), promoter G-57C (rs2267437), promoter G-31A (rs132770), and intron3 (rs132774) polymorphisms with lung cancer risk in a Taiwanese population was studied.

Materials and Methods:

In total, 358 patients with lung cancer and 716 healthy controls recruited from the China Medical Hospital in Taiwan were genotyped by PCR-RFLP method.

Results:

The results showed that there were significant differences between lung cancer and control groups in the distribution of their genotypic (p=3.7E-4) and allelic frequency (p=2.7E-5) in the *Ku70* promoter T-991C polymorphism. Individuals who carried at least one C allele (TC or CC) had a 2.03-fold increased odds ratio of developing lung cancer compared to those who carried the TT wild type genotype (95%CI=1.42-2.91, p=0.0001). In the other three polymorphisms, there was no difference between the case and control groups in the distribution of either genotypic or allelic frequency.

Conclusion:

In conclusion, the *Ku70* promoter T-991C, but not the promoter C-57G, promoter G-31A or intron3, is associated with lung cancer susceptibility.

P557
The Effect of Akt1 Deficiency and Neonatal Immune Activation on Schizophrenia-related Phenotypes in Adult Mice

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Background:

Schizophrenia appears to be a multifactorial disorder with a strong genetic predisposition. Accumulating evidence suggests *Akt1* (protein kinase B alpha) may contribute to susceptibility for schizophrenia. *Akt1* mutant mice also displayed neuromorphological and behavioral abnormalities. In addition to genetic predisposition, early infection-induced disruption in neurodevelopment may cause long-lasting changes, leading to functional deterioration and the onset of schizophrenia in later life. Indeed, immuno-precipitated neurodevelopmental animal models displayed aberrant behavioral functions, and perinatal infection altered the immune response as well as learning in adulthood in rats. Alteration of Akt1 expression was also observed in prenatal immune challenged mice. Given the existing evidence, the objective of this study is to examine whether early infection alone or its interaction with *Akt1* deficiency may be pertinent to the vulnerability of schizophrenia-like endophenotypes and neurochemical abnormalities in adult mice.

Materials and Methods:

Akt1 heterozygous (HET) pups and their wild-type littermate controls received daily injections of saline or polyriboinosinic-polyribocytidylic acid (polyI:C; 5 mg / kg) from postnatal days 2 to 6 to induce antiviral response. Neonatal cytokine expression levels in the brain were measured by cytometric bead array. Adult behavioral functions were measured by a battery of behavioral tasks, including locomotion, Y maze, social preference, social recognition, ultrasonic playback paradigm, tail suspension, and prepulse inhibition in 3-month-old mice. Afterward, adult mice received an additional polyI:C challenge (12 mg / kg) to investigate their behavioral alternations.

Results:

Our preliminary data revealed an increase of cytokine IL-6, TNFα, and IL-1β in the poly I:C-treated neonatal brains, giving evidence of an efficient neonatal immune activation. In adulthood, the additional poly I:C challenge effectively reduced total traveled distance in these mice. Compared to neonatal poly I:C-treated mice, neonatal saline-treated mice also displayed altered ratio of central traveled distance between Akt1 deficient mice and their wild-type littermates. A possible interaction between *Akt1* and neonatal immune activation was found in the social recognition task after the additional poly I:C challenge. These preliminary data hinted a gene-environment interaction. Further behavioral and neurochemical analyses are still in progress.

Conclusion:

Our preliminary data demonstrated the involvement of *Akt1* and neonatal poly I:C challenge in the pathogenesis of schizophrenia-related phenotypes in adult mice. Findings from this study will provide some clues to the understanding of gene-environment interaction in the etiology of schizophrenia.

P558
Early Growth Response-1 Protects Pancreatic β-Cells from Free Fatty Acid-Induced Apoptosis

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Backgrounds:

Early growth response-1 (Egr1), a zinc-finger DNA binding transcription factor, is induced by many environmental signals including growth factors, hormones and neurotransmitters, and is highly associated with growth, cell survival and apoptosis. Previous study has demonstrated that Egr1 mediated responses of pancreatic β-cells to sustained glucose stimulation and indirectly regulate insulin production. Pancreatic β-cells plays a crucial role in glucose homeostasis and its failure is related to diabetes mellitus. Diabetes mellitus and obesity are often accompanied by abnormal lipid metabolism with increased concentration of free fatty acids (FFAs) in the circulation. Chronically elevated levels of FFAs have been shown to result in pancreatic β-cell dysfunction, and decreased β-cell mass associated with increased rates of β-cell apoptosis.

Materials and Methods:

In our study, we used the insulinoma cell line MIN6 treated with palmitic acid (PA), the most abundant FFA in circulation. We examined the expression and roles of Egr1 in MIN6 cells by quantitative RT-PCR, immunoblot or imaging.

Results:

After treatment with PA, Egr1 mRNA levels were increased at 15 min and peaked at 2 hour. Treatment of nifepidine (L-type calcium channel inhibitor) or EGTA (calcium chelator) blocked PA-induced Egr1 upregulation. We found increased phosphorylation of Erk1/2 and JNK, but not p38, after treatment of PA. Treatment of the inhibitor for Erk1/2, but not JNK and p38, attenuated PA-induced Egr1 upregulation. These results suggest that PA induces Egr1 expression through Ca^{2+} influx and Erk1/2 activation. After PA treatment, we found that Egr1-knockdown cells were more susceptible to PA-induced caspase 3 activation. Furthermore, phosphorylation of Akt in Egr1-knockdown cell was decreased, suggesting that the absence of Egr1 downregulates the PI3K/Akt survival pathway.

Conclusion:

These data suggest that Egr1 may attenuate PA-induced apoptosis to maintain functional integrity of β-cells.

P559
Cyclooxygenase-2 (COX-2) Up-regulation As a Novel Prognostic Marker for Poor Clinical Outcome of Upper Tract Urothelial Cancer.

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Background:

There are no reliable biomarkers for diagnosis, prediction of outcome or treatment effect monitoring for upper tract urothelial carcinoma (UTUC), which is a uniquely prevalent cancer in Taiwan. In the present study, 128 primary UTUC specimens of various grades and primary tumor status were examined for the expression of cyclooxygenase-2 (*COX-2*) in tumor and stroma tissues aiming to clarify the association of *COX-2* expression with clinical outcomes in Taiwanese patients with UTUC.

Materials and Methods:

Immunohistochemistry was implemented to investigate *COX-2* expression levels in 128 paired tumor and stroma specimens. The association of *COX-2* expression with tumor progression and prognosis was analyzed.

Results:

Our data demonstrated that positive *COX-2* expression was more frequent in stromal cells (57.0%), than in tumor sites (53.1%), and the up-regulation of *COX-2* was strongly associated with higher cancer-specific death and cancer recurrence rates. In *COX-2*-negative cases, no similar correlation was found.

Conclusion:

COX-2 expression was up-regulated in both stromal and tumor cells of more than half of the studied UTUC patients and the positive expression of *COX-2* in stromal cells may be a potential predictive and prognostic biomarker for UTUC, especially for cancer-specific death and recurrence.

P560
The Specific Haplotype of Cyclooxygenase 2 may Enhance the Risk of Taiwan Ureter Cancer.

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Background:

The association between c*yclooxygenase 2 (Cox-2)* and ureter cancer has never been investigated in the past. In this study, the association of *Cox-2* genotypic polymorphisms with ureter cancer was examined to specifically address this issue.

Materials and Methods:

Fifty-six ureter cancer patients and 436 non-cancer controls recruited from the China Medical Hospital in central Taiwan were genotyped and analyzed. Up to six polymorphic variants of *Cox-2*, including G-1195A, G-765C, T+8473C, intron 1, 5, and 6, were investigated in our study to analyze the association of the genotypes with susceptibility to ureter cancer.

Results:

At first, no significant difference in the distribution between the ureter cancer and control groups was found in each of the polymorphism site investigated. However, our analysis of the joint effect for *Cox-2* G-765C and intron 6 showed that individuals with GC at G-765C and AG+AA at intron 6 presented a higher potential for developing ureter cancer than the other groups.

Conclusion:

Since there was no obvious association between *Cox-2* genotypes and ureter cancer stage or grade, our findings suggest that the C allele of *Cox-2* G-765C together with the A allele of Intron 6 may be responsible for ureter carcinogenesis and may be useful in the early detection and prediction of ureter cancer.

P561

Effects of Chronic Diosgenin Treatment on The Bone of Aging Rats Induced by D-galactose

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Backgrounds:

D-galactose has been known to accelerate aging by causing oxidative stress, and thus induce osteoporosis. Our pervious study has showed that diosgenin can prevent osteoporosis in menopausal rats. The aim of this study was to clarify the effects of chronic diosgenin treatment on the bone loss in D-galactose-induced aging animal model.

Materials & Methods:

Twelve-week male Wistar rats were daily treated with D-galactose (200 mg/kg/day, i.p.) to induce aging animal model and orally co-administrate with the treatment of diosgenin (0, 10, 50 mg/kg/day) for 8-week. Then, rats were sacrificed and their femurs were taken for measuring mechanical and morphological properties.

Results:

The results showed that demonstrated D-galactose had no statistical change in mechanical properties of the bone, but significantly decreased the frame volume ($F(3,35)= 8.102$, $p< 0.001$).Compared with the D-galactose-treated rats, chronic diosgenin treatment significantly decreased the porosity ($F(3,35)= 5.556$, $p= 0.003$), frame density ($F(3,35)= 6.559$, $p= 0.001$) and while at the dosage of 10 mg/kg/day could significantly increase the frame volume ($F(3,35)= 8.102$, $p< 0.001$) to the control.

Conclusion:

The results showed that D-galactose can induce aging animal model and lead to osteoporosis, while after treated with diosgenin can reduced age-related bone loss and it might be potential for clinical applications in osteoporosis treatment.

P562

Effects of DC Electric Field on Synaptic Plasticity and Seizure Activities in Thalamocingulate circuitry

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Background/Aims

Seizure affects 1 % of population, and 30 % among them suffered from drug-resistant epilepsy. Clinical application of transcranial magnetic stimulation, transcranial current stimulation and direct current (DC) electric field stimulation provide non-invasive approaches for the treatment of drug-resistant seizures. Previous studies showed that field stimulation could modulate synaptic plasticity aswell as influence epileptiform activities in various brain regions, such as in motor cortex andhippocampus. However, seldom research focus on the field effect on synaptic transmission within thalamocortical system, which is an important circuitry in sensory processing and in generating epileptiform activities. The medial dorsal (MD) thalamic nucleus is heavily connected to anterior cingulate cortex (ACC) and medial prefrontal cortex (mPFC) and could regulate seizure activities in cortical regions. Seizures generated in mPFC and ACC are often drug-resistant and alternative treatment such as field stimulation needs to be evaluated in this brain region. Therefore, the current study is aimed to investigate the effect of DC field stimulation on the changes of thalamocingulate synaptic plasticity and seizure-like activities generated within this circuitry.

Methods/Statistics

Male C57BL/6J mice were used in study. Previously developed brain slice cutting method preserve the pathway between MD and ACC was used in this study. The local field potentials were recorded with multielectrode array. Data were acquired by the PC based data software MC_Rack at a sampling rate of 10 kHz and analyzed with MATLAB 7.5. Uniform electric fields were generated by passing constant current between two parallel AgCl-coated silver wires placed inside the MEA chamber.

Results

Application of 10 minutes of anodal DC field stimulation could potentiate the synaptic transmission in the MD-ACC pathway. The potentiation effect could last 20 minutes after ceasing application of DC stimulation. The potentiation effect was mediated by the volume changes of extracellular space, which will in turn increase the concentration of neurotransmitter. The potentiation was prevented by the application of furosemide which was used to stabilize the extracellular space. The effect of DC field stimulation on synaptic potentiation was also blocked by the application of APV, the NMDA receptor antagonist. Drug resistant seizure was induced by perfusion brain slice with 4-aminopyridine (250 μM) and bicuculline (5 μM), application of anodal DC enhanced the seizure-like activities while cathodal DC suppressed them. Furosemide also abolished the effect of DC field on seizure-like activities, which indicated that DC field also influence the seizure-like activities by themodulation of extracellular space.

Conclusion/Summary

DC mediated potentiation is caused by increasing concentration of neurotransmitter because application of furosemide and APV could prevent potentiation. Cathodal DC could suppress spontaneous and thalamic-evoked seizure-like activities, while anodal DC application has opposite effects. Field effect on seizure-like activities is caused by the alterations of extracellular potassium concentration which is mediated by NKCC cotransporter.

P563

FAK Promotes Estrogen Receptor α Activation and The Growth of Oral Squamous Cell Carcinoma Cells

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Background:

Estrogen receptor α (ERα) is a DNA-binding transcription factor that plays important roles in promoting cell growth and survival. Previous studies showed that expression of ERα is found in cell lines and tumors derived from oral squamous cell carcinoma (OSCC); however, the functional characterization of ERα in the oncogenesis of OSCC cells and the underlying molecular mechanism has not been determined.

Materials and Methods:

Four OSCC cell lines, including SCC4, SCC25, OECM-1 and SAS, were used for determining the expression and activity of ERα and FAK. For knocking down the expression of ERα or FAK, OECM-1 or SAS cells were infected with lentiviruses carrying shRNA against ESR1 or PTK2 gene, respectively. The cells expressing shRNA against gene encoding firefly luciferases (Luc) were served as control.

Results:

We found that the expression of ERα in four OSCC cell lines. Results from subcellular fractionation and luciferase reporter assay further showed that rapid-growing OECM-1 and SAS cells exhibited high levels of nuclear expression and transcriptional activity of ERα, compared to slow-growing SCC4 or SCC25 cells. Addition of estradiol (E2) in SCC4 and SCC25 cells promoted cell growth, while treatment of tamoxifen, an ER blocker, in OECM-1 and SAS cells inhibited cell growth. Importantly, knocking down ERα expression in OECM-1 and SAS cells by using shRNA resulted in diminished cell growth. We further observed that the expression and activity of focal adhesion kinase (FAK) was correlated with the proliferative capability of OSCC cells. Knockdown of FAK expression in OECM-1 or SAS cells led to reduced ERα phosphorylation at Ser-118 and transcriptional activity as well as retarded cell growth, whereas increased expression of FAK in SCC25 cells promotes ERα phosphorylation and activity as well as cell proliferation in a hormone-deprived condition.

Conclusion:

Our data collectively suggest that OSCC cells expressed functional ERα and its activity can be up-regulated by FAK signaling, thus promoting the proliferation of OSCC cells.

P564

Diurnal Changes of Dopamine D2 and D3 Receptor mRNA Expression in Medial Basal Hypothalamus of Estrogen-Primed Ovariectomized Rats

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Backgrounds:

The tuberoinfundibular dopaminergic neuron (TIDA) activity in the hypothalamic arcuate nucleus exhibits a diurnal rhythm with high in the morning and low in the afternoon. This diurnal rhythm is prerequisite for the afternoon prolactin (PRL) surge in the proestrous and estrogen-primed ovariectomized (OVX+E2) rats. Several endogenous neuronal rhythms acting on their specific receptors in the afternoon have been reported to be involved in the regulation of the diurnal rhythms of TIDA and PRL surge in OVX+E2 rats. Of these neuronal tones, endogenous dopaminergic rhythms acting on D2 and D3 autoreceptor involved in the regulation of the diurnal activities are recently evidenced, suggesting diurnal expressing activities of D2 and D3 receptors in the hypothalamus of OVX+E2 rats.

Materials and Methods:

Here we examined the dopamine D2 and D3 receptor mRNA expressions in the medial basal hypothalamus of OVX+E2 rats in the morning and afternoon using quantitatively RT-PCR assay.

Results:

We found that both D2 and D3 mRNA expression levels show diurnal changes with low in the morning and high in the afternoon (increased to 135 ± 5 % of the morning level for D2 receptor, $n = 3$, $p < 0.01$; increased to 208 ± 13 % of the morning level for D3 receptor, $n = 3$, $p < 0.005$). Disruption of the rhythmic activity of suprachiasmatic nucleus (SCN) by exposure the animals to the constant light for 7 days reversed both the rhythmic mRNA expressions of D2 and D3 receptors with significantly higher in the morning (increased to 151 ± 4 % of D2 control; $n = 3$, $p < 0.005$; to 134 ± 4 % of D3 control; $n = 3$, $p < 0.05$) and significantly lower in the afternoon (decreased to 84 ± 4 % of D2 control; $n = 3$, $p < 0.05$; to 34 ± 3 % of D3 control; $n = 3$, $p < 0.005$). OVX rats without primed with E2 showed no diurnal expressing rhythm of D2 receptor mRNA (to 105 ± 7 %, 100 ± 4 % of control in the morning and afternoon, respectively; $n = 3$) and a reversed expressing rhythm of D3 receptor mRNA (increased to 133 ± 11 % of control in the morning; $n = 3$, $p < 0.05$; decreased to 42 ± 2 % of control in the afternoon; $n = 3$, $p < 0.005$).

P565
食用保健食品對記憶、注意力、衝動控制等能力之動物模式探討

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記憶、注意力與衝動控制是大腦運作之基礎，人類的日常生活非常仰賴這三種基本功能。實驗一使用物件探索作業來測試長期食用保健食品對於大鼠的時序記憶、空間記憶、物體再認等能力之影響。實驗二則使用五選項反應時間作業來測試長期食用保健食品對於大鼠的注意力與衝動控制能力之影響。保健食品共分為 50mg/kg、100mg/kg、200mg/kg、300mg/kg、400mg/kg 等五種劑量溶於糖水中供動物飲用。對照組則飲用相同容量的糖水。實驗一發現，每日服用 100mg/kg 保健食品的大鼠具有優異的空間記憶，並以 100mg/kg 作為主要測試劑量進行後續實驗。實驗二發現，在困難度較高的實驗情境下，實驗組大鼠的正確反應率較高、衝動錯誤次數明顯較少。總結本研究結果證實，長期食用 100mg/kg 保健食品確能提升大鼠的空間記憶、選擇性注意力與衝動控制等認知能力。

P566
Bufalin Induced G2/M Phase Arrest and Triggered Autophagy via TNF, JNK, BECN-1, and ATG8 Pathway in Human Hepatoma Cells

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Backgrounds:
Liver cancer is the fifth most common cause of cancer death worldwide and a threat to the whole human beings. The research of more effective anti-hepatoma drugs is urgently needed. Bufalin is isolated from a traditional Chinese medicine and of less toxicity to the normal cells. Also it has been found growth inhibition in cancer cells.
Materials and Methods:
In this study, we aimed to investigate the efficacy and mechanism of bufalin in Huh7, Hep3B, and HA22T human hepatoma cells. First, the three cells were treated with bufalin, then the proliferation was detected by WST-1 assay and cell cycle was detected by flow cytometry analysis.
Results:
The results showed that bufalin inhibited the proliferation of hepatoma cells and regulate the hepatoma cell death program in a dose- and time-dependent manner with not typical apoptosis. Second, RT-PCR arrays were applied to investigate the autophagy transcriptional response triggered by bufalin and 13 genes were altered and further confirmed by real-time PCR. Third, the translational levels of selected genes were examined by Western blot to reveal the bufalin-induced autophagy cascade.
Conclusion:
Bufalin synergized with JNK pathway to induce the autophagy of hepatoma cells and closely associated with the up-regulation of TNF, BECN-1, MAPK, and ATG8, together with the down-regulation of Bcl-2 and Bid. Our work provided a multi-angle evaluation system for anti-hepatoma pharmacology for pre-clinical drug investigation. In this case, bufalin was capable to induce hepatoma cell autophagy, suggesting a potential regimen for single or combined chemotherapy to overcome hepatoma in clinical practice.

P567
The Biosynthesis Pathway Relationship between Polyamines and Nitric Oxide contents in Rice (Oryza Sativa) Seedlings under Salt Stress

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Backgrounds:
To investigate the correlation between the polyamines(PAs) contents and the nitric oxide(NO) biosynthesis in rice seedlings in salt stress by HPLC and NO fluorescent dyes.
Materials and Methods:
My rice seedlings chose the TK9(台梗九號) and TCS10(台中秈十號). In this study, I used the sodium nitroprussiate dehydrate (SNP), an NO donor , to supply exogenous NO to the seedlings. Another inhibitors were used to control the rice seedlings NO synthesis likes NG-Methyl-L-Arginine Acetate (L-NMMA), an NO synthase inhibitor; tungsten, an NO reductase inhibitor; and 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-1-oxy-3-oxide (cPTIO), an NO scavenger. By high performance liquid chromatography (HPLC), the changes of the PAs contents related to the NO biosynthesis could be measured. And using the cell-permeable NO-binding dye 4-Amino-5-methylamino-2,7-difluorofluorescein diacetate (DAF-FM DA),I observed the NO contents in the rice seedlings roots by fluorescence microscopy and quantification.
Results:
By NO fluorescence quantification, I found the NO relative fluorescence reduced with the increase salt stress. In the same time, the salt stress result in PAs measurement increasing, both in TK9 and TCS10 rice seedlings had similar results.
Conclusion:
My results supported the inference that in salt stress, plants maybe decrease the NO synthesis activity, and transfer the common precursor, argnine, to synthesize the PAs.

P568
Evidence for Inhibitory Effects of Flupirtine, a Centrally Acting Analgesic, on Delayed Rectifier K+ Currents in Motor Neuron-Like Cells

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Backgrounds:
Flupirtine (Flu) is a triaminopyridine that functions as a centrally acting nonopioid analgesic agent with muscle-relaxant and neuroprotective properties. Previous studies showed that Flu may play indirect N-methyl-D-aspartate (NMDA) receptors antagonist. Besides, there are evidences to suggest that Flu has the ability to facilitate the activity of GABAAreceptors accompanied by increased stimulation of Kv7 channels.
Materials and Methods:
In this study, we use NSC-34 cell as our cell model. NCS-34 cell is a hybrid cell line and it was produced by fusion of neuroblastoma cells with mice spinal cord cells.With the aid of patch-clamp recordings, we intend to employ whole cell mode of the patch clamp technique to ensure whether the activity of delayed rectifier K+ channels (IK(DR)) channel is functionally expressed in NSC-34 cells.
Results:
When cells were depolarized from -50 to +40 mV, Flu would decrease the current amplitude from 581±32 pA to 404±26 pA (n=11). Moreover, after addition of different Flu concentration, the results showed that the effects of Flu were concentration-dependent increase in the rate of current inactivation.In addition, we want to examined whether linopiridine, a blocker of IK(M), NMDA agonist, or gabazine, the blocker of GABAA receptor had any effects on Flu-induced perturbations of IK(DR) amplitude. The results show that addition of linopiridine had little or no effect on the inactivation time constant of IK(DR) and the application of NMDA and gabazine had minimal effects on current amplitude.
Conclusion:
We demonstrated that Flu would produce inhibitory actions on IK(DR) in a concentration- and state-dependent fashion.

P569
The Regulatory Role of Calcium-associated Signaling Pathway in Endothelin-1-induced Lipolysis in Adipocyte

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Backgrounds:

Endothelin-1 (ET-1) leads to vasoconstriction by increasing intracellular calcium (Ca2+) level in vascular smooth muscle cells. Studies showed that plasma ET-1 concentration was higher in patients with metabolic syndrome and obesity. ET-1-induced lipolysis may increase plasma free fatty acids and deteriorate insulin resistance. In our previous studies, we have demonstrated that ET-1 induced lipolysis via the endothelin type A receptor (ETAR) and activation of the ERK pathway in rat and 3T3-L1 adipocytes. But it's still unclear whether Ca2+ signaling is involved in ET-1-induced lipolysis. In this study, we would like to investigate the role of Ca2+ and the crosstalk between Ca2+ and ERK signaling in ET-1-induced lipolysis in 3T3-L1 adipocytes.

Materials and Methods:

3T3-L1 fibroblasts were grown and maintained in DME high glucose medium containing antibiotics and 10% FBS in a 10% CO2 environment. The cells were allowed to grow until 2 days postconfluency and then differentiated in the differentiation medium for 3 days. The cells were fully differentiated typically by 10 days after differentiation. Before each experiment, cells were incubated in serum-free medium for 6 h in DMEM-low glucose. Glycerol in culture supernatants was measured by a colorimetric method using glycerol assay kit (Randox Laboratories, Ltd. Antrim, UK). The ERK phosphorylation and ATGL expression were detected by immunoblotting. The HSL phosphorylation was measured with immunoprecipitation.

Results:

In this study, we found that ET-1 significantly increased hormone-sensitive lipase (HSL) phosphorylation and lipolysis, which was totally impaired by ERK inhibitor (PD98059). Ca2+-free medium treatment significantly inhibited ET-1-induced lipolysis and HSL phosphorylation. In addition, U73122 (phospholipase C inhibitor) treatment also inhibited ET-1-indiced lipolysis and HSL phosphorylation, but the inhibitory effect of U73122 on ET-1-induced lipolysis was less than that of Ca2+-free medium. Furthermore, ET-1-induced ERK phosphorylation was diminished by Ca2+-free medium and U73122 treatment.

Conclusion:

The results indicated that ET-1 induced lipolysis through increasing extracellular influx and activating intracellular PLC/IP3/Ca2+ pathway, which elevated intracellular Ca2+ level and subsequently elicited ERK phosphorylation. Activation of ERK signaling by ET-1 caused HSL phosphorylation and lipolysis.

P570
The Identification of Neuropeptide Y Receptor Subtype Involved in Phenylpropanolamine-induced Increase in Oxidative Stress and Appetite Suppression

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Background:

Hypothalamic neuropeptide Y (NPY) and superoxide dismutase (SOD) have been reported to participate in the regulation of appetite-suppressing effect of phenylpropanolamine (PPA), a sympathomimetic agent. This study explored whether Y1 receptor (Y1R) and/or Y5 receptor (Y5R) was involved in this regulation.

Methods:

Wistar rats were treated with PPA for 24 h. Changes in food intake and hypothalamic NPY, Y1R, Y5R, and SOD contents were assessed and compared.

Results:

Results showed that food intake and NPY contents were decreased following PPA treatment, while Y1R and SOD contents were increased and Y5R contents remained unchanged. Moreover, although Y1R or Y5R knockdown by themselves could modify the food intake, Y1R but not Y5R knockdown could modify PPA-induced anorexia as well as NPY and SOD contents. In addition, selective inhibition of Y1R but not Y5R could modulate PPA-induced anorexia.

Discussion:

It is suggested that Y1R but not Y5R participates in the anorectic response of PPA via the modulation of NPY and SOD. Results provide molecular mechanism of NPY-mediated PPA anorexia and may aid the understanding of the toxicology of PPA.

P571
Comparisons of the Behavioral and Neuronal Responses in Rats Exposed to Different Opioids

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Backgrounds:

The fetuses and infant were affected by the use and abuse of illicit drugs which were delivered to the infant across the placenta or by the breast milk. Methadone is the approved drug for opioid replacement therapy in the US. Buprenorphine, the partial opioid agonist, is a new drug for opioid replacement therapy. Less neonatal abstinence syndrome was developed in the infants exposed to buprenorpine than the infants exposed to methadone prenatally. Morphine, methadone and buprenorphine are all opioid agonists. The exposure of opioid agonists prenatally has the potential to disrupt normal development of the central nervous system. The effects may not become clear until childhood, when more complex cognitive functions begin to emerge. The aim of this study is to investigate the differences of behavioral and neuronal responses in rats exposed to opioids prenatally and comparison the effect of restraint stress on the rats.

Materials and Methods:

The neuronal activities in hippocampus and amygdala were monitored with chronically implanted electrodes in prenatal morphine, methodone, buprenorphine and saline exposed rats. The behavioral responses were evaluated by the locomotor activity in the open field test.

Results:

Restraint stress decreased the locomotion of prenatally methadone-exposed rats. Large portion of units in hippocampus and amygdala demonstrated higher firing rate during locomotion than resting in the prenatally opioid- exposed rats. The enhancement of unit activities in hippocampus during locomotion were decreased after the restraint stress in prenatally saline and methadone-exposed rats but not in prenatally morphine-exposed rats. The increase of unit activities in amygdala during locomotion was also reduced after the restraint stress in prenatally methadone-exposed rats.

Conlusion:

The results suggested that effect of restraint stress on locomotion were different in the animals prenatally exposed to morphine and methadone and this difference is also reflected on the neuronal responses of hippocampus and amygdala.

P572
A Functional Circuit Underlying Male-male Courtship Behavior In The Drosophila Brain

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Backgrounds:

Animals present several sensory cues to recognize the same species and suitable mate for reproduction. Courtship, an instinct of animals in the nature, normally occurs in between opposite sexes. Nevertheless, a lot of research proved that courtship and sexual behaviors existed between the same sexes in many species. The involved biologically molecular mechanism has not been clarified.

Materials and Methods:

The GAL4/UAS gene expression system is a precise means of targeted gene expression employed to study male-male courtship behavior in Drosophila. Drosophila also provides a research advantage of large-scale analyses that the analyses and quantification of courtship behavior have been clearly confirmed. In this study, we using tissue-specific GAL4 drivers resulted in tyrosine hydroxylase (TH) expression that was restricted, and expected dopamine (DA) level will be boost to those specific tissues for large amount screening dopamine-mediate male-male courtship activity circuits.

Results:

When the level of dopamine does not perform normally, the courtship behavior among male Drosophila would largely increase. In this study, we demonstrate the existence of critical circuit; in VPL neurons which innervate the calyx of mushroom bodies (Mbs), through DA, enhance the visual cue dependent male-male courtship.

Conclusion:

As such a complicated behavior could be controlled by this simply neural circuitry, it is expected that the outcome would become an effective platform for relevant research on the molecular mechanism of homosexual behavior.

P573
Antagonistic Roles of CD14 and TLR4 in LPS-induced Colonic Epithelial Cell Apoptosis

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Backgrounds:

Luminal exposure to bacterial lipopolysacharride (LPS) induces apoptosis in enterocytes via unknown mechanism. Recent studies in monocytes showed that after LPS binding to CD14, phosphatidylcholine-specific phospholipase C (PC-PLC), sphingomyelinase (SMase) and ceramide activate PKCζ, and recruits TLR4 for complex formation and downstream MAPK and NFκB signals. Human colonic enterocytes from normal subjects constitutively express CD14 but seldom TLR4.

Aim:

To investigate the receptors and signaling pathways involved in LPS-induced epithelial apoptosis.

Methods and Results:

Apical expression of CD14, low levels of MD2 and absence of TLR4 were demonstrated in primary human colonocytes and Caco-2 cells. Luminal E.coli LPS challenge increased cell apoptosis in a time- and dose-dependent manner. Neutralizing anti-CD14 and gene silencing of CD14/PKCζ attenuated the apoptosis, whereas anti-TLR4 had no effect. Pretreatment with a PC-PLC inhibitor (D609), a SMase inhibitor (imipramine), and inhibitory PKCζ pseudosubstrate decreased the LPS-induced apoptosis. Ceramide (C16) was increased after LPS challenge, which paralleled the phosphorylation and membrane translocation of PKCζ. Neither nuclear translocation of NFκB, nor phosphorylation of MAPK, IκB, Akt, and IRF3 was present in LPS-challenged cells. TLR4 overexpression by plasmid transfection inhibited LPS/CD14-mediated apoptosis. Moreover, ex vivo colonic tissues of C3H/HeJ (spontaneous mutation with malfunctional TLR4 signaling) mice were luminally challenged with LPS for 2 hrs on Ussing chambers. The mucosal apoptosis and phosphor-PKCζ levels in enterocytes were up-regulated after LPS challenge in C3H/HeJ mice. Expression of CD14 was confirmed in isolated mouse colonic enterocytes.

Conclusion:

LPS-induced epithelial apoptosis was dependent on CD14-mediated lipid secondary messengers, whereas TLR4 plays an antagonistic role.

P574
Asymmetric Coupling to the Opening and Closing Processes for BK Channel Activation by Voltage and Ca^{2+}

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Backgrounds:

BK channels provide feedback on membrane potential and intracellular calcium concentration, regulating muscle contractility, neuronal excitability and neurotransmitter release. A simplified allosteric gating scheme of BK channel with one Ca2+ site and one voltage sensor on each of the four subunits requires a minimal 50 state two-tiered model (with 25 closed states and 25 open states). It has been previously shown that, with few constrained rate constants, allosteric models of this type can approximate the gating of BK channels. In this study, we explore to what extent such models with highly constrained rate constants can account for the single-channel gating.

Materials and Methods:

Single-channel data were collected over wide ranges of voltage and Ca^{2+} from BK channels in excised inside-out patches of primarily cultured rat skeletal muscle (Rothberg and Magleby, 1990; Rothberg and Magleby, 2000). Symmeric 140 mM KCl solution was used for recording BK channel activities at room temperature (22-24°C). Open and closed interval durations were 2-D binned and simultaneously fit with maximum likelihood method to estimate the most likely gating parameters for describing the 1-D dwell time distributions with sums of exponentials (Gil et al., 2001; McManus and Magleby, 1988; Rothberg and Magleby, 2000).

Results:

An idealized model with independent voltage and Ca^{2+} sensors modulating the opening and closing rates could approximate the gating, but with some obvious differences between predicted and experimental data. The most likely parameters in this model indicated that each of the activated voltage and Ca^{2+} sensors increased the opening rates by ~10-40 folds, with little effect on the closing rates. Adding a tier of flicker closed states and/or allowing specified cooperativity among and between the voltage and Ca2+ sensors improved the description of the data.

Conclusion:

Such highly constrained models provide a means to include the large numbers of states entered during gating of BK channels with multiple sensors per subunit, while limiting the number of gating parameters sufficiently to allow insight into gating mechanism. Thermodynamically speaking, the asymmetric coupling to opening and closing rates for each activated voltage and Ca^{2+} sensor indicates that the major actions of voltage and Ca^{2+} are to destabilize the closed states of the channels, rather than to simply stabilize the open states.

P575
17β-oestradiol aggravates hyperglycemia-induced dysfunction of macrovascular tone regulation in ovariectomized female rats

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Backgrounds:

Our previous studies have demonstrated that macrovascular relaxant response is significantly impaired in diabetic female rats comparing with male rats with diabetes. It is well known that sex hormones are short-term and long-term regulator of vascular tone.

Materials and Methods:

Therefore, we used female Wistar rats divided into four groups, that is control, diabetic female rats (DM), diabetic ovariectomized female rats (OVX+DM), and plus 17β-oestradiol (E2, 1mg/kg/week)-treated diabetic ovariectomized female rats (OVX+DM+E2), to examine whether 17β-oestradiol, one of female sex hormones, play a role in the aggravation of hyperglyciemia-induced macrovascular dysfunction in diabetic female rats. Ovariectomy and E2 treatment were performed for 4 weeks followed by diabetes induction via nicotinamide (180mg/kg) plus streptozotocin (90mg/kg) method for another 8 weeks. After that, rats were sacrificed to remove their thoracic aorta for evaluation of vascoconstriction by phenylephrine and vasorelxation by acetylcholine, clonidine or insulin, and for assessment of basal reactive oxygen species (ROS) production and NADPH-related oxidase activity.

Results:

We found that: (1) Fasting blood glucose was significantly higher in all three DM group than that in control group; (2) phenylephrine, a α1 adrenoceptor agonist, -induced vasoconstriciton was markedly impaired in DM group comparing with that observed in control group; (3) both acetylcholine, a NO-cyclic GMP pathway stimulator, and clonidine, a α2-adrenoceptor agonist and PI3K/Akt pathway stimulator -mediated endothelium-dependent relaxation were significant lower in OVX+DM+E2 group than that in OVX+DM group. However, insulin, another PI3K/Akt pathway stimulator, -induced vasorelaxation of aortic rings was similar between OVX+DM+E2 group and OVX+DM group; (4) Basal endothelium-derived nitric oxide release was significantly reduced in OVX+DM+E2 group than that in OVX+DM group, but the basal ROS production was comparable between these two groups.

P576
The Effects of Cytotoxicity and Apoptotsis of Antrodia Cinnamomea in Cisplatin-resistant Human Non-small Cell Lung Cancer Cells

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Backgrounds:

To investigate a novel anticancer effectiveness by pretreating patient-derived non-small cell lung cancer cells (NSCLCs) with cisplatin follwed by the administration of Antrodia Cinnamomea (AC).

Materials and Methods:

The cisplatin-resistant patient derived NSCLCs were established with a 6-periods of cisplatin treatments at 2 μg/ml and 5 μg/ml respectively. The capabilities of AC in cytotoxicity, migration and levels of active caspase 3, PARP cleavage, mitochondrial cytochrome c, and Bax/ Bcl-2 proteins were evaluated respectively.

Results:

AC showed significant dose-dependent cytotoxicity in patients-derived NSCLCs. The nontoxic dose of AC enhanced the cytotoxicity of cisplatin-resistant patient-derived NSCLCs. The wound healing assay showed significant inhibitory effect in cisplatin-resistant NSCLCs when AC was treated at 20 mg/ml. Additionally, the enhanced apoptotic effect as characterized by increased Bax and decreased protein levels of Bcl-2, and higher levels of PARP cleavage and release of cytochrome c, suggesting that intrinsic pathway of apoptotic processes in AC treatment on cisplatin-resistant NSCLCs at 48hr was triggered.

Conclusion:

Our results provide the first evidence that AC induced signigicant higher cytotoxicity and triggered intrinsic apoptosis in cisplatin-resistant NSCLCs.

P577
Combined Obstructive and Restrictive Ventilatory Insufficiency Induced y Ischemia/Reperfusion of the Pancreas in Rats

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Backgrounds:
Reperfusion of the ischemic rat pancreas induces acute pancreatitis. Proteases released from the pancreas and respiratory burst in the lung both induce lung function changes by inflammatory responses.

Materials and Methods:
Ischemia (I) was induced by clamping the gastroduodenal artery and the splenic artery for 2 hours to induce ischemia of the pancreas, followed by reperfusion (R) for 6 hours. We then observed lung function parameters such as peak flow (PF), maximum expiratory flow rate (MMF) and vital capacity (VC). This protocol resulted in significant elevations of blood concentrations of oxygen radicals ($P < .01$), nitric oxide ($p< .05$), tumor necrotic factor ($P < .05$), amylase ($P < .01$), and white blood cells ($P < .01$) in the I/R group.

Results:
Pulmonary function data showed that reperfusion of the ischemic pancreas induced significant decreases in the PF ($p<.01$), MMF ($p<.001$), and VC ($p<.01$) when compared with the control group.

Conclusion:
Oral administration of oxidative inhibitor of niacin (300 mg/kg), with antioxidant and anti-inflammatory effects, significantly attenuates the I/R-induced obstructive and restrictive ventilatory insufficiency which proved that oxidative stress mediates I/R of pancreas-induced lung capacity and lung mechanics changes.

P578
Resveratrol Rescues Endothelial Dysfunction and Protects Against Focal Cerebral Ischemia-Reperfusion Injury in db/db Mice

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Backgrounds:
Obesity which is a prevalent problem over the world increases the risk of many metabolic and vascular diseases, including metabolic syndrome, type 2 diabetes mellitus, stroke, and cardiovascular diseases. In obese organisms, level of inflammation and reactive oxidation stress (ROS) which damage the vascular endothelial cells are enhanced because of increased adipose tissue volume and the subsequent hypertrophy. Therefore, inhibiting the process of inflammation and ROS by antioxidant is a potential therapy preventing the complication of obesity. Thus, the present study was aimed to investigate whether resveratrol (RSV), an antioxidant, protected against focal cerebral ischemia-reperfusion (FC I/R) injury by up-regulating the superoxide dismutase (SODs) and down-regulating pro-inflammatory cytokines in db/db mice. Moreover, the effects of perivascular adipose tissue (PVAT) on phenylephrine-induced vasoconstriction and acetylcholine-induced vasorelaxation were examined in db/db mice. Whether the vascular dysfunction in db/db mice can be rescued by RSV treatment will be also determined.

P579
Revisiting Behavioral Deficits of ASIC1a Knockout mice

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Backgrounds:
Acid-sensing ion channel-1a (ASIC1a) is a member of ENaC/DEG Na+ channel superfamily. ASIC1a, as a chemosensor, is prominently expressed in central neurons and is thought to contribute to synaptic plasticity, anxiety, learning behavior and fear. However, our recent study challenges the essential role of ASIC1a in hippocampal long-term plasticity, casting the doubt of the role of ASIC1a in hippocampus- and amygdala-dependent behavior. Here, we used conditional ASIC1a knockout to revisit the role of ASIC1a in mice behavior.

Materials and Methods:
- Elevated-plus maze
  Mice were placed in the center of the plus maze, and admitted to freely explore for 10 min.
- Hidden platform water maze
  Mice were placed into the swimming pool pseudo-randomly (excluding target quadrant) during 5 days training, and allowed to search the hidden platform for 60 seconds. Mice were given 2 sessions per day, and 3 trials per session. After 5 days training, the platform was removed, and the probe test was given on day 6.
- Fear conditioning
  Mice were placed into two different contextual conditions (context A & context B) for two days. On day 1, mice were placed into the context A and given 5 tone-shock pairings. After 24 hours, mice were first placed into context A without any tone or foot shock for 3 min to assess the contextual fear. After contextual fear test, mice were placed into context B and receive 3 tones (without foot shock) to evaluate cued fear memory.

Results:
First, ASIC1a-null mice showed normal learning performance like WT littermates, and had the similar ability of memory retention. It suggested that loss of ASIC1a does not impair the hippocampus-dependent spatial memory. Then, ASIC1a-null mice exhibited lower freezing responses than WT in fear conditioning, meaning that the lack of ASIC1a influenced the fear-related behavior. Besides, we used E-maze to assess whether ASIC1a mice have a difference in the innate fear. However, the result reveals that there is no significant difference in time spent or distance traveled in each arm on E-maze between two groups.

Conclusion:
ASIC1a-null mice have normal anxious behavior but display deficits in fear learning and memory. However, in contrast to a previous report, we show that ASIC1a knockout mice exhibit normal performance in spatial navigation.

P580
Amentoflavone Induced Cell Cycle Arrest and Apoptosis in Human Breast Cancer MCF-7 Cells via Mitochondria-dependent Pathway.

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Background:
Amentoflavone, isolated from an ethyl acetate extract of the whole plant of *Selaginella tamariscina*, a traditional herb, may exhibit antitumor activity. The aim of this study was to investigate the anticancer mechanism(s) of amentolavone.

Materials and Methods:
In the present investigation, cell viability was measurement by MTT assay, cell cycle distribution determination by flow cytometry, comet assay for DNA damage investigation, western blotting for determination of protein level and statistical analysis were calculated by Student's *t*-test.

Results:
Cells treated with amentoflavone exhibit a series of cellular alterations related to apoptosis, including DNA and nuclear fragmentation, and deregulation of intracellular reactive oxygen species (ROS) and calcium. In addition, markers of mitochondrial-mediated apoptosis, including the reduction of mitochondrial inner-membrane potential, the release of cytochrome c from mitochondria, and activation of caspase 3, were observed.

Conclusion:
In conclusion, our results present, to our knowledge, the first evidence that amentoflavone induces apoptosis of MCF-7 breast cancer cells, and that this is closely related to mitochondrial dysfunction. Amentoflavone may be a potential therapeutic agent for breast cancer treatment.

P581

Evaluation of the Correlation between the Degree of Stenosis and the Alteration of Blood Flow Field in Internal Carotid Artery by Using Numerical Methods

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Backgrounds:

Stroke is the second leading cause of death in Taiwan and 1/6 of all human beings suffer at least once in their lives. Approximately, 87% of cases of stroke are classified “ischemic”, in which 25-30% of ischemia is caused by internal carotid artery (ICA) stenosis. The atherosclerotic plaque clots within the blood vessels, which causes the thickness of Tunica intima and reduces the blood flow in ICA, following by disruption of blood supply to the brain. Accordingly, higher degree of stenosis is at greater risk of ischemia. Clinically, the degree of ICA stenosis is predicted based on duplex velocity measurements. However, there is a limitation for lower degree of stenosis.

Material and Methods:

Here, in order to provide an additional reference to assessment of mild ICA stenosis, we used computational fluid dynamics (CFD), and an in vitro model of stenosis to evaluate the correlation between the changes of blood flow and the degree of stenosis in internal carotid artery. We established a numerical model with concentric and eccentric stenosis in different degree (10%, 30%, 50%, 70% and 90%) based on the computer tomography image from a healthy adult.

Results:

We found that the numerical simulation of changes in blood flow conformed to the clinical data in previous findings. Moreover, the flow patterns, such as sheer stress, turbulence and recirculation, in carotid bifurcation models are consistent with both numerical and clinical observations in ICA stenosis.

Conclusion:

This study suggest that our numerical model of stenosis may provide a predicted model of mild stenosis by giving the parameter of blood flow, which could be further used as a preoperative evaluation in ICA surgery, such as carotid endarterectomy and carotid artery stenting.

P582

Studies of molecular mechanisms that cause chromosome amplification-associated hepatitis B virus-induced hepatoma

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Background:

Hepatocellular carcinoma (HCC) is the most common malignancy worldwide. Chronic hepatitis B virus infection is one of the major agents to induce HCC. Type II ground glass hepatocytes (GGHs) harboring the pre-S2 mutant large surface antigen (LHBS) in the non-tumorous liver tissues in HCC patients represented pre-neoplastic lesions and were in high risk to tumorigenesis. HBV carriers who displayed pre-S2 mutant LHBS in sera were also found highly prevalent to HCC. We previously found that the pre-S2 mutant LHBS accumulated in endoplasmic reticulum (ER) lumen, inducing ER stress-mediated oxidative stress and DNA damage, which suggested that it caused genomic instability and, ultimately, transformation. The goal of this study was to screen for chromosome gene copy number variations (CNV) in the HBV transgenic mice and HCC patients, and to characterize chromosome amplification/deletions in HBV-induced HCC.

Materials and Methods:

The mouse array comparative genomic hybridization (CGH) analyses in livers of the transgenic mice carrying HBX, pre-S2 mutant LHBS, and both genes were performed. The cDNA microarray data in these respective mice were kindly provided by Dr. Ih-Jen Su (NHRI). Gene expressions in the liver tissues of 3 HBV-, 3HCV-induced HCC, 3 HBV with cirrhosis, and 3 HCV with cirrhosis in patients were analyzed by cDNA microarray, performed by Dr. Chia-Jui Yen (NCKUH). Gene CNVs and expression changes identified by array CGH and cDNA microarray analyses were further confirmed using real-time PCR assays.

Results:

We found that pre-S2 mutant LHBS mice exhibited significantly higher copy number variations (CNVs) than the HBX ones did. The CNV levels in the pre-S2 mutant LHBS mice were equivalent to those in the double transgenic mice, indicating that the pre-S2 mutant LHBS is the main viral factor contributing to genomic instability in HBV-induced HCC. The mouse cDNA microarray analysis also found that the genomic instability-related pathways including DNA damage, apoptosis, and cell cycle regulation, were greatly affected in the pre-S2 mutant LHBS mice, showing disturbed genome integrity in them. Also, some genes on chr. 1q were amplified and over-expressed in HBV-induced HCC. Among these genes, Nek2, Nuf2, Aspm and Cenpf were M phase cell cycle factors. The results of genomic and reverse transcriptase real-time analyses confirmed that Nek2, Nuf2 and Aspm genes were amplified and over-expressed in HBV-induced HCC in transgenic mice and HCC patients.

Conclusion:

HBV pre-S2 mutant LHBS represents an important factor for HBV-induced genomic instability, which is a prerequisite for HCC development. Also, HBV oncoproteins induce chromosome 1q genomic instability, which is believed to play important roles in HBV-associated hepatocarcinogenesis.

P583

Neuroprotective Effect of Histone Deacetylase Inhibitor Valproic Acid in Spinocerebellar Ataxia Type 17 Mice

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Backgrounds:

Spinocerebellar ataxia type 17 (SCA17) is an autosomal dominant neurodegenerative disease caused by expanded CAG repeats on TATA binding protein (TBP) gene. The major clinical symptoms of SCA17 include ataxia, dementia, and chorea. In addition, as in several types of SCAs, SCA17 was also reported to have degeneration and abnormalities in cerebellar Purkinje cells. Histone acetyl transferase, CREB binding protein (CBP) down-regulation was identified in several polyQ diseases, including Huntington’s disease (HD) and SCA1. Valproic acid (VPA), an histone deacetylase (HDAC) inhibitor, was reported to be potential for several neurodegenerative disease models, including HD and Amyotrophic lateral sclerosis.

Materials and Methods:

We have established a mouse model carrying 109-CAG repeat-extended TBP gene which develops many SCA17 phenotypes after 4-week old. VPA treatment was started at 4 weeks old mice with the concentration of 0.026% in drinking water. Mouse motor coordination was evaluated by rotarod task and neuropathology was characterized at 11 and 20 weeks old mice to determine the VPA therapeutic potential.

Results:

Our study identified decreased CBP level in SCA17 mouse Purkinje cells. The reduced CBP could down-regulated acetylation of histones. Our histological data showed up-regulation of histone acetylation after VPA treatment. Moreover, both of behavior and pathological results demonstrated several SCA17 symptoms were rescued, including Purkinje cell degeneration, motor impairment, and loss of body weight from 11 week- to 20 week-old.

Conclusion:

Pathological characterization showed that Purkinje cell degeneration was rescued after VPA treatment. These results suggest that VPA has a potential neuroprotective effect on our SCA17 mouse model.

P584

Screening of Matrix Metalloproteinase Inhibitors from Medicinal Herbal Extracts

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Backgrounds:

Collagen fibers are the major macromolecular structural components in extracellular matrix of human dermis. The most abundant types of collagen in human skin are types I and III, and their fibrils form the mesh largely responsible for the skin's mechanical properties. Collagen damage is due to skin aging and degradation by matrix metalloproteinases (MMPs). The initiative degradation of collagen by MMP-1, whereas MMP-2 and MMP-9 are two gelatinases, which efficiently digest degraded collagen. The loss of collagens gives rise to skin senescence-related wrinkles, fine lines, sagging, and decrease in skin thickness. Chinese herbal medicine has been widely used in cosmetics, moreover, we believe that the extracts of Chinese herb medicine may exhibit the anti-aging capability by inhibition of MMPs’ activities. Therefore, we intended to screen MMP inhibitors from medicinal herbal extracts.

Materials and Methods:

We chose twelve kinds of medicinal herbal extracts to perform the MTT assay, and to be analyzed by gelatin-based zymography. The analysis of zymograms was applied to isolate and screen MMPs inhibitors.

Results:

The experimental results showed that, cytotoxicity was found from the *Salvia miltiorrhiza* and *Morus alba* extracts when 3T3 cells were treated with the concentration of 5.0%, as well as, the survival rates of other herbal extract-treated cells are above 80.0%. When treated with the concentration which increased to 10.0%, the extracts from *Astragalus membranaceus* and *Ligustrum lucidum* showed no cytotoxicities, however, the survival rates of other herbal extract-treated cells were relatively low. On the other hand, the analyses by gelatin-based zymography showed that the extracts from *Salvia miltiorrhiza, Ligustrum lucidum,* and Morus *alba* exhibited the inhibitory abilities toward MMP-2 and MMP-9 with the concentration of 5.0%, moreover, inhibitive capacities remarkably increased when the concentration elevated to 10.0%.

Conclusion:

We suggest that these medicinal herbal extracts with MMP inhibitory activities can be developed as effective anti-aging agents used in skincare cosmetics.

P585

Identification and functional investigations of the interactome(s) of Enterovirus 71 (EV71) VP4 structural protein with cellular proteins

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Background:
Enterovirus 71 (EV71) is a small, nonenveloped virus with positive single strand RNA genome. It is a major causative agent of hand, foot, and mouth disease (HFMD), and known to cause severe neurological disease mainly on young children. EV71 is typified by an icosahedral outer capsid structure consisting of 60 capsomers that is composed of four structural proteins VP1-VP4. The VP1 has a well documented function in receptor binding, and is likely to have multiple roles with other host factors in viral multiplication and pathogenesis. However, the function of other capsid proteins remains largely unknown. A study has shown that the participation of VP4 is required during cell entry of the poliovirus, a close relative of Enterovirus. We therefore speculate that the function of VP4 is equally important as VP1 for viral multiplication and pathogenesis. It will be critical to systemically investigate the host proteins involved in the interactions to VP4 for further functional association studies in EV71 multiplication and pathogenesis.
Materials and Methods:
In this study, we adapted a modified murine stem cell virus (MSCV) packaging vector to express the intact EV71 VP4 fusion protein. This vector allows efficient expression and recovery of bait proteins for tandem affinity purification (TAP) of interacting protein complexes in mammalian cells. By using a modified purification strategy and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), we identified 32 candidate VP4-interacting host proteins.
Result:
We have constructed VP4 expression plasmids encoding VP4 with Tandem tags, and confirmed the expression of TAP-tagged VP4 in RD, SK and 293T cell. By using a combined Tandem Affinity Purification and LC-MS/MS approach, we have found several novel VP4-interacting proteins. Most of them are RNA binding proteins with helicase or splicing activities. We are verifying the interactions of VP4 and its candidate interacting proteins by co-immunoprecipitation and confocal imaging studies.
Conclusion:
Identification of EV71 VP4-interacting proteins may provide critical insights toward the understanding of possible mechanisms involved in EV71 multiplication and pathogenesis. Further investigation on the functional relevance of the VP4-interacting cellular proteins may facilitate the development of anti-viral agents or gene therapy-based therapeutic interventions.

P586

APC Haploinsufficiency And P53 Loss Cooperate To Induce Mucinous Cystic Neoplasms And Invasive Pancreatic Carcinoma.

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Backgrounds:
Mutations of the APC (adenomatous polyposis coli) gene are the most common events in familial adenomatous polyposis (FAP), which are also associated with the development of sporadic tumors of the distal gastrointestinal tract including pancreatic tumor. To define the potential tumor suppressor role of APC in pancreatic cancer development.
Materials and Methods:
We have employed genetic modified mice (GEM) carrying pancreas-specific deletions of APC by Cre-mediated deletion of the PDX-1 gene during pancreatic development. . Most condition deletion of APC in early pancreatic development results in an early embryo lethal.
Results:
In this study, we further found that conditional knockout of p53 combined haploinsufficiency of the APC gene engenders a distinct class of pancreatic tumors, mucinous cystic neoplasms (MCNs), which may culminate to become invasive ductal or acinar adenocarcinomas. Using mouse inflammatory cytokine array analysis, we found that these cysts formed by accumulation of ascites fluid secreted by neoplastic and inflammation cells with high levels of G-CSF, IL-1a, IL-4, IL-6, MCP-1, MIP1-gamma, TIMP-1, KC(keratinocyte -derived chemokine) and sTNFR1. The intracellular mucins in these lesions were further confirmed typically positive for periodic acid-Schiff (PAS) and alcian blue staining. In addition, ovarian-like stroma" was also identified in the area surrounding these MCN lesions. Progression of MCNs induced by homozygous deletion of p53 and inactivation of APC leading to aberrant Wnt/bcatenin signaling pathway may increase liver and stomach metastasis in our Pdx Apc $^{L/+}$ P53 $^{L/L}$ mouse model. RT-qPCR array data of cultured mouse primary pancreatic ductal cells isolated from Pdx Apc $^{L/+}$ P53 $^{L/L}$ revealed a typical MCN signature with increased expression of MUC4 and MUC6 but not MUC5AC.
Conclusion:
In conclusion, our genetic mouse model here demonstrated that APC haploinsufficiency in synergy with p53 inactivation may be a prerequisite for mouse pancreatic MCN development.

P587

A Functional Circuit Underlying Male-male Courtship Behavior In The Drosophila Brain

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Backgrounds
Animals present several sensory cues to recognize the same species and suitable mate for reproduction. Courtship, an instinct of animals in the nature, normally occurs in between opposite sexes. Nevertheless, a lot of research proved that courtship and sexual behaviors existed between the same sexes in many species. The involved biologically molecular mechanism has not been clarified.
Materials and Methods
The GAL4/UAS gene expression system is a precise means of targeted gene expression employed to study male-male courtship behavior in Drosophila. Drosophila also provides a research advantage of large-scale analyses that the analyses and quantification of courtship behavior have been clearly confirmed. In this study, we using tissue-specific GAL4 drivers resulted in tyrosine hydroxylase (TH) expression that was restricted, and expected dopamine (DA) level will be boost to those specific tissues for large amount screening dopamine-mediate male-male courtship activity circuits.
Results:
When the level of dopamine does not perform normally, the courtship behavior among male Drosophila would largely increase. In this study, we demonstrate the existence of critical circuit; in VPL neurons which innervate the calyx of mushroom bodies (Mbs), through DA, enhance the visual cue dependent male-male courtship.
Conclusion:
As such a complicated behavior could be controlled by this simply neural circuitry, it is expected that the outcome would become an effective platform for relevant research on the molecular mechanism of homosexual behavior.

P588

Study on the tumor suppress of APOBEC3B and APOBEC3G in Human Hepatoma Cell Line, Hep3B

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Backgrounds:
To clarify the effect of tumor suppress of cytidine deaminase gene, APOBEC3B and APOBEC3G were subcloned into pLKO_AS2.puro vector and transfected to human hepatoma cell line, Hep3B.
Materials and Methods:
APOBEC3B was subclone to pLKO_AS2.puro vector by *Asc I/Nhe I* restriction cutting site and APOBEC3G was subclone to pLKO_AS2.puro vector by *Nhe I/EcoR* I restriction cutting site.
pLKO_AS2.puro-APOBEC3B and pLKO_AS2.puro-APOBEC3G were transfected to Hep3B cell lines by lipofectamine 2000 (Invitrogen). Protein assay was performed by western blotting and wound healing of cells were observed by phase-contrast microscope. Immunofluorescence and histochemical analysis was observed by fluorescence microscope.
Results:
To evaluate the situation of tumor suppress in Hep3B, the cells growth on culture dish were scratched with plastic pipette tip in 70-80% confluence after transfect with pLKO_AS2.puro-APOBEC3B and pLKO_AS2.puro-APOBEC3G. Our data shows the wound closure was observed in control and pLKO_AS2.puro express only after wound-scratch 72hrs under phase-contrast microscope.
Furthermore, the wound migration in pLKO_AS2.puro-APOBEC3B and pLKO_AS2.puro-APOBEC3G overexpress was not complete after wound-scratch 72hrs under phase-contrast microscope. To clarify where the APOBEC3B and APOBEC3G express locate in cell, FITC stain was apply to the pLKO_AS2.puro-APOBEC3B and pLKO_AS2.puro-APOBEC3G overexpressed culture dish and histochemical analysis was observed by fluorescence microscope.
Our data shows the overexpressed APOBEC3B and APOBEC3G were located in cell nucleus. It seems to suggest APOBEC3B and APOBEC3G may regulate gene express in Hep3B. Furthermore, the effect of mitomycin in Hep3B and how mitomycin regulated the express of APOBEC3B and APOBEC3G are now studying.
Conclusion:
Our results suggest that overexpress of APOBEC3B and APOBEC3G could contribute to inhibit the cell growth of human hepatoma cell line, Hep3B.

P589

Characterization of Voltage-gated K+ Channels in Lateral Subdivision of Central Amygdala (CEl) Neurons

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Backgrounds:

The central nucleus of the amygdala (CEA) is a key brain structure involved in fear memory. The CEA can be divided into different subregions. In the amygdala central nucleus lateral part (CE_l), the majority of neurons are GABAergic neurons. Electrophysiological studies have revealed that these neurons display diverse intrinsic firing properties. We distinguish these neurons with their intrinsic firing properties. At least, two major types of neurons were identified in the CE_l; late-spiking (LS) neurons exhibit a subthreshold ramp phenotype and longer spike latency to the first action potential compared to regular-spiking (RS) neurons. We aim to unravel the molecular determinants underlying the variation of firing pattern among CE_l neurons.

It is known that firing pattern of a given neuron is determined by the morphological architecture and a repertoire of intrinsic membrane conductances. Since activation and inactivation of voltage-gated potassium (Kv) channels can shape the spike waveform and spike precision, their expression is one of the main factors in determining the variance of firing phenotypes. We thus aim to characterize the functional properties of Kv channels by using patch-clamp recordings from CE_l cells and correlate their morphologies.

Materials and Methods:

Acute brain slices were prepared from 21- to 45-day-old wildtype mice. Whole-cell recordings were performed to check firing pattern and rheobase test in current-clamp configuration .We characterized gating and pharmacological properties of somatic K^+ channels in nucleated patches from late-spiking neurons and regular-spiking neurons in lateral subdivision of central amygdala. Nucleated patches were held at -90 mV to make the K^+ channels ready to open. Cell morphological reconstructions were done by *post hoc* staining and the software Neuromantic for more detailed morphological analysis. The biocytin-filled cells were stained with Alexa-594 conjugated with streptavidin or FITC conjugated with avidin, and were scanned by two-photon microscopy.

Results:

Application of a potassium channel blocker 4-aminopyramidine (4-AP) at low concentration (30 μM) can reduced the spike delay of LS neurons but had little effect on RS neurons. However, in nucleated patch recordings, no significant differences of A-type and delayed-rectifier Kv channels were found between both amygdala CEl interneurons.

Conclusion:

The result indicates that 4-AP sensitive K^+ current plays an important role in mediating the spike delay, thus define the firing pattern of CE_l neurons. There is no evidence that somatic Kv channels of CE_l neurons have significant difference on their characterization and compositions. This might suggest that the 4-AP sensitive K^+ current is not located primarily on the soma.

P590

Extract of Wedelia chinesis Inhibits LPS/IFN-γ-Induced C6 Astrocytoma Cell Activation and Protects Against Inflammation-Mediated Differentiated PC12 Neuronal Cell Injury

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Backgrounds:

In the central nervous system (CNS), astrocytes cells play a central role between brain inflammations and infections, because of those cells secrete pro-inflammatory cytokines and chemokine result in progression of CNS diseases including Alzheimer's disease and brain injury. In our preliminary study, it showed ethyl acetate extract of *Wedelia chinensis* (WC-EA) inhibited xanthine oxidase, an inflammatory enzyme, *in vitro*. Experimentally, it has been indicated LPS and IFN-γ can activate astrocytes cells to secrete inflammatory factors. Herb tea or dietary food which suppresses astrocyte activation may possess neuroprotective potential. Whether *Wedelia* chinensis, a popular summer herb tea material in Taiwan, possesses neuroprotective potential is investigated.

Materials and Methods:

We have used the rat C6 astrocytoma cells as the mode system to study the LPS/IFN-γ-induced astrocyte cells activation. First, the cytoprotective dose of WC-EA and the non-toxic dose of LPS/IFN-γ on C6 astrocytoma cells were assessed. In addition, we used the DCFH-DA fluorescent probe and western blot assay to study effect of intracellular oxidative stress and protein expression involving in astrocyte activation, respectively. Besides, TNF-α secretion was measured TNF-α ELISA kit. The cytoskeletal alterations were observed using TRITC-conjugated phalloidin staining of the actin cytoskeleton. Further, the effect of WC-EA on astrocyte activation mediated cytotoxicity on differentiated PC12 cells were measured by MTT assay and LDH assay. .

Results:

In the present study, we founded that WC-EA inhibited LPS/IFN-gamma-induced synapse-like astrocyte cell structure and expression of SSeCKS, a cytoskeleton scaffolding protein, which up-regulated during astrocyte activation in C6 astrocytoma cells. In addition, WC-EA could suppress LPS/IFN-gamma induced intracellular ROS, iNOS expression and TNF-α secretion in C6 astrocytoma cells. Finally, medium with pretreatment of WC-EA in C6 astrocytoma cells significantly decreased cytotoxicity in differentiated PC12 cells as compared to medium with LPS/IFN-gamma-activated C6 astrocytoma cells alone.

Conclusion:

Taken together, these finding indicated WC-EA could protect against LPS/IFN-γ activated C6 astrocytoma cells-induced toxicity in differentiated PC12 cells through inhibiting SSeCKS expression impaired TNF-α secretion and ROS/RNS production.

P591

NRP1 expression and Stat3 activation plays a critical role in oral cancer progression

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Backgrounds:

Oral squamous cell carcinoma (OSCC) is a common neoplasm worldwide. In Taiwan, that is also one of the top ten malignancies, and ranked the fourth leading cause of cancer death in Taiwanese male. Neuropilin-1 (NRP1) has been known to be highly expressed in high grade and metastatic tumors. Signal transducers and activator of transcription 3 (Stat3) is a cytoplasmic transcription factor which is constitutively activated in cancer cells and tumor microenvironment and implicates tumor metastasis, proliferation and apoptosis. Our preliminary results showed that Stat3 activation may positively regulate NRP1 expression in lung cancer cells. In this study, we aim to figure out the role of NRP1 and Stat3 in oral cancer.

Materials and Methods:

We used immunohistochemistry to examine the expression of NRP1 and Stat3 in clinical OSCC specimens. The influence of NRP1 in cancer cell invasion, migration and proliferation was investigated by knocking down the expression of NRP1. Moreover, we established tumor xenografts by subcutaneously implanting SCC15 cells with knockdown of NRP1, Stat3 and shLuci (control) respectively to investigate tumor growth in vivo.

Result:

First we found that NRP1/Stat3/p-Stat3 were higher expression in tumors compared to normal tissues and had colocalization. The expression level of NRP1 is positively correlated with tumor stage in clinical OSCC specimens. Moreover, the expression of NRP1 and p-Stat3 was higher and positively correlated in highly malignant OSCC cell lines. To investigate the influence of NRP1 in OSCC, we knocked down the expression of NRP1 and found the cell migration, invasion, and proliferation was all decreased in NRP1 silencing cells. In addition, we found that silencing the expression of Stat3 leads to a significant reduction in the expression level of NRP1 and IL-6. We analyzed the promoter region of NRP1 and found a potential Stat3 binding site may involve in transcriptional regulation of NRP1. In animal models, we found that knockdown of NRP1 or Stat3 could effectively reduce tumor growth, especially in the shNRP1 group.

Conclusion:

According to these results, the correlation of NRP1 expression levels and Stat3 activation is a novel finding in oral cancer development. In the future, NRP1/stat3 axis can be a new prognosis marker and therapeutic target in oral cancer.

P592

The Association of Guanine Nucleotide Binding Protein *β3* C825T Polymorphism with Colorectal Cancer Formation

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Backgrounds:

Colorectal cancer (CRC) represents the third most common cancer worldwide, and high prevalence in Taiwan region. Emerging studies indicated that single nucleotide polymorphisms (SNPs) are associated with several cancers formation. G proteins, important components of seven transmembrane receptors, are involved in the regulation of intracellular signaling pathways. Recently, C825T polymosphism of GNB3, a G protein *β*3 subunit gene, has been identified to generate a splicing variant GNB3s protein. This splice variant is associated with various malignant diseases.

Materials and Methods:

From January 2006 to June 2006, 84 patients of colorectal cancer and 171 age matched control are enrolled in this study. The genomic DNAs are extracted with QIAamp DNA Mini Kit. The C825T polymorphism of GNB3 is analyzed by polymerase chain reaction followed by restrict fragments length polymorphism (PCR-RFLP) methods. The significant difference is measured by Chi squire test.

Results:

The frequency of the GNB3 825T allele in patients with colorectal cancer was significantly lower than in controls (33.33% vs 62.57%, OR 3.34, p<0.0001). In addition, the frequencies of GNB3 homozygous 825T/T allele and C-carrier (T/C+C/C) genotype in CRC groups was not associated with stages (P = 0.63), tumor size (P = 0.21), distant metastasis (P = 0.74) and survival (P = 0.34). However, that was significantly lower frequency of T/T homozygous allele in the lymph node metastasis CRC patients compared to C-carrier (T/C+C/C) genotype (P = 0.043).

Conclusion:

Our study provides the first evidence that GNB3 C825T polymorphism may be associated with CRC in Taiwan.

P593
Studying the Role of Calprotectin in Oral Cancer and Stromal Cells

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Backgrounds:

Oral cancer is one prevalent male cancer type in Taiwan. Calprotectin is a heterodimer of S100A8 and S100A9. Both proteins belong to a large calcium-binding S100 family and are predominantly expressed by myeloid cells. In addition to their expression in inflammatory cells, calprotectin has been detected in many cancer types and has recently emerged as a pro-inflammatory marker for both acute and chronic inflammatory diseases. Taken together, calprotectin may play a prominent role in inflammation-associated cancer including oral cancer. Although a number of putative functions have been proposed for calprotectin, its biological role particularly in cancer cells remains ill defined. Using IHC staining, we were able to find the increase of S100A9, a subunit of calprotectin, in the stromal tissue adjacent the tumor tissues significantly associated with poor clinical outcome. The purpose of this study is to investigate the role of calprotectin in oral cancer cells, endothelial cells and monocytes. The latter two types are rich in tumor stroma.

Materials and Methods:

Immunofluorescence microscopy using cell type specific markers was used to characterize the types of cells expressing calprotectin in oral cancer tissues. To address the role of calprotectin in oral cancer, both overexpression and knockdown approaches were undertaken. For studying the effect of secretable calprotectin, conditioned media (CM) collected from calprotectin-altered oral cancer cells was used to study their effect on endothelial cell proliferation, migration, and tube formation, and monocyte activation. S100A9 ELISA in patient serum was used to determine if the alteration could be used as a biomarker for oral cancer prognosis.

Results:

Immunofluorescence microscopy showed that calprotectin was not only detected in oral cancer cells but also in their surrounding stromal cells including monocytes expressing CD68+ or C11b+, and neutrophils expressing CD15+ cells, suggesting the possibility of autocrine and paracrine effect of calprotectin on oral tissues. From our preliminary data, S100A8 or S100A9 knockdown inhibited oral cancer cells proliferation. We also found that the serum concentration of S100A9 was elevated in early-stage but not late-stage cancer patients when compared with healthy controls.

Conclusion:

Calprotectin may play a prominent role in oral carcinogenesis. Understanding the potential role of calprotectin in oral cancer and stromal cell will facilitate the development of new therapeutic approaches for oral cancer treatment.

P594
Generate defective interfering particles of enterovirus 71 by trans-complementing 3AB protein in a transformed cell line

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Enterovirus 71 (EV71), a member of the family *Picornaviridae* and the genus *Enterovirus*, is a small non-enveloped virus with a ssRNA genome of about 7500 nt, and is a major causative agent of hand, foot, and mouth disease in children.

Its nonstructural protein, 3A, is required for forming membranous vesicles of replication. Mutations in 3A therefore results in defective viral RNA synthesis. Supplementing a 3A gene cassette at the 5'-end of viral genome is able to rescue a viral genome with the 3A deletion. However, complementation of the 3A lesions in the poliovirus (PV) genome was not achieved by providing the wild-type (wt) gene products in *trans* with cell lysate transient-expression system. The aim of our study is to investigate whether the 3AB lethal mutations of EV71 can be rescued by trans-complementation with the expressed wild type 3AB intracellularly.

In the present study, we found that full length enteroviral RNA with deletion of 3AB can not replicate well and results in dead-end infection in Vero cells. We will further investigate whether the lethal 3AB deletion mutant can be rescued by trans-complementation, and then generate defective infectious particles in a 3AB stably expressing cell line. Since the defective particles can not replicate in normal cells, 3AB deletion was presumed to be a universal attenuation strategy for generating vaccine strains for enteroviruses.

P595
17β-Estradiol Enhances Human Embryonic Kidney 293 Cell Spreading and Migration Pattern in Estrogen Receptor-α-independent Pathways

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Backgrounds:

The incidence of end-stage renal disease is increased in men as well as postmenopausal women. Nevertheless, deficient of estrogen receptor-α (ERα) signaling results in glomerulonephritis in mice models. Evidence implies the regulation of estrogen/estrogen receptor axis may play a protective role in the development of glomerulonephritis, however, the detail mechanisms remain to be investigated. As the regulation of cell motility is vital for kidney development and homeostasis, the purpose of this study was to examine the effects of 17β-Estradiol (E2) on kidney cells with or without ERα using cell-based system.

Materials and Methods:

Human Embryonic Kidney 293 (HEK293) cells, with no detectable endogenous ERα, were used to ectopically express ERα (HEK/ERα), whereas those transfected empty vectors were used as controls (HEK/Vector). After treatment with E2 (10-7 M) for 16 h, the cell motility were detected by using the transwell assay, cell viability were analyzed using the water-soluble tetrazolium WST-1 assay, and the signaling transduction molecules were evaluated by using Western blot analysis, respectively.

Results:

E2 significantly enhanced cell spreading and migration pattern in HEK/Vector cells, suggesting the E2-enhanced motility pattern could occur through ERα-independent pathways. While the downstream target molecules require further explore, the signalings may involve phosphorylated-active states of JNK and p38. In addition, although the overexpression of ERα led to significant increase the spreading and migration pattern which was mimicked by E2 treatment, to our surprise, HEK/ERα cells show opposite effects on the motility pattern as well as the active states of signaling molecules in response to E2. Detail molecular mechanisms need further investigation.

Conclusion:

We speculate that E2 plays opposite roles in HEK/Vector and HEK/ERα cells, detail investigation on the molecular mechanisms may add the information of the current knowledge on end-stage renal disease.

P596
The Functional Role of CCL28 Gene in the Pathogenesis of Lymphoepitheliomatous Nasopharyngeal Carcinoma

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Background:

Nasopharyngeal Carcinoma (NPC) is one of the common cancers of Taiwanese. In our previous studies, we found that CCL28 [chemokine (CC motif) of ligand 28] was highly expressed in lymphoepitheliomatous NPC. So we investigated the relationship between the expression of CCL28 and the pathogenesis of NPC.

Materials and Methods:

For gain function study of CCL28, we constructed CCL28 expression plasmid and transfected into the NPC cell lines which expressed lower endogenous CCL28, then co-cultured with lymphocytes with or without EBV infection, and observed the CCL28 transfected cells' behavior in vitro and in vivo. We also performed immunohistochemical staining for CCL28 in NPC biopsy specimens.

Results:

Expression of CCL28 in NPC cells could attract the co-cultured EBV-- and EBV+-lymphocytes seperately moving towards the NPC cells in culture dish. The over expressed CCL28 also can accelerate NPC cell migration, proliferation, and invasion. Histopathological examination of SCID mice bearing CCL28 transfectants revealed clear invasion with remarkable vascular and distant-metastasis. In 50 cases of NPC biopsy specimens it was found that high expression of CCL28 in lymphoepitheliomatous NPC tumor cells is correlated with the poor survival of patients.

Conclusion:

The CCL28 may play an important role in the formation of lymphoepitheliomatous NPC and promote tumor proliferation; furthermore, it may be the major factor for up-production of IgA antibody anti-EBV-VCA in the EBV+-NPC patients.

P597
Screening of Ovarian Tumor Specific Peptides for Diagnosis and Treatment of Advanced Stage Ovarian Cancer

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Backgrounds:

Ovarian cancer is one of the common gynecologic cancers. There are few specific symptoms appeared in the early-stage ovarian cancer. Most ovarian cancer patients are diagnosed at the advanced stage with widely metastasis. Moreover, the patients who are at late stage accompany malignant cancer cells in their ascites. The patients of many diseases such as hepatocirrhosis, pancreatitis, and gastric cancer may accompany with ascites production. Therefore, to find a more specific ovarian cancer marker should be desirable to improve the diagnosis and therapy in ovarian cancer.

Materials and Methods:

In this study, we established an animal model to obtained ovarian cancer cells that could effectively produce malignant ascites. We used a phage display strategy to screen peptides which have high affinity to bind the ovarian cancer cells in malignant ascitic fluid of mice.

Results:

We have obtained ovarian cancer cells that could effectively produce malignant ascites, called IGROV1-MA. The morphology of IGROV1-MA was different from their parental cells IGROV1. The proliferation rate of IGROV1-MA was higher than IGROV1. To compare the migrative and invasive abilities of IGROV1 and IGROV1-MA, the results were inconsistent in different conditions. We performed five rounds biopanning against the ascitic ovarian cancer cells in vivo and obtained some phages which have high affinity for IGROV1-MA cells. The sequences of peptides have been identified, and a most frequent peptide was been selected for further application.

Conclusion:

Mice which injected ovarian cancer cells, IGROV1-MA could effectively produce malignant ascites. The morphology of IGROV1-MA was different from their parental cells IGROV1. The proliferation rate of IGROV1-MA was higher than IGROV1. We obtained some phages which have high affinity for IGROV1-MA cells. This new peptides may be applied to diagnosis and therapy in ovarian cancers in the future.

P598
DDX3 Regulates Rac1 Signaling and Modulates Cancer Cell Invasion

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DDX3 is a DEAD-box RNA helicase involved in multiple steps of gene expression. DDX3 has been reported to be upregulated in breast epithelial cancer cell lines with highly aggressive phenotypes and hepatocellular carcinoma tissues. Mutation and misregulation of DDX3 have been implicated in a variety of tumors, suggesting that DDX3 is a potential oncogene. Genetic studies further revealed the genetic and functional interactions between DDX3 and β-catenin in the wingless (Wnt) type of medulloblastoma. A recent report further indicates that DDX3 is a biomarker of metastasis of squamous cell/adenosquamous carcinomas. To reveal oncogenic roles of DDX3 in metastasis, we analyzed phenotypes caused by DDX3 depletion in HEK293, cervical cancer HeLa and neuroblastoma N2A cell lines. In these cell lines, DDX3 depletion increased cell-cell adhesion and decreased cell-extracellular matrix (ECM) adhesion, cell migration and invasion. These results suggest that DDX3 may play an oncogenic role in controlling cancer cell invasion/metastasis via modulating cytoskeleton organization. To discover potential targets of DDX3 involved in cell migration, qRT-PCR array analysis of cell motility genes was performed. The result indicates that genes transactivated by the Wnt/β-catenin pathway are down regulated in DDX3 knockdown cells. Further experiments demonstrate that DDX3 depletion decreases β-catenin stability and attenuates Wnt/β-catenin signaling. On the other hand, we performed pathway analysis of DDX3 targets that are regulated at the level of mRNA translation, and identified a group of them involved in the Rac1-mediated signaling pathway. Our following experiments demonstrat that DDX3 may regulate both Rac1 expression and activation by translational control of the Rac1-related mRNA regulon and therefore contributes to β-catenin stability and cancer cell migration. These results reveal molecular mechanisms for DDX3 to control metastasis and shed light onto how DDX3 may contribute to Wnt type tumors.

P599
Rabbit Embryonic Stem Cell Cultured in the Micropatterned to Uniform the Size of Embryoid Bodied

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Backgrounds:

Embryonic stem (ES) cell are normally cultured on feeder cells and maintained in serum- containing media, but these contain some factors causing differentiation. It is known that embryoid body (EB) formation is a key process for inducing differentiation, and size of EBs might be associated with the efficiency of differentiation. Some studies have shown that micropatterned (MP) could create a uniform size of coating area for the attachment of ES cell colonies which would theoretically help to form uniform colonies and then generate a similar size of EBs.

Materials and Methods:

Rabbit ES cells were cultured on feeder layer supplemented with leukemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF). Knockout serum replacement (KSR) was used to replace serum at the beginning of culture. When ES cell colonies appeared, a glass needle was used to remove the differentiated cells surrounding colonies. The MP dishes were prepared using a polydimethylsiloxane template that has micro islands coated with Matrigel, and were then printed on the culture dish to create coating areas. For generating the same size of colonies, undifferentiated colonies from the beginning of culture were trypsinized into single cells by TrypLE medium. Rho-associated protein kinase (ROCK) inhibitor was added to prevent apoptosis, and cells were collected and transferred to the MP-dish one hour after.

Results:

Rabbit ES cell colonies were grown to confluency on the coating area around days 4-5. For EB formation, MP-derived colonies were picked up and EBs spontaneously formed with various sizes after 24 h of culture.

Conclusion:

Rabbit ES cells could be maintained in a feeder-free with MP culture condition. The size of colonies could be well-controlled by this culture system, and remains the capability of EB formation.

P600
Orange-spotted Grouper (Epinephelus coioides) PKR Gene activated by RNA Virus and Interferon

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Protein kinase R (PKR) is a multifunctional serine-threonine kinase involved in translational control by the phosphorylation of eukaryotic translation initiation factor 2 (eIF2a). PKR can be induced by double-stranded RNA (dsRNA) and interferon (IFN), it play an important role in antiviral defense, anti-proliferative effects, cell growth control and various stress response. We have cloned and characterized PKR gene of orange-spotted grouper which was 2390b. p., encoded 618 amino acids. Measuring the expression of PKR in tissues of healthy grouper by qPCR. The result indicated the expression of PKR is higher in skin, muscle and head kidney, and PKR expression greatly increased after virus challenge and poly (I:C) injection. It's worth noting that phenomenon that PKR expression increased was along of the ascension of IFN and the proliferative of virus. Therefore, we could determine that PKR was actived and increased by dsRNA virus and IFN.

Key words:
Grouper, interferon (IFN), protein kinase R (PKR)

P601

Encapsulating RC-1 and combining oligosaccharide targeting molecule with the novel liposome to enhance the therapeutic efficacy for hepatocellular carcinoma

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Backgrounds:

Usually, hepatocellular carcinoma (HCC) is a maglignant tumor due to its poor surveillance and diagnosis, which eventually results in metastasis. Several curative treatments such as resection, ablation and liver transplantation are not suitable for advanced HCC which are conventionally treated with chemotherapy. However, there are no effective anticancer drugs could provide a better survival benefit to metastatic HCC patients, currently. Thus, to discover a novel chemotherapeutic agent has been a critical need in liver cancer therapy.

Materials and Methods:

According to the previous literatures, the specificity protein 1 (Sp1) regulates the hepatoma progress by binding to those specific regions in promoters which involve in both tumorigenesis and metastasis of HCC. Therefore, RC-1, a Sp1 inhibitor was designed to develop as a potential anticancer agent to HCC. Moreover, the YC-1 receptors which are only expressed in hepatoma can bind glycoproteins modified with oligosaccharide residues at their glycan chain terminals. According to these findings, a novel liposome was used for encapsulating RC-1 inside and adsorbing a cluster glycopeptide (oligosaccharide) on its surface for the specific targeting to HCC.

Results:

Here, we attempted to combine the liposome/RC-1/oligosaccharide as an efficient therapeutic compound, and this complex indeed showed a potent proliferative inhibition of HCC cells than free RC-1 treatment. In addition, *in vitro* and *in vivo* studies both proved that the oligosaccharide did deliver the liposome to the YC-1 receptor positive HCC cell (HepG2 cell) while the YC-1 receptor negative HCC cell (Mahlavu cell) didn't.

Conclusion:

These results indicated that the liposome/RC-1/oligosaccharide complex may supply a feasible strategy for the advanced liver cancer therapy.

P602

Association of Glucose-6-phosphate dehydrogenase (G6PD) and HMG-CoA reductase (HMGR-1) in *C. elegans* Embryogenesis

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Backgrounds:

Glucose-6-phosphate dehydrogenase (G6PD) is the rate limiting enzyme in pentose phosphate pathway. G6PD generates NADPH for many essential biochemical reactions in cells. A major obstacle for studying the physiologic function of G6PD is the lack of G6PD-knockout animal model due to the embryonic lethality induced by G6PD deficiency.

Materials and Methods:

Using RNAi knockdown technology, we have created G6PD-deficient nematode *C. elegans* as an animal model. All strains were grown on RNAi plates. Upon young adult stage, 20 hermaphrodities were transferred to new RNAi plates, and incubated in 20 °C refrigerator (10 *C. elegans* in one plate). The egg production was scored every 1.5 days and the hatching percentage was also determined after 12 hours for three days. The following formula was used hatched egg / egg production* 100 %.

Results:

Compared with mock worms, *g6pd* (RNAi) *C. elegans* showed increased germ cell apoptosis (2 folds of mock), decreased egg production (78 % of mock) and reduced hatching (10 % of mock). We further investigated whether altered lipid metabolic genes of NADPH-dependent fatty acid (*fasn-1*) and mevalonate (*hmgr-1*) biosynthesis, play significant roles leading to G6PD-knockdown-induced defective embryonic development in *C. elegans*. Egg production and hatching from *hmgr-1* (RNAi) and *fasn-1* (RNAi) mutants were scored in G6PD knockdown condition. The results showed that *hmgr-1* (RNAi) or *fasn-1* (RNAi) reduces egg production (30 % and 24 % of mock, respectively). In addition, *hmgr-1/g6pd* co-RNAi or *fans-1/g6pd* show any further decreases on egg production nor hatching compared to *g6pd* (RNAi) *C. elegans*.

Conclusion:

In summary, these findings suggest a possible association between G6PD deficiency-induced defective embryogenesis and mevalonate biosynthesis and the detail mechanism remains to be elucidated.

P603

Protein Expression in the Gonad of Female Tilapia (Oreochromis mossambicus) was Affected by Pituitary Adenylate Cyclase-Activating Polypeptide in vitro

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Backgrounds:

Animal reproduction and development are under the regulation hypothalamic-pituitary-gonadal axis. Hypothalamus secrets gonadotropin-releasing hormones that induce pituitary to release gonadotropins which stimulate gametogenesis and steroidogenesis in gonads. Pituitary adenylate cyclase activating peptide (PACAP) is a neuropeptide which can elevate the concentration of intracellular cAMP and calcium in the gonadotroph cell and somatotroph cells. Tilapia is one of the most common commercial fish in Taiwan, but literature concerning the mechanism of proteins regulated in whose reproductive process is still scant. Our previous studies found that the expression of PACAP and its receptor in the gonad of tilapia (*O. mossambicus*) and PACAP was involved in the cAMP-protein kinase A (PKA) signaling pathway and could play important roles in the regulation of reproduction system as an autocrine/paracrine factor in bony fish. In present study the protein expression profiles in tilapia ovaries after administration of PACAP *in vitro* were analyzed by proteomic approaches.

Materials and Methods:

Protein extracted from tilapia testes was divided into 7 groups including fresh tissue, Pre-culture (cultured in DMEM for 8 hours), 4 experimental groups (PACAP, Forskolin [adenylate cyclase activator], H89 [PKA inhibitor], and PACAP+Forskolin, all groups with 10 μM) for additional 2 hours after Pre-culture, and control (no chemical supplemented) and were analyzed by two-dimensional gel electrophoresis. Spot detection and matching were performed using the Phoretix 2D software (Nonlinear Dynamics, UK). Significance values ($P < 0.05$) between groups were compared by a Duncan multiple range test after one-way analysis of variance.

Results:

The present data showed that PACAP treated group had significantly higher protein spot number than those of Pre-culture and H89 treated groups, but lower protein spot number than that of Fresh group. The results confirm that the expression of tilapia ovarian follicles protein in vitro was indeed affected by PACAP. Similar results were observed when compared with those previously treated by steroids and gonadotropins.

Conclusion:

The identification of protein spots will help us to clarify the relationship among gonadotropins, steroids, neuropeptide, and related proteins in tilapia gonads, and a fish follicles protein database could be established, and further could be applied in reproductive endocrine research and fishery development.

P604

To Investigate the Roles of IL-12 Family Cytokines on Islet Graft Rejection and Tolerance

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Backgrounds:

IL-12 family cytokines are mainly produced by antigen presenting cells and have crucial roles in the control of T helper (Th) cell differentiation and function. It has been reported that Th1 and Th17 cells are linked to the graft rejection whereas Th2 and Treg cells are correlated to graft tolerance. In this study, we aim to investigate whether IL-12 family cytokines modulate the differentiation of Th cells and subsequently influence the survival of islet grafts in diabetic recipients.

Materials and Methods:

To investigate the IL-12 family cytokine-regulated dendritic cell and T cell interaction and its subsequent effect on T cell activation and differentiation, we co-cultured bone marrow-derived dendritic cells (BMDCs) from IL-12 knockout, IL-23 or IL-27 knockdown NOD mice with CD4 T cells from BDC2.5 transgenic NOD mice in the presence of antigenic peptide. The proliferative response and cytokine production of T cells were analyzed 3 days after co-culture. To further dissect the differential contribution of these Th subsets in islet graft rejection or tolerance, we transplanted islets into streptozotocin-induced diabetic recipients with IL-12 knockout, IL-23 or IL-27 knockdown background. The normoglycemic recipients were then transferred with BDC2.5 CD4 T cells and blood glucose was monitored daily. The histology of islet grafts and the differential Th subsets were analyzed at the end of the study.

Results:

BMDCs from IL-12 knockout, IL-23 or IL-27 knockdown mice displayed similar capacity to induce T cell proliferation. However, BMDCs from IL-12 knockout or IL-23 knockdown mice showed slightly reduced ability to promote Th1 and Th17 differentiation. In contrast, BMDCs from IL-27 knockdown mice enhanced Th1 and Th17 development. In transplantation experiments, the survival of islet grafts was prolonged in IL-12 knockout or IL-23 knockdown recipients and was reduced in IL-27 knockdown mice after diabetogenic T cell transfer, as compared with the control recipients. These data indicate that IL-12 family cytokines play important roles in controlling T cell differentiation and subsequent islet graft rejection or tolerance.

Conclusion:

Our findings suggest that IL-12 and IL-23 mediate the islet grafts rejection through promoting Th1 and Th17 differentiation. IL-27 acts as a regulatory cytokine to dampen immune responses and reduction of IL-27 leads to increased T cell activation and early graft rejection.

P605

Smad4 loss triggers the phenotypic changes of Pancreatic Ductal Adenocarcinoma

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Backgrounds:

Smad4 has been identified as a gastrointestinal (GI) malignancy specific tumor suppressor gene which was mutated in one third of colorectal cancer and half of pancreatic tumor. Smad4 inactivation by allelic deletion or intragenic mutation mainly occurs in late PanINs or carcinoma stage of human pancreatic ductal adenocarcinoma (PDAC). A number of studies have proposed the potential role of Smad4 mediated anti-tumor effects in cancer biology; however its relevance in PDAC molecular phenotype has not yet been fully characterized.

Materials and Methods:

This study seeks to establish the impact of the signature molecular alterations on Smad4 loss in PDAC by using human PDAC cell linesWe showed that re-expression of Smad4 in Smad4 inactivation of PDAC cells reduced cell proliferation in some, but not all PDAC cells, and significantly suppresses PDAC cells migration.

Results:

Furthermore, we reported that Smad4 transcriptionally activated nestin/TGFb1 pathway and induced several transcriptional factors expression. In contrast, loss of Smad4 leads to increased CD133, EGFR and Ecadherin expression, and exhibits a more epithelial-like phenotype. We further determined how Smad4 loss contributes to the alterations of kinase signaling network (particularly ERK/p38/Akt and TGF-b/ Smad pathways) in vitro.

Conclusion:

This study provides a foundation for investigation of the cellular and molecular basis for this alteration in PDAC, and further provides the detail insight into such genotype-phenotype correlations—and of the associated molecular circuitry driving these processes— which may critical for both the design and assessment of efficacy of targeted PDAC therapies.

P606

Evaluation of the Correlation between the Degree of Stenosis and the Alteration of Blood Flow Field in Internal Carotid Artery by Using Numerical Methods

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Backgrounds:

Stroke is the second leading cause of death in Taiwan and 1/6 of all human beings suffer at least once in their lives. Approximately, 87% of cases of stroke are classified “ischemic”, in which 25-30% of ischemia is caused by internal carotid artery (ICA) stenosis. The atherosclerotic plaque clots within the blood vessels, which causes the thickness of Tunica intima and reduces the blood flow in ICA, following by disruption of blood supply to the brain. Accordingly, higher degree of stenosis is at greater risk of ischemia. Clinically, the degree of ICA stenosis is predicted based on duplex velocity measurements. However, there is a limitation for lower degree of stenosis.

Material and Methods:

Here, in order to provide an additional reference to assessment of mild ICA stenosis, we used computational fluid dynamics (CFD), and an in vitro model of stenosis to evaluate the correlation between the changes of blood flow and the degree of stenosis in internal carotid artery. We established a numerical model with concentric and eccentric stenosis in different degree (10%, 30%, 50%, 70% and 90%) based on the computer tomography image from a healthy adult.

Results:

We found that the numerical simulation of changes in blood flow conformed to the clinical data in previous findings. Moreover, the flow patterns, such as sheer stress, turbulence and recirculation, in carotid bifurcation models are consistent with both numerical and clinical observations in ICA stenosis.

Conclusion:

This study suggest that our numerical model of stenosis may provide a predicted model of mild stenosis by giving the parameter of blood flow, which could be further used as a preoperative evaluation in ICA surgery, such as carotid endarterectomy and carotid artery stenting.

P607

Crystal structure of DNA replication initiation regulatory proteins Spo0J/Soj from Helicobacter pylori

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Backgrounds:

Spo0J (stage 0 sporulation protein J) and Soj, belonging to the ParAB family, are crucial for segregation of chromosome, initiation of sporulation and the progression of cell cycle. Spo0J and Soj form a complex at the chromosomal *parS* locus, which resides near the replication site, *oriC*. The Spo0J-Soj complex will promotes chromosomes separation. Spo0J regulates the ATPase and the DNA binding activities of Soj and thereby to control the initiation of DNA replication.

Materials and Methods:

Gel filtration chromatography was used to determine the oligomerization states of the *Hp*Spo0J, *Hp*Spo0J$_{N240}$ and *Hp*Soj. The DNA-binding activity of *Hp*Spo0J and *Hp*Spo0J$_{N240}$ were examined a 24 bp DNA fragment containing the 16bp Spo0J binding site in gel mobility shift assays. Fluorescence anisotropy assay to measure the binding ability of *Hp*Spo0J$_{N240}$ with specific *parS*-contained DNA or non-specific DNA. Crystal of *Hp*Spo0J$_{N240}$-*parS*$_{24}$ complex was grown in hanging drop at 20 ℃ against the reservoir solution containing PEG 8000 as a precipitates

Results:

Size exclusion chromatography reveals that *Hp*Spo0J assembles as a tetramer and *Hp*Soj forms as a monomer. Electrophoretic mobility shift assays demonstrated that *Hp*Spo0J specifically binds with the *parS* DNA and *Hp*Soj interacts with plasmid pUC19 DNA with a cofactor, ATP. Fluorescence anisotropy assays demonstrated that HpSpo0J specifically binds with the double-stranded parS DNA; however, *Hp*Soj do not bind with single and double-stranded DNA. The HpSpo0J-*ParS* DNA complex has been obtained and the crystals were grown using PEG8000 as a precipitates. Meanwhile, crystals of *Hp*Soj have grown in a precipitates, PEG35000. The structural study of both *Hp*Spo0J and *Hp*Soj are undergoing.

Conclusion:

The gel filtration chromatography data reveals that *Hp*Spo0J formed as a tetramer but *Hp*Spo0J$_{N240}$ was a dimer. The DNA binding activities of *Hp*Spo0J and *Hp*Spo0J$_{N240}$ were examined in the various molecular ratios for proteins and *parS*$_{24}$. The fluorescence anisotropy assay reveals that *Hp*Spo0J$_{N240}$ binds specifically to oligonucleotide duplexes containing the *parS* consensus sequence. The *Hp*Spo0J-ParS DNA complex and soj-ATP have been obtained in crystal, those result describing Spo0J specific parS DNA binding, Soj ATP binding, dimerization, cooperative DNA binding, and ATP hydrolysis by Soj. Spo0J and Soj were important regulators of the initiation of DNA replication.

P608

In vivo reporter gene imaging of cancer stem cells-like population using piggyBac gene delivery system

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Backgrounds:

Cancer stem cells (CSC, or named cancer initiating cells) are considered to be a rare population involved in tumor progression and relapse after therapy. However, it remains debate whether distant disseminated cancer cells in vivo would perform CSC phenotypes.

Materials and Methods:

In this study, we used 4T1 murine breast cancer cells to establish a syngeneic tumor model and found that liver metastatic cells exhibited several biological and molecular characteristics distinct from parental 4T1 cells, including CSC-like phenotypes. The reporter gene imaging was exploited to track the growth of living cancer cells in vivo and followed by ex vivo analysis of the metastatic cells identified by expressed reporter genes. The *piggyBac* transposon system was used to stably deliver multiple reporter genes including firefly luciferase, red fluorescent protein and herpes simplex virus type 1 - thymidine kinase (HSV1-tk) to 4T1 cells. The tumor growth and dissemination in vivo was tracked using the bioluminescent imaging.

Results:

We isolated the liver metastatic cells (named 4T1_L cells) for further analysis. Compared to parental 4T1 cells, these cells exhibited several CSC associated characteristics, including the ability of mamosphere formation, drug resistance, higher tumorigenic potential in Balb/C mice and reduced 26S proteasome activity. We also found that 4T1_L cells exhibited stronger migrated and invasive abilities. The microarray assay showed that the Twist1, a transcription factor involved in epithelial-mesenchymal transition (EMT), was significantly up-regulated in 4T1_L cells. Interestingly, the Twist1 level was suppressed in 4T1 cells but not in 4T1_L cells after exposure to ionizing radiation.

Conclusion:

The current data suggest that disseminated tumor cells may contain CSC and EMT related phenotypes, and this population is resistant to ionizing radiation and chemotherapy. This finding may explain the difficulty of therapy on metastatic tumors, and it would be important to design new strategy to treat this population.

P609
Inhibitory Effect of Cantharidin and Norcantharidin on Murine Melanoma Cells

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Backgrounds:
Compounds Cantharidin and Norcantharidin are potential for inhibiting certain cancer cells. However, their effects on melanoma are still unclear. The aim of this study assayed the effect of Cantharidin and Norcantharidin on regulating cell survival and melanin synthesis in B16 melanoma cells.
Materials and Methods:
Murine B16 melanoma cells were treated with various concentrations of cantharidin and norcantharidin with and/or without α-MSH (10 nM) co-treatment for 72 h. Then the cells were collected and assessed the cell viability, melanin content, cell cycle, as well as the related molecule expression.
Results:
Cantharidin and norcantharidin possessed inhibitory effect on B16 cells, with IC_{50} values are 12.5 μM and 25 μM, respectively. Cantharidin decreased melanin production in a concentration dependent manner. Cell cycle analysis found that the increased-expression of sub-G1 phase in cantharidin-treated B16 cells. Furthermore, tyrosinase and MMP-9 protein levels were also downregulated. Although norcantharidin had cytotoxicity to B16 melanoma cells, it showed no significant change on melanin production, tyrosinase activity, and MMP-9 protein levels.
Conclusion:
Both cantharidin and norcantharidin have inhibitory effects on viability of B16 cells. Cantharidin also downregulated melanogenesis, tyrosinase activity, and MMP-9 expression in B16 cells.

P610
Viral Susceptibility, Transfection and Growth of SPB

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This study investigates the susceptibilities of SPB to fish viruses including giant seaperch iridovirus (GSIV-K1), red sea bream iridovirus (RSIV-Ku), grouper nervous necrosis virus (GNNV-K1), chum salmon reovirus (CSV), and eel herpesvirus (HVA). GSIV-K1, RSIV-Ku, and CSV replicated well in SPB cells, with a significant cytopathic effect and virus production. However, the cells were HVA and GNNV refractory. To examine the ability of SPB cells to stably express foreign protein, expression vectors encoding GNNV B1 and B2 fused to enhanced green fluorescent protein (EGFP) and GSIV ORF35L fused to DsRed were constructed and introduced by transfection into SPB cells. Stable transfectants displayed different morphologies compared with SPB and with each other. EGFP-B1 was predominantly localized in the nuclei, EFPF-B2 was distributed throughout the cytoplasm and nucleus, and granular 35L-DsRed was localized with secreted vesicles. The expression of EFPF-B2 in SPB cells produced blebs on the surface, but the cells showing stable expression of EGFP, EGFP-B1, or 35L-DsRed showed normal morphologies. Results show the SPB cells and the transfected cells grow well at temperatures between 20 and 35 °C, and with serum-dependent growth. SPB cells are suitable for studies on foreign protein expression and virology.

P611
Characterize a Novel Cell Line from the Caudal Fin of Koi Carp Cyprinus carpio

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A continuous cell line (KF-101) derived from the caudal fin of the koi carp *Cyprinus carpio* was established and characterized. The KF-101 cell line multiplied abundantly in L-15 medium containing 10% FBS at 25 °C, and was subcultured for more than 90 passages over a period of 3 years. Immunocytochemistry revealed that the KF-101 cells contain keratin, junction proteins connexin-43 and occludin, and ectodermal stem cell marker Pax-6, but do not contain vimentin. Furthermore, the KF-101 cells reacted with anti-human DARPP-32 and anti-human GATA-4 antibodies, and the labelling was regulated according to the cell cycle. However, the labels of the DARPP-32 and GATA-4 antibodies in the KF-101 cells were the suggested phosphatase-1 inhibitor-1 and GATA-3, respectively. In addition, the KF-101 cells were susceptible to koi herpesvirus but were resistant to eel herpesvirus, seaperch iridovirus, grouper nodavirus, and chum salmon virus. The results indicate that the KF-101 cells are favourable materials for investigating biological and virological development.
Keywords:
epidermis, herpesvirus, Pax-6, stem cell

P612
Exploring The Role of LGR5 in Oral Squamous Cell Carcinoma (OSCC)

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Backgrounds:
The leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) is a well characterized surface marker for multiple type of adult stem cells, including small intestine, colon, stomach, liver, hair follicle and mammary gland. Recently, LGR5 is also found to mark the taste bud stem cells in posterior tongue. Elevated expression of LGR5 has been linked to the development of cancer stem cells in a variety of human malignancies, including colorectal cancer, gastric cancer and esophageal adenocarcinoma. However, the role of LGR5 in oral squamous cell carcinoma (OSCC) has not been reported. In this study, we aimed to explore the role of LGR5 in OSCC pathogenesis and to delineate the mechanism underlying its function in OSCC.
Materials and Methods:
We evaluated the change in DNA copy number, gene expression level and protein expression pattern of LGR5 in OSCC samples using multiple online databases. We established a siRNA-based technology to suppress endogenous levels of LGR5 and analyzed the effects of LGR5 depletion on cell growth, proliferation and downstream signaling in cultured OSCC cells.
Results:
Using the cancer genome atlas database, we discovered that the DNA copy number of LGR5 is significantly amplified in head and neck cancer. The mRNA and protein levels of LGR5 in OSCC tissues were also found to be up-regulated based on the GSE3524 dataset and in Human Protein Atlas database. Depletion of LGR5 suppressed the proliferation and reduced the colony forming ability of cultured OSCC cells. The effect of LGR5 depletion on the cell cycle distribution was further confirmed by BrdU incorporation and flow cytometry.
Conclusion:
Our findings suggest that LGR5 may function as an oncogene in promoting OSCC pathogenesis by regulating the proliferation and cell cycle progression of OSCC cells.

P613
Using a novel liposome-base polymer LPPC as an efficient anti-tumor vaccine

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Backgrounds:

Currently, vaccines are used to improve immunity against the particular disease. Whereas, the success of vaccines relies on their association with selected adjuvants in order to increase their immunogenicity and ensure long-term protection. An ideal adjuvant should enhance the immunogenicity of an antigen, improve antigen delivery and induce the production of immunomodulatory cytokines. In addition, different immune responses have various effects on diseases. Most adjuvants enhance Th2 responses, which generally induce a humoral response critical in the defense against extracellular pathogens. In contrast, The Th1 response activates cytotoxic T lymphocytes (CTL) and Natural killer cells (NK cell), these cells play a major role in the induction of apoptosis for virus-infected cell or tumor cell. It seems that Th1 responses which are induced by adjuvant are much better than Th2 responses for cancer therapy. For this reason, our strategy is to focus on the induction of Th1 immune responses by a novel Th1 adjuvant..

Materials and methods:

Our lab has already developed a cationic liposome complexed with polyethyl- enimine and polyethylene glycol polymer (LPPC) that can absorb various proteins without covalent conjugation. We demonstrated that LPPC is an adjuvant that enhances mouse immunity against antigens and specifically increases Th1-related immune responses. Previously, it was shown that LPPC strongly binds DNA and many kinds of proteins, and that these adsorbed substances cannot be replaced by the addition of other proteins. Therefore, in this study, we use the LPPC to absorb tumor antigen, which enhances the efficacy of anti-tumor response.

Result:

LPPC can slow down the formation of tumor if the mice pre-treated with LPPC-antigen complex.

Conclusion:

The results demonstrate that LPPC is an ideal adjuvant that enhances mouse immunity to against antigen. In the future, we will treated tumor with the LPPC-antigen complex to observe the efficacy of anti-tumor response.

P614
Erlotinib and Its Derivative Modulate Anti-cancer Effect via EGFR-TKI-Independent Signal in NSCLC Cells.

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Backgrounds:

Erlotinib is an epidermal growth factor receptor (EGFR) tyrosin kinase inhibitor. Here, we identify that protein phosphatase 2A (PP2A) is a major determinant mediating erlotinib-induced apoptosis in non-small cell lung cancer (NSCLC).

Materials and Methods:

EGFR wild-type NSCLC cell lines are treated with erlotinib or erlotinib derivative. Cell survival, apoptosis, phosphatase activity and cellular signaling were analyzed by WST1, FACS, Phosphatase activity assay and Western blot assay. *In vivo* efficacy was determined in nude mice with xenograft.

Results:

Erlotinib showed differential effects on apoptosis in 3 human HSCLC cell lines. Erlotinib induce significant apoptosis in the H358 cells, however, H460 cells present resistant to erlotinib-induced apoptosis. Erlotinib inhibit of p-AKT is a dose- and time-dependent in sensitive cells, however, no changed of p-AKT in resistance cells. Adding okadaic acid, a PP2A inhibitor, abolished the effects of erlotinib on apoptosis in sensitive cells; and forskolin, a PP2A activator, enhanced the effect of erlotinib in resistant cells.

TD2-2 is a novel erlotinib derivative that closely resembled erlotinib structurally but lacking inhibition of EGFR kinase activation. TD2-2 abolished the resistant of erlotinib to H460 cells though down-regulation of p-AKT. The effects of TD2-2 exposure are similar to erlotinib in PP2A activity. *In vivo* xenograft data displayed that TD2-2 had strong inhibition of the tumor growth and apoptosis than erlotinib.

Conclusion:

Our analysis indicate that activation of PP2A determines the effects of erlotinib or its' derivative, TD2-2, on tumor suppression in NSCLC cells. PP2A-Akt is the vital mechanism involved in erlotinib and its' derivative which distinguish from traditional EGFR pathway.

P615
Inflammatory Effects on Knee Joint Tissue by Indoxyl Sulfate

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Backgrounds:

Indoxyl sulfate (IS) is one of a number of protein-bound uremic toxins that accumulate in patients with chronic kidney disease. Current conventional hemodialysis is ineffective at removing this toxin, as 90% of IS is bound to albumin and the IS–albumin complex molecule is larger than the dialysis membrane's pore size.

Although IS may impair osteoblast function and induce abnormalities of bone turnover or arthropathy, the effects on knee joint tissue by IS has not been investigated yet. The present studies have been carried out to test the IS effects on synovial fibroblasts, meniscal fibrochondrocytes, and articular chondrocytes.

Uremic toxins have been identified to metabolism by organic anion transporters (OATs) which the roles in joint tissue were unknown. The expression and regulation of OAT1, OAT2, OAT3, OAT4, and URAT1 in synovial fibroblasts, meniscal fibrochondrocytes, and articular chondrocytes following IS stimulation were then examined.

Materials and Methods:

Our results showed a significant upregulation of cyclooxygenase 2 (COX-2) and interleukin-8 (IL-8) in three type cells following IS treatment at a concentration of 100μg/mL for 24 hours by reverse transcription polymerase chain reaction (RT-PCR), real-time PCR, and western blot/immunocytochemistry.

Results:

COX-2 was increased 11.52±4.95, 4.21±0.89, and 3.95±0.35folds in synovial fibroblasts, meniscal fibrochondrocytes, and articular chondrocytes respectively. IL-8 showed 5.87±2.32, 2.98±1.00, and 2.31±0.93 fold in synovial fibroblasts, meniscal fibrochondrocytes, and articular chondrocytes respectively. A dose dependent manner was also identified IL-6 showed no significant change at the same condition examined. The production of Nitric oxide (NO) determined by Griess reaction was also demonstrated. NO showed 1.62±0.55 and 1.28±0.34 -fold increase in synovial fibroblasts and meniscal fibrochondrocytes respectively.

Conclusion:

Our results showed that IS may induce inflammatory response and oxidative stress in synovial fibroblasts, meniscal fibrochondrocytes and articular chondrocytes. OAT4 may play an important role in IS metabolism in synovial fibroblasts, meniscal fibrochondrocytes and chondrocytes.

P616
Functional Characterization of Glucose Transporter 3 in Head and Neck Cancer initiating Cells

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Background:

Head and Neck squamous cell carcinoma (HNSCC) is a lethal cancer with clinical, pathological, phenotypical and biological heterogeneity. Cancer initiating cells (CICs) exhibit self-renewal and promote tumor progression capacity. We have also identified the subpopulation of head and neck cancer initiating cells (HN-CICs), and observed the upregulation of Glucose Transporter 3(Glut3) in HN-CICs by differential systemic analyses. However, the role of Glut3 in HN-CICs metabolism alternation remains unclear. Herein we determined the critical role of Glut3, a novel CICs marker, in the maintenance of stemness characteristics and tumorigenic phenotype of HN-CICs.

Methods:

Stable overexpression and knockdown of Glut3 expression in HNSCCs and HN-CICs was achieved by lentiviral-mediated system. Consequently, we elucidated the stemness properties and tumorigenicity of HNSCCs and HN-CICs with Glut3 down-regulation. Consequently, we investigated the role of Glut3 in metabolism regulation of cancer initiation cells.

Results:

Lentiviral knockdown of Glut3 significantly reduced the self-renewal ability and cancer initiation cell marker Grp78 expression in HN-CICs. Additionally, down-regulation of Glut3 enhanced the differentiation capability but inversely diminished "stemness" gene expression of HN-CICs. Of note, knockdown of Glut3 lessened tumorigenicity of HN-CICs both in vitro.

Conclusion:

We showed that Glut3 contributes to the maintenance of stemness and tumorigenicity of HN-CICs. In addition, the expression of Glut3 was involved in HN-CICs metabolism. Overall, silencing Glut3 might be a potential therapeutic target for HNSCC by eliminating CICs.

P617
Development of High-Throughput Methods on Screening Zebrafish Mutants Created by TALEN-Mediated Gene Knockout Technique

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Backgrounds:

Transcription activator-like effector nucleases (TALENs) is a new genetic engineering tool which can induce DNA double strand break at a unique site of genome. TALEN is recognized target genome by DNA binding motif repeats and cleavage target site by FokI restriction endonuclease. Although several fast and reliable assembly methods have been published in the recent years, however, a comprehensive and high-throughput screening procedure has not yet been published. Here we combined high resolution melting analysis (HRMA), high resolution capillary electrophoresis (HRCE) and qPCR method to report the TALENs' activity and to screen TALENs-mediated mutants in zebrafish.

Materials and Methods:

TALENs were assembled by using GoldenGate or FLASH methods by Zgenebio company. Later, we synthesized the corresponding mRNA by in vitro transcription with SP6 RNA polymerase. Around 100 pg TALEN mRNAs were injected into one-cell stage of zebrafish embryos. At 24 hpf, we isolated the genomic DNA from 20 TALENs-injected embryos and calculate the mutation rate by using combinational methods of HRCE and qPCR methods. In addition, we adapted HRMA to screen putative founders and performed Sanger sequencing to elucidate the possible insertion/deletion (indel) patterns.

Results:

In total, we validated the in vivo activity of 15 TALEN pairs and found 10 out of 15 TALEN pairs (67%) got positive activity in zebrafish. In addition, by extracting genomic DNA from founder's fin clip and F1 progeny, we found the somatic mutation rate is well correlated with the germ line mutation rate which detected by HRMA.

Conclusion:

Our results indicate the TALEN pairs designed by Zgenebio company get high in vivo activity in zebrafish. For one targeting gene, we routinely designed two to three TALEN pairs in order to get 100% successful targeting rate. In addition, pre-screen the somatic indels in founder generation can significantly reduce the mutation screening efforts. In summary, we take the advantages of HRMA, HRCE and qPCR methods to streamline the pipeline on screening genetic mutants created by TALEN-mediated gene knockout technique.

P618
The Effect of MiR-124 Overexpression in Anti-tumor Drugs Sensitivity

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Backgrounds:

MicroRNA has been known to play a critical role in regulating various physiological mechanisms, including growth and development, abnormality of which may be involved in the initiation and progression of human cancer. Previous studies have shown that microRNA-124 (miR-124) participates in not only regulation of early neurogenesis but also suppression of tumorigenesis. The miR-124 was reported to be attenuated in several tumors, such as glioma, breast cancer and hepatocellular carcinoma

Materials and Methods:

This hypothesis was initially suggested by the clues that overexpression of miR-124 is associated with reduced DNA repair capacity. This study then examined which target genes involved in DNA repair were regulated by miR124, using target prediction bioinformatics database, miRanda and TargetScan. We used the reporter assay to analysis the miR-124 binding site of target genes. Further experiments demonstrated whether overexpression of miR-124 affects cell survival caused by DNA damaging agent.

Results:

Overexpression of miR-124 increases the anti-tumor drugs sensitivity in cell survival assay. This reporter assay resulted in ATM Interactor (ATMIN, also called ASCIZ) and poly(ADP-ribose) polymerases (PARP1). MiR-124 regulated protein expression of ATMIN and PARP1 by binding to the 3-untranslated regions (3'-UTR) of the mRNAs of these two genes. The DNA repair capacity in homologous recommendation and strand break repair were reduced by miR-124 overexpression.

Conclusion:

Functionally, overexpression of miR-124 inhibited DNA repair and enhanced cellular sensitivity to multiple DNA-damaging agents via ATMIN and PARP1. These findings support miR-124 is a potential therapeutic agent to improve the efficacy of chemotherapy with DNA-damaging agents.

P619
Mangosteen Prevented Neurotoxicity in Hippocampal Slice Culture and Improved the Cognitive Function in Triple Alzheimer's Disease Mice

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Backgrounds:

Alzheimer's disease (AD) is a progressive neurodegeneration with an increased β−amyloid (Aβ) and hyperphosphorylated tau protein in the brain, accompanied with cognitive impairment. Promising therapeutics to prevent and cure AD is an unmet need. Polyphenols identified from several plants have shown protective effects on Aβ related neurodegerative disorders *in vitro* and *in vivo*. Mangostin is a known polyphenolic xathone derived from Mangosteen (*Garciniamangostana Linn*), which has the bioactivities of anti-inflammation and anti-oxidation. In this study, we evaluated the pharmacological effect that Mangosteen exerts on prevention of oligomer Aβ42-induced neurotoxicity in an *in vitro* culture system and improvement of cognitive function in 3×Tg AD mice model.

Materials and Methods:

Various doses of mangostin and Mangosteen were pretreated to oligomeric Aβ42-incubated hippocampal slice culture. Propidium iodide staining, BDNF and IL-6 ELISA were performed to assay the pharmacological effects. Mangosteen diet was administered *ad libitum* to young 3×Tg AD mice until 12-month old of age. Several behavioral tests including spontaneous activity (locomotor), elevated plus maze and Morris Water Maze were performed to analyze the neuropharmacological influence.

Results:

We found that Mangosteen treatment in hippocampal slice culture showed reduced neurotoxicity with high concentration and even prevented Aβ42-induced neurotoxicity with low dose. In addition, the low dose pretreatment of Mangosteen elevated the level of BDNF in slice tissue and IL-6 in medium. Furthermore, during the in vivo treatment, mouse body weight and blood glucose were unaffected under Mangosteen diet treatment. There were no effects on motor function and anxiety behavior identified in mice after Mangosteen diet. However, Mangosteen diet improved the short-term spatial reference memory and spatial working learning and memory in 3×Tg AD mice.

Conclusion:

Mangosteen treatment prevented neurotoxicity in in vitro culture system and improved short-term spatial learning and memory ability in 3xTg AD mice model. Further pathological study may help us to understand the mechanism.

P620
Identification of Therapeutic Potential of HDACi Compounds with SCA17 Slice Culture and Transgenic Mice

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Background:

Spinocerebellar ataxia type 17 (SCA17) is a polyQ neurodegenerative disease caused by abnormal CAG repeat expansion of TATA-box binding protein (*TBP*) gene. The CAG trinucleotide expansion results in the mutant polyQ TBP protein misfolded and accumulated in the cells, which further caused the neuronal lose, especially the cerebellar Purkinje cells. Ataxia, motor dysfunction, and dementia are clinical symptoms of SCA17. As the role of TBP being critical in transcription initiation, improving the transcriptional activity by histone deacetylase inhibitors (HDACi) becomes an attractive approach for SCA17 treatment

Materials and Methods:

SCA17 mouse cerebellar slice culture was used to screen HDACi compounds for their efficiency in reducing the TBP aggregation within the Purkinje neuron. SCA17 transgenic mice were used to validate the in vivo effect of HDACi compoud identified from the slice culture.

Results:

We have evaluated several HDACi compounds and found that NC108 could significantly reduce the TBP aggregation on SCA17 cerebellar slice culture. In the *in vivo* study, we found the SCA17 transgenic mouse phenotypes were ameliorated by NC108 injection.

Conclusion:

NC108 could be a potential HDACi compound in the treatment of polyQ-mediated SCA17 disease.

P621

Neural Tube Closure Defects Lead To the Loss of Asymmetry In Zebrafish Epithalamus.

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Backgrounds:

Zebrafish epithalamus exhibits structure and gene expression asymmetries in multiple levels. In developing WT epithalamus, the Nodal antagonist genes *lefty 1* (*lft1*) and the Nodal effector gene *pitx2* are expressed in the left side of the pineal complex anlage. Later, the parapineal forms to the left of the pineal organ and instructs the asymmetric development of the habenular nuclei. The left and right habenula display differences such as higher neuropil density and *kctd12.1* gene expression on the left, and higher expression of the *kctd12.2* gene on the right. Previous studies have demonstrated that disrupting the formation of midline structures such as the notochord, neural tube floor and brain dorsal midline causes the sidedness of asymmetric structures to become random.

Material and Methods:

Zebrafish husbandry, microinjection, whole mount in situ hybridization, and immunostaining protocols were modified from Westerfield, M. (2000), The zebrafish book: A guide for the laboratory use of zebrafish (*Danio rerio*), 4th ed., Univ. of Oregon Press, Eugene. All mutant and transgenic fish lines, probe constructs, morpholino sequences are available on the Zfin database (www.zfin.org).

Results:

To further investigate the role of dorsal midline in brain asymmetry, we took advantage of zebrafish mutants that led to open neural tube. In such mutants, the developing pineal is divided into two widely separated domains, one on the left and one on the right. We found that when the neural tube did not close, the pineal complex and habenula nuclei became left isomerized. *lft1* and *pitx2* were expressed in both sides of the divided pineal anlage. The normally left sided parapineal marker gene *gfi1.2* was expressed in two zones, one on each side. *Kctd12.1* became highly expressed in both the left and right habenula while *kctd12.2* expression was low or undetectable.

Conclusion:

The fact that isomerization is observed only when the epithalamus is physically divided suggests that certain left inducing signal is likely regulated by a reaction–diffusion mechanism. In such model, if the initial gene expression asymmetry is disrupted, a random asymmetry can still form via the interaction between positive and negative feedback mechanisms. However when the distance of the two part become too great for the signals to travel, isomerization is generated.

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P622

MicroRNA-206 Controls Somite Boundary Formation by *Silencing reticulon* in Zebrafish Embryos Through AKT / GSK3β Signaling Pathway

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Backgrounds:

It has been demostrated that *microRNA-206 (miR-206)* is expressed exclusively in skeletal muscle and regulates the proliferation and differentiation of muscle fibroblasts. At early stage of embryonic muscle development, it has been proven that *miR-206* targets *reticulon (rtn)* mRNA, which causes somite boundary defects when overexpressed. However, the molecular regulatory mechanism of how Rtn affects the development of skeletal muscle is unknown.

Materials and Methods:

Antisense mropholino oligonucleotides (MO) were injected to 16 hpf zebrafish embryos, and transcriptional regulation were observed via whole mount *in situ* hybridization (WISH). Then, we used immunostaining and Western blot to observe somite boundary defects and protein level expression.

Results:

Firstly, injecting *miR-206*-MO which specifically inhibits endogenous *miR-206* could cause an increase in the expression of Rtn protein, and it was found by immunofluorescence staining that actin filament crossing-over between somites occurred in the embryonic muscles (55% of 95 injected embryos). Secondly, through WISH we found that overexpression of Rtn had no influence on the expressions of segmentation clock-related genes, including *deltad, her1, fgf8* and *tbx24*, indicating that the effect of Rtn over the formation of somite boundary is independent from the segmentation clock-related genes. We further applied Western blot and found that both the expression of Fibronectin and the phosphorylation level of Focal adhesion kinase (FAK) at Tyrosine 397 decreased, indicating that overexpression of Rtn causes abnormal expression and aggregation of Fibronectin, thus leading to defect of somite boundary. In addition, to clarify the molecular mechanism of actin filament crossing-over between somites, we inhibited the embryonic endogenous *miR-206* or excessively expressed the *rtn mRNA*. It was then shown by Western blot that the expression of Protein kinase B (AKT) and Glycogen synthase kinase 3β (GSK3β) remained constant, but the phosphorylation level of AKT and GSK3β as well as the expression of Snail increased, and the expression of E-cadherin decreased as well, suggesting that overexpression of Rtn could influence the epithelial mesenchymal transition, leading to occurrence of actin filament crossing-over between somites.

Conclusion:

Our analysis indicated that *miR-206* controls the production of the extracellular matrix Fibronectin by fine tuning *rtn* expression which regulates E-cadherin through AKT / GSK3β signaling pathway to control the normal formation of somite boundary at early stage of zebrafish development.

P623

Development Of A Novel Cell-based Sandwich ELISA For Quantitative Analysis Of Polyethylene Glycol(PEG) And PEGylated Molecules

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Backgrounds:

PEGylated molecules (nanoparticles, micelles, liposomes and proteins) are highly regarded as the third generation of therapeutic agents, with an expected market of ten billion US dollars within five years. The successful translation of PEGylated nano-molecules to the clinic depends on accurate methods for the qualitative and quantitative analysis of PEGylated nano-molecules. However, simple and sensitive methods to directly measure the concentrations of PEGylated nano-molecules for pharmacological studies remain unavailable.

Materials and Methods:

This is, in turn, creating great demand for the quantification of free PEG and PEGylated molecules in vivo for both drug development and clinical applications. However, a simple, sensitive and low-priced method to directly measure the concentrations of PEG for pharmacological studies was still unavailable. Here, we developed an anti-PEG cell-based sandwich ELISA by expressing anti-PEG antibody or anti-methoxyl-PEG antibody on cell surface to trap methoxyl-PEG or PEGylated molecules.

Results:

The anti-PEG cell-based sandwich ELISA can employed to measure methoxyl-PEG or PEGylated molecules (small-molecule drugs, proteins, nanoparticles and liposomes) at concentration as low as nanogram (ng) level, even in the presence of 20% serum. These cell-based ELISA kits are simple, sensitivity, popularization and inexpensive in which general laboratories can afford and easily used. Finally we show that the sandwich ELISA could accurately measured the half-life of methoxyl-PEG or PEGylated molecules *in vivo.*

Conclusion:

We believe that development and validation of an optimal, standardized and versatile platform to quantify PEGylated nano-molecules in biological samples will promote its widespread adoption to replace the traditional, cumbersome and inconvenient methods for the preclinical and clinical testing of PEGylated molecules.

P624

從缺

P625
LGR5 Regulates Proliferation and Cell Survival in CRC Cells by Targeting Wnt Signaling

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Backgrounds:

The leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) is a newly identified surface marker for intestinal stem cells and its expression level was commonly elevated in human colorectal cancers (CRCs). Our previous study demonstrated that the expression of LGR5 was significantly increased in the hyperplastic section and adenoma located adjacent to normal colon mucous. Expression of LGR5 continued to increase from adenoma to carcinoma of CRC and its levels were positively associated with tumor stages. These data suggest that LGR5 may be involved in CRC initiation and progression. However, the role of LGR5 in CRC pathogenesis has not been well established.

Materials and Methods:

In this study, we aimed to investigate the pathological role of LGR5 in CRC tumorigenesis and to elucidate the mechanism underlying LGR5-mediated CRC pathogenesis. We established a siRNA-based technology to suppress endogenous levels of LGR5 and analyzed the effects of LGR5 depletion on cell growth, proliferation and downstream signaling in cultured CRC cells.

Results:

Depletion of LGR5 suppressed the proliferation and reduced the colony forming ability of several cultured CRC cells. In addition, depletion of LGR5 in CRC cells caused an increase in the fraction of apoptotic cells as analyzed by Annexin V/PI staining and DNA fragmentation assay. Furthermore, depletion of LGR5 suppressed the activity of Wnt/β-catenin signaling and reduced the expression of c-myc and cyclin D, two Wnt/β-catenin targets in CRC cells. Treatment of Wnt3a significantly reduced the growth inhibition and apoptotic cell death induced by LGR5 depletion in CRC cells. Taken together, these data suggested that LGR5 may be involved in CRC tumorigenesis by targeting Wnt signaling and regulates cell proliferation and survival.

Conclusion:

Our findings suggest that LGR5 plays an important role in CRC pathogenesis and has the potential to serve as a diagnostic marker as well as a therapeutic target for CRC patients.

P626
Microarray Analysis of Resistant HCT116 Colon Cancer Cell Lines and the Effect of Traditional Chinese Medicine BP002

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Background:

Chemoresistance is one of the obstacles in the treatment of cancer. In this study, a systemic approach was used to identify the possible mechanisms underlying the development of 5-flurouracil-induced resistance on HCT116 colon cancer cells.

Materials and Methods:

In order to reverse 5FU-resistant HCT116 subclones to its naïve state, it is necessary to discover a potential Chinese medicine in the role of adjuvant treatment. We screened 40 Traditional Chinese Medicines and by using MTT assay, we found that BP002 was most effective in the combine treatment with 5FU. In addition, a predictive marker, Cep55, was selected according to our microarray experiment and a RT-PCR experiment confirmed this gene was down-regulated. Finally, western blot results explain the mechanism of Cep55 in these subclones.

Results:

BP002 alone did not have the toxicity to the resistant sub-clones, but greatly inhibited cell proliferation after adding 5FU. The microarray analysis revealed that most of the genes were involved with cell cycle regulation. The selected predictive marker Cep55 is a cell cycle-related gene and is down-regulated in HCT116 5-FU subclones. The down expression of Cep55 is able to slow down the growth rate of subclones by increasing the level of DNA repair genes.

Conclusion:

BP002 was demonstrated to be the potential adjuvant drug for 5FU resistant subclones and the predictive marker Cep55 is one of the factors in chemoresistance. With these findings, we hope to provide the best treatment option in the clinical studies.

P627
Involvement of TGIF in the Low-level Arsenic Trioxide-induced Malignant Transformation of HaCaT Cells

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Backgrounds:

Arsenic is a well-known poison and carcinogen in humans. Ironically, it also has been used in traditional Chinese medicine for more than 2400 years to effectively treat some human non-carcinogenic ailments and cancers. Arsenic trioxide (ATO) is a multi-target drug that has been approved by the Food and Drug Administration as the first-line chemotherapeutic agent for the treatment of relapsed and refractory acute promyelocytic leukemia. Currently, phase II trials are being conducted with arsenic-based drugs for the treatment of various forms of cancer. However, the toxic effects of ATO on human are also concerned clinically. Thus, the paradox of arsenic, its role in carcinogenesis and its potential for clinical therapy, should be elucidated to improve its therapeutic effects.

Materials and Methods:

The mechanisms of which TGIF involved in the low-level ATO-induced malignant transformation of HaCaT cells were elucidated in vitro by using pharmacologic inhibitors, RT-PCR, Western blot, soft agar, migration/invasion, and gelatin zymography assay.

Results:

Low-level ATO could stimulate HaCaT cell proliferation, MMPs activation, and anchorage-independent growth, which included c-Src-Y416 and EGFR-Y845 phosphorylation, and ROS produciton. TGIF expression was involved in the signalings. Moreover, overexpression of TGIF, as well as low-level ATO induced cadherins changes, MMPs expression, invodopodia formation, increase of migration/invasion activities and anchorage-independent growth.

Conclusion:

We suggest that low-level ATO induces malignant transformation of HaCaT cells. TGIF expression plays an important role in the phenomena.

P628
Guei-Chih-Fu-Ling-Wan Modifies Immune Responses and Inhibits Tumor Growth in Orthotopic Mouse Bladder Tumor Model

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Backgrounds:

Transitional cell carcinoma (TCC) is the most common tumor type in the urinary system, after surgery to remove the tumor part will combine with Bacillus Calmette-Guerin (BCG) to prevent the recurrence. However, the BCG treatment caused severe side effects like as hepatitis, abscess or pneumonitis. Thus, in this study we want to develop the substitute drug, the traditional Chinese medicine Guei-Chih-Fu-Ling-Wan (GFW), to better treatment of the bladder tumor.

Materials and Methods:

We treated murine bladder cancer cell MB49 with different doses of GFW in vitro, using flow cytometry and western blot to validate the cell and found that cells became autophagy or apoptosis after treatment. In vivo, we established the orthotopic bladder cancer model to investigate the efficiency of GFW treatment.

Results:

In vitro, cell survivals were gradually decreased after GFW treatment and with dose dependent under 1.5mg/ml, however, most cell died when GFW dose were greater than 1.5mg/ml. And, the underlying mechanism of cell death after GFW treatment is through autophagy and apoptotic pathway under the low and high dosage of GFW, respectively. In vivo, we established the orthotopic bladder cancer model to directly monitor the limiting ability of tumor size and regulation in immune system of GFW. Mice were implanted with 5x105 MB49 cells in bladder, administration of GFW in a 176 mg/ml dosage within the bladder tumor once every two days, after 10 days, hematuria was observed both in control and GFW treatment. However, after 24 days, the tumor sizes were reduced after GFW treatment, and also increase the percentage of CD4+ T lymphocytes in spleen in vivo.

Conclusion:

GFW treatments caused the cell death of MB49 bladder tumor in vitro, and reduced the orthotopic bladder tumor in vivo.

P629

Regulation of Human Hepatocellular carcinoma Progression by Epithelial membrane protein 3 (EMP3)

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Backgrounds:

Epithelial membrane protein 3 (EMP3) is a trans-membrane signaling molecule with important roles in the regulation of differentiation, proliferation and invasion of cancer cells, but its function in hepatocellular carcinoma (HCC) progression remain unclear.

Materials and Methods:

Immunohistochemistry, immunofluscence and western blot analysis was performed for EMP3 in human hepatocellular carcinoma tissues and four HCC cells. Cell viability of knockdown EMP3 to human SK-Hep-1 and Huh-7 cells lines was examined using MTT assay. Cell cycle distribution was determined by flow cytometry. Cell motility, migration and invasion was examined by wound healing assay, migration and invasion assay. Expression of cell cycle-regulated proteins, MMP-9 and uPA was examined at mRNA (RT-PCR), activity (Gelatin/Casein zymography) and protein (western blot) levels.

Results:

Our data showed that EMP3 protein and mRNA expression are higher in HA22T/VGH, SK-Hep-1 and Huh-7 cells than in HepG2 cells, and it was significantly up-regulated in HCC tissues compared the non-tumor HCC tissue. The inhibitory effects of EMP3 on HCC cells growth were associated with the G0/G1 cell cycle arrest concomitant with a marked inhibition of SKP2 and cyclin E as well as the induction of the p27. EMP3 knockdown in HA22T/VGH and SK-Hep-1 cells decreased cell motility, migration and invasion. Knockdown of EMP3 also decreased the protein and activity levels of MMP-9 and uPA in HCC cells. Moreover, EMP3 knockdown decreased Akt activity-promoted migration and invasion. In addition, shEMP3 cells treatment with an Akt inhibitor LY294002 in HCC cells inhibited the migration and invasion. Conversely, Akt overexpression in shEMP3 cells produced the opposite effect.

Conclusion:

EMP3 may be a novel oncogene in HCC, and its downregulation may effectively suppress HCC tumor growth and metastasis. Targeted inhibition of EMP3 may be a novel therapy for HCC progression.

P630

Exploring Anti-inflammatory and Anti-cancer Activities for Different Parts of Alpinia Nantoensis

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Backgrounds:

Natural products containing a diversity of metabolites have been the most important source of leads for the development of drugs. Extracts from plants of the genus *Alpinia* have been widely used as folk medicines for relieving inflammatory syndromes of common cold and gastrointestinal disorders. The application of *Alpinia* plants also have been implicated in chemoprevention and chemotherapy given that their extracts exhibited strong anti-oxidant and anti-cancer activities. *Alpinia nantoensis* is a folk plant endemic to Taiwan but the information on bioactivities for its metabolites is limited so far. In this study, the anti-cancer and anti-inflammatory activities for extracts from rhizome, stem, and leaf parts of this plant were investigated.

Materials and Methods:

The model of lipopolysaccharide (LPS)-stimulated nitric oxide (NO) production in RAW 264.7 macrophage was used to assess the anti-inflammatory for extracts of A. nantoensis. The anti-cancer activities were observed in MDA-MB-231, MCF-7, and A549 human cancer cell lines. The cell growth curves were used to measure the cancer proliferation rate. The sphere formation assay was used to test the stemness potential of cancers. The wound healing and Boyden chamber assays were used to assess the cancer migration and invasion abilities. The western blot assay was used for both qualitative and quantitative analysis of protein expression.

Results:

All parts of extracts significant decreased LPS-stimulated NO production in RAW 264.7 macrophages, and the calculated IC_{50} values for extracts of rhizome, stem, and leaf were 3.35 ± 0.39 μg/mL, 6.71 ± 0.53 μg/mL, and 1.1 ± 0.27 μg/mL, respectively. The rhizome part of extract was shown strongest but moderate of cytotoxicity on MDA-MB-231, MCF-7, and A549 cells. The calculated IC_{50} values for them were 28.13 ± 2.43 μg/mL, 42.22 ± 3.17 μg/mL, and 68.64 ± 3.22 μg/mL, respectively. Low concentration of rhizome and leaf parts of extracts significantly inhibited the sphere formation of MCF-7 cell and inhibited the cell migration and invasion of MDA-MB-231. The expression of phospho-AKT was markedly decreased in MDA-MB-231 cells treated with 3 crude extracts might be one of molecular mechanism underlying the anti-cancer activities.

Conclusion:

Our results demonstrated the extract of A. nantoensis has powerful biological activities and worth continuing to explore its active constituents for supporting health benefits or treating inflammatory or malignant diseases.

P631

Study of the inhibitory effects and mechanisms of Duchesnea indica on invasion and migration of lung cancer cells

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Backgrounds:

Cancer has ranked the number-one cause of death entire the world. Particular lung cancer death rate is the fastest growing. Although therapeutic advances, the cure rate for lung cancer still low. In the cancer patients, metastasis is often fatal cancer caused by one of the reasons. The epithelial-to-mesenchymal transition (EMT) in lung cancer cells is considered pre-requisite for acquiring invasive/migratory phenotype, and subsequent metastasis. In the recent study, there were some functions Chinese herb medicine have been reported to have various anti-carcinogenesis properties, such as anti-metastasis, anti-proliferation, and anti-angiogenesis.

Materials and Methods:

The purpose of this study is to explore the inhibitory effect of Duchesnea indica extract (DIE), extracted by 50% ethanol, on the invasion, motility and migration of A549 cells by Boyden chamber invasion assay, motility assay, and would healing assay. We examined the effect of DIE on factors (such as MAPKs and PI3K/Akt pathways) of cancer metastasis and EMT by Western Blot. We treated A549 cells with various concentrations of DIE (0, 25, 50, 75, and 100 μM), and then subjected cells to gelatin zymography and casein zymography to investigate the expression of matrix metalloproteinases (MMPs) and urokinase-type plasminogen activator (u-PA).We also induced the cell morphology transition by using TGF-β1, and then to observe whether the phenotype will be upside down from mesenchymal to epithelial using a microscope.

Results:

In this study, we have demonstrated that DIE could inhibit invasive and migratory potential, reduce the u-PA and MMP-2 activities, and have a negative influence on cell spreading of a highly metastatic lung cancer cell line at non-cytotoxic concentration. Further investigation showed that higer migration ability is due to epithelial-mesenchymal transition (EMT) occurred in A549, when we treated DIE detection that EMT phenomenon might be reverse. DIE induced up-regulation of epithelial marker such as E-cadherin and decreased the mesenchymal markers such as vimentin and N-cadherin. DIE also inhibited p-Akt and p-ERK1/2 protein expression.

Conclusion:

Taken together, our findings showed a strong anti-migratory and anti-invasive efficacy of DIE against human lung cancer A549 cells, which was in-part through promoting E-cadherin expression and decreasing the level of N-cadherin, p-Akt, p-ERK1/2.

P632

Transient Relaxation of Human Fallopian Tube Fimbriae by Ovulated Follicular Fluid, An Early Step of Oocyte Pick-up

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Background:

The fimbriae are free hanging fringe structures of the fallopian tube infundibulum which is connected to the ovary by a band structure called fimbriae ovarica. The function of the fimbriae is to move along the ovarian surface to the site of dominant follicle and “catch” the oocyte cumulus during ovulation. The mechanism by which the ocyte cumulus is caught is elusive. We hypothesized that at the moment of ovulation, the motion of tube fimbriae is controlled by the ovulated follicular fluid to accomplish the “catch” activity.

Materials and methods:

Normal fallopian tubes were collected from gynecological operations. Multiple strips of the fimbriae, proximal fallopian tube and frimbria ovarica were dissected and studied for relaxation/contraction activities with a Radnoti Glass Technology tissue bath system. Briefly, sliced tissues were suspended with appropriate pulling weight and were incubated in Kreb buffer. Follicular fluid, ions and reagents were added to the tissue bath reservoirs. Data were collected and analyzed by BSL PRO software.

Results:

Fallopian tube fimbriae and, to a less extent, the proximal tube were transient relaxed by treatment with follicular fluid when compared to its untreated baseline, but the fimbriae ovarica was not responded. The relaxation response was fully inhibited by calcium blocker (felodipine) and partially inhibited by tyrosine kinase inhibitor (imatinib). Increasing the calcium concentration induced a transient contraction of proximal fallopian tube and the fimbriae. The relaxation activity in the follicular fluid was not affected by boiling, indicating non-protein factors were involved. Treatment with NO donor (nitroglycerin) or adrenaline (isoproterenol hydrochloride) could not induce a relaxation. The results suggest a heat-resistant relaxation activity in the follicular fluid acting on the fallopian tube fimbriae.

Conclusion:

A Calcium dependent, transient relaxation of the fimbriae of human fallopian tube but not the fimbriae ovarica was observed after follicular fluid treatment. This relaxation may induce a negative pressure in the infundibulum of fallopian tube, which is sealed at its junction to the uterus (the interstitial portion), to facilitate a suction of oocyte cumulus to the tube; while the fimbriae ovarica, which holds still the infundibulum to the ovary, is not affected. The study for the first time unveiled the very early step of human oocyte pick-up.

P633

Development of Multi-level Targeting Magnetite Nanoconjugates As a Comprehensive Oral Cancer Therapeutic Strategy

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Backgrounds:

Recent reports suggest that combined chemical and physical approaches in cancer therapy may have synergistic advantage such as integrated chemotherapy and hyperthermia. Such approaches are especially effective when multiple modalities are delivered simultaneously under cellular and molecular targeting based manner. Today, the concept of applying nanoparticles to integrate multiple therapeutic functions has become an emerging trend in cancer treatment. In addition, previous studies reported that CD44 plays multiple functions in oral squamous cell carcinoma (OSCC) progression. CD44 can be triggered to internalize by extracellular matrix or antibody and to form the transcriptional complex. Their nuclear translocation could turn on cancer reprogramming genes. In this study, we use this internalization pathway to design a novel hyperthermo-chemotherapy that shuttled the oligonucleotide magnetite nanoparticles from cell membrane to the nucleus.

Materials and Methods:

The designed nanoparticle includes a high-saturation magnetization magnetite nanocrystals core conjugated with polynucleotide loaded with 5-fluorouracil (5-FU) shells and anti-CD44 mAb. We used OSCC cell lines and normal cells in targeting internalization study. We utilized radiofrequency (RF) to induce magnetic hyperthermia (MHT) and evaluated the efficacy in HSC-3 bearing NOD/SCID mice.

Results:

In vitro study discovered the internalization of CD44 in OSCC cells are significantly higher than the normal cells. OSCC cells are more susceptible to the nanoparticles derived cytotoxicity. We further confirmed that combined RF and targeted nanoparticles could further augment cancer cells cytotoxicity in a time-dependent manner. In vivo evaluation showed only a single dose of I.V. injection of the magnetite nanoparticles could cause significant tumor regression when combined RF exposure.

Conclusion:

This novel therapy consists of two stages: First, the CD44 induced internalization of the nanoparticle could augment both extra-and intracellular MHT by RF in oral cancer. Second, the 5-FU released during MHT provides effective chemotherapy to retard tumor re-progression. We anticipate such achievements could inspire further nano drug delivery strategy to advance oral cancer therapeutics.

P634

Scale regeneration serves as an alternative model to study bone fracture in high vertebrates

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Backgrounds :

Fish scale is a dermis-derived structure, which mainly constitutes collagen and calcium, also contains osteoblasts, osteoclasts, and bone matrix. Once scales are removed from skin, regeneration events can be completed within short time. The previous studies on fish scale regeneration are largely based on compositional or histological analysis. To date, it is still lacking a good in vivo model to dissect the molecular mechanism on controlling scale regeneration.

Materials & Methods:

The specific aim of this research is to analyze the regeneration dynamics of zebrafish scale at histological and molecular levels. To facilitate scale regeneration observation, we created Tg(osx:nlsGFP)^{cy25} to illuminate scales and bones. After scale amputation, the regenerating scale displayed a strong green fluorescence due to the reactivation of osterix transcription. Combining histological and morphological observation, some interesting events of scale regeneration were revealed as wound healing, mesenchymal condensation, scale protruding and scale mineralization. To explore gene expression profiling, we performed microarray on different scale regeneration time points and did gene clustering analysis.

Results :

Data show one gene cluster's expression level well correlated with scale regeneration activity after clustering with STEM software. After classified with DAVID software, we discovered genes involved in spliceosome pathway are highly enriched. Other genes like proteasome, DNA replication, cell cycle are also up regulation. Side-by-side comparison with data collected from rat femoral bone fraction also display similar enriched pattern. In addition, administration of Forsamax, a clinical drug used to prevent osteoporosis, can significantly accelerate scale regeneration in vivo.

Conclusion :

This result shows gene expression profiles during zebrafish scale regeneration is similar with the mammalian bone fracture. From the results, we suggest zebrafish scale regeneration might serve as an alternative and simple model to study human bone fracture mechanism.

P635

The potential effects and the molecular mechanism of omega-3 fatty acids of Acetaminophen -induced liver injury in zebrafish model.

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Backgrounds:

Omega 3 fatty acids in dietary fish oil are reported to have anti-inflammatory and injury effects in humans, but the biochemical basis and the molecular mechanism for these beneficial health effects is still not well understood. Acetaminophen (APAP) is one of the most widely used pharmaceutical analgesics; an overdose is currently the most frequent cause of acute liver failure in many countries. Because conflict theory exists about omega 3 fatty acids on APAP induced liver. We aimed to investigate the potential effects and the molecular mechanism of ω-3 fatty acid to APAP-induced liver failure in zebrafish model.

Materials and Methods:

Wild-type zebrafish (AB line) were supplemented for 7 days with encapsulated fish oil. Four kinds of liver-specific omega-3 transgenic fish strains. Individually carrying Δ4, Δ5, Δ6 desaturase and elongase gene were received formulated diet. Advanced works will be carried out in the aspect of liver injury from blood biochemical analysis, histological, immunohistochemical examination, reverse transcriptase polymerase chain reaction and western blot methods.

Results:

We also established the zebrafish model for APAP liver toxicity and established four kinds of liver-specific omega-3 transgenic fish strains. Individually carrying Δ4, Δ5, Δ6 desaturase and elongase gene.

Conclusion:

Zebrafish could be used as a universal preclinical model organism to test omega-3 fatty acids on Acetaminophen -induced liver injury in vivo.

P636

Identification of migration related genes (S100A9, C8orf30A and IL8) in esophageal squamous cell carcinoma

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Backgrounds:

Esophageal cancer (EC) is classified as esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EA). The prognosis of ESCC is poor and the 5-year survival rate is less than 10% because no reliable biomarker has been identified.

Materials & Methods:

In this study, ESCC CE81T cell and its derivate CE81T-1 with higher migration activity were used to identify ESCC migration related biomarkers. CE81T-1 cell with increasing migration activity after Transwell™ screening of CE81T cell was established and followed by microarray analysis to identify migration related genes. Furthermore, S100A9 protein was detected in ESCC tumor tissues by IHC staining. To study the function of S100A9, overexpression ectopic S100A9 or siRNA was used together with MTT, Transwell™ and ECIS™ assays.

Results:

S100A9 was the most down-regulated gene, and C8orf30A and IL-8 were the most up-regulated genes. In 60 pairs of ESCC specimens, the mRNA expression of S100A9 was significantly lower in the tumor parts as compared with the adjacent normal tissues (P = 0.0228). In contrast, the mRNA expression of IL-8 was significantly higher in the tumor (P = 0.0061). Furthermore, C8orf30A expression was significantly correlated with ESCC metastasis status (P = 0.0358) and associated with poor survival (P = 0.036). Furthermore, S100A9 protein was highly expressed in well differentiated ESCC tumor tissues. Functional studies revealed that S100A9 plays a suppressive role in migration and proliferation of ESCC cells.

Conclusion

Altogether, we reveal that C8orf30A has the potential to be a novel biomarker of prognosis for ESCC metastasis and survival. Furthermore, IL-8 and S100A9 genes have the potential for ESCC diagnosis.

P637
Misfolded HLA-B27 proteins degrade by proteasomal and lysosomal degradation systems

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Backgrounds:
Human leukocyte antigen B27 (HLA-B27) is associated with Ankylosing spondylitis (AS), which is a chronic, progressive inflammatory rheumatic disease involving primarily the sacroiliac joints and the axial skeleton. Over the past 30 years, the exact pathogenic role of HLA-B27 in AS and other spondyloarthropathies has yet to be determined. In Taiwan, susceptibility to AS is determined by homozygosity for HLA-B2704, and HLA-B2706 shows the weakest association with disease.

Materials and Methods:
The 293T cells were transfected with pcDNA3.1-HLA-B2704 or -HLA-B2706 for 48 h. The splicing of Xbp-1 transcripts, a signature for ER stress, was determined by RT-PCR. The expression of GRP78 was detected by Western blot and Q-RT-PCR. The HeLa B2704 and B2706 stable cells were used to study the degradation of B27 proteins after treating with MG132, a proteasome inhibitor, and/or chloroquine, a lysosome inhibitor. The co-localization of B27 and LC3, an autophagy marker, was detected by immunostaining and confocal microscope.

Results:
Our data showed that overexpression of HLA-B27 proteins would trigger ER stress in 293T cells. This study also investigated how the IRE-1 pathway, one of the ER stress sensors, modulates the fate of misfolded HLA-B27. The RT-PCR showed that the splicing of IRE-1 downstream factor Xbp-1 was much obvious in HLA-B2704- and HLA-B2706-expressing cells than that of control. However, the expression of B2704 in stable HeLa cells was hardly detected by Western bolt. After treating stable HLA-B2704 and –B2706 HeLa cells with MG132 and chloroquine, the expression levels of HLA-B27 proteins were clearly detected and increased with a dose-dependent manner. Twenty-four hrs after transfecting HeLa cells with pcDNA3.1-HLA- B2704 and -HLA-B2706, the cells were treated with starvation and/or CQ for 24hr. Immunostaining with LC3 and c-myc (B27) antibodies showed the colocalization of LC3 and B27 proteins in transfected cells.

Conclusion:
This study showed that HLA-B27 indeed induced ER stress in cell line systems and sequentially triggered ubiquitin-proteasome system and autophagy-lysosome system to degrade misfolded HLA-B27 proteins.

P638
Analysis of the Mechanism of GEF-H1 in Mesenchymal Stem Cells Osteogenic Differentiation

I-Husan Huang

Dexamethasone (Dex) is a kind of steroid compound for inducing mesenchymal stem cells osteogenic differentiation, which promotes stress fiber and focal adhesion (FA) formation at the initiation of this process. However, the mechanism of this process is not entirely understood. Guanine nucleotide exchange factors H1 (GEF-H1), which promote the exchange of GDP for GTP, is RhoA GTPase specific activator. Our data showed that Dex stimulated the recruitment of GEF-H1 into FA. In addition, GEF-H1 deletion decreased ability of osteogenic differentiation, inhibited stress formation and altered FA composition. Thus we considered that dexamethasone-induced FA recruitment of GEF-H1 promotes osteogenic differentiation. To test this hypothesis, we compared GEF-H1 KD FA composition with control. We found that NM IIB is one GEF-H1-dependent recruitment protein. Here we demonstrated that GEF-H1 interacts with NM IIB at FA and GEF-H1 deletion inhibited NM IIB recruitment into FA. It revealed that the interaction between NMIIB and GEF-H1 is essential for MSCs osteogenic differentiation.

P639
The Chemopreventive Effects of Ginkgo biloba Extracts against UVB-induced Corneal Phototoxicity

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Backgrounds:
UVB irradiation activates multiple inflammatory responses leading to ocular surface damages. This study analyzed the chemopreventive effects of Ginkgo biloba extracts (GK) against UVB-induced corneal phototoxicity in a mouse model.

Materials and Methods:
ICR mice were randomly divided into 5 groups: (1) blank control, (2) with UVB exposure but without GK supplement, (3) with UVB exposure and with GK at 0.4 mg/kg of body weight, (4) with UVB exposure and with GK 2mg/kg of body weight, and (5) with UVB exposure and with GK at 10 mg/kg of body weight. All groups were subjected to corneal surface evaluations, including smoothness, opacity, topography, and the extent of staining, together with conjunctival PAS staining in order to determine the UVB-induced corneal and conjunctival injuries, with or without various doses of GK treatments.

Results:
UVB exposure lead to corneal surface damages, including reduced smoothness and transparency, impaired cornea sensitivity and visual acuity. The results showed that GK was effective in the prevention of UVB-induced corneal and conjunctival surface damages in a dose-dependent manner. Multiple proinflammatory factors, including NF-κB-p65, COX-2, MMP-9 were also reduced by GK in a dose-dependent manner.

Conclusion:
In summary, Ginkgo biloba extracts may help to prevent UVB-induced corneal damages and conjunctival degeneration. Results of this study support that Ginkgo biloba extracts may be used as a prophylactic agent prior to excessive UVB exposure.

P640
從缺

P641
Grifola Frondosa Cultivated in Taiwan Suppress The Cancer Cell Viability

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Backgrounds:

Grifola frondosa (GF) also named Maitake mushroom, has been cultivated artificially in Japan, China and recently also in Taiwan. It's contained the GF polysaccharide which regulates several biological functions, including anti-cancer. However, the anti-cancer effect of GF cultivated artificially in Taiwan remains unknown. Therefore, the purpose of this study is to investigate the anti-cancer activity of GF, LG1 which is cultivated in Asia University Taichung, Taiwan.

Materials and Methods:

The GF was extracted by hot (100℃) and cold (25℃) water named LG1H and LG1C, respectively. Following, the cell viability was then assessed by MTT assay.

Results:

Results showed that both of the LG1H and LG1C inhibited cell growth in J82, and Hep-GA cells, a human bladder cancer and liver cancer, respectively. Moreover, the anti-cancer of PC-3, a human prostate cancer was also suppressed following LG1C, but not LG1H treatment.

Conclusion:

In summary, among these cell line tested, LG1 showed the highest anti-cancer effect on J82 cells.

P642
Exploring the Molecular Mechanisms of Anti-Proliferative Effect for TF-7 on Human Breast Cancer Cells

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Backgrounds:

Deregulated AKT activity is frequently observed in many cancers and plays a key role in tumor progression. It occurs either from constitutively activation of the receptor tyrosine kinases (RTKs) or due to gain- or loss-of-function in positive regulator PI3K or negative regulator PTEN. In this study, we investigated anticancer activity for a small molecular TF-7 which was newly identified from the root part extract of an herbal plant.

Materials & Methods:

The human breast cell lines MDA-MB-231 and MCF-7 were used to evaluated the chemical responses. MTT assay was used to test the cell viability. The abilities of migration and invasion of MDA-MB-231 were determined by wound-healing and transwell migration assays and matrigel invasion assay, respective. The stemness of MCF-7 was measured by sphere formation assay. Phspho-specific antibodies were used to detect the signaling transduction by western blot assay. The chemical effect on cell-cycle distribution was determined by DNA content stain and following flow cytometry analysis.

Results:

We found the calculated IC_{50} values for TF-7 were about 50 μM for both MCF-7 and MDA-MB-231 cells indicating TF-7 processes only moderate toxicity. Worth noting is that TF-7 was capable of inhibiting MDA-MB-231 cell migration and invasion in the micromolar concentration range. Further investigation into the molecular mechanism of TF-7 reveals the inhibition effect of this compound on AKT phosphorylation at low concentration. Under conditions of either resting or growth factors stimulating, we proved TF-7 is able to inhibit the autophosphorylation of both RTK EGFR and non-RTK Src.

Conclusion:

Our analysis indicated that the results of this study supported TF-7 is a potential inhibitor of tyrosine kinases, and further investigation on the target specificity is of particular importance for it to be considered as a lead to development for tumor targeted therapy.

P643
Mechanism of METCAM/MUC18-promoted progression of human breast cancer cells

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Backgrounds:

Our previous studies suggest that over-expression of METCAM/MUC18, an Ig-like cell adhesion molecule, promotes tumorigenesis and progression of human breast cancer cells. To understand the mechanism, in this study we further investigated the effect of possible clonal variation and the dosage effect of METCAM/MUC18 on the progression of human breast cancer cell line SK-BR-3 in immunodeficient mice.

Materials & Methods :

We have further obtained several G418-resistant clones of SK-BR-3 that expressed different levels of METCAM/MUC18. These clones will be injected at subcutaneous site or orthotopic site (at the mammary fat pads) into female nude mice and the induction of tumors and possible metastasis will be monitored. The tumor sizes at different times will be measured by a digital caliper (tumorigenesis). At the end point, mice will be euthanatized and various cavities will be surgically opened to check for possible metastatic lesions in various organs. The lysates of tumors and/or metastastic lesions will be prepared and analyzed by Western blotting to determine the effect on various tumorigenesis and metastasis-pertinent downstream effectors.

Results:

We expect that the expression dosage of METCAM/MUC18 will have a proportional effect on promoting the tumroigenesis and metastasis of the SK-BR-3 clones. We also expect that the expression of an apoptosis index (Bax2) will be decreased, but the expression of anti-apoptotic indexes, proliferation indexes, aerobic glycolysis, angiogenesis indexes, and migration-related pathways will be increased.

Conclusion:

We anticipate that METCAM/MUC18 expression will promote the progression of SK-BR-3 human breast cancer cells via up-regulating various progression-pertinent pathways.

P644
The Immune Regulation of Tumor Angiogenic Switch: A Microarray Study

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Background:

Angiogenic switch (AS) is the key step for tumor rapid expansion. Recent studies have demonstrated that anti-tumor T cell immunity could modulate tumor microenvironment to inhibit AS. However, the immune targets remain unclear. Previously, we have established an Epstein-Barr virus- encoded oncogene N-LMP1 tumor mouse model. Tumor was induced on day0 by fragment transplantation, and it grows exponentially after day14. By using non-invasive dynamic contrast enhanced-MRI (DCE-MRI), we have identified an AS period between day7-day14. By vaccination using irradiated tumor single cells prior to tumor induction, the AS period was found significantly inhibited in vaccinated mice. Further analyses indicated that CD4 T cell immunity is responsible for the immune control. This study was designed to understand the immune impact by quantitative analysis at the gene expression level after vaccination using affymetrix microarray.

Materials and methods:

We collected three types of tissue samples: (1) day0 tumor fragments without transplantation, (2) day7 tumor, and (3) day7 tumor from vaccinated mice. Identification of genes differentially expression between (1)-(2), (2)-(3) and (1)-(3) were performed with GeneChip® Operating Software (GCOS) and further analyzed by MetaCore.

P645
Investigating the Interaction Between the AdeRS Proteins and the Promoter of *AdeABC* Operon of *Acinetobacter baumannii*

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Backgrounds:

Expression of the RND-type efflux pump AdeABC in *Acinetobacter baumannii* is regulated by the AdeRS two-component system. The AdeRS two-component system provides a signal transduction mechanism by which bacteria monitor and respond to various signals, including environmental stimuli, cell-cycle cues and cellular differentiation signals .The two component system uses the transfer of phosphoryl groups to control gene transcription and protein activity. We want to confirm the regulation of the AdeRS two component system by demonstrating the interaction between the AdeR protein and the promoter of AdeABC operon by electrophoresis mobility shift assay (EMSA).

Materials and Methods:

The *A. baumannii* clinical strains were found with mutations (Met197→Iso) (Gly200→Cys) on their response regulator. First, the 806-bp of *adeR* was PCR amplified from *Acinetobacter baumannii* DNA with primers adeR-F_NdeI and adeR-R_HindIII. The PCR product was cloned into pET41b. The resulting plasmid was transformed first into DH5α and then into BL21(DE3). The DNA probes were labeled with biotin and then was applied for demonstrating the interaction between the mutant adeR and the promoter by EMSA.

Results:

In the present study, we found the different promoter binding capacity and effect on the downstream AdeB protein expression between wild type and mutant AdeR proteins. We will further demonstrate the AdeS could activate AdeR and stimulate the expression of AdeABC proteins. Successfully grading the AdeS stimulation effect might explain the overexpression of AdeABC proteins in multi-drug resistant isolates of *Acinetobacter baumannii.*

Conclusion:

We have confirmed that the mutant adeR strongly interacts with the adeABC promoter by EMSA. Successfully further grading and comparing the binding effect of wild type adeR might explain the overexpression of adeABC proteins in multidrug resistant isolates of *Acinetobacter baumannii.*

P646
Activation of FGFR2 Signaling in Breast Cancer Reduces DNA Double Strand Break Repair Capacity via Mre11 Inhibition

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Backgrounds:

Fibroblast growth factor receptor 2 (FGFR2) is a member of receptor tyrosine kinase family, which has been implicated to regulate oncogenic pathway of various types of cancer. In breast cancer, the overexpression and constitutive activation of FGFR2 has been implied to be an unique subgroup of triple negative breast cancer (TNBC), however, the mechanisms underlying this tissue specificity has remained unknown. Given breast tumorigenesis is particularly associated with DNA double-strand break repair (DSBR) and known breast cancer genes, including *BRCA1 and BRCA2*, play important roles in DSBR, the present study tests the hypothesis that FGFR2 is involved in DSBR.

Materials and Methods:

To determine whether FGFR2 was participated in DSBR, the I-Sce-induced repair assay and Luciferase-based plasmid repair assay were applied to evaluate the impact of *FGFR2* overexpression on homologous recombination (HR) and non-homologous end-joining (NHEJ) repair activity, respectively. The identification of FGFR2-reduced Mre11 expression was validated by immunoblotting and real-time RT-PCR, and the Luciferase-based Mre11 promoter assay was used to screen the candidate transcription factor downstream of FGFR2. Further analysis of FGFR2 signaling pathway and its correlation with Pit-1 transcription factor were estimated by chromatin-immunoprecipitation and *in vitro* kinase assay.

Results:

Supporting to the hypothesis, our findings show that the expression of Mre11, which is a major component involved in DSBR, is down-regulated by *FGFR2* overexpression, which is further linked to decreased DSBR activity. The dissection of *Mre11* promoter reveals the Pit-1 transcription factor is a regulator in FGFR2-attenuated *Mre11* expression. FGFR2 downstream ERK directly interacts with and phosphorylates Pit-1 at Thr 75, which elevates its binding affinity toward *Mre11* promoter and inhibits *Mre11* expression. Moreover, the FGFR2 stable clone shows not only lower *Mre11* expression but also weaker γ-H2AX after IR damage, which implies that FGFR2 may increase the sensitivity to chemotherapy.

Conclusion:

This study suggests that the FGFR2-ERK-Pit1 pathway participated in regulating DSBR via affecting expression of Mre11, which yields important insight to understand breast tumorigenic role of FGFR2 and potential therapeutics of TNBC.

P647
Dietary glutamine decreases lung inflammation and the receptor for advanced glycation end-products (RAGE) expression in acid and lipopolysaccharide-induced acute lung injury in mice

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Backgrounds:

Glutamine (GLN) has improved the outcome in clinical and experimental sepsis. However, the mechanisms of GLN remain unclear, and may depend upon the route of GLN administration and on the models of acute lung injury (ALI). The aim of this study was to investigate GLN's protective effect on direct acid and liposaccharide (LPS)-induced ALI in mice.

Materials and Methods:

Female BALB/c mice were divided into two groups, a control group and a GLN group (4.17% GLN supplementation). After a 10-day feeding period, ALI was induced in half of the mice by intratracheal administration of hydrochloric acid (pH 1.0; 2 mL/kg BW) and LPS (5 mg/kg BW). All animals were sacrificed 3 hours after ALI challenge. Serum, lungs, and bronchoalveolar lavage fluid (BALF) were collected for further analysis.

Results:

The results of this study showed that ALI-challenged mice had a significant increase in the myeloperoxidase (MPO) activity, and in the levels of IL-1beta, IL-6, and TNF-alpha of the lung than did unchallenged mice. Compared with the control group in ALI-challenged mice, GLN treatment reduced the levels of receptor for advanced glycation end-products (RAGE) and IL-1beta in BALF, with a corresponding decrease in their mRNA. The GLN group also had markedly lower in mRNA of cyclooxygenase-2 (COX-2) and NADPH oxidase-1 (Nox-1).

Conclusion:

We suggest that the benefit of dietary GLN may be partly contributed to an inhibitory effect on RAGE expression and pro-inflammatory cytokine production at an early stage in acid and LPS-induced ALI.

P648
Analysis of anti-Herpes Simplex Virus type 1 Activities from *Polygonum Multiflorum* Extracts and Their Effects on Expression of Alzheimer's Disease Markers

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Backgrounds:

Herpes simplex virus type 1 (HSV-1; herpesviridae) is an enveloped DNA virus and a risk factor for Alzheimer's disease (AD). *Polygonum multiflorum* is applied for anti-aging in Chinese medicine. In the present study, anti-HSV-1 activity of *P. multiflorum* extracts (PM) and effects of HSV-1 infection on AD markers, amyloid precursor protein (APP) cleavage and tau proteins phosphorylation, were evaluated.

Materials and Methods:

The anti-HSV-1 activity was determined by plaque reduction assay. The virus titer was analyzed by plaque forming assay. Cell viability and HSV-1 structure proteins expression such as gB were determined by alarmar blue test and Western blotting, respectively. Both APP cleavage and tau proteins phosphorylation, in HSV-1 infected SH-SY5Y cells were determined by Western blotting.

Results:

PM blocked HSV-1 replication in Vero E6 cells in a dose-dependent manner with IC50 108 ±13.8 μg/ml. The inhibitory effect of PM was not related to direct cytotoxicity. The results demonstrated that PM added at 0 to 8 hr postinfection time reduced HSV-1 replication in Vero E6 cells. PM has 20% inhibitory activity on virus adsorption and entry. HSV-1 gB proteins expression in Vero E6 cells was attenuated by PM. HSV-1 infection induced tau protein phosphorylation and increased APP-F35 fragments production in SH-SY5Y cells.

Conclusion:

P. multiflorum contained anti-viral components that inhibited HSV-1 replication by blocking of gB proteins synthesis. The APP cleavage and tau proteins phosphorylation in SH-SY5Y cells could be induced by HSV-1 infection. In future, effects of PM on AD markers expression will be studied.

P649
The Role of Apoptosis and Autophagy in Pathogenesis of Spinalcerebellar Ataxia Type 2

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Backgrounds:

Spinocerebellar ataxia type 2 (SCA2), one of the most common ataxias worldwide, is caused by the expansion of a CAG triplet repeat located in the N-terminal coding region of the *ATXN2* gene. Alleles of the *ATXN2* gene that carry 13–31 CAG-trinucleotide repeats are present in normal individuals. Contrariwise, alleles with a CAG triplet repeat number of >31 and up to approximately 200 are present in patients with SCA2. However, mutant Ataxin-2 presents abnormal folding that gives rise to the formation of aggregates, which might trigger a series of events that lead to programmed cell death. Although the detail mechanism of pathogenesis is yet to be defined, neurotoxin, especially reactive oxygen species (ROS), released from aggregated mutant proteins, may play a role in the pathogenic process.

Materials and Methods:

In this study, the lymphoblastoid cell lines (LCLs) isolated from SCA2 patients were utilized to compare with the wild-type lymphoblastoid cells. Cell cycle and apoptosis were measured using floe cytometry assay, immunoblot. Autophagy was characterized by the increase of Atg8 (LC3 II) and the formation of acidic vesicular organelles (AVOs).

Results:

To investigate whether the mutant Ataxin-2 accumulation is caused by molecular chaperone dysfunction, heat shock induction strategy was employed and then analyzed by western blot. Interestingly, results found the autophagy marker protein, LC3 II were higher in SCA2 patients. Electron micrographs showed that only the cells expressing expanded Ataxin-2 contained aggregated protein and autophagic vacuoles.

Conclusion:

Based on the above observations we hypothesized that the aggregated mutant Ataxin-2 proteins may generate ROS in mitochondria, which subsequently up-regulate LC3 II expression levels and ultimately lead to autophagy and cell death.

P650
Using Heat-shook-stress-responsive Cells in Brain to Study Translational Inhibition

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Backgrounds:

When cells encounter endoplasmic reticulum (ER) stress which cause by an accumulation of misfolded proteins in the ER, unfolded protein response (UPR) well regulate the expression level of C/EBP homologous protein (CHOP) which play an important role in cell survival or apoptosis. It has been reported the uORF sequence located in 5`UTR of human chop gene (huORFchop) inhibits the rate of chop translation. However, underlying molecular mechanisms is still unknown and there is still no in vivo animal model available.

Materials & Methods:

To study the mechanism of huORFchop mediated translational control in vivo, we use the zebrafish transgenic line, termed huORFZ, harboring a construct in which the uORFchop sequence is added to the leader of GFP and is driven by a cytomegalovirus promoter. The GFP appeared only when huORFZ embryos were treated with ER stress.

Results:

Through using the heat-shock to induce ER stress, the number of GFP cells in the 72 hpf huORFZ embryo brain was depending on the heat-treated time and it was also known as the dose-dependent effect. Immunohistochemistry showed that these heat-induced GFP^+ cells with longer process were GS+ glia cells, but not HuC/D+ neurons, suggesting there was some difference between GS+ glial cells and neurons, and huORF^{chop} mediated translational inhibition was only repressed by heat shock in GS^+ glia cells. These indicated that brain tissue responds to heat-shock in a cell-type specific manner. Interestingly, the GFP signal first appeared in Ventricular zone of brain and TUNEL assay identified these brain GFP^+ cells as non-apoptotic cells. BrdU assay showed that GFP+ cells proliferated in 24 hr after heat shook. Moreover, Lineage tracing by heat-induced GFP showed a few GFP^+ cells had neurons-specific HuC/D marker in 9 dpf embryo. Now, we are developing a Laser capture Microdissection to harvest brain neurons and GFP^+ cells, and using microarray analysis to find out which factors are involve in regulating huORFchop.

Conclusion:

Based on the above results, we demonstrated that repression of huORFchop mediated translational inhibition have cell-type-specific response to heat shook. These heat-induced brain GFP+ cells are GS+ redial glia-like cells which may have proliferational and deffriancational potential. Therefore, we suggested that stress-induced factors involved in huORFchop mediated translational control under heat-shock stress.

P651
The role of tumor associated macrophage on drug resistant in non-small cell lung cancer

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Backgrounds:

Increased evidence suggests that chronic inflammation is associated with cancer development, metastasis, invasion and drug resistant. The possible mechanisms by which inflammation can contribute to carcinogenesis include lymphocytes infiltration, cytokines and chemokines secretion, tissue remodeling and angiogenesis, etc. The inflammatory mediators facilitate the communication between tumor cells and tumor-associated host stromal tissue, thereby accelerating tumor progression. Tumor-associated macrophages (TAM) are key regulators of the link between inflammation and cancer. Previous studies found macrophage in non-small cell lung cancer (NSCLC) specimens by immunohistochemistry staining, and also discovered interplay of cancer cells and macrophages would induce secretion of certain cytokines and chemokines(MCP-1, IL-6, IL-8, IL-10, and TNF-a). However, the mechanism of cytokines and chemokines involved with tumor drug resistant in NSCLC is still obscure. It is also unclear the regulatory mechanisms in change of macrophages from M1 to M2 phase affected by tumor cells.

Materials and Methods:

we attempt to investigate the roles of TAM-mediated molecules using different characteristics of NSCLC cell lines (H2126, CL1-1, H1437, H23, H838, CL1-5, and H2009) as the study model. Furthermore, we used both cytokine antibody array to assay the secretion characteristics of cytokines. cDNA microarray was used to analyze gene expression during treatment.

Results:

We found macrophage-cultuered medium enhances chemoresistance rate of cisplatin and gemcitabine after treatment in NSCLC cell line. Following, We found 221 genes upregulation and 186 genes downregulation in which associated with adhesion molecule, inflammation cytokines and proliferation using microarray analysis after treatment in NSCLC cell line. Therefore, we used cytokine array, RT-PCR and Flow Cytometry to confirm those data. Consequently, we also found that macrophage-cultured medium could reduce cell viability through arresting cell cycle.

Conclusion:

These results suggested that macrophage-cultured medium regulate cytokines and chemokines to arrest cell cycle for chemoresistance enhancement.

P652
EZH2-mediate Regulation of Cancer-cell Migration

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Background:

EZH2 is a catalytic subunit of Polycomb repressive complex 2 (PRC2), which trimethylates histone H3 on lysine 27, resulting in repressing gene transcription. It has been known that EZH2 plays a pivotal role in cancer progression and metastasis. Previously we have demonstrated that EZH2 can be phosphorylated by cyclin-dependent kinase 1 (CDK1) at Thr487. The phosphorylation of EZH2 at Thr487 disrupts EZH2 binding with other PRC2 components SUZ12 and EED, and thereby inhibits its methyltransferase activity. [supported by NSC99-2632-B-039-001-MY3 and NSC101-2325-B-039-002].

Materials and Methods:

Overexpression wild-type EZH2 and mutant EZH2 in breast cancer cell line, MCF7. Transwell chamber were used in migration and invasion assay.

Result:

Moreover we found that cells expressing EZH2 T487A mutant showed higher migration and invasion ability than those expressing wild-type EZH2. In this study, we further explore the role of phosphorylation of EZH2 at Thr487 in cancer cell migration and invasion.

Conculsion:

Phosphorylation of EZH2 at Thr487 enhanced cancer cells migration and invasion ability.

P653
The Effect of Anti-cancer for *Zingiber zerumbet* Extracts in Oral Cancer Cells

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Background:
Several reports on the bioactivity of zerumbone, a major constituent of *Zingiber zerumbet*, have been published, including findings of anti-cancer and anti-inflammatory activities, but other ingredients of *Zingiber zerumbet* have barely been discussed. There are three extracts (Z7-Z9) were prepared by refluxing with acetone, methanol, or hot water from our colleague. The aims of this investigation are to determine the effects and molecular mechanisms of anti-cancer effects of *Zingiber zerumbet* extracts.
Methods:
In cancer cell cytotoxcity analysis, the oral cancer cells (Cal27 cell line) was incubated the *Zingiber zerumbet* extracts with different concentrations (1, 10 and 50μg/ml). Cell proliferation was determined by MTS assay. Propidium iodide (PI) and annexin V-FITC staining combined FACScan laser flow detection system were applied to analyze cell cycle and apoptosis.
Results:
The anti-cancer activities with IC50 values are 60, 30 and 10 μg/ml for Z7, Z8 and Z9, respectively. Z9 absolutely does not contain zerumbone, contrary to Z7 containing predominant level by HPLC assay. Z7 exhibited the strongest induction of apoptosis and cell cycle arrest at G2/M phase in oral cancer cells. However, the hot water extract Z9 exhibited the strongest cytotoxicity on human Cal27 oral cancer cells than the other extracts.
Conclusion:
Our analysis indicated that the acetone-extracted Z7 and methanol-extracted Z8 contain zerumbone. And the zerumbione-cantained extracts are effective for induction of apoptosis. However, the hot water-extracted Z9 exhibited the strongest cytotoxicity on human Cal27 oral cancer cells. Our data indicated that there are unknown ingredients in *Z. zerumbet* for cytotoxicity .

P654
Mushroom body α´β´ neurons responsible for distinct olfactory memories in *Drosophila*

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Background:
To investigate the role of synaptic output from mushroom body (MB) α´β´ neurons during intermediate-term memory formation in *Drosophila.*
Material and methods:
[Brain image] Fly brain were imaged under a Zeiss LSM 710 confocal microscope with a 40× C-Apochromat water-immersion objective lens, or a 63× LCI Plan-Neofluar objective lens.
[Behavioral analysis] Using MB α´β´ neurons specific GAL4 driver combined with UAS-*shibire*^{ts} transgene to block the neurotransmission output in α´β´ neurons at different time period. Olfactory associative memory was measured by training adult flies in a T-maze with the Pavlovian conditioning procedure.
Results:
Using MB α´β´ neurons specific GAL4 driver combined with UAS-*shibire*^{ts} transgene, we identified that neurotransmission from α´β´ subset is specifically required for acquisition of 3-hour ASM. However, blocking the synaptic output in α´β´ neurons during memory consolidation or retrieval disrupts ARM.
Conclusions:
These observations elevate the different dynamic roles of MB α´β´ neurons underlying ASM and ARM in *Drosophila.*

P655
The Role of PAI-2 in LPS-induced Septic Shock in Mice

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Backgrounds:
Innate immunity is the first defense when host encounters pathogens or senses danger signals released from damaged tissues. Recently, a novel system named the inflammasome, a multiple protein complex for caspase-1 activation, has been characterized. Activation of the inflammasome can lead to the cleavage of pro-inflammatory cytokines IL-1β and IL-18 into their active forms. Further studies showed that the inflammasome plays a critical role in host defense against bacterial and viral infection in vivo. On the other hand, dysregulation of inflammasome activity is associated with a variety of human diseases including autoimmune diseases and metabolic diseases. Thus, tight control of inflammasome activation is important; however, its positive and negative regulation is poorly understood. Previous studies demonstrated that mice with Ikkβ deletion in myeloid cells (IkkβΔ) are more susceptible to LPS-induced septic shock, and blocking IL-1 receptor (IL-1R) activation by injecting mice with IL-1R antagonist (IL-1ra) completely protected both WT and mutant mice against LPS-induced mortality. In addition, PAI-2, a NF-κB- and p38 MAPK-mediated gene, suppressed IL-1β production in Ikkβ-deficient BMDMs (bone marrow-derived macrophages) after LPS treatment, suggesting that PAI-2 inhibits endotoxin-induced IL-1β in macrophages.
Materials and Methods:
To further elucidate the physiological role of PAI-2 in LPS-induced septic shock, we generated transgenic mice that specifically express PAI-2 in myeloid cells, then crossed this transgenic mice with IkkβΔ mutant mice to see if myeloid-deleted Ikkβ mice with restored PAI-2 expression, can resist LPS-induced septic shock.
Results:
We found that LPS-induced IL-1β production caused by Ikkβ deficiency was suppressed by PAI-2 in BMDMs. In addition, PAI-2 expression decreased plasma IL-1β in IkkβΔ mice after LPS challenge. We also demonstrated that PAI-2 transgenic mice showed a decrease in circulating IL-1β level upon E. coli infection.
Conclusion:
Our data indicated that PAI-2 can protect LPS- and E. coli- induced IL-1β production.

P656
Development of High Sensitive Mutation Detection Panel of *KRAS, BRAF* and *PIK3CA* Genes

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Background:
Mutations in the genes in epidermal growth factor receptor (EGFR) signaling pathway play an important role in colorectal cancer progression and affect the efficiency of EGFR-targeted therapy. *KRAS, BRAF* and *PIK3CA* are frequently mutated genes in this pathway. A sensitive detection method for these gene mutations will be helpful for prediction and prognosis of targeted therapy.
Materials and Methods:
We established a panel of sensitive assays for detecting mutations in *KRAS, BRAF*, and *PIK3CA* genes by using peptide nucleic acid (PNA) probes and melting analysis.
Results:
The assay detected as low as 0.1% mutants in the wild-type background. The mutation spectrum was compared with that by conventional polymerase chain reaction (PCR) plus direct sequencing. We found that the established panel detected more mutations than conventional PCR plus direct sequencing. We have used the assay to detect mutations in colorectal cancer samples. The inconsistency of the two methods was possibly due to genetic heterogeneity of cancer tissues, which makes the ratio of mutant alleles lower than the detection limit of the conventional method.
Conclusion:
We suggest that our assay panel is a more proper assay in detecting gene mutations in the clinical laboratories.

P657
Histology-directed Expression Profiling and Imaging of Oral Cancer by MALDI-TOF Mass Spectrometry

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Backgrounds:

Squamous cell carcinoma (SCC) of oral cavity is an aggressive cancer and a high recurrence rate has been found after treatment, therefore an urgent need for better diagnostic methods for analysis of oral cancer in relation to prognosis.

Materials and Methods:

We examined surgically resected frozen specimens using matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-MS).

Results:

We found 25 clearly distinguished cancer spectral clusters as classifiers to classify images pattern I of neoplasia, pattern II of normal epithelium, pattern III of keratin and pattern IV of tumor environment/immunity. The potentials of image pattern I and IV to discriminate tumor from normal cell were highlighted. Image pattern I and III were characteristic of tumour invasion and metastases. Unique MALDI expression profiles in hyperplasia, mild dysplasia, moderate dysplasia, severe dysplasia, carcinoma in situ and cancer were associated with tumor progression.

Conclusions:

These results suggested that direct expression profiling and pattern imaging have the potential to supplement morphology and provide molecular detail to improve SCC diagnosis, treatment and prognosis.

P658
Cytokeratin 17 Expression in Areca-Associated Oral Squamous Cell Carcinoma

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Backgrounds:

Cytokeratin (CK)13 and 17 are known to be useful for the diagnostic markers for oral squamous cell carcinoma (OSCC). The aim of this study was to evaluate CK 17 expression on the various subsites and pathological parameters of OSCC, in addition to combined assessment of clinical risk factors: areca.

Materials and Methods:

Tissue microarray, immunohistochemistry, and western blotting were performed for surgically resected OSCC specimens.

Results:

A total of 94 cases showed that CK13 in normal epithelium and CK17 in cancer lesions were significance in various subsites and pathological parameters of OSCC. CK 17 was significantly expressed in well-differentiated OSCC compared to moderately/poorly differentiated OSCC, and correlation with not-buccal subsite without areca ($p<0.05$). Moreover, CK 17 was significantly higher expressed in cases of neck lymph node metastasis without extracapsular spread (ECS-negative) than those with extracapsular spread (ECS-positive) ($p<0.01$), indicating CK 17 expression correlated with the tumor differentiation of OSCC might have important implications for ECS of lymph node metastasis.

Conclusions:

These results suggested that CK 17 expression might be a high sign for the grading of OSCC involving subsites and areca chewing.

P659
Effect of H_2O_2-Induced Human Peripheral Blood Mononuclear Cells (PBMCs) Pretreated Toona Sinensis On Oxidative Damage

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Backgrounds:

Toona sinensis (TS) is used in Chinese traditional medicine and is believed to possess strong immunomodulatory property. In this study, we evaluated the effect of non-cytotoxic concentrations of aqueous leaf extracts of TS against H_2O_2-induced oxidative damage in human peripheral blood mononuclear cells (PBMCs).

Materials and Methods:

The PBMCs were obtained from healthy person by the BD vacutainer CPT™ Cell Preparation tubes with Sodium Citrate. For all experiments, PBMCs were pretreated with different concentration of TS extracts (0, 50, 75, 100, 150 μg/mL) for 30 min. before the treatment with 1.25 mM H_2O_2 for 2 hours. The assays include trypan blue exclusion assay, MDA assay, superoxide anion assay and GSH/GSSG assay.

Results:

We found that the LC50 of H_2O_2 for PBMCs was 1.25 mM for 2 hours. The supplementation of the TS extracts improved the PBMCs viability and there was a dose-dependent protection against H_2O_2-induced cytotoxicity. Besides, the supplementation of TS extracts significantly inhibited superoxide anion generation and MDA formation. Furthermore, the levels of GSH and GSH/GSSG ratio were decreased, and GSSG was increased after the treatment of H_2O_2; however they were recovered when the PBMCs were supplemented with TS extracts before the treatment of H_2O_2.

Conclusion:

In conclusion, Toona sinensis extracts might protect PBMCs from H_2O_2-induced oxidative stress by increasing GSH, and reducing lipid peroxidation and superoxide anion.

P660
Multiple broad-spectrum antibiotics used to induce the generation of vancomycin intermediate *Staphylococcus aureus:* A case study

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Backgrounds:

Staphylococcus aureus is a common causative agent of wide variety clinical infections, from local skin and soft tissue infections to deep seated abscesses and osteomyelitis, even more deadly infections such as life-threating septicemia and endocarditis. Currently, the majority of nosocomial infections of *Staphylococcus* aureus often showed methicillin /oxacilin-resistance. As the result, vancomycin has become the most commonly used drug for the treatment of MRSA (methicillin-resistant *Staphylococcus aureus*) infection. However, since 1996 the susceptibility to vancomycin has tend to diminished worldwide and clinical isolates including vancomycin-resistant *Staphylococcus* aureus (VRSA) and vancomycin-intermediate *Staphylococcus* aureus (VISA) were reported constantly. The inpatient of this study was from Chang Gung Memorial Hospital repeatedly infected with MRSA. Although variety of broad-spectrum-antibiotic treatment has been implemented, MRSA remained in patient's body system.

Materials and Methods:

Even worse, the antibiotic-resistance seemed to increase overtime. E-test, Staphylococcal Cassette Chromosome mec (SCC*mec*), Panton–Valentine leucocidin (PVL), and Multilocus Sequence Typing (MLST) techniques were used to elucidate whether drug susceptibility was related to genotypes among seven MRSA clinical isolates from this patient.

Results:

As the result, from the ST's point of view, these isolates belong to the same sequence type. Furthermore, from the MIC values of the E-test, the drug-resistance of these MRSA turned out to be stronger along the use of antibiotics.

Conclusion:

Linezolid can better inhibit *S. aureus* than vancomycin and daptomycin. As the result, from the ST's point of view, these isolates belong to the same sequence type. Furthermore, from the MIC values of the E-test, the drug-resistance of these MRSA turned out to be stronger along the use of antibiotics.

P661
ACE2 and MMP-9 Activity as Potential Biomarkers in Pleural Effusions

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Background:

Pleural effusion is common problem, but the rapid and reliable diagnosis for specific pathogenic effusions are lacking. Recent studies showed the renin-angiotensin system (RAS) and matrix metalloproteinases (MMPs) involved in the mechanism of pleural fibrosis and may be as potential biomarkers in pleural effusions diagnosis.

Materials and Methods:

The major components of RAS, MMPs and immune cytokines, including angiotensin converting enzyme (ACE), angiotensin converting enzyme II (ACE2), matrix metalloproteinases-2 (MMP-2), matrix metalloproteinases-9 (MMP-9), transforming growth factor-β1 (TGF-β1) and tumour necrosis factor-α (TNF-α) activities, were measured and compared in the patients with transudative and exudative effusions. The exudative effusions were come from the patients with tuberculosis, pneumonia and adenocarcinoma.

Results:

Increased ACE and equivalent ACE2 activities, resulting in a significantly increased ACE/ACE2 ratio in exudates, were detected compared to these values in transudates. MMP-9 activity in exudates was significantly higher than that in transudates. Advanced analyses showed significantly increased ACE and MMP-9 activities and decreased ACE2 activity in pleural fluid are features of pleural space infection in patients with pleural tuberculosis. However, the expression of TGF-β1 and TNF-α in pleural effusion were no difference with exudates and transudates. The results indicate that increased ACE and MMP-9 activities found in the exudates were mainly contributed from a higher level of both enzyme activities in the tuberculous pleural effusions.

Conclusion:

Interplay between ACE and ACE2, essential functions in the RAS, and abnormal regulation of MMP-9 probably play a pivotal role in the development of exudative effusions. Moreover, the ACE/ACE2 ratio combined with MMP-9 activity in pleural fluid may be potential biomarkers for diagnosing tuberculous pleurisy.

P662
Capsaicin-, Substance P-, and Histamine-induced Changes in Tracheal Venule Leakage and Blood Pressure in Spontaneously Hypertensive Sprague-Dawley Rats

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Backgrounds:

Intravenous (i.v.) application of a high dose of capsaicin (Cap), substance P (SP), or histamine produces formation of many endothelial gaps in the venules of tracheal mucosa, that results in an extensive plasma leakage in normotensive Sprague-Dawley (SD) rats.

Materials and Methods:

The present study investigated whether hypertension could have an influence on vasoactive agent-induced plasma leakage and blood pressure. The femoral artery of spontaneously hypertensive SD rat was cannulated for monitoring arterial blood pressure and femoral vein cannulated for injecting Cap (90 microgram/ml/kg, over 2 min), SP (3 microgram/ml/kg, over 20 sec) or histamine (2 mg/ml/kg, over 20 sec) for producing vascular permeability. The tracer dye was Evans blue (50 mg/ml/kg). Rats of control group received i.v. injection of Evans blue, over 10 sec, followed by vehicle of vasoactive drug. Five min after application of Evans blue and blood pressure recording, rats were perfused with saline and phosphate-buffered paraformaldehyde. Evans blue content in the airways, including trachea and main bronchi, was extracted with formamide and determined with an ELISA reader.

Results:

Venule leakage as expressed by Evans blue content was low in Cap-treated rats, while the degree of venule leakage remained high in SP- or histamine-treated rats. Application of Cap resulted in a triphasic change in arterial blood pressure but there was no significant difference between normotensive and spontaneously hypertensive SD rats. Application of SP or histamine resulted in a marked drop of arterial blood pressure in spontaneously hypertensive rats than that in the normotensive rats. The change in heart rate of normotensive rats after histamine application was higher than that of hypertensive rats.

Conclusion:

It is suggested that desentization of C-fiber neurons to capsaicin developed in the airways of spontaneously hypertensive SD rats, that led an decrease in vascular permeability. However, hypertension did not inhibit SP- and histamine-induced permeability in venule endothelium.

P663
Triptolide inhibits tumor function in Tumor Microenvironment

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Background:

Head and neck squamous cell carcinoma (HNSCC) has the high rate of recurrences and of advanced disease. Tumor associated macrophage (TAM) respond to the presence of stimuli in different parts of tumors with the release of a distinct repertoire of growth factors, cytokines, chemokines, and enzymes that regulate tumor growth, angiogenesis, invasion, and metastasis. Triptolide (TPL) is a Chinese medicinal herb which has been used successfully in treating inflammatory diseases. Here we determined whether TPL would lead to anti-cancer effect on tumor microenvironment of HNSCC.

Materials and Methods:

U937 cells were coculture with SAS cells in a noncontact system. The cytokines expression was detected by ELISA and cell proliferation was detected by Methylene blue. RNA levels were detected by PCR. Protein levels were detected by western blot. In vivo experiment used xenograft (NOD)/SCID mice.

Result:

In our study, TPL could inhibit the growth of SAS cell coculture with U937 cell in vitro and in vivo. TPL inhibited the invasion, migration ability and angiogenesis on SAS cell coculture with U937 cell. The cytokines expression of IL-6, IL-8, TNF-α were induced by coculture system and the TPL repressed the cytokines expression. The enzyme expression of COX-2 was increased when SAS cell coculture with U937 cell and TPL reduced the expression.

Conclusion:

These data indicates that Triptolide can suppress the invasion, migration ability, angiogenesis and cytokines expression in coculture system.

P664
Establishment of Human Dental Pulp Derived Induced Pluripotent Stem Cells (hDP-iPSCs) for Tooth Regeneration

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Backgrounds:

Defined sets of transcriptional factors can reprogram human somatic cells into iPSCs. The potential of iPSCs to develop into dental epithelial cells when coculturing with dental mesenchymal stem cells is explored in this study.

Materials and Methods:

Normal human teeth were collected from adults and splitted therefore. The pulp tissues were gently separated from the crowns and roots and then digested in a solution of collagenase type I and dispase. Single-cell suspensions were obtained by passing the cells through a strainer. Isolated dental pulp cells (DPCs) were seeded on dishes with medium, and then incubated at 37°C in 5% CO_2. Flow cytometry was used to confirm DPCs cell types. The expression pattern of endogenous stemness genes were measured in DPCs by reverse transcription polymerase chain reactions. *Oct4, Sox2, Klf4* and *Glis1* transcription factor genes were introduced into DPCs for the purpose of generating DPCs-iPSCs.

Results:

Flow cytometry data show that DPCs are CD34/CD45- and CD44/CD90/CD105+ cells, indicating their mesenchymal cell characteristics. DPCs also expressed an individual-specific endogenous stemness gene expression pattern, suggesting their heterogeneity in cell components. In addition, immunofluorescence staining showed that DPCs-iPSCs expressed stemness markers such as Oct4, Nanog, SSEA3, SSEA4, Tra-1-60 and Tra-1-81, indicating that DPCs are successfully reprogrammed into the pluripotent stage.

Conclusion:

In this study, dental pulp stem cells expressed mesenchymal cell markers and were successfully reprogrammed into iPSCs. Further study will explore their odontogenic ability.

P665
The Effects of Toona sinensis leaf extracts on renal cell carcinoma cells

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Background:

Tonna sinensis (TS) is a tranditional Chinese medicine and it has been reported to have anti-oxidant, anti-diabetic, anti-angiogenic, and anti-cancer potential. Many studies have demonstrated that TS inhibits cancer cell proliferation and cause apoptosis, but there are no studies concern the effects on renal cell carcinoma (RCC). In this study, the effect of TS-induced apoptosis was studied in cultured human RCC cell lines (786-O and A-498).

Materials and methods:

The seven crude extracts were prepared by aqueous and alcoholic (50% and 25%) extraction methods using standard protocol. The anti-proliferative effects of the TS extracts were evaluated by MTT assays. The apoptosis of the RCC cells were confirmed by annexin V-propidium iodide (annexin V-PI) staining. The migration and invasion of cancer cells were examined by wound healing and transwell assays.

Results:

All seven TS extracts reduced the growth of two RCC cell lines. Using annexin V-PI staining and MTT assay, we demonstrated that all seven TS leaf extracts (500 μg/ml) induced RCC cells apoptosis and inhibited cell proliferation. In addition, the wound healing and transwell assay revealed that all TS extracts (250 μg/ml) blocked the migration and invasion of 786-O and A-498 cells.

Conclusion:

These findings suggested that the extracts of TS leaf might have the chemoprevention potential to against human renal carcinoma.

P666
Autism-related gene *Dlgap2* mutant mice display abnormal olfactory phenotype

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Backgrounds:

Olfactory system, a relatively primitive brain region, plays an important role in the establishment of social behaviors in mammals. However, the molecular mechanisms regarding olfaction and social behavior are still largely unclear. Clinical research has suggested an association between DLGAP2, encoding a postsynaptic protein, and impaired social performance in patients of autism spectrum disorder (ASD). It is clear that Dlgap2 is highly expressed in the olfactory system in rodents, the roles of Dlgap2 in olfaction and social behavior can thus be elucidated.

Materials and Methods:

Using the cre/lox system, the Exon 6 of *Dlgap2* gene was deleted and *Dlgap2* knockout mice were subsequently generated. The structure and function of the olfactory system were examined with morphological and behavioral means, respectively.

Results:

In *Dlgap2* knockout mice, olfactory detection and discrimination were comparable to those in wildtype littermates. However, they demonstrated hyperactivity towards social odors. In the morphological aspect, the dendritic length in superficial pyramidal cells of the piriform cortex was longer in *Dlgap2* knockout mice while the spine density in mutants was lower.

Conclusion:

Our discovery revealed the role of *Dlgap2*, an autism-related gene, in dendritic development of neurons in the piriform cortex. Dendritic deficits in the olfactory cortex might disturb the detection or interpretation of social signals which might account for social odor-induced hyperactivity in *Dlgap2* knockout mice.

P667
Activation Galanin Receptor 2 Plays the Role in Mechanical Allodynia Following Lysophosphatidylcholine Treatment of the Median Nerve

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Backgrounds:

Galanin receptor 2 (GalR2) which is one of galanin receptors modulates neuropathic pain, but regarding its expression and function after median nerve injury is still lacking.

Materials andMethods:

In this study, we used lysophosphatidylcholine (LPC) injection into rat median nerve for inducing demyelination, then with immunocytochemistry, drug treatment and behavioral test to investigate the changes in GalR2 expression in the cervical 6 dorsal root ganglion (DRG) and its role in the median neuropathy.

Results:

In this study, we found that the percentage of GalR2-LI neurons in the C6 DRG peaked at 1 week after LPC treatment, also double labeling for NF200, ATF-3, VR1, Galanin and NPY were increased comparing to saline group, but decreased in for SP. These results implied that LPC treatment upregulated GalR2 expression in the A-type and injured DRG neurons further containing NPY. Moreover, we employed LPC treatment along with GalR2 agonist (AR-M1896) or antagonist (M-871) intraplantar application to examine their effect on median neuropathic pain and c-Fos expression in the operated side cuneate nucleus (CN). The mechanical allodynia level and the number of c-Fos-LI neurons in the M-871 group were dramatically attenuated, whereas those in the AR-M1896 group had increased compared to the saline group.

Conclusion:

These results indicated that activation LPC-upregulated GalR2 may be possibly via promoting NPY release to evoke c-Fos expression in the CN and to transmit LPC-induced tactile hypersitivity.

These evidences could provide a new therapeutic target for neuropathic pain after axonal degeneration.

P668
RANKL Gene Expression in Monocyte Increased by Silica to Induce Osteoclast Differentiation

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Backgr ounds:

Silica is commonly applied to biomaterials of bone tissue engineering and can be absorbed by osteoclast, but the relationship between silica particles and osteoclast remain unclear and biocompatibility and toxicity of these materials usually limit their applications. We investigated the bioeffect of silica particles on osteoclast using monocyte cell line of RAW 264.7 cells.

Materials and Methods:

In this study, the silica particles were washed 5 times in PBS. RAW 264.7 cells were treated with silica particles (0, 10, 30 and 50ppm) for 24hr, and evaluated the survival rate using MTT assay. The gene expression of receptor activator of nuclear factor kappaB ligand (RANKL) and reactive oxygen species (ROS) were measured. The effects of silica particles on preosteoclast differentiation were analyzed by flow cytometry. To observe the silica particles uptake, silica particles were labeled with fluorescein isothiocyanate (FITC), and the particles of silica-FITC distribution were counted in osteoclast.

Results:

Treatment with particles resulted in dose-dependently increase in ROS and preosteoclast number. The culture of RAW 264.7 cells indicated that silica was able to enhance gene expression of RANKL as well as to increase the differentiation into preosteoclasts. The flow cytometry data found that the percentage of preosteoclast differentiation was increased by silica particles. The uptake of particles associated with culture time and tartrate resistant acid phosphatase (TRAP) in osteoclast.

Conclusion:

The results suggest that silica may stimulate the differentiation of RAW 264.7 cells into preosteoclasts possibly via increasing the gene expression of RANKL induced by ROS.

P669
The Effect of Gender on Aging and Inflammation-Induced DA Neuron Death in C57BL/6 Mice

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Backgrounds:

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by the loss of dopaminergic (DA) neurons in the substantia nigra (SN). The mechanisms responsible for the loss of DA neurons in PD are poorly understood. Because DA neurons are highly susceptible to microglial activation and pro-inflammatory cytotoxic factors, it has been suggested that microglia activation predisposes DA neuron degeneration. Another characteristic of PD is gender differences. Epidemiological studies reveal that both incidence and prevalence of PD are 1.5–2 times higher in men than in women. The objective of this study is to investigate the effect of gender on aging and inflammation-induced DA neuron death.

Materials and Methods:

To determine the gender effect on normal aging, brains of male and female C57BL/6 mice at the ages of 3, 6 and 9 months were harvested to investigate DA neuron loss and microglia activation in the SN. To determine the gender effect on inflammation, mice of both sexes were challenged by a low dose of intra-peritoneal LPS (0.15 mg/kg) injection. The saline treated group served as control. The LPS-induced microglia activation, determined by the Iba-1+ area, and number of DA neurons were examined 24 h after the LPS administration.

Results:

Our data showed that the DA neuron loss was more prominent in the aged male mice than aged female mice. Although the degrees of microglia activation were increased in both genders of aged mice, the intensity of microglia activation was much more pronounced in male than in female mice. LPS-induced microglia activation was present in the SN of male mice but not in the female mice. These results suggested that the microglia in the male mice are more sensitive than that of female mice when exposed to inflammatory challenge.

Conclusion:

Although DA neuron loss and microglia activation in the SN are evident in both male and female C57BL/6 mice during aging, these changes are less dramatic in the female mice. The protective effect of female gender is ovary dependent.

P670
Compression reduces the transcortical projection of underlying cortex

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Background:

Meningioma is a common tumor that compresses rather than infiltrates the brain. It's usually diagnosed after symptoms occur. Its non-infiltrating nature lets it appeared non-threating and regularly results in delayed treatment. Using a rat epidural bead implantation model we found earlier that compression reduced the dendritic arbors and spines of somatosensory cortical pyramidal neurons in a matter of days and the reduction of dendritic spines was not reversible after decompression. This shows that the compressed cortex has quickly undergone structural remodeling before functional or behavioral deficits can be noticed. Alterations of the dendrites of pyramidal neurons imply that the compressed cortex changes its output. Here we asked whether the contralateral cortex was affected in case of unilateral cerebral compression since layer III pyramidal neurons are primarily contralateral projecting. In addition, we further asked whether this contralateral cortical effect could be reversed after decompression.

Materials and methods:

We used the same epidural bead implantation method that we developed earlier to focally compress the somatosensory cortex of rat on one side. The effects of short and long-term compression were investigated by allowing the bead-implanted animals to survive for a week and a month respectively before tracer application. To trace contralateral projection, the anterograde tracer miniruby was injected into the compressed cortex and allowed to survive for 10 days before been sacrificed for examination based on our preliminary studies that the tracer took time to transport to the contralateral cortex. To study the effect of decompression, rats of the 1 week or 1 month focal compression groups were decompressed for 2 weeks and then the anterograde tracer was injected into the previously compressed cortical area. Animals were sacrificed for examination 10 days after tracer application as stated before.

Results:

Compression for 1 week reduced the commissural projection of underlying cortical neurons. Sustained compression for 1 month reduced the projection further. Decompression of the short-term (1-week) compression animals resulted in near complete recovery of the commissural projection. However, only partial recovery of the contralateral projection was identified for the long-term (1-month) compression ones.

Conclusion:

Our results show that focal compression could affect contralateral cortex in a week through a reduction in the excitatory inputs received from the compressed cortex. Prolonged compression of the cerebral cortex for a month is sufficient to cause long-persisting and likely unrecoverable changes of the contralateral projection from the compressed cortex. Reduction of commissural projections will no doubt affect the coordination of the two hemispheres. This in addition is an alarming finding as meningioma is often unnoticed and left or chosen to be un-treated for months or years after it occurs. (Supported by NSC-101-2320-B-320-001-MY3 and TCIRP 101004)

P671
A stereological and cellular analysis on the effects of hydrocephalus on cerebral cortex and hippocampus

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Background:

Hydrocephalus, a result of oversecretion, obstruction of pathway, or malabsorption of cerebrospinal fluid, often causes abnormal dilatation of the cerebral ventricles and lead to mental retardation, poor fine motor skills, and memory deficits. Expansion of the ventricle compresses the brain and is likely accountable for the above-mentioned functional abnormalities. It appears to cause no apparent neuronal death in the cerebral cortex and hippocampus but the status of the surviving neurons remains largely unclear. Here we use the kaolin-injection method to induce hydrocephalus in rats and a special large area frozen section and mounting method to section the brain in situ with skull. Stereological reconstruction of the brain was conducted for subsequent analysis of the volume and surface area of cortex and hippocampus. In addition, we use intracellular dye injection method for studying how hydrocephalus affected the soma-dendritic features of the cortical and hippocampal output neurons, namely pyramidal neurons.

Materials and methods:

To induce hydrocephalus, Kaolin was injected into the cisterna magna of postnatal day 21 SD rats and survived for a week to develop into the acute phase of hydrocephalus. They were anesthetized and decapitated. The brains with skulls removed were immediately immersed in liquid nitrogen for sectioning using a large-area frozen sectioning and tape-transfer slide mounting technique in the coronal plane serially. Sections mounted on slides were stained and those from the anterior commissure to the caudal end of hippocampus were serially reconstructed in the 3-dimensional plane with Neurolucida® for estimating the volume and surface area of cerebral cortex and hippocampus. For intracellular dye injection, animals were perfused with half-strength fixative and the brain removed and prepared into 350-μm-thick slices. Layer III and V pyramidal neurons of the somatosensory cortex and hippocampal CA1 pyramidal neurons were injected with Lucifer yellow under visual guidance in an upright fluorescence microscope. The soma-dendritic arbors of the injected neurons were reconstructed 3-dimensionally and analyzed accordingly. Dendritic spine densities on these neurons were also analyzed at high magnification.

Results:

The brain was characteristically hydrocephalic with enlarged ventricles a week after kaolin injection. Stereological analysis shows that hydrocephalus reduced the volume of cerebral cortex and hippocampus to 80% and 77%, respectively. The surface area of cerebral cortex was however increased by 6% while that of the hippocampus reduced by 7%. Individual cell-wise, cortical and hippocampal pyramidal neurons had distorted dendritic arbors with reduced dendritic lengths. In addition, their dendritic spines also decreased. Western analysis confirmed that the reduction of dendritic spines represents reduced excitatory connection as PSD95, a postsynaptic glutamatergic postsynaptic marker, to these cortical and hippocampal output neurons.

Conclusion:

In short, using brain in situ frozen section technique we demonstrated for the first time that hydrocephalus affects cerebral cortex and hippocampus profoundly. It thinned and spread the cortex but compacted the mass of the hippocampus as expected from their intracranial location. It in addition shortened the dendritic arbors and reduced the dendritic spines on cortical and hippocampal output neurons. These are likely causes for some of the symptoms. (Supported by TCIRP 101004-04Y1)

P672
Micro-CT As a Tool in Evaluation of Fracture Healing by Rat Tibial Osteotomy

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Backgrounds:

To develop the rat tibial osteotomy as an animal fracture model on evaluation of fracture healing and test the micro computed tomography (Micro-CT) on measurement of callus formation and mineralization with better 2D image resolution (~9μm) and 3D fracture site reconstruction combined with quantitative analysis.

Materials and Methods:

Wistar rats over 300g were assigned to three groups: group I sham operation, group II tibia osteotomied with intact fibula, group III both tibia and fibula fractured. A standard simple closed transverse fracture was made at the proximal tibial shaft near the metaphysic and a 0.9mm Kirschner wire was inserted into the intramedullary canal for fixation. The fibula was fractured manually in group III. X-ray images were taken at 25, 40 days and animals were sacrificed after 40 days. The micro-CT system were used to obtain serial quantified 2D transverse images of osteotomied tibia and reconstructed to longitudinal 2D or 3D images. After CT scanning, samples were decalicified and processed for standard paraffin 4μm tissue sections, and then H&E stained. Data expressed means±sd were analyzed by ANOVA. Significance was defined at $p<0.05$ level.

Results:

Radiographically, all fractures showed forming of hard callus at 25 days, and bridging of the fracture line at 40 days. Few animals occurred distal tibial fragments due to deviation of K-wire axis that may not fit in curved rat tibia. Micro-CT measured callus volumes showed that the volume of hard callus was larger than soft callus at 25days in groups II & III and the cortical bone did formed at 25 days which may not easily determined in H&E sections. The volumetric bone mineral density (vBMD) of group III is significantly lower than group II, which is same as the group I at 40 days.

Conclusion:

Our research support the rat tibial osteotomy is a good animal model in evaluation of fracture healing with merits of less invasive surgical technique, good life quality after surgery, and low costs. Fixations with intramedullary wire and intact fibula have great influences on stability of tibia. The rotation of fibula fractured tibia is easily observed in micro-CT image which will prolong the duration of early phase of fracture healing. Poor fixation may cause delayed union, so it is necessary to control the fibula stayed intact as possible. Quantitative CT-base analyses of callus structure can provide quick, reliable metrics of callus mineralization of healing, especially temporal changes in 3D callus structure that traditional X-ray film and histomorphometric analyses can't offer.

P673
Study the Effect of N-terminal peptide of Protease-Activated Receptor-2 at Melanocyte Release Melanosome.

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Backgrounds:
Keratinocytes and melanocytes are located at the epidermis. Melanocytes produce melanosomes transfer to surrounding keratinocytes. The process decides the skin color and protects skin from UV damage. There are four hypotheses have been proposed, including: exocytosis, cytophagocytosis, fusion and membrane vesicle transport. Recently, a series studies used the method of microporous membrane filter-separated melanocytes and keratinocytes in co-culture system to demonstrate the hypothesis of membrane vesicle transport is more reliable. However, the regulations of melanosome movement between melanocytes and keratinocytes have not been well documented. The protease-activated receptor-2 (PAR-2) is a seven trans-membrane G-protein-coupled receptor expressed only on keratioincytes but not on melanocytes. PAR-2 is reported to be involved in melanosomes transfer through increased keratinocytes phagocytosis of melanosomes. PAR-2 can be activated through the cleavage of its N-terminal domain by trypsin or mast cell tryptase. This cleavage exposes a new N-terminal, which then acts as its ligand. In our previous studies, we confirmed that PAR-2 cleavaged N-terminal peptides might be the chemotaxin from keratinocytes to induce the melanosomes transfer process of melanocytes.
Materials and Methods:
In this study, RPMI 7951 melanoma cells were observed by electron microscopy and Fontana-Masson staining to verify whether the RPMI-7951 melanoma cell line is capable of releasing melanosomes. Then, RPMI 7951 cell were cultured with oligo-synthetic PAR-2 cleaved N-terminal peptide. The culture medium was collected to isolate pigment globules and measured the melanin content.
Results:
The Fontana-Masson staining showed that RPMI 7951 cell could produce melanosomes. And the microporous membrane cultured system also confirmed that RPMI 7951 melanoma cells could release melanosomes. Then, RPMI 7951 cells were cultured with oligo-synthetic PAR-2 cleaved N-terminal peptide for 72hr. The culture medium was collected and measured the melanin content. The data show that melanin content of RPMI 7951 cell treated with N-terminal fragment of PAR-2 were higher than untreated group.
Conclusion:
In the melanosome transfer process, recent studies show that melanosomes were released by membrane vesicle transport and the pigment globules were ingested by the keratinocytes. In our study, we demonstrated the RPMI-7951 melanoma cell line was able to release melanosomes through Fontana-Masson staining analysis and, conformed the N-terminal fragment of PAR-2 play a important role in melanocytes releasing melanosome. And further, this model system will be applied to test other drug or chemical.

P674
從缺

P675
The Role of Hepatic Vagal Nitroxidergic Neurons in the Pathogenesis of Cirrhosis and Hyperplastic Nodule Induced by CCl4 in Rabbits

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Backgrounds:
In this study, we investigated the expression of neuronal nitric oxide synthase (nNOS) and nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d), two specific enzymes for nitric oxide (NO) synthesis, in the pathogenesis of liver damage induced by carbon tetrachloride (CCl4) intoxication in the rabbit.
Materials and Methods:
We specifically studied the liver-innervated nitroxidergic neurons that originate in the nodose ganglion (NG), nucleus of solitary tract (NTS) and dorsal motor vagal nucleus (DMV) by means of Serum biochemistry, H&E stain, NADPH-d histochemistry, nNOS immunohistochemistry and densitometric measurement.
Results:
Our data from densitometric analysis showed that CCl4 resulted in a significant down-regulation of NADPH-d/nNOS in the NG, NTS and DMV neurons (45.75%, 60.13% and 57.09% decrease, respectively) in the acute liver injury stage at 6 weeks after treatment, but the NO synthesis was slowly restored in the three nuclei (31.42%, 49.64% and 48.90% decrease, persistently) in the chronic liver regeneration stage at 17 weeks. These findings were corroborated by serum biochemistry and hepatic histopathological examinations.
Conclusion:
It is suggested that the severe loss of NADPH-d/nNOS activity in the hepatic vagal nitroxidergic neurons may play a deteriorated role in the formation of massive hepatocyte necrosis and subsequent cirrhosis following CCl4 poisoning, whereas the moderate recovery of NO production may facilitate the development of hyperplastic nodule although it is thought to work in vain for the liver repair.

P676
The Mechanism between Proliferation and Differentiation in Cardiomyocyte

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Backgrounds:
Cardiomyocytes are traditionally viewed as terminal differentiated cells and being permanently withdrawn from the cell cycle. Recent studies showed that cardiomyocytes still have the ability to proliferate, but that the proportion of cardiomyocytes with proliferative activity is very low. The underlying mechanism for cardiomyocytes proliferation and differentiation remains as an enigma. The purpose of this study is to explore mechanisms underlying the inhibition of cell proliferation in cardiomyocytes.
Materials and Methods:
In present study, rat cardiomyoblast H9c2 cell line was used as a model and cells were cultured in 1% HS (horse serum) to induce the differentiation . Previous studies indicated that the expression levels of cell cycle regulatory protein, cyclin A2, decreased, and the differentiation marker, myogenin, increased after induction. From the literature, researchers suggested that ubiquitin-proteasome system may play an important role in the regulation of expression level of these proteins among the differentiation process in cardiomyocytes.
Results:
Treatment of proteasome inhibitor (MG132), a reversible and cell-permeable proteasome inhibitor, resulted in a decrease of myogenin and an increase of cyclin A2 in protein levels. Similar results were obtained when inhibiting the expression of myogenin. We also found that the proportion of cardiomyocytes at G2/M phase was also increased suggesting the cell cycle in progression.
Conclusion:
Taking these data together, we conclude that cardiomyocytes not entering the cell cycle may be modulated by the signal controlling between proliferation and differentiation.

P677
Knockdown Of TDAG8 Reduces Inflammatory Pain

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Backgrounds:
Chronic inflammatory pain results from the direct activation of nociceptors in the skin or soft tissues in response to tissue injury. The damaged and immune cells release inflammatory mediators such as proteases, ATP, proton to cause direct excitation or modulation of nociceptors. High local proton concentrations (tissue acidosis) is mainly responsible for inflammatory pain. Tissue acidosis results in direct excitation or modulation of nociceptive sensory neurons by activating proton-sensing receptors. These receptors are divided into proton-sensing ion channels and proton-sensing G-protein-coupled receptors (GPCRs). It was previously found that all four proton-sensing GPCRs, including OGR1, GPR4, G2A, and TDAG8, are expressed in pain-relevant loci, the dorsal root ganglia (DRG) and TDAG8 has increased expression in inflamed DRG. However, it remains unclear whether TDAG8 is directly involved in inflammatory pain.

Materials and Methods:
In this study, I used shRNA-mTDAG8-B1 to knockdown TDAG8 in vivo and in vitro to investigate the roles of TDAG8 in inflammatory pain.

Results:
The results show that knockdown of TDAG8 decreased TDAG8 protein expression and reduced intracellular cAMP levels and intracellular calcium increase. Decrease of TDAG8 expression completely inhibited acid-induced pain but partially inhibited inflammatory pain.

Conclusion:
TDAG8 is involved in acid-induced pain and inflammatory pain.

P678
Proteomic analysis of the protein profiles in the late stage of repair processes of SCI rat

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Acidic fibroblast growth factor (aFGF), acts as a potent neurotrophic factor, could stimulate the survival of neurons and re-growth of neurite in the injured spinal cord area. However, the molecular mechanism of protective effect of aFGF in vivo is still not clearly or fully understood. Previous studies showed that significant improvement in the locomotor behavior analysis resulted from of the aFGF-treated rats after contusive spinal cord injury (SCI).

SCI will initiate the different pathological changes along with time, it is therefore, we would like to propose that aFGF may play different role at different time course during the repair processes. Proteomic and bioinformatic approach were adapted to investigate the protein profile changes of the damaged spinal cord tissue of the SCI rats treated either with or without aFGF at 28 days after contusive injury. Result of proteomic analysis indicated that all these differentially expressed proteins were categorized into the function of oxidative stress, anti-apoptosis effect, neuronal transporters, and glucose metabolism, etc. Hence, the protective effect of aFGF at the time course came from a set of teamwork of multiple factors that may attenuate secondary injury for providing a better condition recovering from the damage.

In this study, our results provide a better insight into the SCI regeneration mechanisms in the late stage of repair processes of SCI rat. We hope that our experimental results could provide insight into the molecules basis of the repair process of SCI that may be useful for clinical application for the SCI patient.

P679
Screening of Anti-bacteria Herbs Deriving from Taiwanese Folk Medicinal Plants

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Backgrounds:
To find out new antibiotics from local plants' extractions those can against *Escherichia coli (E. coli), Klebsiella pneumonia (K. pneumonia) and Propionibacterium acnes (P. acnes).*

Materials and Methods:
We prepared crude extracts from seven types of plants used in traditional Chinese herbalism in *Southern Taiwanese, Vitis Amurensis, Artemisia argyi, Kyllinga brevifolia rottb, Hydrocotyle nepalensis Hook, Blumea laciniata (Roxb.) DC., Hibiscus rosa-sinensis Linn and Abutilon indicum (L.) Sweet.* The extracts were obtained by using the cold soaking process with acetone(Ace), ethyl acetate(EA), ethanol(EtOH) and hexane(Hex), respectively. The extracts were used to investigate their antibacterial activity by paper disc diffusion assay on E. *coli* ATCC 25922, *K. pneumonia* ATCC 700603, *P. acnes* ATCC 11827 and *P. acnes* ATCC 6919.

Results:
At the end of herbal extraction, 54 extracts were obtained. The results of the antibacterial tests showed that the Ace-, EA-, EtOH- and Hex- extracts of Artemisia *argyi, Kyllinga brevifolia rottb, Hydrocotyle nepalensis Hook and Blumea laciniata (Roxb.) DC.* were effective for *P. acnes* ATCC 6919, and the diameter of growth inhibition was 8.5-15 mm. Simultaneously, the Ace-, EA-, EtOH- and Hex- extracts of *Artemisia argyi, Kyllinga brevifolia rottb* and *Hibiscus rosa-sinensis Linn* were effective for *P. acnes* ATCC 11827, and the diameter of growth inhibition was 8.5-12 mm. The best antibacterial activity was the EtOH- and Ace-extracts of *Vitis* amurensis for P. acnes ATCC 11827, the diameter of inhibition zone were 18 and 16 mm, respectively.

Conclusion:
Our results indicated there was no herbal extracts have an anti-bacterial effect against E. coli ATCC 25922 and K. pneumonia ATCC 700603. But, 18 and 16 of 54 extracts were effective for antibacterial activity against P. acnes ATCC 11827 and 6919, respectively.

P680
Nanogold-based carriers for the drug delivery of butylidenephthalide into human brain glioblastoma cells

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Backgrounds:
The use of chemical synthesis, such as chloroauric acid (HAuCl4) as the carrier to deliver the gene or drug into cells have been widely used in previous report. However, there are some disadvantages, such as chemical reagent residues in the synthesis process and caused biological toxicity effect. Recently we have developed a novel nanogold-based carrier by physical synthesis method, while fabricated by building poly(ethyl glycol)(PEG) and conjugated with flourescein isothiocyanat (FITC) for carrying butylidenephthalide (BP) into human brain glioblastoma cells (DBTRG) to assess the effectiveness of drug delivery capacity.

Materials and Methods:
In this study, gold nanoparticles (AuNPs) were capped by poly(ethyl glycol) (PEG) and conjugated with butylidenephthalide (BP) via the reaction of 3(3,(dimethylamino)propy)carbodiimide hydrochloride (EDC). Conjugation of the BP onto AuNPs was confirmed by the UV-Vis spectroscope, Fourier Transform Infrared spectrometer (FTIR) and Scanning Electron Microscope (SEM) and Dynamic Light Scattering (DLS) analysis. Cellular biological function was examined by using MMT, immunofluoresence staining (IF) and uptake test.

Results:
A schematic illustration of AuNPs (≈ 5 nm diameter), coated with PEG to improve stability and conjugated with FITC for fluorescence. The size was increased to ~ 86 nm based on DLS analysis. We then transfected DBTRG cells with the AuNPs-BP in vitro. AuNPs-BP had significant cellular toxicity effect in DBTRG cells while compared to AuNPs alone treatment group. The exposure (transfection) time of 2~3 h was required for achieving a better uptake of AuNPs-BP by the DBTRG cells.

Conclusion:
DBTRG transfected with AuNPs-BP may provide more efficacious cell-based therapy for brain tumor yet a better understanding of the mechanisms regulating the cellular uptake and intracellular trafficking of AuNPs-BP is required.

P681

Relationship between stability of human Dmc1 filament and Dmc1-mediated homologous recombination

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In eukaryotes, Rad51 and Dmc1 are two important DNA recombinases responsible for homologous recombination, a key event of double-strand DNA repair in mitosis and DNA cross-over in meiosis. Because of restricted level of protein expression, Rad51 is thought to participate in both mitosis and meiosis, and Dmc1 is only in meiosis. In contrast to their expression differences, both Rad51 and Dmc1 share a high similarity in terms of their DNA binding, ATPase activity and catalyzing DNA exchange. Rad51 possesses an ATPase activity and it has been well documented that inhibiting Rad51 ATPase activity stabilizes Rad51 onto DNA and facilitates Rad51-mediated recombination reaction. In contrast to Rad51, it remains largely unknown regarding the functional role of Dmc1 ATPase activity in Dmc1-mediated recombination. We are motivated to decipher the correlation between stability of Dmc1 filament and activity of Dmc1-mediated recombination. Here, we used purified human Dmc1 (hDmc1) wild type and DK mutant or hDmc1 wild type in different ATP analogues to build up a serial of *in vitro* assays. Thus, we can mimic a situation of ATPase inhibition to evaluate the stability of hDmc1 on ssDNA. Interesting, unlike Rad51, the stabilization has no improvement for Dmc1-mediated recombination. Our recent progress towards understanding the role of ATPase activity on Dmc1-mediated recombination will be presented.

P682

HBx Enhances CD44v6-dependent Invasion through miR10b Regulated Ca^{2+} Homeostasis and ECM Degradation on HBx-induced Metastatic HCC Cell Lines

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Backgrounds:
Hepatitis B virus (HBV) is one of the main aetiological agents for hepatocellular carcinoma (HCC), which has an elevated mortality rate due to its high incidence of metastasis. The genome X of HBV that produces transcriptional trans-activating protein kinase was formly reported to be associated with hepatocarcinogenesis. Therefore HBx may contribute to the acquisition of metastatic properties of HCC by increasing their ability to bind hyaluronan acid (HA) through expressing different CD44 in the outer margin of the tumors. The current study aims to elucidate the role of HBx regulating CD44 variant during HCC metastatic processes.
Materials and Methods:
We used HBx-transfected HCC cell lines to confirm the role of HBx for HCC invasion via enhancing calcium pathway (calpain1/calmodulin/calcium receptor) and CD44v6 expression by mean of immunoblotting and ELISA assay. The metastatic ability of HBx-induced HCC cell lines was investigated using migration/invasion assay.
Results:
Our results confirmed that HBx is able to promote metastatic ability in HCC cell lines. Also, we found that there was an increased protein expression of calcium pathway and CD44 variant on HBx-induced HCC cell lines. Furthermore, it is also found that HBx epigenetic-regulats metastatic modification of CD44 variant and calcium homeostasis through the miR10b, which functions as a specific modulator in invasion, and in enhancing metastatic ability.
Conclusion:
We thus conclude that HBx appears to significantly promote HCC invasion through calpain1/ calmodulin modulation and CD44 variant, which might contribute to the metastatic ability of HCC. Additionally, HBx alters the expression and function of miR10b, which may be a key element for regulating ECM interaction and degradation on CD44v6-induced metastasis. Inhibition of miR10b might be a potential approach to arrest the progression of HBV-related metastasis.

P683

Strategy and analysis of microRNA regulation on syndecan-1 expression in breast cancer cells

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Backgrounds:
Syndecan-1 (SDC1) is one of the members in heparan sulfate proteoglycans and its expression is associated with breast cancer malignancy. The purpose of this study was to investigate the possible posttranscriptional regulation of SDC1 by microRNAs (miRNAs). The downregulation of miRNAs that led to upregulation of SDC1 protein expression was pre-assumed and the mechanisms were explored.
Materials & Methods:
The analysis of reported GEO databases (GSE7842) comparing normal human tissues and patient tissues for different miRNA expressions were performed. The RNA22-based algorithm was used to predict and screen the down-regulated microRNAs, which targeted at 3'-untranslated region (3'UTR) of SDC1 gene. A novel approach of multiplex PCR was used to validate the miRNA-target interactions in vitro. One of potential miRNA, miR-122, was validated for its regulation on SDC1 expression by molecular biology and biochemical approaches.
Results:
Comparing the miRNA expressions between the tissues from breast cancer patients and normal tissues, 28 miRNAs were significantly downregulated and 15 of them were predicted to target 3'UTR of SDC1 gene by bioinformatics tool - RNA22. These miRNA targets were further analyzed by novel multiplex PCRs to confirm the miRNA-3'UTR interaction in vitro. Of them, miR-122 (hsa-miR-122-5p) was shown to regulate SDC1 expression. Luciferase assay, miRNA overexpression, and miRNA sponge expression demonstrated the direct regulation of SDC1 expression by miR-122. The miR-122-mediated SDC1 downregulation affected breast cancer cell activities (proliferation and migration) and suppressed of MMP-9 expression.
Conclusion:
We provided the strategy to explore the miRNA-target interactions by combining bioinformatics analysis and multiplex PCR. Of these potential miRNAs, we showed miR-122 would be potential tumor suppressor genes for breast cancer cells through downregulation of SDC1 expression.

P684

Avicennia marina Leaf Extracts Inhibited Proliferation and Induced Apoptosis in Breast Cancer Cells

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Backgrounds:
The leaf and seed of Aicennia marina *Forssk.*(*A. marina*) are used in traditional medicine to treat skin diseases. The main goal of this study is to investigate the effect extract of Avicennia marina on anti-cancer
Materials and Methods:
The A. marina extracts were extracted with ethanol, Ethyl acetate or water. The cell viability was evaluated by MTT assay. The effect of *A. marina* extracts on the induction of apoptosis in breast cancer cells by DNA fragmentation assay and flow cytometry. After treatment with *A. marina* extracts at the indicated concentrations, cells were harvested and protein levels of caspase-8, caspase-3, and PARP were determined by western blotting.
Results:
The antitumor activity of *A. marina* extracts were evaluated in NIH 3T3, HBL100, AU565, BT483, and MDA-MB-231 cell lines. We found that ethyl acetate extracts from *A. marina* have higher anticancer activity on human breast cancer cells compared with other extracts. Further studies revealed that ethyl acetate extract from *A. marina* induced cell apoptosis by caspase-3 activation.
Conclusion:
The experimental results suggest that the important cytotoxic components could be isolated from ethyl acetate extract of *A. marina*. Further investigations are underway in this regard.

P685
Fungal Immunomodulatory Proteins LZ-8 and GMI Activate NLRP3 Inflammasome via Stimulating ROS Generation

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Backgrounds:

The inflammasome is a multi-protein complex which mediates the processing and production of proinflammatory cytokines IL-1β and IL-18. Various danger signals of microbial, endogenous, or environmental origins can stimulate the assembly of inflammasomes, resulting in inflammatory responses that can clear infections or, in some cases, cause diseases to the host. The NLRP3 inflammasome complex is composed of NLRP3, ASC, and procaspase-1, in which caspase-1 is autoactivated and cleaves IL-1β and IL-18 into mature forms of cytokines. LZ-8 and GMI are fungal immunomodulatory proteins (FIPs) derived from Ganoderma lucidum and Ganoderma microsporum, respectively. FIPs are known to stimulate the activation of dendritic cells and T lymphocytes, and in animals, FIPs exhibit adjuvant and antitumor functions. In this study, we investigated the immunostimulatory mechanisms of FIPs and found that LZ-8 and GMI can stimulate inflammasome activation and IL-1β production in murine macrophages.

Materials and Methods:

Peritoneal and bone marrow-derived macrophages (BMDMs) from C57BL/6, ASC$^{-/-}$, NLRP3$^{-/-}$, and caspase-1$^{-/-}$ mice were stimulated with LPS in the presence of absence of FIP co-treatment. At 6 h after stimulation, mRNA levels of NLRP3, ASC, caspase-1, and caspase-11 were analyzed by quantitative RT-PCR, and the production of IL-1β was analyzed by immunoblotting and ELISA. To determine the involvement of reactive oxygen species (ROS) in FIP-induced inflammasome activation, ROS production was measured by dihydrorhodamine 123, and BMDMs were stimulated in the presence of N-acetyl-cysteine (NAC).

Results:

LZ-8 and GMI stimulated IL-1β production in peritoneal macrophages and BMDMs, which was strongly enhanced when cells were co-stimulated with LPS. Compared with LPS stimulation, FIPs stimulated very low levels of IL-1β, NLRP3, caspase-1, and caspase-11 mRNA expression, suggesting that FIPs primarily function as a second signal for inflammasome assembly. FIP-stimulated IL-1β production in LPS-primed BMDMs was markedly attenuated when cells lacked the expression of NLRP3, ASC, or caspase-1, indicating that FIPs activate the NALP3 inflammasome. FIPs stimulated ROS generation in BMDMs, and blocking ROS production by NAC significantly inhibited FIP-induced IL-1β production.

Conclusion:

Our findings provide evidence that FIPs are new type of protein ligands that activate NLRP3 inflammasome via stimulating ROS generation. This activity may be responsible for FIPs' immunostimulatory functions and should be taken into consideration when using FIPs as immunomodulatory agents.

P686
An AMP kinase activator AKA1 suppresses inflammatory responses of RAW264.7 induced by lipopolysaccharide

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Backgrounds:

Activation of Adenosine monophosphate kinase (AMPK) has been reported to inhibit inflammatory responses induced by lipopolysaccharide (LPS) in vitro and in vivo. An AMPK activator AKA1 has been demonstrated to ameliorate inflammatory responses of injured skin. However, whether AKA1 possesses anti-inflammatory activity on activated macrophage, a main player in inflammatory responses, still remain sketchy. Therefore, the present study was aimed to investigate effects of AKA1 on LPS-stimulated murine macrophage RAW264.7 and the underlying mechanisms.

Materials & Methods:

RAW 264.7 cells were used as cell model. After treated with AKA1 at serial concentrations (0, 0.1, 0.5 and 1 μM) and followed LPS (1 μg/mL), the cells and the cultured medium were separately collected. Expression level of mRNA was analyzed by RT-PCR and real-time quantitative PCR (qPCR). Kinase activation was demonstrated by immunoblot using specific antibodies. Nitric oxide (NO) and reactive oxygen species (ROS) were determined by using Griess reagent and 2',7'-dichlorofluorescein diacetate (DCFDA), respectively.

Results:

AKA1 significantly suppressed mRNA levels of pro-inflammatory TNF-α, IL-1β, IL-6, MCP-1, iNOS and COX-2 in RAW264.7 cells stimulated with LPS. In addition, AKA1 also reduced production of NO and ROS by the stimulated cells. Further investigation showed that AKA1 reduced activation of Erk1/2 and mTOR but insignificantly affected phosphorylation of Thr172 at AMPKα and Ser79 at ACC.

Conclusion:

Our findings show that the AKA1 robustly inhibited expression of pro-inflammatory components and reduced production of NO and ROS, suggesting AKA1 possesses potent anti-inflammatory activity against LPS-induced inflammatory responses. Moreover, mTOR signaling is possible involved in the anti-inflammatory activity of AKA1.

P687
Sphingosine 1-Phosphate (S1P)-Induced COX-2/PGE_2 Expression via Pyk2 and c-Src-Dependent MAPKs/AP-1 Cascade in Human Tracheal Smooth Muscle Cells

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Backgrounds:

Sphingosine-1-phosphate (S1P) has been shown to involve in regulate cyclooxygenase-2 (COX-2)/prostaglandin E_2 (PGE_2) axis that are important inflammatory mediators in airway diseases. However, the mechanisms regulating COX-2 expression by S1P in airway inflammation remain unclear. Here we will establish the mechanisms underlying S1P-induced COX-2 expression in human tracheal smooth muscle cells (HTSMCs).

Materials and Methods:

HTSMCs were used in the study. To investigate the mechanisms of S1P-induced COX-2 expression, Western blot, RT-PCR, real-time PCR analyses, promoter luciferase assay coupled to use pharmacological inhibitors or transfection with siRNAs were performed. PGE2 release was detected by an ELISA kit.

Results:

First, we demonstrated that S1P induced COX-2 protein and mRNA expression, promoter activity, and PGE2 generation which were attenuated by the inhibitor of S1PR1 (W123), S1PR3 (CAY10444), Src (PP1), Pyk2 (PF431396), MEK1/2 (U0126), p38 (SB2021290), or JNK1/2 (SP600125) and transfection with siRNA of c-Src, Pyk2, p42, or p38α. Moreover, pretreatment with PP1 or PF431396 significantly inhibited S1P-stimulated p44/p42 MAPK, p38, and JNK1/2 phosphorylation. Pretreatment with AP-1 inhibitor (Tanshinone IIA) and si-RNA of c-fos or c-Jun significantly reduced the COX-2 expression induced by S1P. Pretreatment with W123, CAY, PP1, PF431396, U0126, SB202190, and SP600125 significantly inhibited the AP-1 activity and c-Fos expression in HTSMCs.

Conclusion:

We demonstrated that S1P-induced COX-2 expression and PGE2 generation is mediated through S1PR1/3-dependent Pyk2, c-Src/p42/p44 MAPK, JNK1/2, and p38 MAPK pathways, leading to activation of AP-1 in HTSMCs.

P688
A Novel Cell Penetrating Peptide for Liposomal Drug Delivery

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Background:

Cell penetrating peptides (CPPs) may facilitate cellular uptake of various molecular cargos including proteins, polypeptides, and small-molecular compounds. Many cationic CPPs can bind to glycosaminoglycans (GAGs) such as heparan sulfate (HS) and chondroitin sulfate (CS) located on cell surface by electrostatic interactions. Recently, we have identified a 10-amino acid motif derived from human eosinophil cationic protein (CPP*ecp*), which penetrates cells through association with GAGs clustering on cell surface.

Materials and Methods:

To characterize bronchial epithelial Beas-2B cell binding activity of CPP*ecp*, the key residues on CPP*ecp* region in recombinant maltose binding protein fused ECP (MBP-ECP) were individually replaced with amino acid of similar or opposite property through site-directed mutagenesis and assayed by cell-ELISA. Membrane strip assay was performed to identify specific lipids interacting with CPP*ecp*. Besides, *in vitro* cytotoxicity assay in lung adenocarcinomic A549 cells was carried out to evaluate enhancement of liposomal formulated drug (LFD) delivery in the presence of CPP*ecp*.

Results:

When Arg^{34} or Lys^{38} was point mutated into amino acid with similar or opposite property, Beas-2B cell binding activity of mutant MBP-ECP was lower than that of wild type. CPP*ecp* interacted with specific membrane lipids including PI3P, PI4P, PI5P, PI (3,4) P_2, PI (3,5) P_2, PI (3,4,5) P_3 and sulfatide, indicating that it also possessed plasma membrane binding activity. Besides, cytotoxicity of LFD in the presence CPP_{ecp} was higher than that of LFD along in A549 cells, suggesting synergistic effect of our CPP_{ecp}.

Conclusion:

Our CPP_{ecp} possessed not only cell binding activity, but also membrane lipid binding activity and enhancement in LFD effect by CPP_{ecp} might facilitate novel design for drug delivery.

P689

Intracellular and *in vivo* biodribution of a glycosaminoglycan-binding cell penetrating peptide

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Backgrounds:

Sulfated glycosaminoglycans (GAGs), heparan sulfate and chondroitin sulfate are the major components assisting in growth factor-receptor interaction. Previous studies revealed that blocking of heparan sulfate expression inhibited tumor progression including growth, metastasis, invasion, and angiogenesis on several cancer models *in vitro* and *in vivo*. We have recently characterized a dual-functional peptide derived from human eosinophil cationic protein (CPP*ecp*) NYRWRCKNQN, with GAG-binding and cell penetrating activities.

Materials and Methods:

Characterization of the interaction between sulfated GAGs and CPP*ecp* was investigated by fluorescence assisted carbohydrate electrophesis (FACE). Cellular binding assay was performed employing flow cytomtery after incubation with FITC-labeled CPPecp at 4 °C for 1 h. Beas-2B cells were treated with 10 μM eGFP-CPP*ecp* at 37 °C for 1 h, and the cell lysate was separated by cell fractionation. Western blotting was used to determine eGFP-CPP*ecp* in cytosol or endosome. To investigate the biodistribution of CPP*ecp in vivo*, eGFP-CPP*ecp* was intravenously injected into Balb/c mouse from tail vein, and harvested 1 and 3 h after administration for dissection and immunohistochemistry (IHC) staining with anti-eGFP antibody.

Results:

Heparan sulfate and chondroitin sulfate demonstrated extremely high binding affinity to CPP*ecp*, suggesting that sulfated group was critical for CPP*ecp* binding. In addition, screening of in vitro binding on various gastrointestinal cell lines demonstrated that CPP*ecp* bound to cells rich in heparan sulfate on the cell surface. Cellular binding activity of recombinant eGFP-CPP*ecp* on CT-26 cell line, an epithelial colon cell line appeared to be dose-dependent. In addition cellular biodribution of eGFP-CPP*ecp* was in endosome rather than cytosome. When eGFP-CPP*ecp* was intravenously administrated into Balb/c mouse, in vivo biodribution of eGFP-CPP*ecp* was determined mainly in lung, intestine, and colon tissues.

Conclusion:

Our novel eGFP-CPP*ecp* possesses high potential for *in vivo* molecular targeting to specific tissue in mouse model, which may facilitate development of a novel tool for practical application in translational medicine.

P690

Resistin-induced expression of SDF-1 through coordinated activation of p38 MAPK and NFκB in gastric epithelial carcinoma

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Backgrounds:

The stromal cell-derived factor-1 (SDF-1)/CXC receptor 4 (CXCR4) is involved in the carcinogenesis of human gastric cancer, where it stimulates angiogenesis and favors metastasis of tumor cells to distant organs. Obesity has been shown to be associated with the risk of tumor development and plays a key role in the development of gastric cancer. A limited number of studies have investigated the association of resistin and SDF-1 with gastric cancer. Herein, we investigated the molecular mechanisms by which resistin influences the expression of SDF-1 in gastric carcinoma cells.

Materials and Methods:

Real-time PCR and western blotting analyses were performed to clarify molecular alterations. The human gastric cancer cell lines were exposed to doses of resistin and SDF-1 expression and secretion was found to be increased. Inhibition of Toll-like receptor 4 (TLR4) by competitive antagonist inhibited resistin-induced SDF-1 expression. Pharmaceutical inhibitors and short hairpin RNA (shRNA) were used to demonstrate that the activation of p38 pathway is critical for resistin-induced SDF-1 expression mediated by TLR4.

Results:

Using promoter activity and transcription factor enzyme-linked immunosorbent assay, we found that such an effect correlates with induced expression of SDF-1 was mediated by NF-κB in gastric epithelial carcinoma. The results revealed that inhibition of p38 activation blocked SDF-1-induced expression and SDF-1 promoter activity in gastric cells. Inhibition of p38 activation also blocked resistin-increased NF-κB-DNA-binding activities by analysis of chromatin immunoprecipitation assays.

Conclusion:

In summary, we hypothesized that resistin-induced SDF-1 upregulation by activation of TLR4, p38 and NF-κB might explain a new role of resistin in obesity and gastric cancer.

P691

Anticancer Effects and Molecular Mechanisms of *Ganoderma tsugae* Extract on ErbB2- overexpressing Cancer Cells

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Background:

Ganoderma, also known as Lingzhi or Reishi, has been used for medicinal purposes in Asian countries for centuries. It is a medicinal fungus with a variety of biological properties including immunomodulatory and anti-tumor activities. This study aimed to verify the suppression effect and the underlying mechanisms of *Ganoderma tsugae* (GT), one of the most common species of *Ganoderma,* on ErbB2-overexpressing cancer cells.

Materials and Methods:

The quality control of extract of GT (GTE) was verified by a comprehensive PhytomicsQC, the proliferation test of ErbB2-overexpressing cancer cells by MTT assay, soft agar colony formation assay, and xenografted tumor-bearing nude mouse model *in vitro* and *in vivo*, the distribution of cell cycle by flow cytometry, and the expression profiles of relevant proteins by Western blot analysis and immunohistochemical staining (IHC).

Results:

We show that a quality assured GTE inhibited the growth of ErbB2-overexpressing cancer cells *in vitro* and *in vivo* and enhanced the growth inhibitory effect of antitumor drugs (e.g., taxol and cisplatin) in these cells. We also demonstrate that GTE induced cell cycle arrest by interfering with the expression of cell cycle regulatory proteins (e.g., cyclins D1 and E). Furthermore, it is also shown that the GTE-mediated suppression of cell growth involved the inhibition of the ErbB2 signaling pathway.

Conclusion:

This study suggests that GTE may be a useful adjuvant therapeutic agent in the treatment of cancer cells that highly express ErbB2.

P692

Dysregulation of Multiple Metabolic Pathways in Diet and STZ-treated Rats Revealed by Metabolomics Approach

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Metabolomics, defined as global low-molecular-weight metabolite profiling, is an emerging field in the post-genomic era. This platform relies mostly on modern LC-MS and nuclear magnetic resonance (NMR) spectroscopy as the tools. It provides top-down, unbiased, semi-quantitative (or even quantitative) information in biochemical pathways, network and their interactions. Using metabolomics approach, we have carried out an animal study of high-fat, high-fructose diet fed, and STZ-induced diabetes rats to look into the urine, plasma, and tissue metabolomes based on UPLC-TOFMS. Disturbances in amino acid catabolism (particularly in branched-chain amino acids, BCAAs), lipid metabolism (particularly in PUFAs, phosphatidylcholine and cholesterol metabolism and accumulation of catabolic metabolites), and impairment in coordination ranging from glycolysis, β-oxidation, and citric acid cycle were observed. From mild metabolic disturbances (high-fat and high-fructose feeding groups) to severe impairment (STZ-induced DM) in multi-metabolic pathways, this animal study concludes that impaired BCAA catabolism occurs in an early stage. It is followed by disturbed lipid metabolism in driving impairment in citric acid cycle and energy metabolism.

P693
Antibacterial Mechanism of Novel Cationic Antimicrbial Peptides against Multidrug-Resistant *Acinetobacter baumannii*

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Backgrounds:

Acinetobacter baumannii is a nonfermentative Gram-negative bacterium, which causes a wide variety of severe nosocomial infections including pneumonia, bacteremia, urinary tract infection and wound infection in immunocompromised patients with increasing frequency and high mortality rate. Moreover, its remarkable ability to acquire resistance against most of commercially available antibiotics has led to global threat to human health. Recent studies indicated that antimicrobial peptides (AMPs) are being evaluated as a promising solution to combat multidrug-resistance microbial infections.

Materials & Methods:

In our previous studies, we have designed and synthesized a series of novel cationic AMPs with high antibacterial activity and selectivity against a broad spectrum of Gram-positive and Gram-negative bacteria. In the current study, we seek to evaluate their potency against wild-type (wt) and several multidrug-resistant (MDR) *Acinetobacter baumannii* strains. Proteomic approaches were applied to reveal the differences in protein profiles between wt and MDR strains. MIC analysis was performed to evaluate the antibacterial efficacy of these AMPs against wt and MDR strains. Furthermore, cell infection experiments were conducted to verify the protection mechanism of AMPs against the MDR strains.

Results:

MIC analysis showed that our AMPs were potent against MDR strains (MIC values of 4-8 ug/mL), as compared to that of the wt (8-16 ug/mL). Two-dimensional gel electrophoresis was performed as triplicates in three independent experiments. Image analysis revealed several protein spots significantly altered among wt and MDR strains. These protein spots have been in-gel digested by trypsin and then subjected to LC-ESI-Q-TOF MS/MS analysis. Cell infection studies are now under investigation.

Conclusion:

In the current study, a series of novel cationic AMPs were confirmed to possess potent antibacterial activity against *Acinetobacter baumannii* MDR strains. These findings would provide support for future application of these novel cationic AMPs as potential therapeutic agents for treatment of MDR bacterial strains.

P694
The Generation Of *TNFAIP3* Deficient Zebrafish Model

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Backgrounds:

A20 (also known as tumor necrosis factor alpha-induced protein 3, *TNFAIP3*) is an intracellular ubiquitin-editing protein that plays a key role in the negative feedback regulation of NF-kB signaling in response to multiple stimuli. Moreover, A20 also regulates tumor necrosis factor (TNF)-induced apoptosis, inflammation and immunity. Recent genetic studies demonstrate, polymorphisms in the A20 gene locus have been identified as risk alleles for multiple human autoimmune diseases, such as Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus, psoriasis and type 1 diabetes. A20 also confirmed important tumor suppressor gene in several human B-cell lymphomas.

Materials and Methods:

In zebrafish, A20 gene is expressed in multiple tissues. We are interesting on building animal model to study A20-deficiency related diseases. In this study, we adapted transcription activator-like effector nucleases (TALENs) technology to induce error-prone repair that can result in insertion or deletion mutations (indels). Later the putative founders were screened by combinational methods of real-time PCR, high resolution capillary electrophoresis, high resolution melting assay and Sanger sequencing.

Results:

We used solid phase method to assemble TALEN pairs on targeting exon 3 of A20 gene. After injecting 100 ng of A20 TALEN mRNA, approximately 95% of the embryos developed normally. The *in vitro* surrogate reporter and *in vivo* activity assay showed our designed A20 TALEN pair is functional and can create indels at zebrafish A20 locus. We would like to introduce myeloid lineage-specific GFP line into A20 null background to see whether the inflammatory response is activated in A20-deficiency fish or not.

Conclusion:

Our study is the first report on creating A20-deficient animal model by using zebrafish. This A20-deficient lower vertebrate model is useful to study the pathological mechanisms of rheumatoid arthritis, psoriasis and understanding A20 gene function in the future.

P695
Roles of Tid1 and CHIP on Lipopolysaccharide Stimulated H9c2 Cardiomyoblast Cell Hypertrophy and Apoptosis.

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Background:

It is well known that lipopolysaccharide (LPS) not only participates in cardiac inflammatory response but also result in cardiovascular collapse and death during bacterial sepsis. Moreover, Tid1 plays a major role in preventing dilated cardiomyopathy (DCM). CHIP (carboxy terminus of Hsc70 interacting protein), a co-chaperone of Hsp70 and Hsp90 controls the function of Tid1, regulates protein folding-refolding and E3 ubiquitin ligase activity. Previous reports have indicated that, ASK1-CHIP-HSP70 complex played a critical role in inhibition of cardiomyocyte apoptosis and cardiac dysfunction. However the function of Tid1 and CHIP on LPS-induced cardiac apoptosis and hypertrophy remains unclear.

Materials and Methods:

H9c2 cardiacmyoblast cells were transfected with Tid1 or CHIP for 24 h and then treated with lipopolysaccharide for 12 h. To understand how Tid1 and CHIP regulate LPS-induced cardiac hypertrophy and apoptosis. The western blotting, Immunofluorescence, Actin stain and TUNEL assay were analyzed separately.

Results:

From the IHC experiment, we found Tid1 and CHIP protein were increased in different stage of human myocardial infarction tissues. Especially, CHIP was induced by LPS, Isoproterenol and TNF-alpha treatment. Our data suggest that Tid1 and CHIP may involve in cardiac healing and remodeling. After over-expression of HA-Tid1-S and/or HA-CHIP suppressed NFATc3, BNP, TNF-α, cytochrome c and caspase 3 protein level and thus reduced LPS-induced-hypertrophy and –apoptosis. But all the effects were further reversed by CHIP siRNA. Moreover, we even found that CHIP binds direct with NFATc3 and further enhanceed NFATc3 protein degradation via polyubiquitination.

Conclusion:

Our study reveals that CHIP might act as an E3 ligase of NFATc3 and mediates its proteasomal-degradation; which further inhibits LPS-induced hypertrophy and apoptosis in cardimyocyte cells. More studies using siRNA and inhibitors will be done to prove the above mentioned mechanisms.

P696
Anti-inflammatory effects of newly synthesized n-3 eicosatrienoic acid in LPS-activated murine RAW264.7 macrophages

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Backgrounds:

Eicosatrienoic acid (Δ11,14,17-20:3; ETrA) is a unusual naturally-occurring n-3 polyunsaturated fatty acid (PUFA) found mainly in fish oil. ETrA is originally elongated from α-linolenic acid (Δ9,12,15-18:3; ALA), and can also be metabolized to eicosatetraenoic acid (Δ8,11,14,17-20:4; ETA), eicosapentaenoic acid (Δ5,8,11,14,17-20:5; EPA) and juniperonic acid (Δ5,11,14,17-20:4; JPA). Although the metabolism of ETrA has been studied, there are few reports regarding how ETrA might affect inflammatory processes. The objective of this study was to synthesize ETrA from ALA-riched flaxseed oil, and then to determine the effect of ETrA and other n-3 PUFA on the n-6 PUFA composition and inflammatory response of murine RAW264.7 macrophages to lipopolysaccharide (LPS).

Materials and Methods:

ETrA was taken up, incorporated and metabolized to JDA by macrophages, and the proportions of both fatty acids increased in cellular phospholipids in a dose- and time-dependent manner.

Results:

The incorporation of ETrA into cellular phospholipids decreased the proportions of LA, DGLA and AA as well, and reduced the proportion of total PUFA and monounsaturated fatty acids (MUFA). When cells were stimulated with LPS, ETrA, ALA, EPA or DHA suppressed the production of nitric oxide (NO), prostaglandin E2 (PGE2), interleukin-6 (IL-6) and tumor necrotic factor-α (TNF-α), respectively. The modulation of NO and PGE2 was due, in part, to the modified expression of inducible nitric oxide synthase (iNOS) and type II cyclooxygenase (COX-2).

Conclusion:

This study shows that like other n-3 PUFA, ETrA can modulate the metabolism of PUFA and alter the responsiveness of macrophages to inflammatory stimulation.

P697

A Novel Cell Penetrating Peptide for Attenuation of House Dust Mite-Induced Asthma

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Background:

Asthma is a common chronic inflammatory disease characterized by reversible airflow obstruction and airway hyperresponsiveness. Bronchial epithelial cells are the initial cell type as a protective barrier against environmental factors of infectious diseases. They play an active role in antimicrobial defense and may release several cytokines and chemokines that modulate lung inflammation. Eosinophil cationic protein (ECP) is secreted by activated eosinophils and serves as a biomarker for asthma. Recently, we have identified a novel cell penetrating peptide derived from a unique heparin binding motif of ECP (CPP*ecp*).

Materials & Methods:

Regulatory effect of CPP*ecp* on inhibiting asthmatic airway inflammation was investigated by *in vitro* measurement of inflammation related gene expression profiles in bronchial epithelial cells (Beas-2B) by real-time PCR. In addition, BALB/C mice are sensitized and challenged by house dust mite (HDM) as an *in vivo* asthma animal model. Physiological functions including pause enhancement (Penh), lung histopathology, cell counts in bronchoalveolar lavage fluid (BALF), serum mite-specific antibodies in the absence and presence of CPP*ecp* were analyzed.

Results:

CPP*ecp* down-regulated gene expression level of a few inflammation related proteins, indicating that CPPecp played a critical role in regulating inflammatory response of bronchial epithelial cells *in vitro*. Furthermore, administration of CPP*ecp* in asthma mice showed significant reduction in airway hyperreactivity and decrease in asthma-related cytokine expression, suggesting that CPP*ecp* reduced HDM-induced airway inflammation *in vivo*. In addition, intranasal administration of eGFP-CPP*ecp* in vivo mainly accumulated in pulmonary bronchus, suggesting that CPP*ecp* possessed specific targeting to bronchial epithelia.

Conclusion:

Our data demonstrated that CPP*ecp* attenuated development of allergic airway inflammation and airway hyperresponsiveness *in vitro* and *in vivo*, possibly through inhibition of inflammation related protein expression in the inflammatory lung tissue.

P698

Molecular Cloning, Protein Expression and Activity Assay of Triterpenoid Tailoring Glycosyltransferases from Medicago truncatula, *Arabidopsis thaliana* and *Streptomyces antibioticus*

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Backgrounds:

To enlarge the diversity of triterpenes products produced from oxidosqualene cyclases (ERG7) mutants, and to explore the potential bioactivity for pharmatheutical application of unnatural saponins, the glycosyltransferases (GTs)-mediated glycosylations on diverse array of triterpene were investigated.

Materials and Methods:

The triterpene-glycosyltransferases genes from *Medicago truncatula, Arabidopsis thaliana* and *Streptomyces antibioticus* were designed and acquired by artificial synthesis with optimized codon for heterologous expression. The GTs were subcloned into various expression vectors and transformed to E. coli strains for protein expression and purification. The protein identities were configuation by MALDI-TOF MS/MS. The activity of GTs on various substrates was studied by HPLC-MS spectrum.

Results:

We have successfully cloned and expressed four GTs including UGT71G1 (*Medicago truncatula*), UGT73K1 (*Medicago truncatula*), UGT80A2 (*Arabidopsis thaliana*), and OleDA242V/S132F/P67T (*Streptomyces antibioticus*) in *E. coli* system. The homologous GTs were obtained via various chromatographic purifications. The activities of GT's against its respective native substrates were characterized by HPLC-MS. The activity assay and mass spectrum characterization of UGT71G1 showed that five quercetin-monoglycosides with different glycosylated position and one quercetin-diglycoside were produced. In parallel, a β-estradiol-tetraglucoside was produced by the glycosyltransferation of OleD-ASP.

Conclusion:

Four GTs were subcloned and functional expressed in E. coli, and two of these GTs possessed glycosyltransferase activities on quercetin and β-estradiol, respectively. In the future, the triterpene products isolated from (ERG7) mutants will be subjected to glycosyltransferases tailoring reactions and the produced triterpene saponin products will be evaluated for their biotechnology applications.

P699

A Study of Molecular Mechanism on VFT Extracts to Inhibit λ-carrageenan Induced Acute Inflammation in Mice

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Backgrounds:

Inflammation is involved in the pathogenesis of many diseases, including cancer, atherosclerosis and cardiovascular disease. Standard medical practices cannot effectively control or treat inflammatory diseases; this has contributed to increased use of natural products. This study was to investigate possible anti-inflammatory mechanisms of the VFT extract.

Materials & Methods:

The plant (VFT) belongs to *Vitaceae*. We extracted its ingredients by 70% ethanol. The anti-inflammatory effect was evaluated by λ-carrageenan-induced mouse paw edema(Vinegar et al.,1969).

Results:

The VFT_{EtOH} (100, 200 and 400 mg/kg) significantly decreased edema paw volume at 4th to 5th hours after λ-carrageenan had been injected. These results were indicated that the anti-inflammatory mechanism of VFT_{EtOH} may be due to decline the levels of NO and MDA in the edema paw through increasing the activities of SOD, GPx and GRd in liver. Additionally, VFT_{EtOH} also decreased IL-1*β*, IL-6, NF*κ*B, TNF-α, COX-2, and iNOS levels.

Conclusion:

This study demonstrated the possible mechanisms on the anti-inflammatory effects of VFTEtOH and provided evidence for the classical treatment in inflammatory diseases.

P700

A Novel Glycosaminoglycan Binding Peptide for Epithelial Cancer Targeting

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Background:

Glycosaminoglycans (GAGs) are linear polysaccharides, composed of repeating disaccharide units that link to the core protein as proteoglycans. They are modified at different sites along their lengths and structures on the plasma membrane and in the extracellular matrix. GAGs play multifunctional roles in cell growth, differentiation, morphogenesis, migration and bacterial/viral infection due to their anchor for GAG binding molecules in the cellular microenvironment. Accumulated reports regarding altered expression of GAGs in various cancers indicate the importance of GAGs as pharmacological biomarkers for diagnosis and progression. Recently a non-toxic GAG binding peptide (CPP*ecp*) derived from a core GAG-binding motif on human eosinophil cationic protein has been discovered and characterized.

Materials and Methods:

FITC-tagged CPP*ecp* was applied to demonstrate its binding preference on cancer cell and normal lines employing flow cytometry. *In vitro* tissue array analysis was in parallel used to characterize eGFP-CPP*ecp* targeting, heparan sulfate expression and chondroitin sulfate expression on lung epithelial cancer types. For biodistribution of CPP*ecp*, eGFP-CPP*ecp* was intravenously administrated into xenograft lung cancer-bearing mouse by tail vein and harvested 1 h post-injection for dissection and immunohistochemistry (IHC) staining.

Results:

Binding assay demonstrated that CPP*ecp* preferentially bound to cancer cell lines than normal cells by flow cytometry. *In vitro* tissue array analysis revealed that our CPP*ecp* highly recognized lung, colon and small intestine tissues with high expressions of heparan sulfate and chondroitin sulfate. Lung tissue array illustrate that CPP*ecp* highly recognized lung epithelial cancer types which typically expressed high level of heparan sulfate and chondroitin sulfate in parallel. *In vivo* assay showed that tail vein administration of eGFP-CPP*ecp* targeted to H460 and H1299 lung tumor bearing sites in xenograft mouse models after 1 h injection.

Conclusion:

Since lung cancer ranks the most cancer-related deaths worldwide in recent years and most people with early-stage lung cancer do not have any symptoms, our CPP*ecp* demonstrates high potential to be developed as a novel lung cancer-targeting agent specifically targeting cell surface GAGs for early diagnosis or prognostic purposes.

P701

Functional Identification and Fluorescence Analysis of Tryptophan Residues in H^+-pyrophosphatase of *Closteridium tetani*

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Backgrounds:

To identified the role of Tryptophan residues in H^+-pyrophosphatase of *Closteridium tetani*.

Materials and Methods:

In this study, three intrinsic tryptophan residues, Trp-75, Trp-365 and Trp-602 in H^+-PPase from *Clostridium tetani*, were utilized as internal probes to report specific local conformational disturbations by monitoring fluorescence quenching by N-bromosuccinimide and acrylamide. Functional role of Tryptophan residues was identified by site-directed mutagenesis and Trypsin proteolysis.

Results:

In this study, three intrinsic tryptophan residues, Trp-75, Trp-365 and Trp-602 in H^+-PPase from *Clostridium tetani*, were utilized as internal probes to monitor local conformational state of periplasm domain, transmembrane region and cytoplasmic domain, respectively. Upon the substrate analogue Mg-imidodiphosphate binding (Mg-IDP), local structural change prevents modification of tryptophan residues by N-bromosuccinimide (NBS) especially at position Trp-602. Following Mg-P_i binding, Trp-75 and Trp-365, but not Trp-602, were slightly protected against NBS attack, suggesting that only is catalytic region exposed to more hydrophilic environment and presumably ready for next catalytic cycle. The analyses of Stern-Volmer relationship and steady-state fluorescence anisotropy also indicate that local structure around Trp-602 exhibits higher solvent exposure and structural variations. Furthermore, Trp-602 was identified as an essential residue involved likely in stabilizing structure of catalytic region by site-directed mutagenesis and trypsin proteolysis analyses. The fluorescence of variants with only single tryptophan residue remained could be used as intrinsic probes for investigating structure/function relationship of H^+-PPase.

Conclusion:

This study shows that tryptophan of *Ct*H^+-PPase participates in stabilizing structure to perform proper functions by its bulky and hydrophobic side chain especially of Trp-602. The fluorescence of H^+-PPase variants with only single tryptophan could be also employed as intrinsic probe for further investigating structure/function relationship of this essential proton pump in many lethal infectious bacteria.

P702

Transcriptional Response of S-adenosylhomocysteine Hydrolase Genes from *Methanohalophilus portucalensis* FDF^{1T} under Salt Stress

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Backgrounds:

S-adenosylhomocysteine hydrolase (SAHH) catalyzes the reversible hydrolysis of SAH to form adenosine and L-homocysteine. The *Methanohalophilus portucalensis* FDF1^T could *de novo* synthesis betaine as osmolyte, through three steps S-adenosylmethionine-dependent methylation, to protect cell from salt and osmotic stresses insult. An efficient removal of betaine synthesis inhibitor, SAH is necessary for cell to maintain osmolyte betaine level while cell encounter the salt stress. Genes of bacterial/eukaryal type SAHH1 and archaeal type SAHH2 were located in betaine synthesizing gene cluster (*sams1-sahh1-gsmt-sdmt-adk-sahh2*) in strain FDF1^T. Transcriptional analysis of *Mpsahh1* and *Mpsahh2* were investigated to reveal their expression under salt stress.

Materials and Methods:

Early exponential phase cultures of strain FDF1^T grown at 2.1 M of NaCl containing defined medium were salt up-shocked (2.7 & 3.2 M) or down-shocked (1.2 M) at 37°C for 1 hour. Total RNAs were purified and RNA-seq, qRT-PCR and Northern hybridization were performed.

Results:

The transcriptomic analysis of betaine synthesizing gene cluster of strain FDF1^T revealed that all associated genes were up-regulated by increasing salt concentrations. Most profoundly, the transcriptional level of *Mpsahh1* was 6 to 400 folds higher than all salt stress response related genes. In contrast to the high transcriptional levels of the bacterial/eukaryal type *Mpsahh1*, the transcriptional levels of archaeal type *Mpsahh2* were about 2.16, 2.25, and 419.05 fold lower under salt up-shocked (2.7 M) condition with the quantization of Northern hybridization, qRT-PCR and RNA-seq, respectively.

Conclusion:

Transcriptional analysis results indicated the bacterial/eukaryal type *MpSAHH1* plays a major role in maintaining the proper cellular betaine level by efficient removal the inhibitor SAH.

P703

Fabrication of A Hybridization Microfluidics Chip for Pathogen Identification

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Backgrounds:

Rapid identification of causative pathogens is important for clinical diagnoses and treatment and to improve clinical outcomes. The conventional bacterial identification depends on the colonies growing on the cultured medium and examining their morphological and the expressed biochemical characters. The whole process costs around 4 to 5 days. Based on the bacterial 16S rRNA can be phylogenetically categorized their lineages to genus. We can artificially synthesize a bacterial specific probe on the basis of the well documented RNA database. A polymethyl methacrylate (PMMA) microfluidics device is fabricated for rapid specific probe-DNA hybridization for the purpose of bacterial identification.

Materials and Methods:

The hybridization device is made by PMMA sheets, where micro channels and a hybridization chamber are cast by a laser beam. The depth of cutting on PMMA sheet is controlled by varying laser beam moving speed and its power. Several pieces of PMMA sheet are used to form a 3D micro-channels geometry. These sheets are bound by UV/O3 light exposure at initial, then thermal binding by compressing the sheets with applied force. The depth of hybridization is designed to have a proper dimension that a nylon membrane is placed in the chamber properly, causing the microfluidics passing though fibers of the membrane.

Bacterial cells were directly inoculated in 50 μl PCR reaction mixtures. After 35 amplification cycles, 10 μl of PCR samples were directly loaded into the fabricated PMMA chip at speed 15 μl/min. In the following step the fluorescence labeled specific probe was injected into the chip. The high salt buffer was used to wash the non-hybridized probe out of the chip. The migration of the fluorescence labeled probe was recorded under fluorescence microscope.

Results:

Edwardsiella tarda PCR expressed 16S rDNA bound on the circumference of the chip-embedded membrane was confirmed by sybr-stain at initial. The fluorescence labeled probe ET996-HEX was followed to inject into the chip for hybridization. Then, high salt buffer comes in and squeezes the non-hybridized probe flow through from the rim of the membrane into the center outlet of the chip. The probe that caught by the bound DNA stayed on the periphery of the membrane. The emitted fluorescence on the border of the membrane clearly indicated that the hybridization was successful.

Conclusion:

The success of hybridization would implicate that the specific probe represented the corresponded genus or species of the infected bacteria. The time of whole operation process including PCR (3 hr) and PMMA manipulation (less than 1 hr) would perform within 4 hours. The new designed PMMA device could achieve the goal of rapid bacterial identification in comparison with conventional method (4-5 days)

P704

Therapeutic Strategies Targeting Tau Aggregation for Alzheimer's Disease

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Backgrounds:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with abnormalities in folding and accumulation of amyloid-β and tau-associated neurofibrillary tangles (NFTs). The aggregation of tau in AD correlates with the clinical progression of the disease and inhibition or reversal of tau aggregation may protect the affected neurons.

Materials and Methods:

Wild type and pro-aggregation mutant ΔK280 of repeat domain of tau (tauRD, Gln244-Glu372 of tau441 isoform, Khlistunova et al., 2006) was fused with DsRed and used to generate Tet-On 293 and SH-SY5Y cell clones as screening platforms. Inhibitors that retard or block ΔK280 tau aggregation were distinguished by increasing fluorescence on Tet-On 293 cells. For those compounds/herbs with putative aggregate inhibitors, IC50 cytotoxicity and the mechanisms of neuroprotection were determined.

Results:

Upon induction with doxycycline, red fluorescence in ΔK280 tauRD-DsRed 293 cells was significantly reduced compared to that in wild type cells. As a positive control, congo red increased red fluorescence more effectively in ΔK280 tauRD-DsRed 293 cells than in wild type cells. Without significantly reducing cell numbers, treatment of NC009-1, -2, -3, -6, -7 (indolylquinoline compounds), NTNU-003, -008 (GSK-3 inhibitor-like compounds), NH021, NTNU-043, -057, -059 (herbal extracts) resulted in significant increased fluorescence on Tet-On ΔK280 tauRD-DsRed cells. For SH-SY5Y cell clones, retinoic acid treatment generated cells resemblance to adult neurons. Fluorescent microscopy examination revealed that neurite outgrowth (including total outgrowth, processes and branches) was significantly reduced in ΔK280 tauRD-DsRed SH-SY5Y cells compared to that in wild type cells.

Conclusion:

We identified several aggregate inhibitors using ΔK280 tauRD cells. The neuroprotection mechanisms of the identified lead compounds/herbs are currently examined.

P705

Androgen Receptor Signal Promotes Cell Focal Adhesion through β-Integrin and Downstream AKT Phosphorylation in Hepatocellualr Carcinoma Cells

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Backgrounds:

Hepatocellular carcinoma (HCC) ranked 2nd cancer incidence in Taiwan with high morbidity and mortality. Our previous data showed androgen and androgen receptor (androgen/AR) signal promotes HCC growth, but suppresses cancer metastasis. However, the cellular molecular mechanisms are still in large. Extracellular matrix (ECMs) regulate cytoskeletal reorganization, growth, survival, and mobility of the cells. Integrins (ITGs) are transmembrane receptors that introduce the ECM extracellular signals into cells. The differentially expressed ITGs in normal and cancer lesions are recognized as hallmark during cancer development. The aim of this study is to investigate androgen/AR bimodal function in HCC progression through regulating β1-integrin and the downstream signals.

Materials and Methods:

HCC cells from wildtype ($AR^{+/y}$) and AR knockout (L-$AR^{-/y}$) tumor primary cultures, human HCC, SKhep1, stably transfected AR cDNA cells were introduced. Cell adhesion, migration, apoptosis, and β1-integrin small hairpin interfereing RNA were also carried out to test hypothesis.

Results:

Using HCC cells from $AR^{+/y}$) and hepatic L-$AR^{-/y}$ mice tumors, we found cancer cells lost of AR leads could reduce static cell adhesion on ECMs. While expressing AR in the human HCC cells, similar phenotype can be reproduced. Further mechanistic studies showed α5β1ITG mediates androgen/AR functions on cell adhesion in either primary mouse or human HCC cells. Furthermore, using shRNA β1ITG and small molecular signal blockers, we found androgen/AR-α5β1ITG signal in cell adhesion and migration, were through β1ITG-FAK signal to promote cell adhesion, yet, it could also go through β1ITG-AKT signal to mediated cell migration suppression.

Conclusion:

This report is the molecular and cellular level study regarding androgen/AR biphasic function on HCC progression.

P706

Chemoinhibitory Effect of *Hibiscus* Anthocyanins Extract on the Metastasis in B16 Melanoma Cells

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Backgrounds:

The character of Roselle is transliterated from English alphabet. *Hibiscus sabdariffa* Linnaeus or Roselle is a member of Malvaceae family. Roselle is an attractive plant believed to have originated from India, and is widely grown in the tropic, also cultivated in the Sudan and Eastern Taiwan. The main active ingredient in Roselle contained protocatechuic acid, anthocyanins, flavonoids and isoflavones. Cancer metastasis is one of the lethal causes in the late stages of cancer. Many papers indicate that metastasis of cancer cells often invade surrounding normal tissues by local or whole body circulation and lymphatic system to increase the difficulty of treatment. We could not stop the development of cancer; however, trying to block or delay tumor metastasis would be a potential direction of development. This study is observe the effect of anthocyanins isolated from roselle (*Hibiscus* anthocyanin, HAs) to inhibit tumor metastasis.

Materials and Methods:

The use of cell toxicity tests to observe that HAs inhibited B16-F1 cells growth or sensitivity in vitro. From the MTT data, we choice appropriate concentration (0-3 mg/ml) to process wound healing assay, Boyden chamber assay and Western blotting. Further investigation revealed that the anti-metastatic effect of HAs was evident in a C57BL/6 mice model.

Results:

The experiments are demonstrated that HAs exhibited an inhibitory effect on the migration ability with the increasing dose in B16-F1 cells. Additionally, data showed that the inhibition of tumor metastases and tumor growth induced by HAs treatment in a C57BL/6 mice model. The results implied anti-metastatic effect of HAs display in vitro and in vivo.

Conclusion:

Taken together, we suggested that the HAs can promote suppression of tumor metastasis and angiogenesis. Hopefully the preliminary results in this study could be applied in the future health food development.

P707

Identification of Protein Kinases Involved in HCV NS5A Phosphorylation

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Backgrounds:

Chronic hepatitis C virus (HCV) infection is worldwide pandemic often resulting in cirrhosis and liver cancer. A number of direct acting antivirals (DAA) target enzymatic proteins of HCV including nonstructural protein 2/3 (NS2/3), a serine protease and NS5B, RNA-dependent RNA polymerase. NS5A is a phosphorylation protein with no apparent enzymatic activity. Because NS5A is involved in many aspects of HCV life cycle, it is a good nonenzymatic antiviral target. Using protein mass spectrometry, we identified an NS5A phosphorylation site (named S1) in HCV-infected Huh7.5.1 cells. Alanine mutation of S1 phosphorylation site fully disrupts virus replication by reporter virus and reporter replicon assays, suggesting blocking NS5A phosphorylation at this site could be a potential therapeutic strategy for HCV infection. Our aim in the present study was to identify protein kinases involved in NS5A S1 phosphorylation.

Materials and Methods:

We used three algorithms, GPS, PPSP and NetPhosK, to predict potential kinases involved in NS5A S1 phosphorylation. Based on our predictions, we used W7 and KN93 to inhibit calmodulin and calmodulin-dependent kinase respectively in HCV (J6/JFH1 strain) infected Huh7.5.1 cell. . After the drug treatments, immunoblotting was performed to measure the phosphorylation level of S1 site using a phosphospecific antibody. In addition, quantitative RT-PCR was performed to measure HCV RNA level after drug treatment.

Results:

Two out of three prediction algorithms showed that Ca2+/calmodulin dependent protein kinase (CaMK) could be a potential kinase for NS5A S1 phosphorylation. Taking a conservative approach, We found that calmodulin inhibitor W7 reduced NS5A S1 phosphorylation in HCV-infected Huh7.5.1 cells in a dose-dependent manner as revealed by immunoblotting. In addition to reducing NS5A S1 phosphorylation, W7 also reduced HCV RNA level in HCV-infected Huh7.5.1 cells by quantitative RT-PCR assay. We next tested a CaMKII-specific inhibitor KN93. Like W7, KN93 also reduced NS5A S1 phosphorylation and HCV RNA levels in HCV-infected Huh7.5.1 cells in a dose-dependent manner.

Conclusion:

In summary, we found that NS5A S1 phosphorylation and HCV RNA level could be decreased by the calmodulin inhibitor W7 and the CaMKII inhibitor KN93.

P708

The Inhibitory Activity of *Zanthoxylum* armatum Extract Against the Filamentous Growth and Biofilm Formation by *Candida albicans*

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Backgrounds:

Biofilm-associated *C. albicans* infections are clinically relevant due to their high levels of resistance to traditional antifungal agents. In this study, we investigated the effect of *Z. armatum* extract on filamentous growth and biofilm formation by *C. albicans.*

Materials and Methods:

C. albicans were routinely propagated in yeast peptone dextrose medium at 30°C. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were measured using the broth microdilution method. For filamentous growth and biofilm formation, budding cells were harvested and suspended in RPMI 1640 at 37°C. After incubation for 4 h, the percentage of germ tube-forming cells was calculated based on optical microscopy observations. The biofilm formation was estimated by crystal violate staining and XTT assay after 24 and 48 h growth.

Results:

The extract of *Z. armatum* exhibited antifungal activity against *C. albicans* with a MIC of 1 mg/ml and MFC of 6.25 mg/ml. *Z. armatum* extract was significantly inhibited the yeast cells switched to hyphal growth at the sub-MIC concentration. In the present of 0.3 mg/ml *Z. armatum* extract, the germ tube formation was inhibited by 100% and 90% at 4 h and 24 h, respectively. The biofilm formation by *C. albicans* was also significantly reduced by *Z. armatum* extract in a dose-dependent manner and build up mainly with yeast form. In addition, *Z. armatum* extract disturbed the biofilm growth while not affecting the planktonic yeast growth at the same time.

Conclusion:

The results demonstrated that *Z. armatum* can significantly interferes with the morphological switch and biofilm growth of *C. albicans.*

P709

Comparison extraction solvents on inhibitory effects of free radical and lipid peroxidation on rat kidney/liver mitochondria of Ampelopsis cantonensis root and resveratrol

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Backgrounds:

Ampelopsis cantonensis root is a famous native Taiwanese botany for reducing pain, anti-inflammatory and hepatitis therapy. For the liver disease and kidney failure are prevalent in the world, in this study, the protection of ROS damage on mice kidney/liver mitochondria from five different solvent extracts of A. cantonensis were investigated.

Materials and Methods:

From the HPLC quality control analysis of each extract, resveratrol is one of the main polyphenol constituent. Lipid peroxidation (LPO) caused by oxidative stress is a general mechanism that oxygen free radicals cause tissue damage and using ferrous ion as a ROS inducer. Antioxidant capability of each extract and resveratrol were displayed on radical scavenging of DPPH, superoxide anion, hydrogen peroxide, xanthine oxidase, ferrous ion chelating, and ferric-reducing ability of plasma.

Results:

LPO inhibitory effects on kidney/liver mitochondria, the most efficiency is acetone extract (IC50 28.6±1.2 and 11.5±0.7 μg/ml, respectively) resveratrol and trolox (IC50 0.6±0.09 and0.51±0.06 ;7.4±0.4 and 9.03±0.9μg/ml, respectively).

The results showed all extracts having high activity to scavenge DPPH and superoxide anion. The high potency agents were water extract and resveratrol (EC50 4.72±0.41and 59.9±0.3; 17.62±0.51 and 148.9±12.4μg/ml respectively). Compared the polyphenol content and resveratrol among each extract, water extract is a high polyphenol content agent (phenolic content: 373.8±5.43 equivalent gallic acid μg/mg; Flavonoid content: 5.85±0.06 Rutin μg/mg; Flavonol content: 70.8±3.7 Epicatechin μg/mg). Otherwise, acetone extract is the highest resveratrol content :7.44 mg/g.

Conclusion:

Ampelopsis cantonensis and resveratrol were high potential LPO inhibitors on liver and kidney mitochondria injury. Conclusion the above evidences based suggested Ampelopsis cantonensis is a polyphenol rich natural bioresource of antioxidant and kidney/liver damage protective phytochemicals.

P710

Rapid Identification of Bacteria from Positive Blood Culture Bottles by Use of Matrix-Assisted Laser Desorption-Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) Fingerprinting

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Aim:

We evaluated the reliability of the Bruker Daltonik's MALDI Biotyper system in species-level identification of bacteria directly from blood culture bottles.

Material and Methods:

An Saponin-lysing procedure was used to prepare a bacterial pellet from positive blood cultures for direct matrix-assisted laser desorption-ionization time of flight (MALDI-TOF) mass spectrometry analysis. A total of 399 blood culture positive samples were analyzed by MALDI Biotyper system (MBT; Bruker Daltonics, Germany) and compared with VITEK 2 (bioMérieux, Marcy l'Étoile, France).

Result:

Identification results were concordant with those of the conventional culture-based method for 179(93.7%) gram-negatives and 81(64.8%) gram-positive species. Only 4 (21.1%) yeast species could be rapid identified by MALDI-TOF MS. Results were available in in less than 2 hours, suggesting that this approach is a reliable, time-saving tool for routine identification of bacteria species causing bloodstream infection.

Conclusion:

Identification was obtained for 93.7% of the pellets tested. Moreover, turn-around time is sharply reduced for communication of identification to the clinician. This fast and accurate method is promising.

P711

Functional and Mechanistic Comparisons of Two Unusual Deoxyhexose Reductases Likely Involved in NDP-Furanose Formation.

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Backgrounds :

Bioactive natural products are often decorated with deoxysugars in structure, therefore determining the target specificity and potency of the molecules. These glycosylated metabolites produced by nature exhibit a wide spectrum of biological activities, including antibiotic, anticancer and immunosuppressive activities, etc. Therefore, the origin and mechanism for the synthesis of these deoxysugars adapted by nature have become an important subject of research. Ketoreductases acting on sugar nucleotide diphosphates have been found crucial in maturation and/or structural scaffold formation of these sugar donors utilized for the glycosylation. In light of these points, from *Nocardia* species we have recently identified two unusual deoxysugar reductase genes likely involved in the formation of the activated furanoses, which may donate structurally unique furanose moieties as important building blocks found in the skeletons of bioactive secondary metabolites and/or microbial cellular glycoconjugates. Sequence analysis of the two genes revealed both contain $NAD(P)^+$/NAD(P)H binding motif but their exact enzymatic functions are ambiguous. To resolve exact roles of these two genes in NDP-sugar biosynthesis, we thus carry out functional and mechanistic comparisons of these two enzymes, which were cloned, expressed and purified in the heterologous host, *Escherichia coli.*

Materials & Methods:

Two unusual reductase genes are located in different regions of *Nocardia sp.* chromosome, and have been cloned into the T7-*lac* promoter system for high-level overproduction of His-tagged fusion protein. Screening and subsequent selection of optimal expression conditions were used for large-scale protein purification throughout Ni-affinity chromatography.

Results:

Both genes displayed high similarity to the streptose biosynthesis gene of streptomycin with N-terminal NAD(P)H binding domain classified as short-chain dehydrogenase/reductase (SDR) family. Our *in vitro* cell-free assay of the enzymes revealed that they indeed required the NAD(P)H as the coenzyme for reduction activity as traced by HPLC and UV-VIS spectrophotometer. Interestingly, both enzymes displayed relaxed substrate specificity towards NDP-ketohexoses of different (D/L-) configuration, whereas both showed different kinetic and product patterns. By coupling with other NDP-sugar modification enzymes cloned from different microorganisms, the *in vitro* tandem enzymatic assays on both enzymes have allowed us to reveal the enzyme activities, which further shed new light on molecular mechanism and specificity of the reductases as disclosed in many genomes of microbial species.

Conclusion:

The experimental results revealed both enzymes display reductase activity required for NDP-furanose biosynthesis, thereby serving as useful biocatalytic tools for utilization in generation of bioactive glycosides and/or cellular surface glycoconjugates. The information gained from this study can also allow us to resolve relationship between sequence and function. Subsequent structural determination by X-ray crystallography of these two enzymes is underway for further resolution of key residues responsible for the difference in kinetic and activity patterns.

P712

MicroRNA-3906 Maintains Fast Muscle Intracellular Calcium Homeostasis through Fine Tuning the Homer-1b in Zebrafish Embryos

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Background:

Previously we reported an intronic microRNA (miR), *miR-In300* or *miR-3906*, which locates at the first intron of zebrafish *myf5* gene, suppresses the transcription of *myf5* through silencing *dickkopf-related* protein 3 (*dkk3r/dkk3a*) during early development such as 16 hpf when *myf5* is highly transcribed (*Hsu et al.*, 2010). However, when *myf5* mRNAs are gradually reduced to undetectable level in mature somites at late developmental stage, *miR-3906* is still predominant. The line of these evidences give a clue that (1) *miR-3906* may have its own promoter which can transcribe *miR-3906* at late stage; and (2) *miR-3906* plays an unknown function in muscle development at late stage since Dkk3a is not detected anymore in muscle at that stage.

Materials & Methods:

1). Searching for *miR-3906* promoter by luciferase activity assay;
2). Searching for the putative target genes of *miR-3906* by Labeled miRNA pull-down (LAMP) (Hsu *et al.*, 2009) assay, microarray, and luciferase activity assay.
3). Using q-PCR, Whole mount *in situ* hybridization (WISH) and Western blotting for examining gene expression.
4). Detection of intracellular calcium concentration ($[Ca^{2+}]$) by Calcium Green-1 and Fura2-AM assays.
5). Observing the muscle structure of zebrafish by Phalloidin staining and transmission electron microscopy.

Results:

Firstly, we constructed plasmids in which the luciferase gene was driven by various lengths of upstream segment of *miR-3906*. After luciferase activity assay was performed in embryos, we found that a motif located at +303/+402 was able to activate luciferase activity, suggesting that +303/+402 *cis*-element possesses a promoter activity when *myf5* mRNA does not exist. Secondly, we performed LAMP assay from extracts isolated from embryos at late stage such as 32 hpf to find the target gene(s) of *miR-3906* and determined the biological function at late stage. We selected five muscle-specific genes out of 633 putative genes to examine which gene(s) might be the target of *miR-3906*. We constructed plasmids in which each 3' untranslated region (3'UTR) was ligated into the downstream of luciferase gene, and found that *miR-3906* enabled to silence reporter gene fused by *homer-1b*-3'UTR. Using WISH, q-PCR and western blotting, we confirmed that *miR-3906* negatively modulates the expression levels of homer-1b mRNA and *Homer-1b* protein. Thirdly, we found that the expression levels of fast muscle-specific gene *fmhc4* and calcium-sensitive gene *atp2a1* were increased, but slow muscle-specific gene *smhc1* was unchanged, in the *miR-3906*-MO-injected and *homer-1b*-mRNA-injected embryos. The downregulated expressions of f*mhc4* and *atp2a1* induced by excessive *miR-3906* or absent *homer-1b* could be rescued by adding exogenous *homer-1b* mRNA. Fourthly, we observed that the sarcomeric actin arrangement and Z disk were disorganized in the fast muscle of *miR-3906*-MO-injected and *homer-1b-mRNA*-injected embryos. They also exhibited abnormal swimming abilities. Furthermore, we found that the [Ca2+]i in the fast muscle cells of either knockdown of miR-3906 embryos or overexpression of homer-1b embryos was increased 82.9-97.3% higher than that of control embryos. In contrast, the $[Ca^{2+}]$i of the excessive *miR-3906* and absent *homer-1b* embryos was decreased 22-29.6% lower than that of control group.

Conclusion:

Taken together, we concluded that *miR-3906*, which is transcribed from its own promoter at late developmental stage, controls $[Ca^{2+}]$i homeostasis in fast muscle through fine tuning *homer-1b* expression during differentiation in order to maintain normal muscle development during embryogenesis.

P713

Arecoline Induces Apoptosis by Increase of Oxidative Stress and TNF-α in Human Acute T Leukemia Cell Line Jurkat

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Backgrounds:

Arecoline, an alkaloid-type natural product found in betel nuts, has been reported to have antitumor activity. However, it is not known whether it has any effect on human acute T cell leukemia. Therefore, this study was designed to investigate arecoline's effects on human T-cell leukemia cell line, Jurkat.

Materials and Methods:

Jurkat was treated with arecoline, and then examined cell viability detected by tryphan blue , cell cycle progress, reactive oxygen species (ROS) levels and mitochondrial membrane potential (ΔΨm) detected by flow cytometry, as well as DNA laddering detected by electrophoresis. In addition, the apoptosis-related proteins were examined by western blot, and their RNA levels were examined by real-time PCR.

Results:

Our results showed arecoline could inhibit Jurkat cell growth in a dose- and time- dependent manner, but it has no effect on the human peripheral blood mononuclear cells (PBMC). In addition, arecoline induced apoptosis of Jurkat as demonstrated by DNA fragmentation and by sub G1 cell population using flow cytometry. Western blotting showed Bcl-2 was down-regulated, while Bax, cytochrome *c*, caspase-3, caspase-8, endonuclease G were up-regulated after 48 h treatment of arecoline. A significantly increased generation of 89 KDa PARP cleavage fragment was found under this condition. Moreover, after 36 hour exposure of arecoline, intracellular ROS, TNF-α were increased, whereas mitochondrial membrane potential (ΔΨm) was lost.

Conclusion:

These results suggest apoptotic induction of arecoline may be through both extrinsic and intrinsic pathways in Jurkat cells. It shows arecoline has the potential to be against acute T cell leukemia.

P714

The Mechanisms Of High Glucose-Suppressed ATP-Binding Cassette Transporter A1 Expression

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Backgrounds:

Hyperglycemia is a risk factor for the development of both diabetes and atherosclerosis. The ATP-binding cassette transporters A1 (ABCA1) mediates macrophage cholesterol efflux via interacting with apolipoprotein-AI (apo-AI) and promotes reverse cholesterol efflux, which in turn prevents atherosclerosis. Although it has been reported that patients with type 2 diabetes had decreased ABCA1, the underlying mechanisms remain unclear. MicroRNA-33 (miR-33) has been demonstrated to enhance ABCA1 degradation and inhibit cellular cholesterol efflux by targeting to ABCA1 3'-UTR. This study aims to investigate the role of miR-33 in high glucose-suppressed ABCA1 expression and its underlying mechanisms.

Materials and Methods:

In clinical study we detected miR-33a expression in ~100 human serum samples provided from the department of internal medicine, Taichung veterans general hospital. Furthermore, we detected ABCA1 mRNA and protein stability in RAW264.7 macrophages by using western blot and RT-PCR. To investigate the effect of high glucose on miR-33-mediated ABCA1 expression, we used the experiments of ABCA1 3'-UTR reporter assay and quantitative RT-PCR.

Results:

In clinical studies, we found that serum miR-33a expression positively correlated with fasting glucose concentrations and negatively correlated with HDL-cholesterol levels in human subjects. In addition, high glucose treatment decreased the ABCA1 mRNA and protein stability, thereby decreased ABCA1 expression in RAW264.7 macrophages. We also found that the miR-33 expression was increased by high glucose treatment and subsequently bound to the 3'-UTR of ABCA1 mRNA.

Conclusion:

We provided the evidence that high glucose suppresses ABCA1 expression through activating miR-33 expression.

P715

Anchorage-independent growth of melanoma cells contributes to loss of invasiveness through syndecan-1 downregulation

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Backgrounds:

Anchorage-independent survival is one of key features in tumor metastasis. However, whether anchorage-independent growth contributed to specific gene alteration and metastasis features remained to be clarified. Syndecan-1 (SDC1) is one of the members in heparan sulfate proteoglycans and plays roles in various cellular activities. The purpose of this study was to explore the role of SDC1 in melanoma cell invasiveness associated with anchorage-independency, and the mechanisms involved.

Materials & Methods:

We generated the anchorage-independent melanoma cells by suspension culture. The adherent and suspended melanoma cell proliferation and invasion *in vitro/in vivo* were compared. Microarray analysis was used to identify the potential genes correlated with the loss of laminin-binding ability. The roles of *SDC1* were confirmed by *SDC1* gene overexpression and silencing. Cell signalings involved in SDC1 gene regulation were identified by western blot and qRT-PCR analysis along with the treatments of kinase inhibitors.

Results:

The suspended melanoma cells showed characteristics of slower proliferation and loss of invasive ability *in vitro*, but active lung metastases *in vivo*. Specific loss of laminin binding ability in suspended melanoma cells was observed, and downregulation of integrin α6β4 and SDC1 were revealed by microarray and qRT-PCR analysis. Manipulation of SDC1 level affected laminin-binding, cell migration ability, and matrix metalliproteinase-2 (MMP-2) secretion of melanoma cells, which explained the loss of invasiveness under suspension. Further investigation showed protein kinase C delta (PKCδ) was activated and responsible for SDC1 downregulation in suspended melanoma.

Conclusion:

We showed the anchorage-independency led to loss of laminin-binding and MMP-2 secretion, which were contributed by SDC1 downregulation through PKCδ activation.

P716

Protein arginine methyltransferase 1 facilitates erythroid differentiation via modulating p38α MAPK signaling

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Protein arginine methylation is a pivotal posttranslational modification involved in various cellular processes; however, its role in erythropoiesis is poorly understood. Erythropoiesis generates circulating red blood cells and is essential for maintaining vital body conditions. Despite extensive studies, the molecular events regulating erythropoiesis are still not complete.

This study showed that the increase in protein arginine methyltransferase 1 (PRMT1) levels significantly promoted erythroid differentiation in leukemic K562 cells as well as in human primary hematopoietic progenitor CD34+ cells. The shRNA-mediated knockdown of PRMT1 suppressed erythroid differentiation. The methyltransferase activity-deficient PRMT1G80R mutant failed to stimulate differentiation, indicating the requirement of arginine methylation of target proteins. This study further showed that a specific isoform of p38 MAPK, p38α, promoted erythroid differentiation, whereas p38β did not play a role. The stimulation of erythroid differentiation by PRMT1 was diminished in p38α- but not p38β-knockdown cells. PRMT1 appeared to act upstream of p38α, since expression of p38α still promoted erythroid differentiation in PRMT1-knockdown cells, and expression of PRMT1 enhanced the activation of p38 MAPK. The stimulatory effect of PRMT1 remained when MKK3, a upstream kinase of p38α, was knocked down, suggesting that PRMT1 might directly target p38α. Further analysis showed that PRMT1 was associated with p38α in cells by co-immunoprecipitation and that PRMT1 directly methylated p38α in in vitro methylation assays. Taken together, these findings unveil a novel link between PRMT1 and p38α in regulating the erythroid differentiation program and provide evidence suggesting a novel regulatory mechanism for p38α through arginine methylation.

P717
Characterization of the Anti-cancer Effects of BMVC Derivatives

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Backgrounds:

G-quadruplexes are special DNA secondary structures formed by non-Watson-Crick base-pairing of guanines. G-quadruplexes are widely distributed in chromosomes such as telomeres or promoters of some oncogenes. Because telomerase cannot utilize G-quadruplex structure as substrate to extend telomeres and formation of G-qrudplex structures at the promoter regions suppress the expression of several oncogenes, G-quadruplexes stabilizer has potential to be developed into anti-cancer agent. Previously we have identified small compound BMVC that stables G-quadruplex structures and inhibits tumorigenesis. Here we further characterize the anti-cancer properties of several BMVC derivatives. It is known that BLM is a helicase that catalyzes the resolving activity of G-quadruplex structures. The role of BLM in BMVC derivatives-induced senescence was also analyzed.

Materials and Methods:

Two series of BMVC derivatives were synthesized through the collaboration with Dr. Chang, Ta-Chau (Academia Sinica) and Dr. Chen, Chao-Tsen (National Taiwan University). The telomerase inhibitory activities of these compounds were analyzed by TRAP assays. Anti-proliferation and senescence-induction activities of these compounds toward cancer cells were analyzed using resazurin test and senescence associated-β-Gal assay, respectively.

Results:

We found several BMVC derivatives effectively inhibit telomerase activity. They effectively induced cancer cells into senescence. One of these compounds showed selective cytotoxic toward cancer cells. Moreover, knocking down BLM did not appear to affect the DNA damage response induced by BMVC derivatives.

Conclusion:

BMVC and its derivatives have potential to be further developed into anti-cancer drugs. BLM might not involved in resolving BMVC derivative-stabilized G quadruplex structures.

P718
Expression and purification of recombinant cell penetrating peptides employing starch binding domain

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Background:

Peptides play an important role in regulating most human physiological processes. They are small, easily optimized, and can be quickly investigated for therapeutic potential. However, relatively small size and lack of tertiary structure of most functional peptides make them susceptible to rapid protease degradation.

The 11.7 kDa N-terminal starch binding domain of Rhizopus oryzae glucoamylase (RoSBD) has been demonstrated to effectively adsorb onto raw starch and other soluble oligosaccharides. RoSBD has been developed as a purification tag in our laboratory, and several recombinant RoSBD-fused proteins have been successfully overexpressed and purified in bacteria, yeast, and mammalian cell expression systems.

In this study, we used RoSBD as a purification tag and fused at the N-terminus of two different cell penetrating peptides (CPP), CPPecp and KLA-TAT. CPPecp is a 10-amino-acids peptide that can penetrate into cells with no cytotoxicity; KLA-TAT is a 25-amino acid peptide which also contains cell penetrating activity but is toxic to cells.

Materials and Methods:

Escherichia coli Rosetta (DE3) was used to express SBD-CPPecp and SBD-KLA-TAT. Recombinant SBD-CPPecp and SBD-KLA-TAT were purified by amylose column chromatography. Functional assays were carried out by ELISA, cytotoxicity assay, and Western blotting analysis.

Results:

CPPecp and KLA-TAT fused with RoSBD tag could be successfully expressed in E. coli system. RoSBD improved the expression level and solubility of these peptides. Approximately 25 mg and 3 mg of SBD-CPPecp and SBD-KLA-TAT of 83.8% and 73.5% purity could be obtained from 1 L culture, respectively. Function of these peptides with or without SBD tag were examined by ELISA, cytotoxicity assay, Western blotting and also flow cytometry.

Conclusion:

The major contribution of this study includes successful expression of functional peptides by fusion with SBD-tag in E. coli expression system.

P719
Hydrogen-rich Water Against Aβ-induced Cell Death Through AKT/SIRT1/FOXO3a Modulating in Human SK-N-MC Cells

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Backgrounds:

Alzheimer's disease(AD) is the most common neurodegenerative disorder causes dementia. Several lines of evidences suggest amyloid-beta(Aβ) induced reactive oxygen species(ROS)leading neuronal cell death. Additionally, Silence information regulator 1(SIRT1)and Forkhead box protein O3a(FOXO3a)are new important signaling molecules factors which are ROS- modulating proteins. In recent studies, showed hydrogen-rich water(HW), containing a huge amounts of molecular hydrogen, known to be ROS scavenger, might also carry an antioxidant property. Besides molecular hydrogen can pass through blood-brain barrier, therefore, HW has a potential to become protective daily drink against brain injuries caused by oxidative stress. However, the mechanism of how HW inhibit cell death induced by Aβ still unclear. The aim of this study is to investigate whether HW protects cell death by modulating AKT, SIRT1 and FOXO3a.

Materials and Methods:

In this study, we first investigate the neurotoxicity induced by Aβ. Then, we measure the ROS scavenge ability of HW. Next we observed the apoptosis phenomenon between control and HW-treated group. We also observed the inhibition of caspase and activation of SIRT1 and P-AKT and P-FOXO3a.

Results:

Our results showed that HW inhibited ROS formation, inhibited cell apoptosis and activated SIRT 1, P-AKT and P-FOXO3a activities.

Conclusion:

We suggest that the mechanism of the protective effects of HW against cell death may be by the activation of vita-gene and also by activation of AKT/SIRT1/ P-FOXO3a signal pathway.

P720
The Pathophysiological Role of Hepatoma-Derived Growth Factor in the Development of Non-alcoholic Fatty Liver Disease

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Background:

Hepatoma-derived growth factor (HDGF), a nuclear oncoprotein highly expressed in both fetal hepatocytes and hepatoma tissues, has been demonstrated to play a profibrogenic role in hepatic fibrogenesis through aggravating TGF-β1 signaling. This study examined the role of HDGF in the development of non-alcoholic fatty liver disease (NAFLD).

Materials and Methods:

C57BL/6 adult male mice were fed with high fat diet for 4 wk and subsequent choline-deficient diet (CDD) for 8 wks to induce NAFLD. Liver sera and tissues were subjected to biochemical, histopathological, and molecular examinations. Adenoviral-mediated HDGF over-expression or knockdown was used to evaluate its possible prophylactic effect.

Results:

Elevation of total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C) in sera confirmed the successfulness of NAFLD induction. Histologically, oil red staining data showed a typical pattern of fatty droplet deposition in liver tissues. Quantitative PCR (qPCR) and Western blotting detection indicated that HDGF, TGF-β1, COL1A1, and PPAR-γ were up-regulated in steatotic liver after 6 wk of CDD feeding. Adenovirus-mediated HDGF gene modification did not affect AST, ALT, and TG serum levels in NAFLD mice. HDGF siRNA delivery significantly suppressed TC level. Oil read staining and morphometical analysis revealed that both HDGF over-expression and gene silencing suppressed the area of and the averaged size of the fatty droplets deposited in NAFLD livers, suggesting that HDGF might play a role in fatty acid synthesis and transportation, thereby ameliorating fatty liver retention in livers.

Conclusion:

These findings suggest that HDGF modifications ameliorate fatty acid deposit during NAFLD development and that HDGF is functionally involved in lipogenesis of liver metabolism. HDGF gene modifications may be regarded a therapeutic modality for NAFLD treatment.

P721
Gold Nanoparticle-Based RT-PCR Assays for Detection of *Japanese encephalitis virus* NS1

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Backgrounds:

Japanese encephalitis virus (JEV), a mosquito-borne neurotropic flavivirus, causes severe diseases of the central system in humans. Real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR) and capture IgM and IgG enzyme-linked immunosorbent assay (ELISA) are routinely recommend for diagnosis of *Japanese encephalitis virus*. Recent, gold nanoparticles have been demonstrated to improve the efficiency of PCR by its excellent heat transfer property. We describe the use of gold nanoparticle-based RT-PCR and real-time quantitative RT-PCR assays for detection of JEV.

Materials & Methods:

We tested the effect of gold nanoparticles on two different PCR systems, including conventional PCR, and reverse-transcription PCR (RT-PCR) assays for diagnosis in the acute phase of JEV infection.

Results:

Gold nanoparticles increased the amplification yield of the PCR product and shortened the PCR time compared to the conventional reaction. Gold nanoparticles improved sensitivity of JEV NS1.gene-specific application using PCR systems, showing that addition of 1.66 nM or 0.83 nM gold nanoparticles shortens, PCR cycles from 35 to 25 cycles. The gold nanoparticles-based RT-PCR assays was able to detect low levels (1–10 000 copies).

Conclusion:

The assays described here were simple, sensitive, and rapid approaches for detection and quantitation of JEV in tissue cultured samples as well as clinical samples.

P722
Apple Polyphenol Promotes Wound Healing in Ovariectomic Rats

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Backgrounds:

The decrease of estrogen in menopause is an important factor to cause skin aging in women. The skin wound healing would decelerate due to skin aging. In previous studies, many researchers focused on this issue to find the ways to promote wound healing in chronic diseases or aging. Apple polyphenol is proved to possess a good antioxidative effect, and it is beneficial for human. In this study, we address an issue to explore the effect of apple polyphenol in promoting wound healing in the ovariectomic rat.

Materials and Methods:

The rats were ovariectomized and convalesce for four weeks. The uniform burn wound on the rat back skin is artificial made in this study. The apple polyphenols was mixed with base cream to treat on the wound via dressing for ten days.

Results:

In histochemical stain, adipocyte under the skin wound increase, skin lesion appears and the hair follicle becomes rare and irregular. After treating with apple polyphenol for 10 days, the adipocyte showed decrease, skin repairs, and hair follicle recovers to line regularly, it shows a re-epidermalization and promote wound healing significantly as comparing to the control.

Conclusion:

Our present results show the apple polyphenol possesses a good effect in wound healing under menopause.

P723
Effect of *Houttuynia cordata* Extracts on Cancer Cell Motility and Invasion

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Backgrounds:

Cancer has been the leading cause of death in Taiwan and around the world. Chemotherapy is often used to treat cancer patients, but this treatment frequently has severe side effects and high cytotoxicity. Therefore, to identify a new drug or compound with low toxicity and high efficacy for cancer therapy is imperative and beneficial to the patients. Botanical extracts or compounds have been used in health care and as herbal medicine for a long time and exhibit a low toxicity. Among them, *Houttuynia cordata* (HC) has received much attention because its extracts have been wildly used for anti-virus, anti-bacteria, immunostimulant, diuretic, anti-cancer and anti-inflammation in Southeast Asia. In this study, we are investigating the effect of HC extracts on cancer cell motility and invasion, and identifying the molecular mechanisms in which the effective extracts of HC can inhibit cancer cell migration and invasion.

Materials and Methods:

To isolate the effective fractions of HC extracts, we used ethanol as a solvent to extract the botanical compounds from dry HC and fractioned the extract by HPLC. A549 (lung cancer), MDA-MB-231 (breast cancer), KM12-SM (colon cancer), PC3 and CWR22Rv1 (prostate cancer) cells were used as cell models to examine the effects of HC extracts on the invasion and migration of human cancer cells, using transwell assays.

Results:

The fractions 8 and 9 of *Houttuynia cordata* (HC-8 and HC-9) can effectually inhibit the cancer cell invasion and migration including A549, MDA-MB-231, PC3, CWR22Rv1 and KM12-SM cells. Interestingly, HC-8 had no significant effect on the motility of prostate epithelial PNT2 cells. Moreover, HC-8-inhibited A549 and PC3 cell motility was at least in part due to its inhibitory effect on decreasing the activities of FAK and Akt, indicated by their phosphorylation levels. Further, the protein levels of EGFR and HER2 were also decreased by the treatment. In addition, HC-8 was able to reduce the gelatin zymographic activity, down-regulate the gene expression of MMP-9, and differentially up-regulate the expression of TIMP1~4.

Conclusion:

HC-8 and HC-9 can inhibit the cell migration and invasion of human cancer cells, at least in part due to their inhibitory effects on EGFR, HER2, FAK and Akt, as well as suppression of MMP proteolytic activity.

P724
In Vivo Detection on Protein-Protein Interaction Between H^+-PPase and 14-3-3 Proteins

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Backgrounds:

Several 14.3.3 binding motifs have been identified by DNA sequence of H+-translocating pyrophosphatase (H+-PPase). This work is geared to explore the protein-protein interaction of H+-PPase by split-ubiquitin-based yeast-two hybrid.

Materials and Methods:

Integral membrane protein H^+-PPase is fused to C-terminal ubiquitin part on the bait vector and 14-3-3 proteins to N-terminal part on the prey vectors. When bait and prey come together, ubiquitin would be reconstituted and be recognized by protease. The transcription factor fused on C-terminal ubiquitin part would be released and move into nucleus of yeast to express reporter genes. The β-galactosidase assay suggested the interaction between 14.3.3 and H^+-PPase.

Results:

Some proteins of 14-3-3 family are identified as the interacting proteins of H^+-PPase. The expression level of reporter genes indicates the extent of interacting between proteins interested. Different truncated parts of H^+-PPase including several conserved regions are supposed to interact with these 14-3-3 proteins.

Conclusion:

The report suggests that split-ubiquitin-based yeast-two hybrid system was set up for seeking interacting protein of membrane-embedded H+-PPase. Various 14-3-3proteins have been screened as the interacting proteins to H+-PPase recognizing one or more putative residues on the enzyme.

P725

Conformational Changes at Entrance of Proton Transport Pathway in Proton-translocating Pyrophosphatase upon Substrate Binding Observed by Nanobiotechnique

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Backgrounds:

Homodimeric proton-translocating pyrophosphatase (H+-PPase) is indispensable for many organisms in maintaining organellar pH homeostasis. This unique proton pump couples the hydrolysis of inorganic ortho-pyrophosphate (PPi) to proton translocation across the membranes.

Materials and Methods:

H+-PPase consists of 14-16 relatively hydrophobic transmembrane domains (TMs) presumably for proton transport and hydrophilic loops primarily embedding a catalytic site. Several highly conserved polar residues locating at/near the entrance of transport pathway in H+-PPase have been found to be essential for proton pumping activity. In this investigation, single molecule fluorescence resonance energy transfer (smFRET) was employed to dissect the action at the entrance of proton transport pathway in homodimeric Clostridium tetani H+-PPase (CtH+-PPase) upon ligands binding.

Results:

The efficiency of energy transfer from donor and acceptor labeling on two introduced cysteine residues at TM6 of both subunits (219-219 pair) was decreased, while that at TM16 (642-642 pair) was increased upon binding with substrate analogue, imidodiphosphate (IDP). This result indicated that the presence of substrate analogue mediated two sites at entrance of proton transport pathway moving toward each other. Moreover, smFRET analyses on variants with mutation at residue rendering as the first proton-carrying residue identified that the structural action at TM6 and 16 on the entrance of proton transport pathway is presumably relevant to proton translocation of H+-PPase.

Conclusion:

The present smFRET study demonstrates that substrate analogue induces a possible squeezing at the entrance of proton pathway in homodimeric H+-PPase upon substrate binding. Further treatment with hydrolyzed product, Pi, brought no significant change in smFRET results from control, indicating the conformation of the pathway entrance presumably restores to its original state after hydrolysis reaction. Moreover, smFRET determination of R169K variants implicated that the squeezing at the pathway entrance is plausibly essential for the initial event of proton translocation. A working model is accordingly proposed for elucidating substrate mediated squeezing at entrance of proton pathway in H+-PPase. Notwithstanding, the significance of this initial event of proton transport in H+-PPase deserves further elucidations.

P726

Green alga *Chlorella* hydrogenase mutants that have high O_2 tolerance

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Backgrounds:

Green algae have a photosynthetic system similar to plants but can produce hydrogen by hydrogenase (HydA, encoded by hydA) using sun light under anaerobic condition, as O_2 is a strong inhibitor of HydA enzymatic activity. The catalytic H-clusters of the HydA are located in close proximity to O_2 accessible area, termed the gas channel. The mutated amino acid residues around the gas channel could narrow the channel and in turn lower the access of O_2. In this study, we attempted to mutate gas channel so that the mutated HydA can have high O_2 tolerance to enhance hydrogen production in *Chlorella sp*. DT (DT).

Materials and Methods:

The amino acid sequence of DT *hydA* was compared with those of other green algae. The residue of V265, around the gas channel of proximal to the active site, was replaced with W by site-direct mutagenesis. The *hydA* V265W mutantions were confirmed by sequencing. The DT cells were transformed with *hydA* mutation carriers of pHyg3-hydA-V265W. The total hydrogen extracted from the top of bottle was analyzed by GC. The measurement of hydrogenase activity was followed by MV assay.

Results:

The *hydA* transcript fragments of 0.7 kb were observed in DT-V265W mutants but not in the wild type by PCR analysis under aerobic condition. By western blotting analysis, using anti-HydA, the detectable signal of HydA, a protein band of 48kDa, was visualized in DT-V265W mutants but not in the wild type under aerobic condition. Under illumination and S-deprived conditions, hydrogen production of the wild type could be detected at very low O_2 concentration. The hydrogen production of the DT-V265W mutants would have been measuring at different O_2 concentrations under the same condition.

Conclusion:

The observations of *hydA* transcript by PCR and expressed HydA protein by western blotting in certain DT-V265W mutants under aerobic condition suggested that HydA could be expressed in these DT-V265W mutants. The hydrogen production and oxygen resistance of the potential mutants would still need further examination.

P727

Exploring The α-to-β Structural Conversion Mechanism for Mouse Prion Protein

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Backgrounds:

Prion diseases, also called Transmissible Spongiform Encephalopathies (TSE) are not only fetal but also infectious neurodegenerative disorders. The critical molecular event in the pathogenesis of prion diseases is the structural conversion of a normal cellular prion protein, PrPC, into a misfolded, infectious form, PrPSc. The overall structure of the prion protein transits from α to β-dominant state, giving rise to protein aggregation and the formation of toxic amyloid fibrils. Up to now, the structural transition mechanism of the prion protein still remains unclear. Recently, our lab found that the recombinant mouse prion protein (mPrP) in the disulfide-bond reduced state could spontaneously convert, in a neutral and non-denaturing condition, from native α-helical structure to β-rich conformers including β-form oligomers, amorphous aggregate, even amyloid fibrils. This finding provides us an opportunity to dissect the conversion process in details.

Materials and Methods:

To examine the structural state of three α-helices in mPrP in this structural conversion process, site-directed spin-labeling technique (SDSL), electron spin resonance spectroscopy (ESR), analytical ultracentrifugation (AUC), transmission electron microscopy (TEM), circular dichroism spectroscopy (CD), and small molecule fluorescence resonance energy transfer (smFRET) combined with single molecule technique were employed.

Results:

We found the spin-spin interaction in the α-state of N174R1/N181R1, in which both spins are located on helix2. And the distance between these two spins is 1.24nm. However, we could not obtain distance information in the β-state by using cwESR and the spin-spin interaction disappears indicating the helix2 is unfolded or extended after structural conversion. In addition, We observe the spin-spin interaction in α state of the S132R1/Q217R1 due to intensity decrease of ESR spectrum. And the distance between loop (S132R1) and helix3 (Q217R1) is 10.1 Å as we expected. However, we could not obtain distance information in the β-state by using cwESR. Seeking for other approaches, pulsed-ESR results demonstrate no regular distance existing in the β-state between those two spins.

Conclusion:

Helix 2 spin-labeling results suggested that helix 2 is intact in the α-PrP state and unfolded after conversion into β-PrP state. Indicating that helix 2 is involved in the structural conversion process.

Besides, the ESR data of S132R1/Q217R1 in the α-PrP state showed a 10 Å distance between these two spins which correspond to the previous result of NMR. Moreover, the FRET data of Atto-532 /Alexa-647 double-labeled S132C/Q217C showed a 4 nm distance between these two fluorophores. The conformational dynamics of this protein in different solvent conditions are on-going.

P728

Expression and purification of recombinant Alpha-1-acid glycoprotein employing starch binding domain

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Background:

Rhizopus oryzae glucoamylase (*Ro*GA) contains two functional domains which are connected by a linker region. A starch binding domain (*Ro*SBD) is located at the *N*-terminus, and a catalytic domain (*Ro*CD) is at the *C*-terminal end of *Ro*GA. *Ro*SBD processes high affinity to insoluble raw starch and soluble polysaccharides. It has higher affinity to amylose resin at pH 5~8, but lower affinity at pH 10~11. In this study, *Ro*SBD was used as a purification tag for recombinant protein engineering.

Alpha-1-acid glycoprotein (AGP) is an acute-phase protein mainly synthesized in hepatocytes. It has a low p*I* value of 2.8~3.8 and a high carbohydrate content of 45%. Three major functions of AGP, anti-inflammation, immunomodulation and drug binding have been reported. AGP not only binds drug but also has regioselectivity for chiral compounds. Different drug binding sites on AGP have been identified, and they are not completely separated but partially overlapped. In the drug binding pocket of AGP, His, Lys, Trp and Tyr play important roles in the binding process.

Materials and Methods:

RoSBD was fused at *N*-terminus of AGP. *Ro*SBD-AGP was expressed in *Pichia pastoris* system and purified by amylose column chromatography. AGP was cleaved from recombinant SBD-AGP and characterized by SDS-PAGE and Western blotting analyses. The binding constants of AGP to different drugs were measured by tryptophan fluorescence quenching assay.

Results:

A recombinant protein SBD-AGP has been successfully expressed in *Pichia pastoris* KM71. Approximately 13.1 mg AGP of 81% purity could be obtained from 1 L. SBD-AGP was able to bind chiral chemicals chlorpromazine hydrochloride, progesterone, and warfarin employing tryptophan fluorescence quenching assay, which may facilitate further process in large scale purification of chiral drugs.

Conclusion:

The major contribution of this study includes achievement of high level expression of SBD-AGP in *Pichia pastoris* system and identification of drug-binding activities of recombinant AGP.

P729

Design, Construction and Characterization of Multifunctional Fusion Proteins for Biofuel Production

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Backgrounds:

Lignocellulosic biomass from plants is a source of bioethanol. Two major polysaccharides, cellulose and hemicelluloses (xylan), in plant cell wall can be enzymatically degraded into fermentable glucose and xylose, respectively, by endoglucanase, exoglucanase and β-glucosidase for cellulose and by xylanase and β-xylosidase for xylan. Due to high cost of enzyme preparations as one of the challenges in bioethanol industry, consolidation of different activities into multifunctional enzymes represents a practical strategy.

Materials and Methods:

In this study, a thermophilic bifunctional endoglucanase/xylanase from *Clostridium thermocellum* (*Ct*Cel5E) was fused with a mesophilic β-glucosidase from *Clostridium cellulovorans* (*Cc*BglA) or *Trichoderma reesei* (*Tr*Bgl2 P172L) to improve efficiency and convenience for biofuel production. The purified fusion enzymes were assayed and their hydrolytic products were analyzed by thin-layer chromatography (TLC).

Results:

Fusion enzymes retained all the three activities (tri-functional enzyme) and showed an increase on their endoglucanase and β-glucosidase activity, and synergistically enhanced glucose production compared with mixture of the single enzymes. The fusion provides another advantage by preventing reformation of cellobiose from glucoses. When applied the fusion enzymes to a realistic substrate, alkaline-pretreated rice straw, the major hydrolytic products were completely glucose and small xylooligosaccharides. Furthermore, in the presence of β-xylosidase from *Sulfolobus solfataricus P2* (*Sso*3032), we acquired glucose and xylose as major products.

Conclusion:

These studies illustrate that the trifunctional fusion enzymes with a supplement of β-xylosidase can synergistically convert biomass into fermentable glucose and xylose. By providing efficiency and reduced processing cost in enzyme hydrolysis, this strategy is thus applicable to biofuel industry.

P730

Mulberry leave extracts inhibit diabetic nephropathy in a type 2 diabetes animal model

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Backgrounds:

Diabetic nephropathy (DN) is a major cause of end-stage renal disease (ESRD) and the mortality rate due to this disease is continuously progressing worldwide. 8 to 10% patients of type 2 diabetes will suffer from diabetic nephropathy. Here, we design this study to elucidate the role of mulberry leave extracts (MLEs), a traditional Chinese medicine as an anti-diabetic agent, in the pathogenesis of DN.

Materials and Methods:

100mg/Kg streptozotocin and 240mg/Kg nicotinamide were administered to C57BL/6J mice with high fat diet and 1%, 3% of MLEs. The plasma glucose concentration, body weight, oral glucose tolerance, insulin tolerance, albuminuria and renal echo were monitored every two weeks. After 8 weeks later, mice were sacrificed and the kidneys were assayed by HE stain and immunohistochemistry (IHC).

Results:

The results from the oral glucose tolerance (OGTT) and insulin resistance (ITT) tests, MLEs decreased the plasma glucose and improved the insulin sensitivity in type II mice. The results showed that MLEs ameliorated the renal atrophy in DM mice compared with the control group using renal echo detection. In addition, diabetes-caused glomerular atrophy was recovered under treatment with MLEs in diabetic mice. Besides, MLEs improved the MES-13 cells proliferation and ECM synthesis enhanced by high ambient glucose.

Conclusion:

Our results indicate that MLEs has beneficial effects in diabetic nephropathy.

P731

2-Methoxyestradiol Induces Apoptosis and Synergistically Enhances Cytotoxicity of Arsenic Trioxide in Human Urothelial Carcinoma Cells

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Backgrounds:

2-Methoxyestradiol (2-ME), an endogenous derivative of 17β-estradiol, has been reported to elicit anti-proliferative response in various tumors. In this study, we aim to investigate the effects of 2-ME on cell proliferation, cell cycle and apoptosis in human urothelial carcinoma (UC) cell lines.

Materials and Methods:

We used two high-grade human bladder UC cell lines (NTUB1 and T24). After treatment with 2-ME, the cell viability and apoptosis were measured by MTT assay and Flow cytometry (FACS) with Annexin V-FITC and PI labeling. DNA fragmentation was analyzed by agarose gel electrophoresis. Flow cytometry with PI labeling was used for cell cycle analyses. The protein levels of caspase activations, poly (ADP-ribose) polymerase (PARP) cleavage, phospho-histone H2A.X, phospho-Bad and cell cycle regulatory molecules were measured by western blotting. The effects of the drug combinations were analyzed using the computer software, CalcuSyn.

Results:

We demonstrated that 2-ME effectively induces dose-dependent cytotoxicity and apoptosis in human UC cells after 24 h exposure. DNA fragmentation, PARP cleavage and caspase-3, 7, 8, 9 activations can be observed with 2-ME-induced apoptosis. Decreased phospho-Bad (Ser136 and Ser155) and mitotic arrest of cell cycle in the process of apoptosis after 2-ME treatment was remarkable. In response to mitotic arrest, the mitotic form of cdc25C, phspho-cdc2, cyclin B1 and phospho-histone H3 (Ser10) were activated. In combination with arsenic trioxide (As2O3), 2-ME elicited synergistic cytotoxicity (combination index<1) in UC cells.

Conclusion:

We conclude that 2-ME significantly induces apoptosis through decreased phospho-Bad and arrest at mitotic phase in bladder UC cells. The synergistic antitumor effect with As2O3 provides a novel implication in clinical treatment of UC.

P732

Nelumbo nucifera Leave Extracts Suppresses 2-acetylaminofluorene-induced Liver Cancer in Rats

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Backgrounds:

Liver cancer is one of the most frequent human cancers worldwide. Therefore the liver cancer research gradually is also important in chemoprevention. The extract of *N. nucifera* leaves (NLE) have been reported to exhibit several pharmacological effects, including antioxidant, anti-HIV and hypolipidemic, anti-obesity and antibacterial activities. However, the role of NLE as an anticancer agent has not been established.

Materials and Methods:

The effects of NLE on the animal models of rat liver carcinogenesis using 2-acetylaminofluorene (AAF) diet were studied. Male wistar rats were given 0.03% of AAF and administered NLE at doses of 0.5, 1 and 2% for 24 weeks. Further we investigated the mechanism by western blotting, immunohistochemistry and enzyme activities in the liver of AAF-induced rat. And we also utilized flow cytometry, western blot, and immunoprecipitation assays to confirm the inhibitory effect of NLE on human hepatocellular carcinoma cell line.

Results:

In this study, we found that dietary administration of NLE had makerly suppressed AAF-induced liver cancer incidence in wistar rats. Our results showed that AAF treatment led to a significant decrease of body weight and an increase of liver/body weight and serum biomarkers for hepatic injury and hepatocarcinogenesis. Interestingly, the NLE supplement significantly lowered the liver/body weight and the biomarkers but did not affect the body weight. On the other hand, we observed the serum levels of some important enzymes in liver such as ALT, AST, α-FP and γ-GT which were decreased by NLE treatment after AAF-induced liver cancer. NLE increased detoxifying and antioxidant enzyme in AAF-induced liver and reduced liver of the damage. Further investigation revealed that a NLE supplement increased the expression of glutathione S-transferase-R and -μ, the level of transcription factor for protection from oxidative stress, Nrf2, and the level of downstream targets regulated by Nrf2, including glutathione peroxidase, superoxide dismutase-1, and catalase. Furthermore, we demonstrated that NLE could inhibit cell growth and induce cell death in human hepatocellular carcinoma cell by annexin V/PI stain. We presented evidence that NLE promoted AMP-activated protein kinase (AMPK/ACC) pathway, which is sufficient to induce autophagy. These results indicated that the NLE not only induced apoptosis, but also controls non-apoptotic programmed cell death that depends on the autophagy genes.

Conclusion:

These results confirmed that induced cell death was sufficient to elicit tumor regression following NLE treatment. Overall, our studies indicated that NLE has an antitumor activity and significant potential as a chemotherapeutic agent.

P733
RIG-I Plays a Critical Role in Negatively Regulating Stemness Properties in Hepatoma Cell Lines

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Backgrounds:
Hepatocellular carcinoma (HCC) is the third most common cause of death from cancer worldwide due to its high rate of recurrence. Cancer stem cells are considered to cause relapse and metastasis by eliciting new tumors. Retinoic acid-inducible gene I (RIG-I) has been identified as a pattern recognition receptor in innate immune defense for sensing intracellular viral RNAs, like hepatitis C virus which is one of the major risks of HCC. Thus, our study was to investigate the role of RIG-I in liver cancer stem cells.
Materials and Methods:
We used Huh7 and its RIG-I-mutant cell line in this study. Cancer stem cell markers expressed in these two cell lines were compared by western blot and reverse-transcription polymerase chain reaction. Sphere formation assay was used to investigate self-renewal ability of these two cell lines and cells transfected with full length, constitutively active, or dominant-negative RIG-I construct as well. And then we injected 1000 cells for each two cell lines into NOD/SCID mice subcutaneously to inspect their tumorigenicity in vivo.
Results:
In our present study, we found cancer stem cell markers exrpressed higher in the wild-type Huh7 compared with the RIG-I-mutant cell. We also observed solely RIG-I-mutant cells possessed the self-renewal ability. After transfection of dominant-negative mutant of RIG-I to Huh7, the sphere-forming ability was rescued as expected; and RIG-I-mutant cell transfected with full length or constitutively active construct showed suppressed ability of forming sphere. In addition, the RIG-I-mutant cells formed tumors in our mouse model whereas the wild-type cell, Huh7, showed no tumors formed.
Conclusion:
Our study revealed the potential of RIG-I to suppress the stemness properties in the HCC cell lines. In terms of these results, RIG-I may provide a new possible remedy target of liver cancer.

P734
Evaluation of Bioactivities on Spine/Pedicellae Toxin Extracted from Taiwan Common Sea Urchin Species

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Backgrounds:
Sea urchin is a high diversity species which belongs to the phylum of Echinodermata, often causes penetrated injury to animal. According to the morphology, the sting can be divided into spine and pedicellae. This research aimed to investigate the potential toxic effect of Taiwan's common sea urchin species.
Materials and Methods:
Different species of sea urchins were collected by marketing and SCUVA diving during June 2009 to July 2011. Sea urchins were determined by the appearances or by direct-sequence analysis of 16S DNA with the primer set 16Sech5/16Sech3. Six different species, *Tripneustes gratilla* (TG), *Toxopneustes pileolus* (TP), *Diadema savignyi* (DS), *Coelopleurus maculates* (CM), *Anthocidaris crassispina* (AC) and *Eucidaris metularia* (EM), were used here to extract spine/pedicellae toxins. Briefly, approximate 5 g spine/pedicellae were homogenized and extracted by ddH_2O in 4oC. After 24 hr, the extracts were centrifuged in 10,000g in 4 oC and the supernatants were collected which was considered as crude toxin extract and stored at -80 oC for further examinations.
Protein concentrations of crude toxin extracts were determined by BCA protein quantification method. Then, crude toxin extracts were tested for mouse bioassay, haemolytic activity and cytotoxicity (human osteogenic sarcoma, MG63). Further, we used AnnexinV to determine the apoptosis/necrosis stage of MG63 cells affected by each extract.
Results:
We successfully amplified 16S DNA genes by the primer set 16Sech5/16Sech3. By direct-sequence analysis, 6 species were identified. The protein concentrations of crud toxin protein were from 0.09 to 4.10 mg/ml. In mouse bioassay, no animal was sacrificed in this experiment, but in the TG and TP group, mouse showed the symptoms of uncomfortable, slow moving and trembling. Only TG group showed slight haemolytic activity in this experiment. In cytotoxicity test, TP showed cytotoxicity to MG63, other group showed non toxic effect in this experiment. Further, we examined the potential pathway of different extracts effect on MG63 by AnnexinV and result indicated TP extract stimulated MG63 cell to necrosis.
Conclusion:
Our result indicated crude toxin extracts from Taiwan common sea urchin species showed slight haemolytic activity toward mouse blood cell. The species TG and TP, which belong to Toxopneustidae showed toxic effect on ICR mouse and TP also showed cytotocicity to MG63. Overall, the sea urchins in Taiwan were with slight toxicity, and the toxicity effect from TG and TP were still under investigating.

P735
Effect of Penconazole, Propiconazole and Triadimefon on Androgen Receptor with Whole Rat Embryo Culture

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Backgrounds:
Penconazole, propiconazole, and triadimfefon were most common triazole pesticides in Taiwan. They were developed to inhibit the ß-tubulin assembly in mitosis, it is likely to disturb the synthesis of steroid hormone in mammals. A few reports showed that triazole chemicals antagonized the aromatase, which transfer testosterone into 17ß-estradiol in mammals.
Materials and Methods:
This study aimed to investigate the effect of these three pesticides on androgen receptor (AR) in whole rat embryo culture (WREC) on gestation day (GD) 10.5. The concentrations of WREC were pencoazole, 0.004, 0.008, 0.016 ppm; propiconazole, 0.005, 0.01, 0.02 ppm; triadimefon, 0.005, 0.009, 0.019 ppm. The culture period was 48 hours. After evaluation of embryo development it was fixed in formalin or kept in HBSS for immunohistochemistry (IHC) and Western blot (WB), respectively.
Results:
The positive control of carbendazim, benomyl and testosterone propionate (AR) showed positive effect for its receptor. Results showed that these three triazoles induced expressions of AR with IHC in WREC. This result basically meets the principle that triazoles were designed to disrupt the synthesis of steroid hormone. We will further detect these effects by Western blot in WREC. Also, we need to study the antagonistic effects by adding the antagonists for the receptor activity. By the way we need to quantify the androgen expression in IHC and WB.
Conclusion:
It seems that these three triazoles induced expressions of AR in WREC. WREC can be used as a robust method of endocrine disrupting screening for androgen receptor.

P736
Hydroquinone-Induced miR-122 Down-regulation on The Connection of ADAM17-mediated TNFα Shedding

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Backgrounds:
Hydroquinone (HQ) is one of benzene metabolites *in vivo* and has been proved to be associated with myelotoxicity and hemotoxicity of benzene exposure. Previous studies showed that HQ elicits the secretion of TNFα from hematological cells, and HQ-induced TNFα production is related to HQ toxicity *in vivo*. Nevertheless, the mechanism responsible for HQ-induced the production of soluble TNFα (sTNFα) was not elucidated in these studies. To address that question, the present study was conducted.
Materials and Methods:
After treatment of human leukemia K562 cells with 50 μM HQ for suitable time intervals, the expression levels of TNFα, ADAM17 and miR-122 were detected using RT-PCR, real-time PCR, western blotting analyses, flow cytometer or ELISA assay. Moreover, regulation of miR-122 expression in HQ-treated cells was analyzed by promoter luciferase assay, chromatin immunoprecipitation assay and DNA affinity purification assay.
Results:
HQ increased the release of sTNFα into the culture medium of K562 cells. Meanwhile, HQ induced ADAM17 up-regulation in K562 cells. HQ treatment increased ADAM17 mRNA stability, while HQ did not affect ADAM17 promoter luciferase activity. Knock-down of ADAM17 by siRNA suppressed HQ-induced increased sTNFα production, suggesting a causal relationship between ADAM17-mediated TNFα shedding and sTNFα production. HQ elicited down-regulation of miR-122 expression, and miR-122 promoter luciferase activity was suppressed by HQ treatment. HQ-induced AP1 activation was demonstrated to genetically down-regulate miR-122 expression. Transfection of miR-122 repressed HQ-induced increase in ADAM17 mRNA stability and sTNFα production.
Conclusion:
Our data indicate that HQ-induced miR-122 down-regulation reduces ADAM17 mRNA decay, leading to ADAM17 up-regulation in K562 cells. Consequently, HQ treatment increases ADMA17-mediated TNFα shedding and the production of sTNFα.

P737

Hibiscus Sabdariffa Leaf Polyphenolic Extract Inhibits TNF-alpha-induced Migration And Proliferation of Vascular Smooth Muscle Cells.

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Background:

Atherosclerotic plaque is generated partly by proliferation and migration of vascular smooth muscle cells (VSMC), are always accompanied by markedly induced expression of proflammatory cytokines, especially tumor necrosis factor-alpha (TNF-α). Previous studies have indicated *Hibiscus sabdariffa* L. leaf extract, rich in polyphenols, has antioxidant and anticancer effects. In this study investigations were conducted to examine the mechanism of the anti-atherosclerotic potential of *H. sabdariffa* leaf polyphenolic extract (HLP).

Materials and Methods:

We utilized wound-healing and Boyden chamber assay to analyze the effect of HLP on TNF-α-induced VSMC A7r5 cell migration. To highlight the mechanisms of anti-migration effect of HLP, the activities and expressions of molecular proteins were measured by zymography, real time-PCR and Western blotting. In addition, the effect of HLP on the TNFα-induced cell growth was measured by trypan blue assay and BrdU incorporation, the distribution of cells in the cell cycle by flow cytometry, and the expressions of cell-cycle regulatory proteins by Western blotting and immunoprecipitation.

Results:

Herein, we demonstrated A7r5 cells pre-treated with TNFα triggered migration and proliferation, and affected the activity of MMP-9. Non-cytotoxic doses of HLP abolished the TNFα-induced the MMP-9 secretion and cell migration via inhibiting the Akt/AP-1 pathway. On the other hand, the results showed HLP induced p27 expression, inhibited Rb phosphorylation, and thereby blocked the G1 to S transition of cell cycle in the TNFα-treated cells.

Conclusion:

Our data showed HLP inhibited TNFα-induced both migration and proliferation of A7r5 cells. These results suggested HLP might serve as a potential anti-atherogenic agent.

P738

Anti-metastatic effect of *H. polyphenol*-rich extracts (HPE) on human colon cancer cell via CD44/MET signal pathway

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Backgrounds:

Colorectal cancer (CRC) is the most prevalent form of cancer not only Taiwanese people but also worldwide. In view of the largest cause of death of patients is cancer metastasis, we need for more effective anticancer agents. Since the prevention and treatment of colorectal cancer metastasis to provide a significant challenge, the edible plants are increasingly being considered as sources of anti-cancer drugs. H. polyphenol-rich extracts (HPE) is cultivated in the Sudan and Eastern Taiwan and it had been demonstrated previously to possess that can anti-cancer. However, the HPE inhibition metastasis molecular mechanisms remain largely unresolved in colorectal cancer.

Materials and Methods:

The first, We confirm that the concentration of HPE does not affect cell survival by MTT assay.

HPE inhibited DLD-1cells (human colorectal adenocarcinoma cell) migration and invasion by wound-healing assay, transwell, and boyden chamber. In addition to, Western blotting is used to analyze of the molecular mechanisms.

Results:

Our data showed that HPE inhibited DLD-1 cells migration and invasion in a dose-dependent manner by wound-healing assay, transwell, and boyden chamber. Additionally, Western blot assay showed HPE significantly inhibited the expression of p-FAK, p-MET, Paxillin, Cdc-42, CD44, MMP-7 and restrained the level of RhoB. Those data demonstrated the HPE were obvious inhibited with metastatic of colorectal cancer cells.

Conclusion:

These results indicated HPE blocking DLD-1 cell metastasis pathway by way of CD44/MET signal pathway which in turn led to the reduced MMP-mediated cellular events in cancer cells, those provides a new mechanism for anti-metastatic of human colon cancer therapy.

P739

Synthesis and Cytotoxicity Activity of Newly 9-O-Substituted Lipophilic Berberine Derivatives

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Backgrounds:

Berberine, an isoquinoline alkaloid, has a wide range of biochemical and pharmacological effects. Investigate the newly 9-*O*-substituted berberine derivatives as anti-cancer agents and lipophilic substituted structure-activity relationship studied.

Materials and Methods:

Berberrubine obtained from berberine at 190 °C under vacuum, react with alkyl or terpenyl bromide to afford a series of newly 9-*O*-substituted berberine derivatives. The effects of berberine derivatives on the cell viability were determined using MTT assay. To further extend our cytotoxicity study, we examined the apoptosis effect of these derivatives. Flow cytometry was used to obtain the apoptotic rate and the cell cycle distribution. Hoeschst 33258 staining analysis to identification HepG2 cell nuclear condensation.

Results:

We synthesize a series of newly 9-*O*-substituted berberine derivatives. We found that the lipophilic substitute of 9-*O*-alkyl- and 9-*O*-terpenyl berberine derivatives plays a role in inhibiting the human cancer cell growth and its activity could be maximized with the optimized substitute type and chain length. These compounds showed either comparable or better cytotoxic activity against human cancer HepG2 cell line than berberine. Further, in cell cycle distribution, annexin V-FITC/PI and Hoeschst 33258 staining analysis it induced apoptosis in HepG2 cells at low concentration for 24 h.

Conclusion:

Newly 9-*O*-substituted lipophilic berberine induced liver cancer cell HepG2 apoptosis and arrest cell cycle at S phase. Lipophilic 9-*O*-substituted berberine could be potential candidate for new anticancer drug development.

P740

從缺

P741
The Effects of Di-(2-ethylhexyl) phthalate on Tumor Progression in Lung Adenocarcinoma cells

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Backgrounds:

The phthalates, including di-(2-ethylhexyl) phthalate (DEHP), are synthetic chemicals that are widely used as plasticizers. Plasticizer incident was one of the most serious food safety issues that ever occurred in Taiwan and the exposure of the phthalates has been suggested to adversely affect the normal organ development and could cause hepatocellular tumorigenesis and increase cancer malignancy in animal study; however, the mechanisms are still unclear.

Materials and Methods:

We have developed a chronic exposure model in which human lung epithelial BEAS-2B cells and lung adenocarcinoma cells (CL1-0 and CL1-5) were continuously exposed to DEHP in culture over a prolonged time period (10 to 20 passages). After the chronic exposure, the cells were evaluated for migration assay, anchorage dependent colony forming assay, chemo-resistance test and cDNA microarray analysis.

Results:

Here, we provided the evidences to show that chronic exposure of DEHP can promote cell migration significantly in lung adenocarcinoma cells. However, there is no effect of DEHP on cell proliferation, colony formation ability, and resistance to chemotherapy. Genome-wide transcriptomic analysis with Q-PCR validation showed that several metastasis-related pathways might contribute on the mechanisms of DEHP-induced tumor progression, including cell adhesion signaling (*e.g., cadherins, pro-cadherins*, and *integrins*); as well as, cell migration/invasion-related proteinase cascades, which may cause the following activation of metalloproteinase (MMPs) and may lead to increase the tumor metastasis. To analyze the possible transcriptional regulation of the promoter regions of these DEHP-target genes, we suggest that several nuclear receptors (e.g., estrogen receptor or aryl hydrocarbon receptor) might be the targets of phthalate on promoting the progression of tumorigenesis.

Conclusion:

According to our findings, DEHP exposure can enhance genes related to cancer metastasis and cell-adhesion, which might be associated with phthalates-induced tumor progression. Here, we provide possible mechanisms for modulating lung adenocarcinoma progression after chronic DEHP exposure.

P742
Nelumbo Nucifera leaves Extract Inhibits Adipocytes-induced breast cancer metastasis.

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Backgrounds:

Breast cancer is the most common cancer in women in the Taiwan. Then breast cancer deaths accounted for 28.4% of all the cancer deaths, and have been associated with obesity. Previous studies, has been confirmed Nelumbo nucifera leave extract (NLE) can effectively reduce the fatty liver, atherosclerosis, hyperlipidemia and anti-oxidative properties. Thus, we investigated the relationship between obesity and breast cancer, and evaluated the inhibitory effect of NLE on the adipocytes-induced breast cancer progression.

Materials and Methods:

First, we used the conditioned medium (CM) from adipocyte to induce the proliferation and migration in human breast cancer cell line. MTT and cell count assay were used to observe the effect of CM-induced breast cancer cells proliferation. A number of in vitro metastasis models were used to study the migration and invasion of breast cancer cells by CM. Second, we used the NLE to inhibit the CM-induced cell growth and mobility. And the inhibitory mechanism of NLE on CM-induced breast cancer cell was studied by Western blot analysis.

Results:

Adipocyte secreted conditioned medium noticeably induced breast cancer migration. NLE diminished the CM-induced migration number of breast cancer cell by boyden chamber. A direct correlation was found between adipocyte and breast cancer cell migration. Similarly, we used of ECIS assay, wound healing assay and transwell assay identical results were found. Under the CM treatment, phosphorylated p38 MAPK, ERK and JNK levels of MCF-7 cells treated with NLE can significantly inhibited the protein expression were measured by Western blot analysis. Furthermore, we found that NLE suppressed several cytokines from adipocyte-secreted CM, which regulated a variety of cellular functions involved in inflammation, tumorigenesis, and development. These data indicate adipocytes provide a major component of the microenvironment for rapid tumor growth and migration, and this increase was inhibited significantly by NLE treatment.

Conclusion:

In summary, our findings suggest that adipocytes act as major mediators of breast cancer metastasis. The present data demonstrate that NLE as a novel potential therapeutic agent for the migration of human breast cancer cell.

P743
The Evaluation of Risk Factors for Scheduling of Controlled/Illicit Drugs

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Backgrounds:

Drug abuse and misuse not only endanger our health, but also pose threats to public health and result in great social costs. Recently, the drug abuse situation has been worsening in Taiwan. Some of the new abused drugs have not been controlled by the United Nations or Taiwan. If these drugs were not properly scheduled and managed, they might inflict a new trend of drug abuse.

Materials and Methods:

Twelve experts on the fields of addiction, abuse and social hazards were invited to participate in the risk assessment of 30 commonly abused drugs/substances in 2011 and 2012, respectively. Some of the drugs/substances have not been scheduled as controlled drugs. The Delphi approach was adopted and the harms of these drugs/substances were assessed according to three legislative aspects (i.e., addiction, abuse and social hazard), which were further subdivided into the indicators including euphoria, withdrawal, pharmacokinetic characteristics, dependence, international abuse prevalence, national abuse prevalence, different regulations among different countries, infectious hazard, criminality, poly-drug use hazard and social cost.

Results:

According to the risk assessment results evaluated by all the experts, heroin posed the highest risk in both 2011 and 2012. Among the top ten items with greatest harms, ketamine and flunitrazepam were schedule III drugs and alcohol was a legal substance. Interestingly, the harm scores of ketamine and flunitrazepam were higher than some schedule II drugs such as methadone and PCP. The scores of methadone, zolpidem and alcohol were higher in 2011 than in 2012, implying different professional background of experts may affect the evaluation results. Such a difference in harm scoring is more obviously observed in the items of schedules III and IV. The severity of abuse trend and difference of control schedule in various countries may also significantly influence the assessment results.

Conclusion:

Proper drug scheduling is the first step in the management of drug use problems. Facing the threats of new designer drugs, it is imperative to implement a rational and effective scheduling system for appropriate management. This study provides a mechanism to scrutinize, and hopefully to improve, the current evaluation process for drug scheduling.

P744
Nelumbo Nucifera Leaf Extract Attenuate Ethanol-induced Liver Injury and Obesity Via Anti-oxidative Stress and Inhibiting Lipogenesis in C57BL/6J Mice

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Backgrounds:

The roles nucifera leaf extract (NLE) play in preventing alcoholic liver disease remain unknown. We determined whether treatment with NLE prevents experimental alcoholic-induced liver injury and obesity.

Materials and Methods:

Six groups of C57BL/6J mice were fed on Lieber-DeCarli regular diet with or without ethanol of 36% energy for 6 weeks. Meanwhile, mice were treated with 0.5, 1.0 and 2.0% NLE respectively. Liver samples was based on the triglyceride (TG) and total cholesterol (TC) synthesis-related proteins and proinflammatory production was determined by histopathological, immunohistochemistry and western blot evaluation. The quantitative concentrations of antioxidant enzymes were measured as markers of oxidative stress. The perimeter adipose of tissue size and weight are for indirect assessment of obesity and fat location pointers.

Results:

The plasma biomarkers and hepatic content analysis showed that NLE inhibited the TG, TC, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels. Furthermore, treatment with NLE lessened the expression of TG and TC synthesis-related proteins. The antioxidant defense and proinflammatory mediator analysis also exposed the inhibitory effect of NLE on alcohol-induced injury. The attenuated size and weight of the perimeter adipose tissue demonstrated that NLE is capable of reducing body fat and preventing weight gain and offers a weight control utility in alcohol-induced obesity.

Conclusion:

Our results revealed that NLE significantly reduced the lipid accumulation, prevented oxidative stress, facilitated anti-inflammation, suppressed lipid synthesis, increased fatty acid transportation and stimulated fatty acid oxidation by regulating the activation of AMPK. These findings suggest that NLE could potentially be developed as a natural agent for preventing alcohol-induced liver injury and obesity.

P745
Cytotoxic Effects of Nanosilver Particles on Embryonic Development in Mouse Two-cell Stage

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Backgrounds:
Silver nanoparticles (Ag-NPs) have existed in nature since the beginning of earth history. They have been widely used as an antimicrobial coating in various antibacterial products. However, the biological side effect on mouse early embryos remains unknown. In the present study, we investigate the cytotoxicity of Ag-NPs on mouse two-cell stage embryos.
Materials and Methods:
The mouse two-cell stage embryos were exposed to 3, 6 and 12μM Ag-NPs. Cell proliferation, apoptosis detection, early embryonic outgrowth and generation of reaction oxygen species (ROS) were evaluated by Hoechst staining, TUNEL assay, embryo transfer and DCF-DA staining, respectively.
Results:
Two-cell stage embryos treated with 6 and 12μM Ag-NPs exhibited significantly damage embryonic morphology. Percentage of two-cell stage embryo development to blastocyst stage decreased at 6μM Ag-NPs or more concentration. The results also showed that Ag-NPs inhibited cell proliferation and significantly induced cell apoptosis at 6μM Ag-NPs. Ag-NPs retarded early embryonic outgrowth at 3μM Ag-NPs. Two-cell stage embryos treated with Ag-NPs increased ROS level in dose- and time- dependent manner.
Conclusion:
The results reveal that low concentration Ag-NPs has potential to induce embryo cytotoxicity. Ag-NPs-induced cytotoxicity maybe related to generation of ROS. Further study show that NAC can prevent effect of Ag-NPs on generation of ROS.

P746
Study on The Effects of ketamine and its Metabolites on Human Kidney Cell

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Backgrounds:
According to previous study, the complications of ketamine include renal impairment with raised serum urea and creatinine levels. The effect of ketamine and its metabolites on kidney remain unknown. In this study, we explore what the extent and the impact on kidney cell line by the ketamine and its metabolites.
Materials and Methods:
Human kidney cell line (HEK293) has been used in our study. To explore the effects in morphological changes and cell viability after treatment with ketamine and its metabolites (norketamine and dehydrogenorketamine), which was detected by MTT assay, Western blot and flow cytometry.
Results:
The viability of HEK293 cells decreased significantly after treatment with ketamine and its metabolites (norketamine and dehydronorketamine) for 24-48 hours incubation period. The expression of NF-κB is increased in ketamine-treated HEK293 cells. On the other hand, not only the 0.36 nM dehydronorketamine-treated cells were in round-shape when compared to the control group and other ketamine metabolites did, but also its growth rate slow down as well.
Conclusion:
The ketamine and its metabolites decreased the viability of HEK293 cells for 20-25%. In addition, we also showed that the ketamine and its metabolites-treated HEK293 cells increased the expression of NF-κB in the 24-48 hours incubation period. In conclusion, the ketamine and its metabolites decreased the HEK293 cells viability probably through the NF-κB-dependent mechanism. The exact mechanism that cause the morphological and the viability changes require much further study.

P747
The antioxidant capacity and whitening effects of extract from the leaves of *Koelreuteria henryi* Dummer

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Backgrounds:
Koelreuteria henryi Dummer, a deciduous tree, is an indigenous plant in Taiwan. In this study, the antioxidant capacity and whitening effects of hot water extracts of *Koelreuteria henryi* Dummer were investigated with a number of established *in vitro* assays.
Materials and Methods:
The fresh leaves of *Koelreuteria henryi* Dummer were extracted by hot water. The antioxidant activities were examined by DPPH radical scavenging activity, ferrous ion chelating activity, reducing power and TEAC. The whitening effects were examined by the assay of inhibits tyrosinase activity. Additionally, we also measured the amount of total flavonoids and total phenolic in the leaves of *Koelreuteria henryi* Dummer.
Results:
The results showed that DPPH radical scavenging activity, ferrous ion chelating activity, reducing power and TEAC all increased with increasing concentrations of leaf extracts of *Koelreuteria henryi* Dummer. Moreover, the EC_{50} values of leaf extracts of *Koelreuteria henryi* Dummer from the tyrosinase inhibiting assay was 0.55 mg/ml. Additionally, the contents of total phenolic and total flavonoids of leaf extracts of *Koelreuteria henryi* Dummer were 8.00 μg of GAE/mg and 24.12 μg of QE/mg, respectively.
Conclusion:
The results of this study demonstrated that the leaf extracts of *Koelreuteria henryi* Dummer contained abundant total phenolic and total flavonoids, which are known to have significant antioxidant activities. Furthermore, we also found that the leaf extracts of *Koelreuteria henryi* Dummer exhibited remarkable antioxidant activity, as well as high inhibiting activity of tyrosinase. These results clearly indicate that *Koelreuteria henryi* Dummer has significant potential as a natural antioxidant agent.

P748
Systemic and intestine specific enhance EPA and DHA biosynthesis to resist cold shock and oxidative stress in zebrafish

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Backgrounds:
The n-3 PUFA include EPA(20:5n-3) and DHA(22:6n-3), is important for human health, but we can't synthesis these essential fatty acid in our body. The major resource of n-3 PUFA were came from deep sea marine fish oil, recently, over-fishing and marine pollution caused the production of PUFA was seriously effect.
Materials and Methods:
In this study, we try to use β-actin and gut-specific expression promoter IFABP link Δ5, Δ6 desaturase, and elongase 5a genes, microinjected these constructs into zebrafish embryos. The transgenic fish F0 were examined at 3-5 dpf by fluorescence microscopy and RT-PCR. We also used high PUFA contained forage to feed fish for 8 months as compare group, then put these fish into cold shock at 8℃ , or ip 900 mg/ml TAA/LPS into adult fish, or treated to embryos. The ROS were assay by DCFH-DA and the oxidative stress was assay by lipid hydroperoxide.
Results:
We have generated 30 fish for IFABP-Fad5, 4 fish for IFABP-Fad6 and 14 fish for IFABP-elongase 5a transgenic fish, and 10 fish for β-actin-Fad5, 13 fish for β-actin-Fad6, and 3 fish for β-actin-elongase 5a transgenic fish. The transgene integration was confirmed by genomic analysis and RT-PCR. The high PUFA contained fish can maintain normal breathing and balance of swimming under cold shock. 900 mg/ml TAA was injected into fish by ip, and the basophilic cytoplasm were shown by HE stained after 2 weeks injection, we also found TAA can induce H2O2, NO and oxidative damage increasing significantly. In the pre-test, high PUFA fish can partially reduce TAA/LPS induced oxidative stress.
Conclusion:
High PUFA synthesis zebrafish are generate by either β-actin or gut-specific expression promoter IFABP drive Δ5, Δ6 desaturase, and elongase 5a genes expression. The transgene were integrated into fish genome, and can express either in whole body or in specific tissue. The high PUFA fish can resist cold shock and TAA/LPS induced oxidative.

P749
Baicalein Enhances p27^{kip} Degradation in Oral Cancer Cells

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Backgrounds:
While most studies in cancer cell lines report that baicalein causes cell cycle arrest by increasing the expression of p27kip, we found that in our preliminary data, the p27^{kip} was decreased rather than increased in baicalein treated oral cancer cells, HSC-3. The reduction of p27^{kip} was concomitant to the reduction of cyclinD1 and CDK4 as well as phosphorylated Rb (p-Rb), indicating its association with phosphrylation of Rb and growth inhibition. Thus, the purpose of the study is to elucidate how baicalein regulates p27kip expression in the oral cancer cell model.

Methods and Results:
Using cell cycle analysis, we found that baicalein treated cells was arrested at S phase at 24hr treatment. Data by Western Blot showed that p27kip was decreased at 12 and 24hr whereas phosphorylated p27^{kip} (p-p27) was increased in baicalein treated cells. The reduction of p27^{kip} in baicalein treated cells was time-correlated to the decrease of Akt and increase of p-Akt. This suggests that baicalein induces the phosphorylation of p27^{kip}, which is mediated by activation of Akt. It is known that the increase of p-p27 eventually leads to the degradation of p27^{kip} and thus, decrease the expression of p27^{kip}. Interestingly, phosphorylated GSK-3β (p-GSK-3β) was also increased at the time when p-Akt was increased and p27^{kip} was decreased, suggesting that GSK-3β pathway might be also involved in the modulation of p27^{kip}.

Conclusion:
Our data indicates that baicalein modulates the degradation of p27^{kip} through Akt pathway in HSC-3. GSK-3β, the downstream protein molecule of Akt pathway might be an indirect effect of Akt on regulating the expression of p27^{kip}. Downregulation of p27^{kip} has been reported to correlate to the malignancy and prognosis of oral cancers. The impact of baicalein on the reduction of p27^{kip} in oral cancer cells shall be determined in terms of migration and invasion, in addition to its effect on growth inhibition.

P750
Down-regulation of glucose-regulated protein (GRP) 78 Potentiates Cytotoxic Effect of Celecoxib in Human Urothelial Carcinoma Cells

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Backgrounds:
Celecoxib is a selective cyclooxygenase-2 (COX-2) inhibitor that has been reported to elicit anti-proliferative response in various tumors. In this study, we aim to investigate the antitumor effect of celecoxib on urothelial carcinoma (UC) cells and the role of endoplasmic reticulum (ER) stress play in celecoxib-induced cytotoxicity.

Materials and Methods:
The cytotoxic effects were measured by MTT assay and flow cytometry. The cell cycle progression and ER stress-associated molecules were examined by Western blot and flow cytometry. Moreover, the cytotoxic effects of celecoxib combined with glucose-regulated protein (GRP) 78 knockdown (siRNA), (−)-epigallocatechin gallate (EGCG) or MG132 were assessed. We demonstrated that celecoxib markedly reduces the cell viability and causes apoptosis in human UC cells through cell cycle G1 arrest.

Results:
Celecoxib possessed the ability to activate ER stress-related chaperons (IRE-1α and GRP78), caspase-4 and CCAAT/enhancer binding protein homologous protein (CHOP), which were involved in UC cell apoptosis. Down-regulation of GRP78 by siRNA, co-treatment with EGCG (a GRP78 inhibitor) or with MG132 (a proteasome inhibitor) could enhance celecoxib-induced apoptosis.

Conclusion:
We concluded that celecoxib induces cell cycle G1 arrest, ER stress and eventually apoptosis in human UC cells. The down-regulation of ER chaperone GRP78 by siRNA, EGCG, or proteosome inhibitor potentiated the cytotoxicity of celecoxib in UC cells. These findings provide a new treatment strategy against UC.

P751
The Combined Effects of Dextromethorphan and Oxycodone on Treatment of Neuropathic Pain in Mice

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Backgrounds:
Neuropathic pain is a chronic condition caused by injury to the nervous system. It reflects both peripheral and central sensitization. Furthermore, there is increasing evidence which indicates that inflammatory and immune mechanisms play a role in neuropathic pain. Oxycodone has been used clinically for over 80 years. The molecular structure of oxycodone has similarities to morphine and codeine. Dextromethorphan (DM) is a N-methyl-D-aspartate (NMDA) antagonist and has a long history as a cough suppressant. The aim of this study was to investigate the effect of oxycodone, DM or in combination of oxycodone and DM on treating neuropathic pain in mice.

Materials and Methods:
A 14-day schedule was used in this study. Drug(s) was/were administered twice a day starting from 2 hours after spinal nerve ligation surgery in C57BL/6J mice. Von-Frey tests were used for measuring mechanical allodynia before and after drug administration every other day.

Results:
We found that SNL induced mechanical allodynia one day after nerve ligation and lasted for 11 weeks. Acute oxycodone (1, 3, 5 mg/kg, s.c.) attenuated SNL-induced allodynia, so did acute DM (20 mg/kg, i.p.) or co-administration of oxycodone and DM. Chronic administration of oxycodone (1, 3, 5 mg/kg, s.c.) attenuated the development of SNL-induced allodynia in a dose-dependent manner. Co-administration of DM (20 mg/kg, i.p.) with oxycodone potentiated the acute and chronic effects of oxycodone at doses of 1 and 3 mg/kg, s.c.

Conclusion:
The combined use of oxycodone and DM may have beneficial effects on early treatment of neuropathic pain. The mechanisms involved are under investigation.

P752
PI3K Plus MEK Inhibitors Reverse Acquired Resistance in Epidermal Growth Factor Receptor Mutation Lung Cancer Cells With.

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Human non-small cell lung cancer (NSCLC) cells with mutated epidermal growth factor receptors (EGFR) are reportedly sensitive to EGFR tyrosine kinase inhibitors (TKIs), such as BIBW2992 (Afatinib). BIBW2992 is known as a next generation TKI which irreversibly inhibits EGFR and HER2 receptor kinases. BIBW2992 is therapeutically active against both EGFR mutations targeted by first generation TKIs (such as gefitinib) and the others which are resistant to the standard treatments. After prolonged treatments, acquired resistance to EGFR-TKIs is frequently observed. Two BIBW resistant PC-9 cells (PC-9/BIBW C3 and B2) harboring EGFR exon 19 deletion were established to explore effective strategies against resistance to BIBW2992. Western blot assay showed that BIBW significantly reduced EGF-induced phosphorylation of EGFR, HER2, AKT and ERK of the PC-9/wt cells, the parental PC-9 cell. However, BIBW slightly attenuated EGF-induced phosphorylation of ERK and AKT in the PC-9/BIBW C3 cells. Due to the limited potentiation of AZD6244 (an MEK inhibitor) on BIBW-induced cytotoxicity including apoptosis and phosphorylation of ERK and AKT of PC-9/BIBW C3 cells and a mild suppression of PC-9/BIBW C3 tumor growth in nude mice, a combination of BIBW, AZD6244 and Wortmannin (an PI3K inhibitor) was employed. Western blot assay showed that Wortmannin indeed augmented the caspase 3 activation by BIBW plus AZD6244. In addition, SRB assay showed Wortmannin augmented the cytotoxicity by AZD6244 plus BIBW, indicating that combined treatment BIBW, AZD6244 and Wortmannin may be therapeutically useful in reversing acquired BIBW-resistance of lung adenocarcinoma cells harboring *EGFR* mutations.

P753

Administration of Marine Organism-Derived Peptide LCY-1 Exerts Antinociceptive Effects and Attenuates Spinal Neuroinflammation in Chronic Constriction Injury-Induced Neuropathic Rats

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Backgrounds:

In recent years, marine organism-derived peptides have been identified as new and potential sources for drug development, particularly in the development of anti-inflammatory drugs. Recently, we found that the marine organism-derived peptide LCY-1 was biologically active, and previous studies have indicated that marine organism-derived peptides have anti-inflammatory properties. Neuroinflammatory processes are known to play a critical role in the development and maintenance of neuropathic pain, for which no effective drugs are currently available.

Materials and Methods:

In the present study, we investigated the anti-analgesic and anti-neuroinflammatory effects of the marine organism-derived peptide LCY-1 on chronic constriction injury (CCI)-induced neuropathy in rats.

Results:

We found that intrathecal (i.t.) injection of LCY-1 produced a significant and dose-dependent reduction of thermal hyperalgesia, mechanical allodynia, weight-bearing deficits, and cold allodynia in neuropathic rats after CCI surgery. Furthermore, the 50% effective dose (ED 50) of LCY-1 was lower than that of gabapentin, a positive control. Additionally, this regimen did not result in any obvious side effects on the external behavior of CCI rats. Moreover, immunohistochemistry analyses have found that i.t. LCY-1 significantly inhibited CCI-induced activation of microglia and astrocytes, as well as the upregulation of the inflammatory mediator tumor necrosis factor- α (TNF-α) and interleukin-1 β (IL-1β), in the ipsilateral spinal dorsal horn. We also observed the upregulation of the anti-inflammatory molecule transforming growth factor-β1 (TGF-β1) in neuropathic rats after i.t. injection of LCY-1 (20 μg).

Conclusion:

On the basis of these experimental results, we suggest that LCY-1 could be used as a potential therapeutic agent for neuropathic pain.

P754

Effects of KLP-1 on methamphetamine- and apomorphine-induced hyperlocomotion and apomorphine-induced stereotypy climbing behaviors in mice.

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Backgrounds:

Previously, we found a patient with intractable motor tic disorder, a spectrum of Tourette syndrome (TS), responsive to the ground leaf juice of *Clerodendrum inerme (Cl)*. The ethanol extract *Cl* leaves (10–300 mg/kg, *i.p.*) dose-dependently inhibited hyperlocomotion induced by methamphetamine (METH, 2 mg/kg, *i.p.*), but did not affect spontaneous locomotor activity, rotarod performance, and grip force. KLP-1 is an active constituent purified from the *Cl* ethanol extract. Dopaminergic hyper-reactivity in the corticostriatal system is believed to play a role in the pathogenesis of TS. We, therefore, further examined effects of KLP-1 in two hyperlocomotion models induced by METH and apomorphine, respectively. Besides, we also examined the effect of KLP-1 on apomorphine-induced stereotypy climbing behaviors.

Materials and Methods:

Male ICR mice (age 6-9 weeks) were habituated in the behavioral room for at least 30 min before the test. Hyperlocomotion was induced by METH (2 mg/kg, i.p.) or apomorphine (1 mg/kg, s.c.) completed the study. The stereotypy climbing behavior induced by apomorphine was videotaped and scored. Locomotor activity was measured by SDI Photobeam Activity system and rotarod performance by SDI ROTOR-ROD system. KLP-1 were injected by either i.p. injection (10-100 mg/kg) or by bilateral intra-cerebellar (i.c.b.) injection since KLP-1 was reported to activate $GABA_A$ receptors, especially the alpha6 subunit-containing $GABA_A$ receptor that is exclusively expressed in cerebellar granule cells.

Results:

KLP-1 given by i.p. injection (10-100 mg/kg), but not by i.c.b. microinjection (10 nmol), effectively inhibited hyperlocomotion induced by METH without affecting spontaneous locomotor activity and rotarod performance and have no anxiolytic effect. However, at the dose of 100 mg/kg (i.p.) KLP-1 exerted significant sedative effect. On the other hand, KLP-1 at 10 and 30 mg/kg (i.p.) did not affect hyperlocomotion and stereotypy climbing behaviors induced by apomorphine.

Conclusion:

In summary, KLP-1 inhibited the hyperlocomotion induced by METH, a dopamine releaser, but not apomorphine, a D2 receptor agonist. This suggests KLP-1 reduces striatal dopaminergic transmission via the mechanism other than blocking D2 receptors or their down stream signaling. Cerebellar alpha6 subunit-containing GABAA receptors might also be involved in the inhibitory effect of KLP-1 on METH-induced hyperlocomotion.

P755

Inhibition of adipogenesis and induction of lipolysis, therapeutically targeting high fat diet-induced obesity

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Background:

To investigate whether any treatments inhibit adipogenesis and induce lipolysis of 3T3-L1 pre-adipocytes, if so, examine the mechanism of action for the treatments of diet-induced obesity.

Materials and Methods:

3T3-L1 pre-adipocytes used to examine the mechanism of KMUP-1 in inhibiting obesity. Four days proliferation of 3T3-L1 pre-adipocytes was performed to attain the full confluence and treated with KMUP-1 (0~20 μM), then quantified by MMT test to examine the possible cytotoxicity. Two days differentiation of 3T3-L1 pre-adipocytes were induced by insulin (1 μg/ml), dexamethasone (0.25 μM) and 3-isobutyl-1 methylxanthine (0.5 mM) (IDM) or ID-KMUP-1 (0~20 μM), and followed by 4 or 7 days post-confluence of differentiated cells quantified by Oil-Red O staining to observe adipogenesis. Then further 2 days' insulin application without KMUP-1 and other inducing agents, extended to day-10, mature adipocytes were treated with KMUP-1 (0~20 μM) for 0~24 hrs to examine the possible lipolysis and apoptosis. Protein expression of p38/p42 MAPK, ERK1/2, NOX2, PPARγ, PDE-3B, C/EBPβ and ROS induced by free fatty acids (FFAs) and isopreoterenol were measured by flow cytometry or Western blotting. HepG2 cell was cultured in DMEM to measure these proteins.

Results:

KMUP-1 inhibited isoproterenolol-, indomethacin- or FFAs-induced p38/p42 MAPK, ERK1/2, NOX2, PDE-3B, C/EBPβ, and ROS restored eNOS, PPARγ and HSL of adipocytes or hepatocytes and inhibited FFAs-, indomethacin-, and isopreoterenol-induced activation of GPCRs in 3T3-L1 preadipocytes .

Conclusion:

Inhibition of adipogenesis and induction of lipolysis is the strategy of treatment toward anti-obesity.

P756

Microtubule-associated Histone Deacetylase 6 is a Potential Target to Interfere with Calcium Store Sensor STIM1-mediated Cancer Malignant Behaviors

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Backgrounds:

Stromal interaction molecule 1 (STIM1) is an endoplasmic reticulum (ER) Ca^{2+} sensor that is mainly located on the ER membrane and STIM1-dependent Ca^{2+} signaling is important for cancer cell proliferation, migration, and angiogenesis. The clinical relevance of STIM1 has been highlighted in breast and cervical cancer. Previous studies indicate that microtubules play a facilitative role in SOCE by optimizing STIM1 membrane trafficking. Histone deacetylase 6 (HDAC6), a member of the class II histone deacetylase that deacetylates microtubule, can alter the microtubule stability and functions.

Materials and Methods:

In this study, we used confocal microscope to study the important role of microtubule on SOCE activation. Surgical specimens were also used to compare the expression patterns of SOCE-related proteins in cervical cancer cells and normal cervical epithelial cells. Moreover, combination of pharmacological and genetic approaches was used to study the role of HDAC6 on SOCE activation. The SOC-mediated Ca^{2+} influx, induced by epidermal growth factor (EGF), was detected by single cell intracellular calcium measurement.

Results:

The confocal images of living cells indicated that microtubule was necessary for STIM1 membrane trafficking. Then, we analyzed the expression levels of STIM1, Orai1, HDAC6 and acetylated alpha-tubulin in cervical epithelial cells with different malignant potential, and found STIM1, Orai1, and HDAC6 were upregulated in cervical cancer cells. Tubastatin-A, a potent and selective HDAC6 inhibitor, inhibited the SOCE activation and STIM1 membrane trafficking in cervical cancer cells but not in normal cervical cells. In presence of HDAC6 siRNAs, the similar results were found. However, HDAC6 did not affect the interaction between STIM1 and microtubule plus-end-binding protein EB1.

Conclusion:

This study highlights the important role of HDAC6 as a potential target to interfere with Ca2+ store sensor STIM1-mediated cancer malignant behaviors.

P757
Targeting Survivin by a novel small molecule inhibitor, YM155, induces autophagic cell death in human breast cancer cells

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Backgrounds:

Despite hormone therapy, targeted therapy and chemotherapy have been developed to target different types of breast cancer; the current breast cancer treatments still have several limitations and undesired side-effects. Thus, it is important to develop novel strategies to treat breast cancer. Survivin is a member of the inhibitor-of-apoptosis proteins family, and it has been shown to play important role in breast cancer development and progression. YM155 is a novel small molecule inhibitor of Survivin. In this study, we aim to determine the effectiveness of YM155 in targeting various types of breast cancer and its molecular mechanism of action in breast cancer cells.

Materials and Methods:

Estrogen receptor (ER)-positive Tamoxifen-sensitive MCF7, MCF7-deridved ER-positive Tamoxifen-resistant MCF7-TamR7, -TamR8, -TamC3 and -TamC6, and the triple-negative metastatic aggressive MDA-MB-231 breast cancer cells were used in this study. MTT cell viability assay was used to determine the effectiveness of YM155 in targeting various types of breast cancer in vitro. To determine whether YM155 induces apoptosis and autophagy in breast cancer cells, Western blot analysis was used to determine the conversion of LC3B and the cleavage of caspase-3. YM155-treated cells were also stained with Monodansylcadaverine to determine the formation of autophagosome. Comet assay was used to determine possible DNA damage induced by YM155.

Results:

YM155 is equally effective in targeting both the parental ER-positive Tamoxifen-sensitive and the MCF7-dervided Tamoxifen-resistant breast cancer cells *in vitro*. YM155 is also effective in targeting the triple-negative MDA-MB-231 breast cancer cells. Surprisingly, Western blot analysis, immunofluorescent microscopy and autophagy/apoptosis inhibition assay revealed that targeting Survivin by YM155 induced autophagic cell death, but not caspase-3 dependent apoptosis, in most of the tested breast cancer cell lines (except TamC3); despite it is widely believed that Survivin inhibits apoptosis through physical interactions with caspase-3. Interestingly, YM155 also induced autophagy-dependent DNA damage in the treated breast cancer cells.

Conclusion:

Targeting Survivin by YM155 induces autophagic cell death in breast cancer cells. Importantly, YM155 is a promising anti-cancer compound that has potential for the management of various types of breast cancer.

P758
Taiwanin E inhibits proliferation and enhances apoptosis in arecoline and 4-NQO-induced oral cancer cells

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Background:

Oral cancers can be life threatening if not diagnosed and treated early. Areca nut chewing is very popular in Taiwan and in other parts of Asia and chronic exposure to arecoline carcinogens in causes genetic changes in the epithelial cells of the oral mucosa. The use of herbs as alternative cancer therapies has attracted a great deal of attention owing to their lower toxicity. Therefore, the purpose of this study was to investigate the anti-cancer effect of Taiwanin E on arecoline and 4-NQO-induced oral cancer cell lines.

Materials and Methods:

The OSCC model in C57BL/6J Narl mouse is generated by 0.5 mg/mL arecoline plus 0.2mg/mL 4-NQO carcinogen in drinking water for 8 and 28 weeks to mimic the etiology of oral cancer patient in Asia. Mice were sacrificed and cells were cultured as T28 cancer cells. Taiwanin E used in this study was extracted from *Taiwania cryptomerioides* Hayata woud. T28 cells were treated with different concentrations of Taiwanin E and analyzed with MTT assay, western blot analysis, flow cytometry, TUNEL assay.

Results:

Taiwanin E significantly inhibited the cell viability of T28 cells in a dose dependent manner, but no cytotoxicity was observed in N28 normal cells. Taiwanin E activated p21 and p27 proteins and reduced cell cycle regulatory proteins like Cyclin D1 and Cyclin E and thus resulted in G0/G1cell cycle arrest in T28 cells. Annexin V-FITC staining and terminal transferase-mediated dUTP nick end-labeling (TUNEL) staining showed Taiwanin E strongly enhanced apoptosis in a dose-and time- dependent manner. Taiwanin E also decreased anti-apoptotic protein Bcl-xL and increased pro-apoptotic protein Bax, and down-regulated p-PI3K, p-Akt survival protein levels in T28 oral cancer cells.

Conclusion:

Taiwanin E inhibited T28 cells proliferation and inhibited survival ability in a dose dependent manner without affecting the normal cells. Further studies will be performed in oral cancer induced nude mice to verify the efficiency of Taiwanin E.

P759
Role Of Cerebral PPARδ In Depressive Rats

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Backgrounds:

Depression is a common psychiatric disorder in children, adolescents, adults, and the elderly. The brain of depression rat has been higher concentration of reactive oxygen species (ROS). PPARδ can be adjusted to clear the ROS. In neuron, PPARδ could protect nerve cells from apoptosis and prevent ROS generation.

Materials and Methods:

In this study, we generate CUMS (chronic unpredictable mild stress) rats to identify PPARδ in hippocampus and assess behavioral changes. The siRNA of PPARδ in hippocampus for further confirm. After sacrificed, the hippocampus is used to detect the PPARδ, apoptotic proteins and ROS level. In this study, we demonstrated that PPARδ is attenuated in hippocampus.

Results:

The ROS level and apoptotic proteins are higher. Moreover, knockdown of PPARδ shows depression like behavior.

Conclusion:

We concluded that decreased expression of PPARδ could lead depression like behavior of rats by increasing ROS generation and apoptosis in hippocampus.

P760
The Effect of cAMP on DNA Damage Agents Induced Acute Myelocytic Leukemia Cell Death

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Backgrounds:

Acute myeloid leukemia (AML) is a type of cancer of blood and bone marrow cells. Although there are many ways to treat AML, drug resistance still remain a big problem in relapse and poor prognosis. The drug-resistant of AML has not well studied. In previous studies, the high level of cyclic AMP (cAMP) in AML patients is associated with the bad prognosis. Moreover, cAMP having an inhibitory effect on DNA damage-induced apoptosis in various types of cancer cells. In this study, we investigate the effect of cAMP on DNA damage agents induced acute myeloid leukemia cell death.

Material and Methods:

In this study, we use HL60 cell line as an acute myeloid leukemia cell model. We choose three different DNA damage agents-cisplatin, doxorubicin and etopside. We use single cell electrophoresis assay (comet assay) to estimate DNA damage and its repair ability. It involves the encapsulation of cells in a low-melting-point agarose suspension, lysis of the cells in alkaline conditions, and electrophoresis of the suspended lysed cells. This is followed by visual analysis with staining of DNA and calculating fluorescence to determine the extent of DNA damage and the tail moment reflects the degree of DNA damage.

Result:

We observed that HL60 cells were treated with the three DNA damage drugs for 4 hours, the tail moment that caused by etopside is the highest. Next, we want to see the time-course of tail moment that caused by three chemotherapy drugs. After HL60 cells were treated with etoposide 15 minutes, the extent of tail moment is reached a peak value, and the value of the tail moment is sustained decline after 15 minutes.

Conclusion:

Our results indicated that the extent of DNA damage in HL60 cells caused by three chemotherapy drugs is different.

P761
Effects of Donepezil on Lipopolysaccharide-Induced Disseminated Intravascular Coagulation in Rats

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Backgrounds:

Disseminated intravascular coagulation (DIC) is a frequent complication of sepsis. Coagulation activation, inhibition of fibrinolysis, and consumption of coagulation inhibitors lead to a procoagulant state resulting in inadequate fibrin removal and fibrin deposition in the microvasculature. As a consequence, microvascular thrombosis contributes to organ dysfunction.

Donepezil is a long acting, reversible acetylcholinesterase inhibitor and is known to improve memory and cognitive function in patients with Alzheimer's disease. Recent studies show that it decreases cytokines level in human. It also has benefits in animal models of inflammation and sepsis.

The present study was designed to evaluate rats with lipopolysaccharide (LPS)-induced DIC defined by the ISTH DIC criteria.

Materials and Methods:

Sepsis-induced DIC was performed by injection Wistar rats with LPS (10 mg/kg, i.v.). Effects of Donepezil were examined on LPS-induced DIC rats. Changes of hemodynamics, homeostasis, blood glucose, hepatic and renal function, and plasma nitrate (an indicator NO) were examined. Animals were divided into four groups, i.e. sham operation (SOP), SOP + Donepezil (1 mg/kg, i.p.), LPS, LPS + Donepezil. At 6 h after LPS, animals were sacrificed and lung, liver, kidney and thoracic aortas were excised to perform the pathological study, iNOS, PAI-1 and HIF-1 expression analysis, superoxide measurement and fibrin deposition.

Results:

Our preliminary results showed that Donepezil seemed have no beneficial effect on the animals with LPS-induced DIC, if it was administered immediately after the injection of LPS.

Conclusion:

Future studies are needed to clarify the administration time-point or dose selection since Donepezil itself has hypotension and hyperglycemia effect in the present study. Both effects of Donepezil might be deleterious in the LPS-induced DIC animals.

P762
Bioactivity of water soluble phenyl N-mustards benzene conjugates against colorectal cancer

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Backgrounds:

Until now, numerous alkylating agents are the major class of frontline chemotherapeutic drugs, such as oxaliplatin against colorectal cancer, cisplatin against lung and bladder cancers, mechlorethamine against multiple myeloma. Nitrogen mustard, the earliest member of alkylating agent family, still play a key role in treatment of patients with Hodgkin's disease and multiple myeloma. Alkylating agents may be used alone or in combination with other chemotherapeutic agents, such as MOPP (mechlorethamine combine with oncovin, procarbazine, and prednisone) for Hodgkin's disease. Since alkylating agents in general nonselectively react and irreversibly bind to DNA as well as protein's thiols, many unpleasant side effects, such as neutropenia and myelosuppression limit the use of alkylating agents in clinic. To resolve these problems, we have synthesis several series of water-soluble N-mustards, which are designed by linking phenyl N-mustard pharmacophore to the DNA-affinic molecule (such as 9-anilinoacridines, acridines or quinolines) via a urea, carbamate or hydrazinecarboxamide linker. DNA-afficnic molecule can bring compound to target site, and urea linker can reduce the chemical reactivity of N-mustard moiety. In this study, we have evaluated the bioactivity of these novel water-soluble N-mustards against colorectal cancer

Materials and Methods:

Cell viability was assessed by using alamarBlue®. DNA cross-linking measurement was assessed by using alkaline agarose gel shift assay and comet assay. Cell cycle investigation was assessed via flow cytometry. Animal model for in vivo study was HCT-116 xenograft model.

Results:

By using alamarBlue® cell viability assay, we demonstrated that human colorectal cancer cell lines were relatively more sensitive to these water-soluble N-mustards as compared to other cancer cell lines. Among them, we evaluated the anticancer activity of BO-2094 in nude mice bearing HCT-116 and H460 xenografts. Our results showed that BO-2094 given 30 mg/kg for 5 days significantly suppressed the growth of human colon cancer HCT116 cells but was less active against human lung cancer H460 cells. Through alkaline agarose gel shift assay and comet assay, we confirmed the potent activity of water-soluble N-mustards on induction of DNA interstrand cross-links. Meanwhile, we observed G2/M arrest in HCT-116 cells treated with these newly synthesized water-soluble N-mustards.

Conclusion:

Our present studies implicate that these water-soluble N-mustards warrant our further development and preclinical studies.

P763
Mechanisms of Tumor Necrosis Factor-α-Induced Vascular Cell Adhesion Molecule-1 Expression in Human Cardiac Fibroblasts

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Backgrounds:

Tumor necrosis factor-α (TNF-α) may exert as a proinflammatory mediator that regulates immune responses and inflammatory processes in cardiovascular system. Several studies demonstrated that expression of adhesive molecules on the cell surface plays a critical role in several inflammatory responses. However, the mechanisms underlying TNF-α-induced VCAM-1 expression related to inflammatory responses in human cardiac fibroblasts (HCFs) remain unclear.

Materials and Methods:

To investigate whether TNF-α induces VCAM-1 expression, Western blot, RT-PCR, real-time PCR, and promoter assay coupled to using pharmacological inhibitors or specific siRNAs were performed. Moreover, the THP-1 monocyte adhesion was detected by adhesion assay in HCFs challenged with TNF-α.

Results:

The data showed that TNF-α induced time-dependently increased in VCAM-1 mRNA, protein expression, promoter activity, and monocytes adhesion, which were attenuated by pretreatment with the inhibitors of TNF-α receptor (TNF-α receptor neutralized antibody), MEK1/2 (U0126), p38 MAPK (SB202190), JNK1/2 (SP600125), and NF-κB (Bay11-7082). TNF-α-stimulated phosphorylation of ERK1/2, p38 MAPK, JNK1/2, and p65 were attenuated by pretreatment with TNF-α receptor neutralized antibody, U0126, SB202190, SP600125, and Bay11-7082. Furthermore, transfection with respective siRNAs also inhibited TNF-α-induced VCAM-1 expression in HCFs. These results suggested that in HCFs, TNF-α induces VCAM-1 expression mediated through the TNF-α receptor-dependent MAPKs signaling pathway linking to activation of NF-κB.

Conclusion:

In the study, we demonstrated that TNF-α-induced VCAM-1-dependent monocytes adhesion is mediated through activation of the TNF-α receptor 1, ERK1/2, p38 MAPK, JNK1/2, and NF-κB pathways in HCFs.

P764
從缺

P765

Inhibition of PGC-1α by MiR-23a Promotes Mitophagy and Cell Proliferation in Gastric Cancer

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Background:

MicroRNAs (miRNA) are 18-22 bases nucleotides in length, non-coding RNA that negatively regulate gene expression through its incomplete or complete binding to the targets mRNA 3'UTR, causing translational repression or mRNA cleavage. MiRNA have been found to regulate tumor progression, such as apoptosis and proliferation, as expected, in the gastric cancer. Our previous data shown the up-regulated miR-27a directly targets the suppressor of cytokine-induced signaling (SOCS6), elevates the level of signal transducer and activator of transcription 3 (STAT3) and lead to the up-regulated miR-23a targeting the peroxisome proliferator-activated receptor-γ coactivatros-1α (PGC-1α), promotes gastric cancer SC-M1 cells proliferation and inhibits cell death. PGC-1α plays an important role in the maintenance of mitochondrial biogenesis and function. Loss of PGC-1α might contribute to the dysfunctional mitochondria, accumulating intracellular ROS level and eventually lead to fragmented mitochondria and apoptosis. Mitophagy is one of the autophagy mechanisms to eradicate dysfunctional mitochondria via lysosomal degradation pathway. The relationship between the mitophagy and the down-regulated PGC-1α in the gastric cancer remains unsolved.

Materials and Methods:

We first indentified miR-23a can directly target PGC-1α by construct PGC-1α 3'UTR into psi-check2 vector, dual-luciferase reporter assay. Then, we transfected miR-23a inhibitor to check the mitochondrial function and quantity by western blotting and mitochondrial function assays. We also used the SC-M1 (DsRed2-mito tracker) cells to investigate the co-localization between mitophagic marker, microtubule associated light chain 3 (LC3), Parkin and DsRed2-mito tracker by immunostaining.

Results:

The western blotting revealed that miR-23a inhibitor can restore the PGC-1α level in the SC-M1 cells, and dual-luciferase reporter assay shown that miR-23a can directly target PGC-1α, indicating that miR-23a can negatively regulate PGC-1α directly. Next, mitochondrial membrane potential (MMP) assay shown no effect after treated with miR-23a inhibitor and mitochondria mass quantification assay shown decrease after treated with miR-23a inhibitor, implicating that miR-23a might negatively affected mitochondria through decrease the mitochondria mass. After knowing that mitochondrial mass were negatively regulated in gastric cancer, autophagic assay were examined. Co-transfection and immunostaining showed that DsRed2-mito tracker was colocalized with Parkin and LC3 after treated with CCCP. Western blotting also revealed that LC3 and Parkin were both elevated after treated with CCCP. All these evidences provided that mitochondrial dysfunction through miR-23a-down regulated PGC-1α might have an effect on mitophagy and cell proliferation.

Conclusion:

MiR-23a might promote mitophagy and gastric cancer cell proliferation by directly target PGC-1α.

P766

Secondary Metabolites From The Stems Of *Capsicum annuum L. var. longum Sendt* cv.

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Backgrounds:

Red pepper, *Capsicum annuum* (Solanaceae), is used as a spice all over the world. Red pepper is studied actively because its pungent principal component, capsaicin, has a dietary effect, analgesic activity, and antioxidant activity.

Materials and Methods:

The air-dried stems of C. *annuum* L. var. *longum Sendt* cv. (12.5 kg) were extracted with MeOH (30 L x 6) at room temperature and the MeOH extract (302.7 g) was obtained upon concentration under reduced pressure. The chemical constituents in the plants of C. *annuum* L. var. *longum* Sendt cv. were separated with column chromatography. However these compounds were obtained and characterized by the comparison of their physical and spectral data (UV, IR, NMR and MS)

Results:

The MeOH extract of its plants were subjected to solvent partitioning and chromatographic separation to afford 19 pure substances.

Conclusion:

Investigation on the MeOH extract of the plants has led to the isolation of 19 compounds, eight amides: *N-trans*-feruloyltyramine (1) , *N-cis*-feruloyltyramine (2) , *N-trans*-caffeoyltyramine (3) , *N-cis*-caffeoyltyramine (4) , *N-p-trans*-coumaroyltyramine (5) , *N-p-cis*-coumaroyltyramine (6) , cinnaretamine (7) and cinnabutamine (8) ; four steroids: *β*-sitosterol (9) , stigmasterol (10) , *β*-sitostenone (11) and stigmasta-4,22-dien-3-one (12) ; three lignans: (+)-syringaresinol (13) , (+)-dia-syringaresinol (14) and *threo*-2,3-bis-(4-hydroxy-3-methoxyphenyl)-3-methoxypropanol (15) [13]; three benzenoids: p-hydroxybenzoic acid (16) [14], *p*-hydroxybenzaldehyde (17) [14] and vanillic acid (18) [14]; one ionone: (+)-abscisic acid (19)

P767

TCTP is essential for β-cell proliferation during development and β-cell adaptation in response to insulin resistance

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Backgrounds:

We examined the role of translationally controlled tumor-associated protein (TCTP), an evolutionarily highly conserved protein implicated in cell growth and proliferation, in regulating both β-cell mass and function.

Materials and Methods:

Mice with β-cell-specific deletions of TCTP were generated by mating *TCTP*$^{f/f}$ mice with RIP-*cre* transgenic mice. Metabolic and islet morphological studies were performed to determine the effects of deleting this gene on β-cell mass and glucose homeostasis.

Results:

The enhanced β-cell proliferation detected both during the perinatal developmental period and in insulin-resistant states in high fat diet-fed mice was found to parallel the expression of TCTP in pancreatic β-cells. Specific knockout of TCTP in β-cells led to decreased β-cell proliferation and growth, reduced β-cell mass, and impaired insulin secretion; together these effects led to hyperglycemia.

Conclusion:

TCTP is essential for β-cell mass expansion during development and β-cell adaptation in response to insulin resistance. Possible molecular mechanisms of TCTP are under investigation.

P768

Vascular Endothelial Growth Factor Receptor 2 Maintains Resting Cardiac Vagal Baroreflex and Heart Functions in Mice

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Backgrounds:

The ability to maintain a stable blood pressure (BP) and heart rate (HR) is essential to normal function in animals, and the cardiac vagal baroreflex is the most fundamental feedback mechanism in this homeostatic process. Augmented afferent baroreceptor discharges upon detection of an increase in BP will induce the nucleus tractus solitarii (NTS) to normalize BP by reducing HR via excitation of the nucleus ambiguus (NA), which sends inhibitory signals to the heart by way of the vagus nerve. We investigated the role of vascular endothelial growth factor receptor 2 (VEGFR2), also known as Kdr/Flk-1, in cardiovascular regulation and heart function under resting conditions.

Materials and Methods:

Kdr^{tm1Jrt} mice (Strain: B6.129-Kdr^{tm1Jrt}/J, stock #002938) were purchased from Jackson Laboratory, and were maintained as heterozygous (Kdr+/-) and wild-type (Kdr+/+) colonies under specific pathogen-free conditions. BP and HR of mice were recorded under conscious state in their home cages using implantable telemeters. The sequence method was used to determine spontaneous baroreflex sensitivity (BRS). Sequential magnetic resonance imaging/diffusion tensor imaging (MRI/DTI) acquisition was performed under isoflurane anesthesia in a 9.4 T Animal MR scanner. Echocardiography was obtained using a 40-MHz linear-array transducer, in association with a digital ultrasound system. Functional parameters of the left ventricle (LV) were measured from M-mode imaging, and strain analysis was determined from B-mode imaging.

Results:

Real-time PCR and Western blot analysis showed a significant reduction of Kdr mRNA and protein in the NTS and heart of Kdr+/- mice when compared to Kdr+/+ mice. The power spectrum of HR recorded 24h detected by radiotelemetry, an indicator for overall functionality of brain stem cardiovascular regulation, showed that compared to the wild-type mice, the degree of variability, was reduced in Kdr+/- mice. Based on tractographic evaluations using MRI/DTI of the brain stem, we found that the functional connectivity between the NTS and NA was reduced in Kdr+/- mice, concurrent with a decrease in cardiac vagal baroreflex and an increase in HR. Echocardiographic analysis further showed that Kdr+/- mice were inferior to wild-type mice in terms of LV ejection fraction , LV fractional shortening, circumferential strain, and radial strain.

Conclusion:

These results demonstrate that VEGFR2 expression in the NTS is required for the maintenance of cardiac vagal baroreflex manifested in the form of functional connectivity between the NTS and NA, and for sustained heart functions under resting conditions.

P769

B1, An Novel synthetic Diamidoanthraquinone Induce Apoptosis through Down-Regulation of Stat3 and NF-κB in Human Lung Cancer Cells

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Backgrounds:

In our previous reports, B1, a novel topoisoperase II inhibitor, demonstrated a significant cytotoxicity through the cell cycle G1 arrest and apoptosis induction in human lung cancer cells. The exploration of agents targeting disregulated epigenetic pathway becomes an expending filed and sheds light on cancer treatment.Epigenetic regulation changes gene expression without alternation of DNA sequence. Histone acetylation is one of most studied epigenetic regulation, and enzymes, such as histone acetyltransferases and deacetyltransferases (HDACs), govern such modification. Agents targeting HDACs have been proved by the US Food and Drug Administration (FDA) for hematological malignance treatment.

Materials and Methods:

Human lung adenocarcinoma cell line A549 was cultured in MEM medium. (Bioresource Collection and Research Center, Taiwan.) Mitochondrial transmembrane potential (ΔΨm) For dissipation was measured by using a fluorometric probe, 3, 3-dihexyloxcarbocyanine ($DiOC_6$). After incubation for 12 h, A549 cells were stained with DiOC6 for 30 min at 37℃ , and analysed by flow cytometry. For cytoplasmic and nuclear lysate extractions, the cells were incubated in ice-cold hypotonic lysis buffer with freshly added protease inhibitor for 15 min. The contents were mixed on a vortex and then centrifuge (800g/5 min) at 4°C. The supernatant was saved as cytoplasmic enrich lysate and stored at -20°C.

Results:

We shows currently that B1 treatment decreases both the cell cycle signals protein including (cyclinE, CDK2 and p Rb) and apoptosis signals protein (Fas, Bcl-xL and Bcl-2). The transcription factor signal transducers and activators of transcription factor 3 (STAT3) and nuclear factor-κB (NF-κB) are two important transcription factors in tumorigenesis. B1 treatment inhibits cytosolic and nuclear transcription factor NF-κB and nuclear pSTAT3 expression.

Conclusion:

B1, induces human lung cancer cell apoptosis may through the regulation of transcript factors STAT3 and NF-κB.

P770

ATP Mediates NADPH Oxidase/ROS Generation and COX-2/PGE_2 Expression in A549 Cells

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Backgrounds:

Up-regulation of cyclooxygenase (COX)-2 and its metabolite prostaglandin E_2 (PGE_2) are frequently implicated in lung inflammation. Extracellular nucleotides such as ATP have been shown to act via activation of P2 purinoceptors, leading to COX-2 expression in various inflammatory diseases including lung diseases. However, the mechanisms underlying ATP-induced COX-2 expression and PGE_2 release remain unclear.

Materials and Methods:

A lung cancer A549 cells were used in the study. To investigate the effects and mechanisms of ATP-induced COX-2 expression, Western blot, RT-PCR, real-time PCR, and promoter assay coupled to use pharmacological inhibitors or transfection with siRNAs were performed. PGE_2 generation was detected by an ELISA kit.

Results:

The data showed that in A549 cells, ATPgammaS induced COX-2 expression and PGE_2 production, which were attenuated by the inhibitors of P2 receptor (PPADS and suramin), PKCs (Gö6983, Gö6976, Ro318220, and Rottlerin), ROS (Edaravone), NADPH oxidase (DPI and apocynin), Jak2 (AG490), and STAT3 [cucurbitacin E (CBE)] and transfection with siRNAs of PKCs, $p47^{phox}$, Jak2, STAT3, and cPLA2. In addition, ATPgammaS-stimulated ROS generation and $p47^{phox}$ translocation were also reduced by pretreatment with the inhibitors of P2 receptor, PKC, and NADPH oxidase. ATPgammaS-stimulated Jak2/STAT3 activation was also inhibited by these inhibitors in A549 cells.

Conclusion:

We demonstrated that ATPgammaS induces COX-2 expression and PGE_2 production via a P2 receptor/PKC/NADPH oxidase/ROS/Jak2/STAT3/$cPLA_2$ signaling pathway in A549 cells.

P771

A Common Naturally Compound A From *Cruciferous* Vegetables in Inhibiting CYP2C, CYP2B6, and CYP3A4 Expression Through a Receptor-mediated Activation

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Backgrounds:

Discrepancy expression and regulation of drug-metabolizing enzymes (DMEs) is a common cause of adverse drug effects in some drugs with narrow therapeutic index (TI). Several important cytochrome P450s (CYP450s), CYP3A4, CYP2C9, and CYP2B6 are predominantly regulated by nuclear receptors (NRs), pregnane X receptor (PXR) and constitutive androstane receptor (CAR).

Materials and Methods:

A pure compound commonly occurring from *cruciferous* vegetables, compound A, exhibits variety of biological functions, including antimicrobial and anticancer activity, however the effect of compound A on the modulation of CYP450 is not well understood.

Results:

Compound A potently and dose-dependently attenuated CYP3A4, CYP2C9, and CYP2B6 induction by blocking the activation of nuclear receptors, especially PXR and CAR.

Conclusion:

Our results may lead to the cautions of the frequency of undesirable food-drug interactions.Here, we established compound A as a novel and natural potent inhibitor of CYP450s and modulate CYP450s expression thus drug efficacies. Modification of CYP450s expression and activity by consumption of compound A could have important implications on drug safety.

P772

Involvement of autophagy in arsenite-induced neurotoxicity in primary cortical neurons

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Chronic exposure to arsenite-contaminated water/food and arsenic therapy reportedly causes neurotoxicity. In addition to our previous studies which showed the involvement of necrosis and apoptosis in arsenite-induced neurotoxicity, the present study focused on the role of autophagy in arsenite-induced neurotoxicity using primary cultured cortical neurons. Incubation with arsenite was found to cause cell death in concentration- and time-dependent manners. At the same time, arsenite elevated HO-1 level, a redox-regulated protein, indicating that arsenite induced oxidative stress. Furthermore, arsenite reduced procaspase-3 and procaspase-12 levels as well as increased active caspase 3 levels, suggesting that apoptosis was involved in arsenite-induced neurotoxicity. As to the involvement of autophagy, our data showed that arsenite elevated LC3-II level (a hallmark of autophagy) which was attenuated by 3-methyladenine (3MA, an autophagy inhibitor) and enhanced by chloroquine (an inhibitor of autolysosome formation), indicating that arsenite induced autophagy. Similar to 3MA-induced attenuation of LC3-II and arsenite-induced cell death, transfection of Atg7siRNA reduced arsenite-induced LC3-II levels and caspase 3 activation. These data indicate that autophagy plays a pro-death role in arsenite-induced neurotoxicity. Furthermore, arsenite concentration- and time-dependently reduced α-synuclein levels (a presynaptic vesicle-related protein). 3MA reduced arsenite-induced reduction in α-synuclein. Co-localization of α-synuclein and autolysosomes further indicates that autophagy-induced degradation of α-synuclein may contribute to arsenite-induced neurotoxicity. The neurotoxic effects of arsenics, including arsenite, monomethylarsonous acid (MMA^{III}), arsenate and dimethylarsinic acid (DMA^{V}) were compared; the cell viabilities of MMA^{III}, arsenite, DMA^{V} and arsenate were 22±6%, 60±2%, 94±2% and 99±2% of control, respectively. Furthermore, the potency in autophagy activation was as followed: MMA^{III} >arsenite>>arsenate and DMA^{V}. Co-incubation with glutathione attenuated arsenics-induced autophagy activation, suggesting that oxidative stress is involved in the arsenics-induced neurotoxicity. Moreover, autophagy appears to be prodeath in arsenics-induced neurotoxicity.

P773
Neuroprotective Effects of Valproic Acid in a 6-Hydroxydopamine Lesioned Parkinson Rat Model

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Backgrounds:
Parkinson disease (PD) is one of common neurodegenerative disorders and characterized by progressive degeneration of dopaminergic (DA) neurons within the substantia nigra (SN). Increasing evidences from animal PD models suggest the involvement of neuroinflammation in the pathogenesis of Parkinson's disease. Valproate (VPA) is a drug commonly used to treat seizures and bipolar mood disorder. Although the underlying therapeutic mechanisms are unclear, a growing body of evidences suggests that VPA has neuroprotective and neurotrophic actions in vitro, partially from the anti-inflammation effect.
Materials and Methods:
In this study, we injected different doses (1.5-15 μg) of 6-hydroxydoapmine (6-OHDA) into rat medial forebrain bundle (MFB) cause dopamine neuron loss in SN, and treated with VPA before and after 6-OHDA lesion.
Results:
The results showed that VPA can protect DA neurons from 6-OHDA toxicity which increase DA neuron number, rota-rod staying time, dopamine concentration in striatum, and brain-derived neurotrophic factor (BDNF) secretion, but no significant change in inflammation cytokine TNF-α or anti-inflammation cytokine IL-10.
Conclusion:
These results suggested that the neuroprotective effect of VPA occurs only when a lesion is presented at low dosage of 6-OHDA, and this protective effect might from stimulate BDNF secretion.

P774
Induction of autophagy by KMUP-3 in cardiac fibroblasts and cardiomyocytes

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Backgrounds:
Autophagy is an evolutionary conserved process involved in the degradation of long-lived proteins and excess or dysfunctional organelles. Autophagy occurs constitutively at low levels under normal conditions in most cells and is an important house-keeping process. Therefore, autophagy is usually thought of as a survival mechanism. In the heart, autophagy has an essential role for maintaining cellular homeostasis under normal conditions and increased autophagy can be seen in conditions of starvation, ischemia/reperfusion, and heart failure. Therefore, we aimed to investigate signal transduction pathways involved in the induction of myocardial autophagy by KMUP-3.
Materials and Methods:
Autophagy will be assessed by immunofluorescence and immunowesternblot of LC3-II, beclin-1 and other Atg in neonatal rat fibroblasts and cardiomyocytes. Treated cells are measured the contents of NO and the expression of eNOS, Akt and mitogen-activated protein (MAP) kinases (ERK, JNK and p38) by Western blotting analysis.
Results:
In this study, we demonstrated that LC3-II, beclin-1, p-Akt, p-eNOS and eNOS were activated in KMUP-3-treated fibroblasts and cardiomyocytes. And the expression of LC3-II and beclin-1 increase with time- and dose-dependent manner. To investigate whether the KMUP-3-induced autophagy was mediated via PI3K-eNOS signaling pathway, cells were treated with or without KMUP-3 in the presence or absence of the PI3K inhibitor wortmannin and NOS inhibitor L-NAME. Pretreatment with wortmannin and L-NAME markedly reduced the KMUP-3-induced expression of LC3-II, p-eNOS, eNOS and as shown by immunowesternblot and immunofluorescence analysis.
Conclusion:
Based on our results, KMUP-3-induced autophagy in cardiac fibroblasts and cardiomyocytes are modulated by PI3K-eNOS pathway.

P775
Vemurafenib is a High-affinity Substrate of Human ABCG2 Protein

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Backgrounds:
Reports have shown cases of drug resistance against vemurafenib, currently the only effective drug against metastatic and unresectable melanoma that carry an activating BRAF(V600E) mutant. Here, we investigate the potential mechanisms in the development of acquired resistance to vemurafenib in BRAF(V600E)-positive cancers.
Materials:
Multiple drug-sensitive, MDR, wild type and BRAF(V600E) mutant cancer cell lines were used in this study. Pharmacological assays were used to determine effect of vemurafenib in all cell lines. Fluorescent probes and FACSort flow cytometry were used to study the effect of vemurafenib on ABC transporter-mediated drug efflux. Biochemical assays were used to study the binding of vemurafenib to ABCB1 and ABCG2 directly.
Results:
We discovered that vemurafenib interacted with MDR-associated ABCB1 and ABCG2 directly, inhibited their functions and restore drug sensitivity in MDR cells overexpressing human ABCG2. More importantly, we revealed that in the presence of functional ABCG2, BRAF kinase inhibition by vemurafenib is significantly reduced in BRAF(V600E) mutant A375 cells, suggesting involvement of this transporter in acquired resistance to vemurafenib.
Conclusions:
Combination chemotherapy targeting multiple pathways could be an effective therapeutic strategy to overcome acquired resistance to vemurafenib for cancers harboring the BRAF(V600E) mutation.

P776
The Role of Eps8 in Ku70-mediated DNA Double Strand Break Repair in Cancer Cells

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Backgrounds:
DNA damage, such as base damage and DNA strand breaks, might result in cell apoptosis or cancer formation. Double strand breaks (DSBs) are the primary cytotoxic lesion induced by ionizing radiation (IR) and DNA-targeting anti-cancer drugs. These conventional chemotherapeutic drugs are used together with surgery or radiation therapy to treat metastatic cancers, but usually cause multidrug resistance (MDR). DNA repair ability might be one of the causes of anti-cancer drug resistance. Cancer cells may activate non-homologous end-joining (NHEJ) or homologous recombination (HR) to repair DSBs against DNA-damaging agents. The Ku heterodimer (Ku70/Ku80) is one of the main component of the nonhomologous end-joining (NHEJ) pathway that repairs DNA double-strand breaks (DSBs). We are interesting in studing Ku70-interacting proteins, such as Eps8, in DSBs repair.
Materials and Methods:
First, we generate HeLa cell expressing Eps8-siRNA or ku70-siRNA with/without ectopic Eps8. Next, we investigate the participation of Eps8 in the repairing pathway of DNA-damaging agents (doxorubicin and cisplatin) induced DSBs. Single cell electrophoresis (comet assay) is utilized to estimate the degree of DNA damage or DNA repairing ability. Tail moment is a damage measure combining the amount of DNA in the tail with distance of migration. We also measure the cell viabilities or chemosensitivity of HeLa cells treated with doxorubicin or cisplatin by MTT assay.
Results:
From the above experiments, we found that attenuation of Eps8 inhibits the phosphorylation of H2AX. Like Ku70, silencing either Eps8 impaired DSBs. However, ectopically expressed Eps8 further worsen DNA damaging agents-induced DNA damage in Ku70 knockdown cells. This phenomenon can't be seen in 261-Eps8. Finally, our preliminary data indicated that the sensitivity of ku70-knockdown cells to the cytotoxicity of doxorubicin and cisplatin is decreased by ectopic Eps8.
Conclusion:
Our data indicated Eps8-mediated DSBs repair might be via a different pathway as compared to Ku70 . However, this pathway has nothing to do with Eps8-mediated chemoresistance in cancer cells.

P777
The Inhibitory Mechanism of Migration by Galectin-4 in Bladder Cancer Cells

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Backgrounds:

Galectin-4 (gal-4), a member of galectins family, is composed of a linker peptide and 2 carbohydrate recognition domains at each end. Previous studies have shown that gal-4 plays inhibitory roles on cell proliferation, migration, and Wnt signaling pathway. Gal-4 is thought as an indicator of differentiation and also considered as a tumor suppressor. However, the underlying mechanisms are still unknown. This study aims to explore the inhibitory mechanism of gal-4 on migration.

Materials and Methods:

T24 cells with ectopically expressed gal-4 were established by transfection of a gal-4 expression vector into cells. Recombinant human gal-4 carbohydrate recognition domains (CRD1 and CRD2) and proteins (CRD1+2) with His-tag were expressed and purified from E. coli and used to treat T24 cells. The cell migration and invasion ability were determined by wound healing and transwell assay. The localization of gal-4 was examined by immunofluorescence staining. The levels of gal-4 and various CRD were determined by Western blotting assay.

Results:

Our preliminary results showed that ectopically expressed gal-4 mainly existed in cytosol but also co-localized with lipid raft. Intriguingly, gal-4 expression significantly reduced the protein levels of integrin βIII, N-cadherin, and beta-catenin expression. Meanwhile, we also observed reduced migration ability of T24 cells with overexpressed gal-4, implicating that gal-4 is inversely associated with cell migration. To confirm the role of gal-4 on cell migration, T24 cells were treated with recombinant gal-4 or CRDs. The present results showed that recombinant gal-4 was up-taken by the cells and distributed on cell membrane and in cytosol. In addition, CRD1 but not CRD2 and CRD1+2 proteins could inhibit cell migration. However, CRD2 and CRD1+2 inhibit cell invasion. Only CRD1+2 was able to reduce integrin and N-cadherin expression.

Conclusion:

Our present results suggested that gal-4 may play certain role to suppress the expression of integrin and N-cadherin and hence reduce the cell migration activity. The exact mechanism warrants our further investigation.

P778
Anti-diabetic and Antioxidant Activities of *Cirsium japonicum* DC. var. *australe* Kitam

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Backgrounds:

Cirsium japonicum DC. var. *australe* Kitam (CJA) which is a folk medicine in Taiwan has been used to the treatment of diabetes and inflammatory symptom. In addition, The DPPH scavenging and XO inhibitory activities of the CJA extract revealed its potential for cosmetic use and drug development.

Materials and Methods:

In this study, the CJA ethanol extract was investigated by bioactivity-guided fractionation. Using chromatography techniques, pure compounds were isolated. In addition, certain active compositions of CJA fractions are tracked and separated distinctly by using DPPH-TLC analysis following with centrifugal partition chromatography (CPC) method. Those of isolated compounds were investigated for their DPPH and ABTS^{+} scavenging activities. Cellular glucose uptake activity and oil red staining assay were taken to evaluate anti-diebatic activity of the CJA crude extract and major compounds.

Results:

Comparing dried and fresh material of the natural product, *Cirsium japonicum* DC. var. *australe* Kitam, only the extract from fresh plant exhibited DPPH and ABTS+ scavenging activities. Great amount of the major active compound, CJA-01 (1) was obtained by using high efficient chromatography technique, CPC. CJA-01 (1) which is the major component of CJA extract showed significant DPPH and ABTS^{+} scavenging activities EC_{50} 0.18 and 0.19 (mg/mL), respectively. In the other hand, the ethanol layer from CJA exhibited potent cellular glucose uptake activity of 3T3-L1 adipocytes. The 3T3-L1 adipocytes were stained with oil-red after cocultured with test samples for seven days. Our results showed that the CJA ethanol layer produced glucose uptake without surplus lipid accumulation.

Conclusion:

This is a university-industry cooperation success on new cosmetic and hypoglycemic candidate discovery. Anti-diabetic and antioxidant activities of the extract from *Cirsium japonicum* DC. var. australe Kitam has been revealed. *In vivo* animal study will be further investigated for the CJA supplement development.

P779
Depletion of 4E-BP1 and Regulation of Autophagy Lead to YXM110-induced Anti-cancer Effects

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Backgrounds:

Natural products have always been a profuse database for developing new chemotherapeutics. YXM110 is a newly synthesized phenanthroquinolizidines that exhibits excellent anti-cancer activity in numerous cancer cells. We aimed to elucidate the anti-cancer mechanisms of YXM110.

Materials and Methods:

Here, we examined the anti-cancer effects of YXM110 both *in vitro* and in vivo. Using cancer cell lines and the assays of protein synthesis, proteasome, RNA microarray, and autophagy to examine the *in vitro* activities of YXM110. The anti-cancer activity was also confirmed after YXM110 treatment in xenograft models.

Results:

Protein level of 4E-binding protein 1 (4E-BP1), which is crucial in cap-independent translation, was decreased significantly after YXM110 treatment via c-Jun N-terminal kinases (JNK)-mediated proteasomal degradation. Moreover, the effects of YXM110 were associated with several characteristics of autophagy, including accumulation of autophagic vacuoles, elevation of Atg12-Atg5 and LC3-II, and levels of GFP-LC3 puncta. The results suggested that depletion of Mcl-1 contributes to YXM110-triggered autophagy, whereas downregulation of lysosomal-related genes could cause autophagy impairment. Furthermore, YXM110-induced cell death were prevented by autophagy inhibitor 3-methyladenine (3-MA) and Atg5 silencing, indicating that YXM110-mediated autophagy impairment lead to cancer cell death. The tumor suppression with autophagy and apoptosis was also observed in YXM110-treated HCT116 xenograft models.

Conclusion:

In summary, this study has identified that YXM110 inhibits cancer cell growth and induces cell death in human cancer cells both *in vitro* and *in vivo*; the overall mechanisms involve protein synthesis inhibition, proteasome degradation of 4E-BP1, autophagic cell death and apoptosis. YXM110 also exhibits excellent anti-cancer activity in multiple drug resistant cell line KBvin. These observations provide a new insight to understand the mechanisms underlying this novel structure and make YXM110 an attractive anti-cancer agent against a broad spectrum of cancers.

P780
Impairment of Inflammatory-Induced Superoxide Dismutase 2 Expression by the PINK1 G309D Mutation

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Backgrounds:

Parkinson's disease is one of the most common neurodegenerative diseases. Mutation in the PINK1 gives rise to familial early-onset Parkinsonism. However, the etiology related to PINK1 is still not clear.

Materials & Methods:

A stable pool expressing recombinant PINK1 G309D mutant in SH-SY5Y cells was established. We further examined the manganese superoxide dismutase (SOD2) expression, protein kinase activation and NFκB nuclear translocation induced by TNFα in the PINK1 G309D mutant cells.

Results:

The SOD2 induction in response to TNFα treatment was impaired by the expression of recombinant PINK1 G309D mutant. TNFα induced SOD2 induction through the NFκB signaling pathway. The phosphorylation of NFκB p65 was inhibited in cells expressing the PINK1 G309D mutant. In addition, the translocation of p65 to the nucleus was also decreased in the PINK1 G309D mutant cells.

Conclusion:

These results indicate a novel pathway by which the defect of PINK1 kinase domain G309D inhibits the inflammatory cytokine-induced SOD2 production. Impairment of SOD2 production may accelerate the dopaminergic neurodegeneration in Parkinson patients with PINK1 defect.

P781

c-Src-Dependent MAPKs/AP-1 Activation is Involved in TNF-α-Induced Matrix Metalloproteinase-9 Expression in Rat Heart-Derived H9c2 Cells

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Backgrounds:

TNF-α plays a critical mediator in the pathogenesis of chronic heart failure contributing to cardiac remodeling and peripheral vascular disturbances. The implication of TNF-α in inflammatory responses has been shown to be mediated through up-regulation of inflammatory genes, including matrix metalloproteinase-9 (MMP-9). However, the detailed mechanisms of TNF-α-induced MMP-9 expression are largely unclear in the heart cells.

Materials and Methods:

Rat embryonic-heart derived H9c2 cells were used in the study. To investigate the effects and mechanisms of TNF-α-induced MMP-9 expression, zymography, Western blot, real-time PCR, and promoter assay were performed.

Results:

We demonstrated that TNF-α could induce MMP-9 mRNA expression associated with an increase in the secretion of MMP-9. TNF-α-mediated responses were attenuated by pretreatment with the inhibitor of c-Src (PP1), EGFR (AG1478), PDGFR (AG1296), PI3K (LY294002), Akt (SH-5), MEK1/2 (U0126), p38 MAPK (SB202190), JNK1/2 (SP600125), or AP-1 (Tanshinone IIA) and transfection with siRNA of c-Src, EGFR, PDGFR, p110, Akt, or c-Jun. TNF-α stimulated c-Src, PDGFR, and EGFR phosphorylation, which were reduced by PP1. In addition, TNF-α-stimulated Akt phosphorylation was inhibited by PP1, AG1478, AG1296, or LY294002. We further demonstrated that TNF-α markedly stimulated p38 MAPK, p42/p44 MAPK, and JNK1/2 phosphorylation via a c-Src/EGFR, PDGFR/PI3K/Akt pathway. Finally, we showed that, in H9c2 cells, TNF-α-stimulated AP-1 promoter activity, c-Jun mRNA expression, and c-Jun phosphorylation were attenuated by PP1, AG1478, AG1296, LY294002, SB202190, SP600125, or U0126.

Conclusion:

These results suggested that TNF-α-induced MMP-9 expression is mediated through a c-Src/EGFR, PDGFR/PI3K/Akt/MAPKs/AP-1 cascade in H9c2 cells.

P782

C-Src-dependent EGF Receptor Transactivation Contributes to ET-1-induced COX-2 Expression in Brain Microvascular Endothelial Cells

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Backgrounds:

Endothelin-1 (ET-1) is elevated and participates in the regulation of several brain inflammatory disorders. The deleterious effects of ET-1 on endothelial cells may aggravate brain inflammation mediated through the upregulation of cyclooxygenase-2 (COX-2) gene expression. However, the signaling mechanisms underlying ET-1-induced COX-2 expression in brain microvascular endothelial cells (bEnd.3 cells) remain unclear.

Methods:

The expression of COX-2 induced by ET-1 was evaluated by Western blotting and RT-PCR analysis. The COX-2 regulatory signaling pathways were investigated by pretreatment with pharmacological inhibitors, short hairpin RNA (shRNA) or small interfering RNA (siRNA) transfection, chromatin immunoprecipitation (ChIP), and promoter activity reporter assays. Finally, we determined the PGE_2 level as a marker of functional activity of COX-2 expression.

Results:

First, the data showed that ET-1-induced COX-2 expression was mediated through a c-Src-dependent transactivation of EGFR/PI3K/Akt cascade. Next, we demonstrated that ET-1 stimulated activation (phosphorylation) of c-Src/EGFR/Akt/MAPKs (ERK1/2, p38 MAPK, and JNK1/2) and then activated the c-Jun/activator protein 1 (AP-1) via $G_{\alpha/i}$ protein-coupled ET_B receptors. The activated c-Jun/AP-1 bound to its corresponding binding sites within COX-2 promoter, thereby turning on COX-2 gene transcription. Ultimately, upregulation of COX-2 by ET-1 promoted PGE_2 biosynthesis and release in bEnd.3 cells.

Conclusion:

These results demonstrate that in bEnd.3 cells, c-Src-dependent transactivation of EGFR/PI3K/Akt and MAPKs linking to c-Jun/AP-1 cascade is essential for ET-1-induced COX-2 upregulation. Understanding the mechanisms of COX-2 expression and PGE_2 release regulated by ET-1/ET_B system on brain microvascular endothelial cells may provide rational therapeutic interventions for brain injury and inflammatory diseases.

P783

Survivin plays a role in DNA repair in cancer cells with UV-induced DNA damage

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Backgrounds:

Survivin is a member of the inhibitor-of-apoptosis family proteins. It forms the chromosomal passenger complex (CPC) together with INCENP and aurora B kinase, and to promote the completion of mitosis. It also inhibits caspase-3 activation through physical interactions. Interestingly, recent studies indicate that Survivin may play a role in DNA repair. In this study, we aim to determine possible molecular role in which survivin plays in the process of DNA repair.

Materials and Methods:

In this study, UV-treated MDA-MB-231 breast cancer cells were used as a model. Human breast cancer MDA-MB-231 cells were treated with UV-radiation at sub-lethal intensity (50 J/m^2) to induce DNA damage. Western blot analysis and RT-PCR were used to examine the effects of UV-radiation on the expression of Survivin and various DNA damage-signaling molecules such as gamma-H2AX and Ku70 in MDA-MB-231 cells. Nuclear extraction assay and immunofluorescent microscopy were used to determine the cellular localization of Survivin. Comet assay was also used in this study to determine levels of DNA damage in cells with different treatments.

Results:

Western blot analysis revealed that sub-lethal UV-treatment transiently up-regulated the expression of Survivin, Ku-70, DNA-PKCs and gamma-H2AX in MDA-MB-231 breast cancer cells. RT-PCR analysis revealed that UV-radiation induced Survivin expression through post-transcriptional mechanism. In addition, immunefluorescent microscopy showed that UV-treatment induced nuclear accumulation of Survivin with puncta formation in the treated cells. At the functional level, targeting Survivin by specific small molecule inhibitor and siRNA further increased the amount of gamma-H2AX present in cells treated with UV (50 J/m^2). Targeting Survivin also decreased the cell viability of the UV-treated MDA-MB-231 breast cancer cells *in vitro*.

Conclusion:

Sub-lethal UV-treatment induces Survivin overexpression in the nucleus and its overexpression seems to be important for the process of DNA repair. However, further studies are needed to determine possible molecular pathways that are regulated by Survivin during DNA repair.

P784

Alpha -Lipoic Acid (α-LA) Inhibits Platelet-Derived Growth Factor (PDGF) - BB - Induced Proliferation on Rat Aortic Smooth Muscle Cells through AMPK Pathway

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Backgrounds:

The aim of this study was to investigate whether α-LA can inhibit the VSMC proliferation induced by PDGF and the possible molecular mechanism of its action.

Materials and Methods:

Rat aortic smooth muscle cells (RASMCs, A10 cells) were treated with α-LA at increasing concentrations 0, 1, 10, or 20 μM for 48 or 72hr as well as PDGF 0, 1, 2.5, or 5 ng/mL for 24, 48, or 72 hr. In order to investigate the anti-proliferative effect of α-LA on RASMCs, cells were treated with PDGF 5 ng/mL for 24 hr and PDGF with α-LA for another 48 hr. Furthermore, the phosphorylated AMP-activated protein kinase (pAMPK), cyclin D1, p21, $p27^{kip1}$ protein levels were measured in cells after the treatment with PDGF-BB and/or α-LA.

Results:

First, the number of cell was significantly increased after treatment with PDGF-BB (5 ng/ml) compared to the non-stimulated group, and α-LA (10-20 uM) significantly inhibited PDGF-BB- induced proliferation of RASMCs in a concentration-dependent manner. Second, PDGF-BB (5 ng/ml) significantly increased the level of cyclin D1 and p21 as well as decreased the level of p27kip1 expression in A10 cells. These effects of PDGF-BB were all reversed by the treatment of α-LA. Last, α-LA increased the phosphorylation of AMPKα; however, decreased AMPKα performance.

Conclusion:

These results suggest that α-LA inhibits PDGF-BB-induced RASM proliferation in association with induction of p27 expression and reduction of cyclin D1 and P21 expression. Further studies are going to clarify the molecular mechanism of α-LA on the PDGF-induced atherogenic effects. This study provides a rationale for the therapeutic use of α-LA for atherosclerosis.

P785

New neolignan derivative from *Machilus zuihoensis* Hayata var. *mushaensis* (Lu) Y. C. Liu

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Machilus zuihoensis Hayata var. *mushaensis* (Lu) Y. C. Liu is a small evergreen large tree, especially distributed in Philippines, Taiwan, India, China, Korea, Japan, Hainan and Malaysia. The *Machilus* species have reported for cytotoxicity, anticancer, antioxidation, antibacterial, antiplatelet, antiaging, antihypertension, anthelmintic activity and treating skin's disease. Phytochemical investigation of the leaves of *Machilus zuihoensis* Hayata var. mushaensis (Lu) Y. C. Liu were investigated by column chromatography. Three compounds, including one neolignanoid, machilolin-B (1), and two steroids, a mixture of *β*-sitosterol (2) and stigmasterol (3) were isolated from the MeOH extract. Among these isolates, 1 was a new compound. The structures of these compounds were established by spectroscopic and chemical analyses.

P786

PKC antagonist combined with UVB radiation may be another therapeutic method for epidermal proliferative disorders

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Backgrounds:

UVB is known to cause major alterations in growth and differentiation patterns of exposed human skin. Phototherapy, in the form of natural sunlight, has been availed for thousands of years for the improvement of numerous skin diseases. Although many data support the efficacy of phototherapy in the treatment of epidermal proliferative disorders, there remains considerable variability in its utilization around the world.

Method:

The keratinocytes are divided into five groups which are treated with bis indolyl- maleimide II (BIM II; PKC antagonist) at doses of $5x10^{-6}$M and irradiated with UVB at doses of 0 (control), 20, 40, 60, and 80 mJ/cm^2, respectively. MTT assay, western blot assay, and apoptosis test is used in the study.

Results:

In the present study, we try to find another therapeutic method for epidermal proliferative disorders. PKC antagonist and UVB radiation is combined and used in the study. The present study is determined in the expression of RKIP, raf-1, ERK1,2, MEK, proliferation and apoptosis in PKC antagonist combined with UVB radiation treated keratinocyte. The results show that the expression of RKIP, raf-1, ERK1,2, and MEK is significantly altered in PKC antagonist combined with UVB radiation treated keratinocyte.

Conclusion:

We now summarize in this communication the data indicating that PKC combined UVB radiation may be another therapeutic method for epidermal proliferative disorders.

P787

Effects of MH102 on 6-Hydroxydopamine Model of Parkinson's Disease

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Backgrounds:

Parkinson's disease (PD) is a chronic degenerative disease in the central nervous system, resulting in dysfunction of motor skills, language ability, and other features of patient. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a nuclear hormone receptor that has been shown to have neuroprotective effects associated with anti-inflammatory and matrix metalloproteinase (MMP) inhibitor properties in various experimental models of neurodegeneration. In vitro studies, PPAR-γ agonists were shown to block microglia and formation of cytotoxic factors from microglia.

Materials and Methods:

The aim of the present study was to investigate the therapeutic potential of the new synthetic biphenolic compound MH102 on alleviating neurotoxicity and motor deficits via activation of PPAR-γ in 6-hydroxydopamine (6-OHDA) PD mouse model. Sevendays after unilateral striatal 6-OHDA lesion induction, mice were administered daily and subchronically with MH102 (0.05-1 mg/kg) for 14 days. Then the apomorphine-induced rotational behaviors and the neuronal toxic responses were determined in the hemiparkinsonian mice.

Results:

Results showed that subchronic administration of MH102 significantly ameliorated apomorphine-induced contralateral rotation and restored the decreased protein expression levels of tyrosine hydroxylase (TH) and dopamine transporter (DAT) in striatum and substantia nigra in 6-OHDA-lesioned mice. Furthermore, MH102 reduced the increases in protein expression of glial fibrillary acidic protein (GFAP) for inflammatory response and inducible nitrous oxide synthase (iNOS) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) related oxidative stress in striatum. In addition, MH102 reversed the reduction of PPAR-γ protein expression in striata of 6-OHDA-lesioned mice.

Conclusion:

Taken together, our data indicate that MH102 may improve the motor dysfunction and neuronal toxicity related to inhibition of neuronal inflammation and oxidative stress, as well as activation of PPAR-γ in 6-OHDA PD mouse model. These results will enhance the potential possibility of MH102 as a new drug for treatment of Parkinson's disease.

P788

Secondary Metabolites From The Leaves Of *Aquilaria Sinensis*

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Background:

Previous phytochemical investigation on Chinese eaglewood revealed characteristic sesquiterpenes and chromone derivatives, but few reports about the chemical constituents of the leaves. The analgesic and anti-inflammatory activities of the ethanol extract of A. sinensis (Lour.) Gilg. Leaves were observed in various experimental models related to nociception and inflammation, so as to provide some evidence for its traditional use.

Materials and Methods:

The specimen of A. *sinensis* was collected from Guansi Township, Hsinchu County, Taiwan in May, 2007. A voucher specimen was identified by Professor Fu-Yuan Lu (Department of Forestry and Natural Resources College of Agriculture, National Chiayi University) and was deposited in the School of Medical and Health Sciences, Fooyin University, Kaohsiung, Taiwan. The leaves (1.2 kg) of A. *sinensis* were airdried and extracted repeatedly with MeOH (6 L× 8) at room temperature. The combined MeOH extracts (31.3 g) were then evaporated and further separated into 4 fractions by column chromatography on silica gel (4.3 kg, 70-230 mesh) with gradients of *n*-hexane/CH_2Cl_2/acetone/MeOH.

Results:

Twelve compounds, including three flavonoids, 5-hydroxy-4′,7-dimethoxy-flavonoid, luteolin-7,3′,4′-trimethyl ether, and 5,3′-dihydroxy-7,4′-dimethoxyflavone, five benzenoids, methylparaben, vanillic acid, p-hydroxybenzoic acid, syringic acid, and isovanillic acid, and four steroids, β-sitosterol, stigmasterol, β-sitostenone, and stigmasta-4,22-dien-3- one were isolated from the leaves of A. *sinensis*.

Conclusion:

Various biochemical characterization properties of A. *sinensis* were demonatrated for the first time in this work.We propose that *Aquilaria Sinensis* are effective nociception.

P789

Involvement of gelsolin in TGF-β induced Epithelial to Mesenchymal Transition of MDA-MB-231 human breast cancer cells.

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Backgrounds:

Increasing evidence indicates that abnormal expression of gelsolin (GSN) is one of the most common disorders in invasive and metastatic breast cancers. TGF-β has been shown to initiate the Epithelial to Mesenchymal Transition (EMT) in breast cancer cells. However, the relationship between the expression level of GSN and the TGF-β signaling for EMT progression is not clear.

Materials and Methods:

MDA-MB-231 human breast cancer cells were treated with 2 ng/ml TGF-β1 for 3 days, and the population of CD44+/CD24- cells were sorted out by flow cytometry. The expression of mRNA and protein content for GSN, and for markers characterizing stem cell function and EMT were measured by real time quantitative PCR and immunoblotting, respectively. Methylation specific PCR (MSPCR) was used to assess the epigenetic modification of GSN gene expression.

Results:

The TGF-β1 enriched CD44+/CD24- cells showed an increase in gene markers for stem cell pluripotency (Oct4, Sox2 and Nanog) associated with an increased expression for mesenchymal cell markers (N-cadherin, and vimentin) and a decreased expression for epithelial cell marker (E-cadherin). In addition, TGF-β1 induced an increased gene expression for GSN but decreased expressions for the two DNA methyltransferases, DNMT1and DNMT3B. MSPCR analysis also showed that TGF-β1 caused a 50% decrease in methylation with concomitant 3-fold increases in unmethylation on the CpG island at the GSN promotor in the CD44+ cells.

Conclusion:

Our data indicated that TGF-β1 induced epigenetic modification of GSN might be responsible for the cancer stem cell signaling in breast cancer cells.

P790

Investigation of the role of glycerophosphodiester phosphodiesterase 2 *(glpQ2)* in *Streptococcus pneumoniae*, TCH8431/19A, causing complicated pneumonia

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Background:

Streptococcus pneumoniae is a common respiratory pathogen leading to community-acquired pneumonia. Inflammation is often activated to clear pulmonary bacteria and symptom alleviates without damaging the lung architecture. However, increased incidence of pneumococcal infections complicated by an empyema, and/or fibrinopurulent stage progression are reported in recent years. The pathogenesis is still elusive.

Choline is an indispensable component in the cell wall architecture among respiratory pathogens. The metabolism of choline production is important in host cell binding, cytotoxicity, and transformation efficiency. Glycerophosphodiester phosphodiesterase (*glpQ*) mediates glycerophosphorylcholine utilization from mammalian cells and two isoforms of *glpQ* genes in *S. pneumoniae* are found. The membrane motif-containing *glpQ* is universal present in *S. pneumoniae* strains. The other form, *glpQ2* which is structurally relative to *glpQ* in *H. influenzae* and *M. pneumoniae*, is sporadic present in *S. pneumoniae* with serotype 19A and 3 frequently associated with complicated pneumonia these years. In this study, we aim to characterize the pathophysiological role of *glpQ2* in complicated pneumonia-causing strain.

Methods:

S. pneumoniae TCH8431/19A strain was isolated from pediatric patient suffering from complicated pneumonia. Deletion of *glpQ2* in TCH8431/19A is used to compare the colony morphology and cytotoxicity against A549 cell line with its parent strain. *Cis*-complementation is also established to confirm the role of *glpQ2*.

Results:

Deficiency of *glpQ2* reveals 59.2% of smaller colony on blood agar plate compared with its parent strain implying that *glpQ2* affects the pneumococcal cell wall architecture. When co-culturing with human lung epithelial cell line, A549, TCH8431/19A results in 40% cell death. However, the cytotoxic effect of the *glpQ2* mutant strains is significantly decreased to 17-21%; in contrast, complementation strains reveal indistinguishable cytotoxicity compared with wild type strain.

Conclusions:

These data indicate that deletion of *glpQ2* interferes with pneumococcal cell wall architecture and cytotoxic effect of complicated pneumonia-causing strain. Our findings suggest that *glpQ2* contributes to pathogenesis of complicated pneumonia via promotion of bacterial cytotoxicity to host cells. The mechanism of *glpQ2* mediating cytotoxicity will be further investigated.

P791

Freshwater Clam Extract Accelerates the Healing of Cutaneous Incision Wound in Rat

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Background:

An excessive inflammatory response results in increased tissue injury and poor wound healing. Freshwater clam extract (FCE) was confirmed had an anti-inflammatory effect. However, there is limited information about FCE effects on wound healing. This study investigated the influence of FCE on the wound healing and inflammatory response in a cutaneous incision model.

Methods:

Sixteen rats were used and divided into 2 groups, the FCE and NS groups. The full thickness skin wounds were created (2 × 1cm^2) on the dorsal surface of the rats. FCE 20mg/kg (dissolved in 1 ml normal saline) or 1 ml NS (normal saline) was applied for oral feeding twice daily for 21days after incision wound was performed. The blood biochemical substances were measured at several time points during the first day after incision, than measured at day5, 10, 15 and 21. On the day21, cutaneous wound tissues were collected for pathological examination.

Results:

There is no significant difference in the levels of WBC, lymphocyte, monocyte, and GRA between FCE and NS group (p>.05) after incision. These data indicated the clean incision wound model was successful. ALT and creatinine levels were also no significant difference. Otherwise, the area of wound in FCE group was smaller than NS group at day21 (2.25% vs.13.75%, p=.029). Histology of repaired incision wounds showed that FCE group had more fibroblast distribution and collagen fiber organization than NS group. The NS group had a greater accumulation of inflammatory cells in granulation tissue.

Conclusion:

FCE produces early wound healing in rat.

P792

Spinal serum- and glucocorticoid-inducible kinase 1 (SGK1) mediates neuropathic pain via kalirin and downstream PSD-95-dependent NR2B phosphorylation in rats

梁淑鈴

The coupling of the spinal post-synaptic density-95 (PSD-95) with the glutamatergic N-methyl-D-aspartate receptor (NMDAR) NR2B subunit and the subsequent NR2B phosphorylation contribute to pain-related plasticity. Increasing evidence reveals that kalirin, a Rho-guanine nucleotide exchange factor modulates PSD-95-NR2B-dependent neuroplasticity. Our laboratory recently demonstrated that serum- and glucocorticoid-inducible kinase 1 (SGK1) participates in inflammation-associated pain hypersensitivity by modulating spinal glutamatergic neurotransmission. Because kalirin is one of the proteins in PSD that is highly phosphorylated by various kinases, we tested whether kalirin could be a downstream target of spinal SGK1 that participates in neuropathic pain development via regulation of the PSD-95-NR2B coupling-dependent phosphorylation of NR2B. We observed that spinal nerve ligation (SNL, L5) in male Sprague–Dawley rats resulted in behavioral allodynia, which was associated with phosphorylated SGK1 (pSGK1), kalirin, and phosphorylated NR2B (pNR2B) expression and an increase in pSGK1-kalirin-PSD-95-pNR2B coprecipitation in the ipsilateral dorsal horn (L4-5). SNL-enhanced kalirin immunofluorescence was coincident with pSGK1, PSD-95, and pNR2B immunoreactivity. Small-interfering RNA (siRNA) that targeted spinal kalirin mRNA expression (10 μg, 10 μL; *i.t.*) reduced SNL-induced allodynia, kalirin and pNR2B expression, as well as kalirin-PSD-95 and PSD-95-pNR2B coupling and costaining without affecting SGK1 phosphorylation. Daily GSK-650394 administration (an SGK1 antagonist; 100 nM, 10 μL, *i.t.*) not only exhibited effects similar to the kalirin mRNA-targeting siRNA but also attenuated pSGK1-kalirin costaining and SGK1-kalirin coupling. We suggest that nerve injury could induce spinal SGK1 phosphorylation that subsequently interacts with and upregulates kalirin to participate in neuropathic pain development via PSD-95-NR2B coupling-dependent NR2B phosphorylation.

P793

The Role of Caveolin-1 in Substratum Rigidity Regulated Focal Adhesion Formation, Cell Spreading and Cell Stiffness

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Backgrounds:
To elucidate the role of Cav-1 in substratum rigidity regulated cell behaviors.

Materials and Methods:
Using atomic force microscopy (AFM) to detect the cell stiffness. Using polyacrylamide gel (PA gel)to generate different substratum rigidity. And use different cell types to confirm the phenomenon.

Results:
Knockdown of Cav-1 by siRNA or shRNA not only reduced the size and number of focal adhesion, but also resulted in cell softening in M10 and NMuMG cells. Disruption of Cav-1 function by MβCD also decreased cell stiffness in a dose dependent manner. In addition, MCF7 cancer cell line and Ras transformed cell NG8 and 7-4 cells exhibited markedly low Cav-1 level and cell softening as well. These cells also lost the stiffness adaptability to substratum rigidity. Overexpression of Cav-1 in 7-4 cells restored not only the cell stiffness but also the adaptability to substratum rigidity.

Conclusion:
This study indicated that Cav-1 can regulate focal adhesion formation, cell spreading, cell stiffness and the adaptability to substratum rigidity.

P794

CX3CL1 Mediates the Pro-inflammatory Properties in the Microparticles Derived from Apoptotic Acute Promyelocytic Leukemic Cells

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Background:
All-transretinoic acid (ATRA) induces acute promyelocytic leukemic (APL) cells to differentiate along the granulocytic lineage and has beenused successfully in treating APL patients. CX3CL1/fractalkine is a chemokine and intercellular adhesion molecule, which also plays an active role in the chemotaxisof macrophages to apoptotic cells. The aim of this study is to determine the role of CX3CL1 in the clearance of apoptotic APL(NB4) cells.

Materials and Methods:
The surface expression of CX3CL1 was determined in the ATRA-NB4 cells which were pre-treated with ultra-violet (UV 2000 mJ/cm^2) before the flow cytometric assay. UV-treated cells were co-cultured with NR8383 phagocytes for phagocytosis assay. Conditioning medium (CM) of UV-treated cells were collected to determine their adhesive activity and migratory activity, as well as the functional activity of microparticles (MPs).

Results:
The percentage of apoptotic ATRA-NB4 cells increased in a time-dependent manner ($P<0.001$)at 4, 16 and 24 hours after UV irradiation, as determined by the surface expression ofAnnexin V and 7-AAD. Phagocytosis of these UV-treated ATRA-NB4 (UV-ATRA-NB4) cells were also increased in a time-dependent manner ($P<0.001$). Increased adhesive activity and transmigration activity were also observed in the CM of UV-ATRA-NB4 cells ($P<0.05$ & $P<0.001$; respectively). The expression of CX3CL1 in the UV-ATRA-NB4 cells were initially decreased 4 hours after UV treatment ($P<0.05$), but thereafter increased significantly at16 hours after UV treatment ($P<0.01$). Western blotting analysis demonstrated that the level of CX3CL1 protein was significantly increased in the UV-ATRA-NB4 cells at the 4 hours after UV treatment. However, the level of CX3CL1 was not detectable in the CM of UV-ATRA-NB4 cells. We further demonstrated that the number of MPs and CX3CL1(+) MPs increased significantly in the CM of UV-ATRA-NB4 cells ($P<0.01$ & $p<0.05$; respectively). Further studies demonstrated that MPs derived from UV-ATRA-NB4 cells were associated with increased adhesive and transmigratory activity, as compared with those MPs derived from UV-untreated ATRA-NB4 cells,and these properties were able to be inhibited when MPs were pre-treated with monoclonal antibody specific to CX3CL1.

Conclusion:
CX3CL1 plays an important role in the clearance of apoptotic ATRA-APL cells.

P795

Metabolic Remodeling Accompanies a Decline in Cardiac Efficiency And Contractile Function In High-Fructose Feeding Animal Model

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Backgrounds:
Cardiac dysfunction is now known to exhibit in type 2 diabetic patient, with high morbidity of cardiomyopathy. In animal model, consumption of high-fructose contributes to insulin resistance and cardiomyopathy. However, previous study shows fructose does not causes cardiac hypertrophy implies the diverse mechanisms with high fat diet induced cardiomyopathy. Thus, the existing disturbances in myocardium independent of obese with cardiac hypertrophic individual are worthy to clarify.

Materials and Methods:
In this study, 8 weeks old male SD rats were fed ad libitum a normal chow diet (C) or high fructose diet (67 % Kcal from fructose) (HFr) for a period of 12 weeks. The cardiac contractility was evaluated by Pressure-Volume catheter. Myocardium lipid profiles were measured by lipidomics.

Results:
The HFr rats developed metabolic syndrome which was characterized by impairment of fasting glucose, hypertension, and hypertriglyceridemia. HFr rats also exhibited a hyperinsulinemia suggesting insulin sensitivity was declined in HFr animals. The appearance and tissue weight of heart were not significant difference compared to controls; however, the HW/BW ration was significantly increased in HFr heart revealed cardiac hypertrophy after normalization of body weight. The myocardium contractile indices (EES, AE/EES, PRSW) were decreased and intraventricular pressure (Max P) was increased in HFr rats compared to controls. These results indicate that impairment of LV systolic factor and mechanical efficiency in HFr insulin resistant rats. Furthermore, there was lipotoxicity effect of the increasing of fatty acids with different carbon chain (C16:1, C18:1, C22:4), which augment myocardium damage. In addition, the HFr increased inflammatory mediator arachidonic acid (AA) also play a role in cardiovascular disease, and synergistically contributed to contractile dysfunction.

Conclusion:
In the conclusion, our results demonstrate HFr-induced systemic insulin resistance may cause cardiac lipid accumulation and lipotoxicity and consequently disturb the mechanical function of the heart.

P796

Hydrogen-rich Water Against Aβ-induced Cell Death Through AKT/SIRT1/FOXO3a Modulating in Human SK-N-MC Cells

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Backgrounds:
Alzheimer's disease(AD) is the most common neurodegenerative disorder causes dementia. Several lines of evidences suggest amyloid-beta(Aβ) induced reactive oxygen species(ROS)leading neuronal cell death. Additionally, Silence information regulator 1(SIRT1)and Forkhead box protein O3a(FOXO3a)are new important signaling molecules factors which are ROS- modulating proteins. In recent studies, showed hydrogen-rich water(HW), containing a huge amounts of molecular hydrogen, known to be ROS scavenger, might also carry an antioxidant property. Besides molecular hydrogen can pass through blood-brain barrier, therefore, HW has a potential to become protective daily drink against brain injuries caused by oxidative stress. However, the mechanism of how HW inhibit cell death induced by Aβ still unclear. The aim of this study is to investigate whether HW protects cell death by modulating AKT, SIRT1 and FOXO3a.

Materials and Methods:
In this study, we first investigate the neurotoxicity induced by Aβ. Then, we measure the ROS scavenge ability of HW. Next we observed the apoptosis phenomenon between control and HW-treated group. We also observed the inhibition of caspase and activation of SIRT1 and P-AKT and P-FOXO3a.

Results:
Our results showed that HW inhibited ROS formation, inhibited cell apoptosis and activated SIRT 1, P-AKT and P-FOXO3a activities.

Conclusion:
We suggest that the mechanism of the protective effects of HW against cell death may be by the activation of vita-gene and also by activation of AKT/SIRT1/ P-FOXO3a signal pathway.

P797
Loss of MeCP2 alters patterns of ultrasonic communication in mouse pups

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Communication deficit is an autistic symptom of Rett Syndrome (RTT), a developmental disorder caused by mutations of a X-linked gene, *methyl-CpG binding protein 2 (MECP2)*. Previous studies showed that ultrasonic vocalization is impaired in pups lacking mu-opioid receptors 1 (MOR1), in addition, we found significant reduction of MOR1 in the striatum of MeCP2 deficient mice, raising the possibility that ultrasonic communication might be altered in pups lacking MeCP2. Here we tested this hypothesis by measuring ultrasonic vocalization in mouse pups carrying deficient MeCP2. The pups of *Mecp2*-null mice and their wild-type littermate controls at age of postnatal day 8 (P8) and P10 were separated from their dams and the pups' ultrasonic calls were recorded in a sound-reduced Styrofoam chamber. Surprisingly, the Mecp2-null pups call more frequently and more sustained, with longer maximal duration of individual call during five-minute recording. Currently the pups of conditional *Mecp2*-knockout mice and pups carrying point mutation of MeCP2 are tested to clarify the roles of MeCP2 in vocal communication in mice. (Supported by NSC100-2320-B-004-001 and NSC101-2320-B-004-003-MY2)

P798
Investigating the Effects of Advanced Glycation End Products (AGEs) on Human Gastric Cancer Cell

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Backgrounds:

To study the molecular mechanism underlying AGEs-stimulated hypoxia-induced factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) expression in human gastric cancer cell.

Materials and Methods:

We treated the human gastric cancer cell, AGS, with different dose of AGEs. The AGEs-stimulated expression of HIF-1α, ERK, phosphor-ERK, Akt, phosphor-Akt, VEGF and RAGE were examined by western blotting in AGS cells. HIF-1α, VEGF and RAGE gene expressions were verified by q-RTPCR. Different kinases inhibitors were examined to figure out the signaling pathway that AGEs-stimulated AGS cell migration. AGEs-stimulated AGS cells migration were also evaluated by transfection with *si*RNA oligonucleotides of HIF-1α and RAGE.

Results:

Our preliminary data implied that AGEs could significantly promote human gastric cancer cell migration but not alter the proliferation. Interestingly, in normoxia condition, AGEs can enhance the hypoxia inducible factor-1α protein expression in a dose and time dependent manners in AGS cells. AGEs treatment can induce the phosphorylation of Akt and ERK, however, pretreatment with the PI3K inhibitor (LY294002) and MAPK inhibitor (PD98059) markedly blocked AGEs-stimulated migration of AGS cells. AGEs also induced VEGF expression but this phenomenon can be reverse by blocking MAPK- and PI3 kinase-activation, respectively. We also found different kinds of AGEs, *N*-(carboxymethyl)lysine (CML) and methylglyoxal (MGO) can markedly promote AGS cell migration. Knockdown of HIF1α and RAGE significantly block gastric cancer cell migration suggested that HIF1α and RAGE might play an important role in AGEs-stimulated AGS cell migration.

Conclusion:

Our study revealed the AGEs can enhance the AGS cell migration through the the PI3K/Akt- and MAPK-dependent pathways, in addition, HIF-1α, VEGF and RAGE might involved the pathogenic molecular mechanism for AGEs in gastric cancer cell.

P799
Correlate between Chronic Hepatitis C Treatment Response and IL28B Gene Polymorphisms to Hepatocellular Carcinoma

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Backgrounds:

Polymorphisms in the IL-28B are important determinants in the spontaneous and drug-induced control of hepatitis C virus (HCV) infection. To assess the association of IL-28 B polymorphisms on the development of hepatocellular carcinoma (HCC) with HCV patients.

Materials and Methods:

All patients were positive for anti-HCV antibody and had detectable HCV RNA. Patients with biopsy-proven chronic HCV and had received combined peg-IFN/ RBV therapy between March 2002 to October 2009 were enrolled. The diagnosis of advanced fibrosis/cirrhosis was determined by histological criteria. Patients underwent liver biopsies within 6 months before the start of therapy. Patients HCV-RNA viral load quantitative analysis; qualitative HCV-genotype and IL28 SNP genotyping analysis.

Results:

The CT and TT genotype of rs12979860 was associated with the development of HCC (p <0.001), and the stepwise logistic regression analysis showed that age greater than 60 years, sex, low platelet count, AFP≥20 ng/ml and liver cirrhosis were associated with HCC onset. The prevalence of HCC in explanted livers was significantly higher among patients with CT/TT genotype, suggesting a protective role of the CC genotype in HCC development (p<0.001). Although no impact could be observed regarding acute cellular rejection, T allele was significantly associated with antiviral therapy failure and faster development of advanced fibrosis after LT.

Conclusion:

Our study analysis indicated that PEGIFN plus RBV could prevent HCC development and improve survival, and the *IL28B* SNP does directly influence hepatocarcinogenesis in chronic HCV infection.

P800
EFFECTS OF IONOMYCIN AND BUMETANIDE ON VOLUME-ACTIVATED K^+ TRANSPORT IN LEUKEMIC CELLS

黃純健

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Backgrounds:

To investigate the effects of ionomycin and bumetanide on the volume-activated K^+ transport in leukemia cells.

Materials and Methods:

Cell culture; The human leukemic cells were maintained in RPMI 1640 medium containing fetal calf serum, L-glutamine, and streptomycin, at 37°C in a 5% CO2 incubator.

K^+ ($86Rb^+$) flux measurements; Leukemic cells in suspension were harvested and then preloaded with $86Rb^+$ (1 μCi/ml) for 30 min. Cells were subsequently washed with HBSS solution. The cells were then exposed to HBSS or calcium-free iso-osmotic solution, HBSS or calcium-free hypo-osmotic solution, or the above solutions with inhibitors. Flux was measured over a 15-min period. Time 0 was defined as the starting point when cells were added into the above solutions. Aliquots of the cell suspensions were removed at various time intervals and immediately centrifuged. Aliquots of the supernatant were removed for scintillation counting.

Results:

Subjected to hypo-osmotic challenge, the cells demonstrated a rapidly increased K^+ flux. This volume-activated K^+ flux was inhibited by calcium-free treatment. However, with the presence of ionomycin, this volume-activated K^+ flux was significantly increased. Treatment with bumetanide significantly inhibited this volume-activated K^+ flux. Furthermore, general protein kinases blocker staurosporine (200 nM) significantly inhibited this volume-activated K^+ flux as well.

Conclusion:

Since the effects of ionomycin and bumetanide are evidently affecting the volume-activated K^+ flux, therefore, the calcium-sensitive K^+ pathway and Na/K/Cl cotransport may mediate the volume-activated K^+ transport in leukemic cells. Furthermore, protein kinases may involve with modulating this volume-activated K^+ transport activity.

P801

Examinations of bilateral and unilateral ventral posterior medial nucleus of thalamus lesions in spatial learning and conditioned place preference in rats

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Backgrounds:
To our knowledge, the ventral posterior medial (VPM) nucleus of thalamus may be related to the numerous learning and memory. However, whether the bilateral and unilateral VPM lesions have a different effect in involving in the spatial learning as well as the conditioned place preference remains unclear.
Materials and Methods:
To address this issue, the present study was designed to examine the Morris water maze and conditioned place preference tasks when rats encountered the damage of the VPM. All rats were respectively given a 0.025 U dose of collagenase type IV (volume, 1.25U) or its vehicle saline by bilateral and unilateral injections to damage VPM. After that, all rats encounter the water maze and conditioned place preference tasks. These two tasks are randomly conducted.
Results:
The water maze test indicated that groups were significant differences among control, unilateral, and bilateral VPM groups. The sessions were significant differences. The interaction of group and session was significant. Furthermore, the post hoc with Tukey indicated that significant differences only in session 1. The conditioned place preference test indicated that there was non-significant among the control, unilateral, and bilateral VPM groups regardless of paired and unpaired sides.
Conclusion:
Altogether, only unilateral lesion of VPM group could impair the spatial learning in Morris water maze task. However, the bilateral lesion of VPM rats did not show any significant difference with the control rats. In summary, why the bilateral VPM lesion is different effect with the unilateral VPM lesion should be discussed.

P802

Resistance of colorectal cancer cells to hypoxia-induced necroptosis is conferred by glycolytic pyruvate scavenging of mitochondrial superoxide

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Depletion of oxygen and nutrients triggers necrotic cell death. Recent findings indicate that increased glucose transporters (GLUTs) and anaerorobic glycolysis, implicated as adaptive responses to hypoxic stress, were found in peri-necrotic regions in human colorectal carcinoma. Receptor-interacting protein (RIP) signaling and mitochondrial reactive oxygen species (ROS) are involved in necrotic pathways.

However, the underlying mechanisms of death resistance conferred by glucose in hypoxic cancer cells remain poorly understood. Our aim is to investigate the signaling pathways of hypoxia-induced necrosis and to explore the glucose metabolic intermediates involved in the mechanisms of anti-necrotic resistance. Human colorectal carcinoma Caco-2 cells were hypoxia exposed with or without glucose, and necrotic death examined using lactodehydrogenase (LDH) activity assay, real-time microscopic imaging, and RIP-1/3 immunoprecipitation and 32P kinase assays. Our results showed that hypoxic challenge in glucose-free media displayed LDH leakage and RIP-1/3 complex formation, which may be inhibited by necrostatin-1 and gene silencing of RIP1. Normoxic counterparts showed no sign of necrosis. Inhibition of mitochondrial superoxide production by antioxidants also decreased LDH leakage. Addition of glucose and pyruvate derivative, but not non-metabolizable analogs, diminished RIP-dependent LDH leakage. Hypoxic cells with glucose showed HIF1α activation and hypoxia-targeted gene (GLUT-1 and -4) expression. Glucose increases ATP and pyruvate levels, whereas pyruvate derivative did not change the ATP level in hypoxic conditions. Pyruvate decreased hypoxia-induced mitochondrial superoxide levels and was involved in anti-necrotic mechanisms. In conclusion, glycolytic pyruvate confers resistance to RIP-dependent necrosis in hypoxic cancer cells through mitochondrial ROS suppression.

P803

Ascorbate Inhibits Nitric Oxide Production and iNOS Expression by LPS Via the inhibition of p38 Mitogen-Activated Protein Kinase in Rat Primary Neuron-Glia Cultures

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Backgrounds:
Sepsis causes excessive production of nitric oxide (NO), inflammatory mediators and depletion of the endogenous antioxidant ascorbate in brain cells leading to disturbance in the prooxidant-antioxidant. Ascorbate administration was found to against oxidative stress in septic models.
Materials and Methods:
In the study, lipopolysaccharide (LPS) was used to primary neuron/glia cultures to model sepsis. We investigated effects of ascorbate on LPS-stimulated mitogen-activated protein kinases (MAPKs) signaling ans inflammatory mediators production.
Results:
LPS (100 ng/ml)-induced the expression of inducible nitric oxide synthase (iNOS) and production of NO, interleukin-6 (IL-6) and macrophage inflammatory protein (MIP) in a time-dependent manner. Time course study also showed that p38 and extracellular-signal-regulated kinases (ERKs) MAPKs were activated by LPS. Inhibitor experiments further demonstrated that LPS-induced iNOS expression and production of NO, IL-6 and MIP were significantly attenuates mainly by p38 inhibitor and partially b3 ERKs inhibitor. Likewise, co-treatment of ascorbate suppressed LPS-induced iNOS expression, NO production as well as attenuated IL-6 and MIP production in a concentration-dependent manner. Cultured cells were treated for 24 h with various concentrations of ascorbate (1, 5, 10 mM) added simultaneously with LPS didn't affect cell integrity as assayed by 3-(4,5-dimethylthianol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) reduction and lactate dehydrogenase (LDH) release. Furthermore, ascorbate not only suppressed LPS-activated p38 and ERKs but also reversed IκBα degradation.
Conclusions:
Asocrbate suppressed LPS-stimulated production of inflammatory mediators in neuron/glia cultures via, at least in part, inhibition of NFκB signaling and MAPK signaling pathways.

P804

Ascorbate Attenuates Methamphetamine-induced autophagy and apoptosis in cultured neuronal/glial cells

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Backgrounds:
Oxidative stress and apoptosis have been demonstrated to be major causes of methamphetamine (METH)-induced neurotoxicity. Moreover, autophagy, an alternative cell death, has also involved in METH toxicity. Little is known about the role of autophagy in the regulation of apoptosis in brain cells. It has been proposed that METH-generated toxicity associated with initiating the apoptotic signaling cascade results in increase the autophagic phenotype in neuronal cells.
Materials and Methods:
We previously demonstrated that ascorbate attenuates METH neurotoxicity. In the study, cultured cortical neuronal/glial cells were treated with METH (5 mM) to induce neurotoxicity. We investigate effects of L-ascorbate on METH-induced oxidative stress, apoptosis and autophagy.
Results:
Results show that levels of reactive oxygen species (ROS) significantly increased in a time-dependent manner after METH treatment. We also found that METH induced the expression of LC3-II, a protein associated with the autophagosome membrane, in a time-dependent manner. Staining of monodansylcadaverine (MDC), a marker for autophagolysosome, revealed that METH- induced the appearance of punctuate structures in the cytoplasm, indicative of autophagy. Pre-treatment of ascorbate not only obviously reduced METH induced ROS production but also attenuates METH-induces LC3-II expression. METH-induced cell damage as revealed by measurement of lactate dehydrogenase (LDH) release, was significantly attenuated by ascorbate. In addition, pre-treatment of ascorbate significantly reduced METH-induced propidium iodide (PI)- positive cells.
Conclusions:
We conclude that ascorbate protects METH-induced neurotoxicity by attenuating ROS production, apoptosis and autophagy. We suggest that ascorbate may serve as a strategy to protect brain against METH neurotoxicity.

P805
Analysis of anti-Herpes Simplex Virus type 1 Activities from *Polygonum Multiflorum* Extracts and Their Effects on Expression of Alzheimer's Disease Markers

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Backgrounds:
Herpes simplex virus type 1 (HSV-1; herpesviridae) is an enveloped DNA virus and a risk factor for Alzheimer's disease (AD). *Polygonum multiflorum* is applied for anti-aging in Chinese medicine. In the present study, anti-HSV-1 activity of P. *multiflorum* extracts (PM) and effects of HSV-1 infection on AD markers, amyloid precursor protein (APP) cleavage and tau proteins phosphorylation, were evaluated.
Materials and Methods:
The anti-HSV-1 activity was determined by plaque reduction assay. The virus titer was analyzed by plaque forming assay. Cell viability and HSV-1 structure proteins expression such as gB were determined by alarmar blue test and Western blotting, respectively. Both APP cleavage and tau proteins phosphorylation, in HSV-1 infected SH-SY5Y cells were determined by Western blotting.
Results:
PM blocked HSV-1 replication in Vero E6 cells in a dose-dependent manner with IC_{50} 108 ±13.8 μg/ml. The inhibitory effect of PM was not related to direct cytotoxicity. The results demonstrated that PM added at 0 to 8 hr postinfection time reduced HSV-1 replication in Vero E6 cells. PM has 20% inhibitory activity on virus adsorption and entry. HSV-1 gB proteins expression in Vero E6 cells was attenuated by PM. HSV-1 infection induced tau protein phosphorylation and increased APP-F35 fragments production in SH-SY5Y cells.
Conclusion:
P. *multiflorum* contained anti-viral components that inhibited HSV-1 replication by blocking of gB proteins synthesis. The APP cleavage and tau proteins phosphorylation in SH-SY5Y cells could be induced by HSV-1 infection. In future, effects of PM on AD markers expression will be studied.

P806
Peroxisome Proliferator-activated Receptor Gamma Analogue Decreased Epileptic Responses

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Backgrounds:
Epilepsy is a general neurological problem with the most extreme form of synchronous brain hyperactivity and increasing interests show that the inflammation might be related to the pathogenesis of epilepsy. Furthermore growing evidence shows that peroxisome proliferator-activated receptor gamma (PPARγ) analogue might have anti-inflammatory effects. To investigate the effects of PPARγ agonist on epileptic responses was the main focus in this study.
Materials and Methods:
Adult male C57BL/6 mice were used and treated with pentylenetetrazole (PTZ) by a single intraperitoneal injection to induce epilepsy. The intensity of epileptic responses was evaluated using a scoring scale. We also used the rosiglitazone as the PPARγ analogue to treat animal through the oral gavage.
Results:
The epileptic inducer, PTZ, showed the dose-dependence in the scoring scale of epileptic responses. Different dosages of rosiglitazone co-administrated with the epileptic inducer, PTZ, could not influence the severity of epileptic responses. However, three-day pre-treatments of rosiglitazone diminished the epileptic responses induced by the PTZ. The initial time, duration, and severity of responses were lessened after co-administration of rosiglitazone.
Conclusion:
These data in this study indicate that the PPARγ analogue, rosiglitazone, attenuates PTZ-induced seizure responses, but the treated timing is the considerate factor.

P807
Effects of Andrographis paniculata extract on antioxidation

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Backgrounds:
Andrographis paniculata Nees., Family Acanthaceae, is a traditional folk medicinal plants, widely used in various applications, and has been used as antiinflammatory, antibacterial, antipyretic and immune promoting traditional therapeutic drugs. Excessive reactive oxygen radicals produced by oxidative stress make the brain hurt easily. It has been known that oxidative stress could easily lead to neurodegenerative diseases and the extract of Andrographis paniculata is a good antioxidant.
Materials and Methods:
We used the human neuroblastoma IMR-32 cells treated with hydrogen peroxide and ethanol oxidation to investigate whether Andrographis paniculata extract has antioxidant neuroprotective effects. To study the protective effects of Andrographis paniculata extract, we treated cells with hydrogen peroxide/ethanol and various concentrations of Andrographis paniculata extract (25, 50, 100, 200 μg/ml) for 24 hours and measured the cell survival rate. We also used ApoGlow™ assay kit to study the mechanism of Andrographis paniculata extract to protect cells from oxidative stress.
Results:
Under the cellular experimental conditions, the use of hydrogen peroxide and ethanol can induce cell death and decrease cell survival rate. However, Andrographis paniculata extract can protect cells from damage and increase cell survival rate. We studied whether the cell death via apoptosis using ApoGlow™ assay kit and found that hydrogen peroxide/ethanol induces apoptosis, increasing ADP/ATP ratio, and Andrographis paniculata extract decreases the adverse effects.
Conclusion:
Integrated our experimental results, Chinese herbal medicine Andrographis paniculata extract protects nerve cells against oxidative stress injury. Maybe it can be used for prevention or treatment of neurodegenerative diseases in the future.

P808
The Impact of P-selectin on High-Fat Diet-Induced Obesity and Insulin Resistance

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Backgrounds:
Chronic inflammation is widely observed in obese individuals, and trigger insulin resistance and type 2 diabetes. P-selectin is a cell adhesion molecule which expressed on the surface of activated vascular endothelial cells and platelet. P-selectin participate the recruitment of leukocytes to injury area in the early stage of leukocyte attachment, and followed mediates inflammation. In addition, P-selectin also activated platelets and contribution to leukocyte rolling.
Materials and Methods:
The present study was aimed to investigate the impact of P-selectin on high-fat diet-induced obesity, adipose tissue inflammation, and systemic insulin resistance. The experimental animal were randomly divided into four groups, wild-type fed with chow diet (C) or high-fat diet (H) and P-selectin KO mice fed with chow diet (PC) or high-fat diet (PH).
Results:
C57BL6c mice fed with high-fat diet for 8 weeks significantly increased body weight gain, hyperglycemia, hyperinsulinemia, and hyperlipidemia. The high-fat fed mice also impaired OGTT (oral glucose tolerance test), enhanced GSIS (glucose-stimulated insulin secretion), and declined insulin sensitivity during insulin tolerance test. Under intravital microscopic observation, the numbers of rolling leukocytes were significantly increased in wild type mice fed with high-fat diet. The results show that high-fat diet-induced obesity, insulin resistance, and leukocyte-endothelium adhesive interaction were significantly ameliorated in P-selectin KO mice.
Conclusion:
Taken together, our results demonstrate that P-sel KO suppressed body weight gain after feeding with high fat diet. Blockade of high fat diet-induced P-selectin production maintained insulin sensitivity via suppression of leukocyte rolling, and followed suppressed inflammation.

P809
Suppression Effect Of Fracture Caused Inflammation By Parecoxib In Conscious Rat Model

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Backgrounds:

Analgesics, such as Cyclooxygenase-2 (COX-2) selective inhibitor Non-steroidal anti-inflammatory drug (NSAID) drug would be given for pain control. The medication is effective but may influence the inflammation reaction and cause the further effect to bone and soft tissue healing. The aim of this study was to investigate the pattern of cytokine release and its acute stage time course after tibial and fibular shaft fracture and further medication influence.

Materials and Methods:

Thirty two Wistar–Kyoto rats were used in the study. The fracture-group rats received left tibial and fibular shaft fractures with consistent three point bending method. Parecoxib was given after the procedures of fracture. The rats were randomly divided into control group, medication group, fracture group, and fracture with medication group. Their physiologic changes were continuously monitored in conscious and unrestrained for 72 hours. The Blood samples were taken from the femoral arterial catheter at 1, 3, 6, 9, 12, 18, 24, and 48 hours after the procedure of fracture.

Results:

Parecoxib suppresses IL-6, IL10 and leukocyte peripheral migration after fracture. TNF-α was also suppressed during initial 6 hours but then rebounded to higher level. Less hemorrhage and leukocyte infiltration of surrounding fracture site muscle are also noted in the medication group.

Conclusion:

Parecoxib suppresses inflammatory cytokines effectively in 12 hours. However, the inflammation reaction flares up after 12 hours.

P810
Effects of Lycium Barbarum Polysaccharide on Male Copulatory Behavior in Rats

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Backgrounds:

Lycium chinense is a well-known traditional Chinese medicine, which has a long history of use in treatment of male infertility. *Lycium barbarum* polysaccharide (LBP) is one of the main active ingredients of *Lycium chinense* extract. This study aims to investigate the effects of LBP on male copulatory behavior in rats.

Materials and Methods:

Sexually experienced adult male Long-Evans rats, 12-16 weeks of age, were randomly divided into four groups, which are given 50, 100, or 200 mg/kg/day of LBP or vehicle (distilled water) via oral gavage for 28 days, and copulatory behavior were measured after 7, 14, 21, and 28 days of oral administration of LBP or vehicle. Approximately 16-18 h after the last behavioral test, the rats were sacrificed and the accessory reproductive organs were collected and weighted.

Results:

Administration of 100 mg/kg of LBP for 7, 14, 21, and 28 days 28 days significantly increased intromission frequency compared to either the control group on the same day or the same group on day 0. Similar results were also observed at the dose of 200 mg/kg of LBP treatment for 14, 21, and 28 days. An increase in ejaculation frequency was seen after treatment with 100 mg/kg of LBP for 14, 21, or 28 days when compared to either the control group on the same day or the same group on day 0. Treatment with 50 mg/kg of LBP for 7, 21, and 28 days, and of 200 mg/kg for 7, and 21 days significantly increased ejaculation frequency when compared to the same groups on day 0. Besides, rats orally administered of 50 mg/kg of LBP for 14 days displayed more ejaculation frequencies than controls. A reduction in post-ejaculatory interval was only seen after administration of 100 mg/kg of LBP for 14 days compared to the vehicle-treated group. No significant differences were found in latencies for mount, intromission and ejaculation. After treatment for 28 days, the weight of seminal vesicle was statistically decreased in the 100 mg/kg treatment group, and there was a significant correlation between ejaculation frequency and seminal vesicle.

Conclusion:

These findings show that LBP (especially at the dose of 100 mg/kg) enhances the copulatory behavior of male rats.

P811
Importance of COX-2 Activation during Adipocyte Hypertrophy and Hypoxia in The Development of Obese Adipose Tissue Inflammation

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Purpose:

Adipose tissue inflammation is crucially involved in obesity-associated cardiometabolic disorders. However, the role of obese adipocyte in the development of adipose tissue inflammation remains unclear. This aim was to determine the role of adipocyte COX-2 activation in the development of obese adipose tissue inflammation.

Methods and Results:

Oral administration of COX-2 inhibitors suppressed the elevated epididymal adipocyte COX-2, TNF-α, MCP-1, RANTES, CCR5, HIF-1α mRNA and MCP-1, RANTES production in high-fat induced obese rats. COX-2 inhibition also significantly attenuated the chemotaxic effect of obese adipocytes and adipose tissue infiltration of macrophages and T cells. 3T3-L1 adipocytes treated with palmitate and/ or hypoxia to mimic the progression of adipocyte hypertrophy and hypoxia in the development of obesity were pretreated with COX-2 shRNA or NS-398 to examine the involvement of COX-2 activation. Adipocyte COX-2 mRNA and PGE2 production duration hypertrophy and hypoxia were causally linked with the pro-inflammatory mRNA levels and adipokine production and subsequently affected synergistically inflammatory reaction of obese adipocytes with RAW264.7 cells or T cells. EP3 instead of other EP receptors was enhanced in obese visceral fats in rats and humans and also palmitate, hypoxia-treated 3T3-L1 adipocytes. EP3 antagonist L-798106 significantly inhibited the augmented pro-inflammatory mRNA and protein levels in the treated 3T3-L1 adipocytes. Adipose COX-2, HIF-1α and TNF-α mRNA levels were higher in obese than in lean subjects. The IL-6 and MCP-1 levels in human obese adipocyte-derived conditioned medium were attenuated by COX-2 inhibition.

Conclusion:

Our results suggest that adipocyte COX-2 activation via PGE2/EP3-mediated signal pathway is crucially involved in the pathogenesis of obesity-associated adipose tissue inflammation.

P812
Electrophysiological characterization of sodium-activated potassium channels in NG108-15 and NSC-34 motor neuron-like cells

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Backgrounds:

Activation of KNa channels requires an intracellular sodium concentration [Na]i exceeding physiological level after Na+ influx. Accumulation of [Na]i developed through Na+ channels, including persistent Na+ channel (INaP). Current studies showed that sodium-activated potassium currents (IK(Na)) was activated by persistent Na+ currents (INaP). However, the electrical properties of KNa channels in motor neurons remains largely unknown. Therefore, the purpose of this study was to characterize the biophysical properties of sodium-activated potassium currents (IK(Na)) in NG108-15 and NSC-34 neuron cells.

Materials and Methods:

RT-PCR experiment to analysis the expression of Slick & Slack. Ion currents were recorded with cell-attached mode or whole-cell mode.

Results:

The results showed that increasing stimulation frequency altered the amplitudes of both INa and IK. With changes in stimulation frequency, the kinetics of INa inactivation and IK activation were correlated at NG-108 and NSC-34 cells. Tef (10uM) increased the amplitudes of both INa and IK throughout the voltage ranging from -30 to +10 mV. However, in cell-attached recordings, signal-channel conductance was not changed in the presence of Tef. Riluzole (10 mM) reversed Tef-stimulated activity of KNa channels. Spike-frequency adaptation was also to be facilitated during treatment with Tef.

Conclusion:

Activation of KNa channel associated directly with intercellular Na+ and participated regulation of action potential firing in motor neuron-like cells.

P813

CORM-2 Attenuates TNF-α-Induced cPLA$_2$ Expression via Inhibition of PKα-Dependent NADPH Oxidase/ROS and NF-κB Cascade

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Backgrounds:

Rheumatoid arthritis (RA) is characterized by chronic inflammatory infiltration of the synovium and elevation of pro-inflammatory cytokines, which are key features of synovial inflammation. Cytosolic phospholipase A_2 (cPLA$_2$) plays an important role in the development of several inflammatory diseases. Although heme oxygenase-1 (HO-1)-derived carbon monoxide (CO) can modulate inflammatory processes, the effects of CO on TNF-α-induced cPLA$_2$ expression in RA synovial fibroblasts (RASFs) are unclear.

Materials and Methods:

RASFs were obtained from patients with RA who underwent knee surgery. The involvement of MAPKs and NADPH oxidase/ROS in TNF-α-induced cPLA$_2$ expression was investigated using pharmacologic inhibitors and transfection with siRNAs and was analyzed by Western blotting and promoter assay. Histologic expression of cPLA2 was evaluated after treatment.

Results:

TNF-α induced TNFR1 and PKCα complex formation. TNF-α-induced cPLA$_2$ expression was mediated through TNFR1/PKCα-dependent signaling pathways, including NADPH oxidase/ROS production, which was attenuated by a ROS scavenger, the inhibitor of NADPH oxidase, PKCα, p38 MAPK, or JNK1/2, transfection with respective siRNAs, and HO-1 induction by CORM-2. TNF-α-induced cPLA$_2$ expression was mediated through recruitment of NF-κB to the cPLA$_2$ promoter region, which was attenuated by NAC and CORM-2. Furthermore, CORM-2 inhibited TNF-α-mediated cPLA$_2$ expression in the ankle synovium of mice.

Conclusion:

In RASFs, TNF-α induced cPLA$_2$ expression via the formation of TNFR1/PKCα complex which stimulated NADPH oxidase/ROS generation and IKKα/β phosphorylation, leading to NF-κB activation. Induction of HO-1 by CORM-2 exerted anti-inflammatory and antioxidant effects which were required in concert to prevent the activation of NF-κB-regulated genes implicated in the pathogenesis of RA.

P814

Estrogen reduces human mesenchymal stem cell-mediated growth and motility in gastric cancer cells

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Background:

Epidemiologic studies have reported that the prevalence of gastric cancer in male is about 2-fold higher than that in female. It also has been suggested that later age at menarche, older menopause, and nulliparity are associated with increased risk of development of gastric carcinoma in women. These findings contribute to the co-relation between reduction of estrogen and development of gastric cancer. Cancer stem cells (CSCs) are reported to be involved in the malignant cancer development. Mesenchymal stem cell (MSC), a type of stem cell, is shown that it might be involved in cancer metastasis. Here we will investigate the role of estrogen in human mesenchymal stem cell-mediated growth and motility in human gastric cancer.

Method and Material:

We culture human gastric cancer cell lines (CS12, AGS) and human bone marrow mesenchymal stem cells (HBM_MSCs) in the co-culture system. We measured the cell proliferation by BrdU proliferation assay and cell survival by MTT assay. The motility of gastric cancer was measured using modified Boyden chamberswith filter inserts for 24-well dishes containing 8-mm pores. IL-8 and IL-8 neutrolizing antibody were used to measure the inhibitory effect of motility in gastric cancer cells.

Result:

HBM_MSCs significantly promoted cell proliferation by MTT assay in CS12 and AGS gastric cancer cells. In the regulation of cell migration, HBM_MSCs also significantly enhanced the motility capacity of CS12 and AGS cancer cells. Treatment of IL-8 neutrolizing antibody (100, 500 and 1000 ng/ml) significantly inhibited HBM_MSCs-upregulated motility of gastric cancer cell. E2 showed the inhibition of HBM_MSCs-induced motility of CS12 and AGS cells, which may be through suppressing IL-8 function.

Conclusion:

This study showed that HBM_MSCs is important factor in regulating cell growth and motility in CS12 and AGS gastric cancer cells. E2 Treatment may significantly reverse the capacity of HBM_MSCs by suppressing IL-8 function.

P815

The Phagocytosis And Polarization Effect Of Trypsin Hydrolysis Whey Protein In Murine Phagocytes.

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Backgrounds:

Whey protein is one of the soluble proteins as byproduct of dairy production; it takes 20% of the whole protein of the milk. The advantage of the whey hydrolysate is the following; low cast, easy to absorb, and contain the functional oligopeptides. Macrophages had been demonstrated to the phenotypes which led to activated status in classical (M1) and alternative (M2) macrophages might be regulated by active oligopeptide.

Materials and Methods:

Whey protein was dispersed in phosphoric acid buffer (pH 7.0) and obtained two separate solutions 0.5% and 1.0%. Whey protein was hydrolyzed by trypsin (0.5%) in 37ºC for 2, 4, 6, 8, 10, 12 hr. RAW264.7 and DC2.4 cells were culture with various whey hydrolysate with pEGFP transform e.coli for further two hour. After quenching with trypan blue buffer, phagocyte was measured with fluorescent reader. Culture supernatants were detected with regulatory cytokines including IL-2, IL-4, IL-6, IL-10, IL-13, IL-33, IFN-γ, TSLP, MCP-1, TNF-α. Culture precipitated cells were monitored with CD68, CD197 for M1 cell and CD68, CD206 for M2 cell.

Results:

Hydrolytic whey protein had promoted phagocytotic immunity effects via modulated type 1 and type 2 cytokines, Cell surface activated form provide modulated cytoskeleton to modulated pseudopodia rearrangement to increased phagocytosis.

Conclusion:

Phagocytosis defense have monocyte,macrophage and neutrophils. It is takes an important part of immunity. In this study, we use macrophage *in vitro* to test immunomodulatory effect of whey hydrolysate, and learn to adjust the phagocytic activity of the cellular and molecular biological pathways. Not only we can learn about specific whey protein peptide and phagocytosis regulation pathway, but also we can further develop the functional peptides.

P816

Attenuation of Capsaicin-induced Pulmonary Chemoreflex Following Cervical Spinal Cord Injury in Rats

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Backgrounds:

High cervical spinal injury usually interrupts bulbospinal respiratory pathways and results in changes of breathing pattern and lung property. These respiratory complications following injury have the potential to influence pulmonary vagal afferent activity. Pulmonary C-fibers are the main chemosensitive afferents in the lung. Activation of pulmonary C-fibers evokes pulmonary chemoreflex (e.g. apnea, hypotension and bradycardia) which is an important reflex to modulate cardiorespiratory responses. The aim of present study was to investigate whether pulmonary chemoreflex was altered following cervical spinal cord injury.

Materials and Methods:

Adult male Sprague-Dawley rats were divided into uninjured and spinal cord injured (C2 hemisection, C2Hx) group. Tidal volume and respiratory frequency were measured under anesthetized and spontaneously breathing condition at 1 day (acute) or 8 weeks (chronic) post-injury. Three doses of capsaicin (0.5, 1.0, 1.5 μg/kg) were randomly injected into the right atrium to stimulate pulmonary C-fibers.

Results:

Capsaicin evoked a dose-dependent apnea in uninjured animals, however, respiratory patterns of C2Hx animals were not significantly influenced by capsaicin administration at acute injury phase. At 8 weeks post-injury, pulmonary chemoreflex could be evoked but intensity of the reflex is significantly attenuated compared to age-matched uninjured animals.

Conclusion:

These results suggest that capsaicin-induced pulmonary chemoreflex is abolished following acute cervical spinal cord injury and gradually recovered at chronic injury phase. Attenuation of pulmonary reflex may enable animals to maintain the essential ventilation when inhibitory reflex is evoked during injury phase, however, this change may be also a risk factor for suffering pneumonia after spinal cord injury.

P817
Regulation of Interleukin-6 by Hypoxia in Endometriotic Stromal Cells

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Backgrounds:

Endometriosis is a common gynecologic disease of reproductive age women. Despite the complicated and largely unknown etiology, a growing body of evidence indicates that cytokines and growth factors are involved in the pathogenesis of endometriosis. Elevated concentrations of several cytokines, such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α in peritoneal fluid of women with endometriosis have been reported. We have previously demonstrated that IL-1β induces expression of cyclooxygenase-2 (COX-2) leading to overproduction of prostaglandin E_2 plays important roles in the development of endometriosis. However, the regulation and pathological function of other cytokines remain largely uncharacterized. Herein, we aimed to investigate the regulation and function of IL-6 in women with endometriosis. Hypoxia is a critical stress during the development and progression of endometriosis. Upregulation of hypoxia-inducible factor-1α (HIF-1α) has been found in endometriotic stromal cells. Since HIF-1α is a master transcription factor in controlling the expression of many stress-related genes, we hypothesized that aberrant expression of cytokines may also under the regulation of HIF-1α. Thus, this study was designed to investigate the functional role of hypoxia in regulation of IL-6 signaling pathway.

Materials and Methods:

Human endometrial biopsy specimens were collected from women undergo laparoscopic operations. After separation, cells were cultured in DMEM/F12 under normoxia or hypoxia or treated with IL-6. Levels of proteins were detected by western blotting.

Results:

Expression of IL-6 was upregulated by hypoxia. Hypoxia-induced IL-6 expression was mediated via downregulation of dual specificity phosphatase-2 (DUSP2) as knockdown of DUSP2 is sufficient to induce the expression of IL-6. In contrast, forced expression of DUSP2 under hypoxic condition abolished hypoxia-induced IL-6 expression. Treatment of endometrial stromal cells with IL-6 triggers phosphorylation of signal transducer and activator of transcription 3 (STAT3). Concordance with this notion, phosphorylated STAT3 was evident in stromal cells isolated from endometriotic lesion compared to those isolated from normal endometrium.

Conclusion:

Levels of IL-6 and its downstream effector, phosphorylated STAT3 were elevated in endometriotic lesion. Aberrant activation of IL-6-STAT3 signaling pathway was due to overexpression of HIF-1α. Our data indicate that hypoxia-activated IL-6-STAT3 signaling pathway may play an important role in the progression of endometriosis.

P818
The Joint Effect of Smoking and *hOGG1* Genotype on Oral Cancer in Taiwan.

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Background:

This study aimed at evaluating the association and interaction among human 8-oxoguanine DNA N-glycosylase 1 (*hOGG1*) genotypic polymorphism, smoking status and oral cancer risk in Taiwan. For this purpose, the well-known polymorphic variants of *hOGG1*, codon 326, was analyzed for its association with oral cancer susceptibility, and its joint effect with individual smoking habits on oral cancer susceptibility.

Materials and Methods:

In total, 620 patients with oral cancer and 620 healthy controls were recruited from the China Medical Hospital were recruited and genotyped.

Results:

The results showed that the *hOGG1* codon 326 genotypes were differently distributed between the oral cancer and control groups (p=0.0266), with the C allele of *hOGG1* codon 326 being significantly (p=0.0046) more frequently found in cancer patients than in controls. We further analyzed the genetic-smoking joint effects on oral cancer risk and found an interaction between *hOGG1* codon 326 genotypes and smoking status. The *hOGG1* codon 326 CC genotype was associated with oral cancer risk only in the smoker group (p=0.0198), but not in the non-chewer group (p=0.8357).

Conclusion:

Our results provide the evidence that the C allele of *hOGG1* codon 326 may have a joint effect with smoking on the development of oral cancer.

P819
The Inhibitory Effect Of Oligopeptides By The Whey Hydrolysate On The Differentiation Of 3T3-L1 Cells.

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Backgrounds:

With the change of dietary habits of the people, suffering from obesity, hyperlipidemia, heart disease and colorectal cancer, and other lifestyle-related diseases of the population went up sharply to become a very important issue. Whey protein is byproduct of cheese production, about 20% of the milk protein, containing the complete amino acid. In this study, we monitored the modulated effect of trypsin hydrolyzed whey protein in inhibition of adipocyte differentiation. And further to understand the oligopeptide to inhibit adipocyte differentiated mechanisms and the process of the peptide of the whey protein.

Materials and Methods:

In this experiment, hydrolyzed whey protein on 37ºC and harvested on 0, 2, 4, 6, 8, 10, 12 hours. And further use the different zeolites membrane by 30, 10, 1 kDa and 300 Da membrane respectively. 3T3-L1 is preadipocytes cell and most commonly used to promote adipocyte differentiation as insulin, isobutyl-methyl-xanthine and dexamethasone for 2 days and then to the single insulin cultivate two days for each change culture medium. Adipogenesis genes including C/EBPβ, PPAR/γ were analyzed by real-time RT-PCR and/or protein expression. Cells were also stained with Oil Red O to detect oil droplets in adipocytes.

Results:

The whey hydrolysate can modulate C/EBPβ and PPAR/γ gene expression to down-regulated preadipocyte differentiation. The adipogenesis inhibitory peptide was purified from whey hydrolysate and was identified to be a oligopeptide having an IC50 value of 0.011-0.037 mg protein/ml. Thus, these results showed the potential anti-obesity effect of the purified whey hydrolysate through control of adiposity.

P820
Effect Of Hyperbaric Oxygenation After Sepsis In Diabetic Individuals

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Backgrounds:

Diabetes mellitus is a metabolic syndrome. Hyperglycemia is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves, blood vessels and immunity system. If occurs of infection in diabetic individual, it is possible to get sepsis. Our previous data showed hyperbaric oxygenation pretreatment could attenuate cardiovascular neural dysfunction in diabetic rats with sepsis, so we tried to study the effect of hyperbaric oxygenation in diabetic individuals with sepsis which is relevant to clinical situation.

Materials and Methods:

We implanted the wireless transmitters in Sprague Dawley (SD) rats. Streptozotocin (STZ, 60 mg/kg) and lipopolysaccharide (LPS, 50 mg/kg) were used to induce diabetes and sepsis. Hyperbaric oxygenation (HBO, 100% O_2, 3 ATA, 60 mins) after sepsis was given. Frequency-domain analysis of telemetric mean systemic arterial pressure (MSAP) and pulse-pulse interval (PP) were applied to quantify the parameters of blood pressure variability (BPV) and heart rate variability (HRV).

Results:

The experimental results demonstrated that: (1) HBO could extend the survival in diabetic rats after sepsis. (2) BPV and HRV were decreased in septic shock. HBO could increase the BPV and attenuate the reduction of HRV in diabetic animals.

Conclusion:

Diabetic animals slowly responded to sepsis. HBO increases vascular sympathetic modulation in middle phase of septic shock. We suggest giving HBO that therapy in diabetic individuals with sepsis earlier might be able to improve survival.

P821
Chronic Treadmill Exercise Exerts Differential Effects On Hypobaric Hypoxia Induced-Neurotoxicity In Rats

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Backgrounds:
Each brain area, with its unique neuronal populations, is specified for distinct functions. Thus, different brain areas manifested differential responses upon various stimuli. For example, the substantia nigra is more sensitive to the systemic immunological insults than other brain regions like the cortex, limbic area and the cerebellum.
The brain is the most vulnerable tissue when exposed to inadequate oxygen (hypoxia). One of the most common hypoxia situations is when people rapidly ascend to the high altitude area without adaptation and thus causes acute mountain sickness (AMS). AMS is associated with hypoxia-induced neuronal death and inflammation. Animal studies reveal that hypoxia causes neuronal injuries in the hippocampus and cortex, hence causes functional and behavioral deficits associated with these brain regions. One protective strategy to attenuate hypoxia- induced neurotoxicity is to precondition neurons with mild hypoxia exposure. Exercise has been regarded as a state of relative hypoxia. In this study, from the preventive medicine point of view, we investigated the effects of treadmill exercise on hypoxia induced-neurotoxicity in brain regions like hippocampus and cortex.
Materials and Methods:
Four –week-old male Wistar rats were trained on treadmill exercise for 4 weeks (13 - 20 m/min, 40 - 60 min/day, 5 days/week) before subjected to hypoxia - insult for 7 hours (the pressure was 0.303 atm in the hypoxia chamber). The sedentary and exercise rats were than sacrificed and perfused after 0 h, 4h and 24h of reoxygenation. Rat brain cortexes and hippocampus were then dissected out for Western blotting and other analysis of various protein expressions associated with oxidative stress and inflammation.
Results:
The expression levels of proteins like iNOS, nNOS and GFAP were analyzed. After hypoxia treatment, the expression levels of those proteins were all increased in both the hippocampus and cortex areas, which peaked at 4h after the reoxygenation, indicating an increase in the oxidative stress and gliosis. Nevertheless, the treadmill exercise training significantly attenuated the increased expression of the above proteins in the hippocampal protein samples, while this attenuation effects were not observed in the cortex samples.
Conclusion:
Our data indicated that the chronic treadmill exercise exerted better neuroprotection effects in the hippocampal area than in the cortex area in terms of cellular oxidative stress and gliosis responses. This might be due to different functions performed in these two brain areas. The detailed mechanisms are still under investigation.

P822
Effects of Catechins Supplementation on Running Exercise-induced Organ Injuries in Rats

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Backgrounds:
Acute running exercise induce reactive oxygen species (ROS) production, and lead to lipid peroxidation, protein oxidative damage and organ injury. Catechins, consisting mainly of epigallocatechin gallate, epicatechin gallate, gallocatechin, and epigallocatechin. These polyphenols have some physiological activities, including antibacterial, anticarcinogenic, antidiabetic, and antiatherogenic effects. In this study, we will investigate the effects of catechins on running exercise-induced injuries.
Materials and Methods:
We purchased forty 8-week-old Sprague-Dawley rats and randomly divide into four groups (ten for each group), including control group (C, the rats receiving saline supplementation), catechins control group (CC, the rats receiving 50 mg/kg B.W. catechins supplementation), exercise group (E, saline supplementation and then subjected to running exercise), and catechins exercise group (CE, receiving 50 mg/kg B.W. catechins and then subjected to running exercise). Following seven days catechins supplementation, the rats subjected to treadmill running (18 m/min, 40 mins), and the rats sacrificed for serum biochemistry measurement and organ histological analysis. The serum biochemistry items including creatine phosphokinase (CPK), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine (CRE), alanine transaminase (ALT), aspartate aminotransferase (AST), and uric acid (UA).
Results:
Catechins supplementation attenuated all the blood biochemistry of exercise group levels except serum creatinine. These results showed that catechins reduce acute running exercise caused organs injuries. According to histopathological observations, there are moderate tubular dilation in the renal tubules and slight hyaline casts deposited in the collecting tubules. There was slight edema appeared in triceps after running.
Conclusion:
Our results suggest that acute running exercise cause muscle, kidney, and liver injuries, and catechins can reduce acute exercise-induced organ injuries.

P823
The Effect of the Tocolysis Chinese Medicine in Zebrafish Embryo Development

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Purpose:
The purpose of this study is to demonstrate the effects of tocolysis Chinese medicine in different embryo developing stages of zebrafish. Three kinds of Chinese medicine were applied in this study.
Materials and Methods:
The drugs were added at one of the follow time courses that 0 hour, 12 hour, or 24 hour after the eggs of Zebrafish (*Danio rerio*) had fertilized. The drug dosages (1000, 750, & 250 ppm) were conversion lower/equal to the human dosage.
Results:
It is found that (1) Xiong Gui Jiao Ai Tang(XGJAT) and Dang Gui Shao Yao San(DGSYS) could not lead to embryo dead, but the higher dose of Bao Chan Wu You Fang(BCWYF) would increase lethal rate. (2) Giving the drugs at 12h, all the dosage of BCWYF could keep the embryo life; and the higher dose had rose the higher hatchling rate, like the control group. (3) All the three kind drugs would delay the embryo development, especially at 0h. (4) Giving the drugs at 12h or 24h that would short the body length of lava.
Conclusion:
It is suggested that XGJAT and DGSYS could use at the embryo early stage, but the application of BCWYF should be very cautiously at the early stage.

P824
MicroRNAs Regulate MCF-7 Breast Cancer Cell by Targeting *NDRG1* During Reoxygenation

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Backgrounds:
Hypoxia is a characteristic feature of solid tumor. Hypoxia tumors were reported to be more malignant and resistant to therapy. Previously, we identified that down regulation of N-myc down-regulated gene 1 (*NDRG1*) promoted migration in a breast cancer cell line MCF-7 during reoxygenation. However, the regulatory mechanism of *NDRG1* remained indefinite. In order to investigate the regulation of *NDRG1*, the purpose of this study is to explore whether *NDRG1* is subject to microRNA regulation.
Materials & Methods:
MCF-7 cells were incubated under hypoxia (0.5% O_2 concentration) for 24 hours and transferred to normoxia. Cells were harvested respectively at 0 (hypoxia control), 1, 12, and 24 hours under normoxia. NanoString® nCounter miRNA Expression Assay was used to examine the transcriptional profile of microRNAs upon reoxygenation. The criteria for choosing differentially expressed microRNAs included that mean P-value at a given time point was < 0.05, and mean value was over 1.5 fold changes. *In silico* analysis using TargetScan was performed to search microRNA binding sites (2^{nd} and 7^{nd} nucleotide of microRNAs) within *NDRG1* 3' untranslated region (UTR). The microRNA candidates were validated by RT-qPCR, Luciferase assay, and Western blot.
Results & Conclusion:
We have identified 28 microRNAs, which were up-regulated during reoxygenation. Among these microRNAs, miR-1282, miR-2276, miR-501-3p and miR-769-3p were predicted to bind in the *NDRG1* 3'UTR. RT-qPCR validated that these four microRNAs were up-regulated during reoxygenation. In the future, Luciferase assay and Western blot will be conducted to explore whether *NDRG1* was regulated by these microRNA candidates during reoxygenation.

P825
Investigation of Dopamine D2 Receptor Activity in Adolescent and Adult Akt1 Mouse Model of Schizophrenia Using MicroPET Imaging

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Backgrounds:

Schizophrenia is a complex neuropsychiatric disorder with genetic and environmental components. The onset of schizophrenia generally occurs in late adolescence or early adulthood. Accumulating evidence from human genetics and animal studies suggest AKT1 is a susceptibility gene for both schizophrenia and methamphetamine use disorder. Methamphetamine can also cause psychotic symptoms indistinguishable from schizophrenia in abusers and aggravate psychosis in schizophrenic patients. However, the effect of insufficient *Akt1* on the regulation of its upstream dopamine D2 receptors remains unclear. Taking advantage of "gene-environment interaction" approach, this study focuses on quantification of dopamine D2 receptor activity in adolescents and adults using Akt1-deficient male mice as a model.

Materials and Methods:

All *Akt1* heterozygous (HET) mice and their wild-type (WT) littermates used in this study were generated from *Akt1* HET breeding pairs. These mice were examined on P48-52 and P90-100 to mimic periadolescent and adult periods in humans, respectively. Each mouse received 6 daily injections of methamphetamine (or saline as vehicle control) to induce behavioral sensitization and their locomotor activity was recorded in an open field on Days 1, 3, and 6. One day after last injection, D2 receptor activity in striatum and prefrontal cortex of these mice was quantified using microPET with ^{18}F-fallypride.

Results:

Injections of methamphetamine significantly induced hyperlocomotion in both adolescent and adult mice. A significantly genotypic effect on methamphetamine-induced hyperlocomotion was also observed, especially after 1st methamphetamine injection. Compared to WT with saline, MicroPET imaging revealed a significant reduction of activity in adult HET mice (especially in caudate putamen) whereas adolescent mice did not exhibit any genotypic alteration. But no genotypic effect was found in both adolescent and adult methamphetamine-sensitized mice after 6-day injections.

Conclusion:

Our data indicated that both adolescent and adult HET mice appeared to be normal in their basal locomotion but less sensitive to acute injection of methamphetamine. The basal dopamine D2 receptors activity appeared to be different between genotypes in adult but not adolescent mice. Repeatedly injections of methamphetamine dampened striatal D2 receptor activity in adult WT but not HET mice. These findings support that Akt1 deficiency predisposes to abnormalities of dopamine-dependent locomotion and D2 receptor activity in adult but not adolescent mice.

P826
The carcinoembryonic antigen as a potential prognostic predictor for breast neuroendocrine carcinoma.

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Background:

Primary neuroendocrine carcinomas of the breast (PNCB) are very rare and tumor markers for this indication are not well defined. We aim at reporting a case and providing a marker useful for prognosis and prediction of tumor recurrence for patients with PNCB.

Materials and Methods:

One 75-year-old woman presented with a slight painful lump in her left breast of more than 6 months duration. The patient underwent serum level of carcinoembryonic antigen (CEA) determination and ultrasonography, mammography and a modified radical mastectomy with axillary lymph node dissection.

Results:

Prior to surgery, the serum level of carcinoembryonic antigen (CEA) (54.4 ng/ml; normal limit <5.0 ng/ml) was significantly elevated. Ultrasonography identified a hypoechoic lesion. Mammography revealed a hyperdense lesion with a well-circumscribed margin. Pathology showed tumor cells with neuroendocrine features, with diffuse immunopositivity for chromogranin and synaptophysin. The tumor cells were also strongly positive for progesterone and estrogen receptor, but negative for HER-2/neu expression. The CEA value gradually decreased to the normal range within one month after surgery. Neither recurrence nor distant metastasis has been detected at 20 months after surgery and hormone therapy with letrozole. The serial CEA levels were within normal limits in the follow-up period.

Conclusion:

The serum CEA level after surgery may be a potential marker for evaluating tumor recurrence or prognosis of patients with PNCB.

P827
Mechanisms of Sphingosine 1-Phosphate-Induced Cyclooxygenase-2 Expression in Human Cardiac Fibroblasts

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Backgrounds:

Sphingosine-1-phosphate (S1P), one of the lipid components, has been shown to regulate expression of cyclooxygenase-2 (COX-2) and contributes to inflammatory responses. However, the mechanisms regulating COX-2 expression by S1P in human cardiac fibroblasts (HCFs) remain unclear. Therefore, we investigated the mechanisms underlying S1P-induced COX-2 expression and prostaglandin E_2 (PGE_2) biosynthesis in HCFs.

Materials and Methods:

To investigate whether S1P induces COX-2 expression, Western blot, RT-PCR, and real-time PCR were performed. PGE_2 release was detected by an ELISA kit. To study the signaling molecules involved in S1P-induced COX-2 expression, the selective pharmacological inhibitors and transfection with siRNAs were performed.

Results:

We found that HCFs express various S1P receptor subtypes including $S1P_1$, $S1P_2$, and $S1P_3$. Time-course incubation of S1P resulted in an increased in COX-2 mRNA and protein expression, the promoter luciferase activity, and PGE_2 production. Moreover, we investigated the intracellular signaling pathways of S1P-induced COX-2 expression in HCFs. The data showed that S1P-induced COX-2 expression and PGE_2 production were attenuated by the inhibitor of $S1P_1$ (W123), Gi protein (GP2A), Src (PP1), MMP2/9i, HB-EGF (CRM-197), EGFR (AG1478), PI3K (LY294002), PYK2 (PF431396), PKCα (Gö6976), JNK1/2 (SP600125), or AP-1 (Tanshinone IIA) and transfection with respective siRNAs. Moreover, S1P stimulated the phosphorylation of Src, EGFR, Akt, PYK2, PKCα, and JNK1/2, which were attenuated by their respective inhibitors. These results suggested that RTKs, PKCs, MAPKs, PI3K/AKT, c-Jun/AP-1 are involved in S1P-induced COX-2 expression.

Conclusion:

We demonstrated that S1P-induced COX-2 expression and PGE_2 release were mediated through activation of the $S1P_1$, Gi protein, c-Src, MMP/HB-EGF, EGFR/PI3K/Akt, PYK2, PKCα, and AP-1 pathways.

P828
The Anticancer Effect of the Fruiting Body and Primordial of *GrifolaFrondosa*

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Backgrounds:

Grifola frondosa (GF), a widely consumed edible and medicinal resource mushroom in Asian countries, exhibits considerable biological functions, such as anti-tumor, anti-mutagenic, anti-hypertensive, anti-diabetic, hypolipidemic, and collagen biosynthesis-enhancing activities. However, the biological function of white GF and its primordial, named LG3 and LG3P, respectively remains unknown. Thus, the purpose of the present study was to investigate the anticancer effects of LG3 and LG3P.

Materials and Methods:

The LG3 and LG3P were extracted by hot (100 ℃) and cold (25℃) water, and cell viability was then assessed by MTT assay.

Results:

Our results showed that both hot and cold water extracts of LG3 obviously suppressed cell viability on C6, J82, PC-3 and DU145 cancer cells. The IC_{50} analysis results revealed that cold water extracts from LG3 possess higher antitumor activity than those extracted by hot water. On the other hand, the antitumor activity was more prominent on prostate cancer cells, PC-3 and DU145 cells. Therefore, the antitumor activity of LG3P was then assessed on these cells. Results indicated that the cell viability of both PC-3 and DU145 cells were inhibited following LG3P stimulation.

Conclusion:

In summary, our results showed that the white GF as well as its primordial parts possess almost same anticancer functions on prostate cancer cells.

P829
Identification of Globo H-positive Cancer Initial Cells from Pancreatic Ductal Adenocarcinoma

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Backgrounds:

Pancreatic cancer is the eighth leading cause of cancer death in Taiwan. At the time of diagnosis, more than 50% of tumor had already metastasized. Existence of pancreatic cancer stem/initiating cells (CSCs) has been considered as a possible link for poor clinical outcomes of pancreatic ductal adenocarcinoma (PDAC). Identification of the biomaker of cancer stem/initiating cells is critically needed for developing diagnosis and targeting therapies for pancreatic neoplasm. The carbohydrate antigen Globo H is commonly found in several types of epithelial cancers and has recently been shown as a therapeutic target for breast and prostate cancer. However, whether Globo H can be used as markers for enriched isolation of pancreatic cancer stem/initiating cells has not been addressed.

Materials and Methods:

Our pilot studies confirmed Globo H was found to express in most Patients with pancreatic cancer. Globo H is expressed in 93% of Ca of ampulla of vater and 92% of PDAC. Among patients with advanced stage of PDAC expressed higher levels of Globo H. Moreover, we also found Globo H was expressed in 5 of 6 pancreatic cancer cell lines we examined.

Results:

Based on using FACS analysis, MTT and invasion assay, we found that Globo H-positive pancreatic cancer cells displayed higher drug resistance, proliferation rate and invasive capability compared to Globo H-negative pancreatic cancer cells. Moreover, using FACS- sorting and tumor-engraft assay, Globo H-positive pancreatic cancer cells were found to express stemness genes, possess sphere-forming ability and have higher tumorigenic potentials. Importantly, injections of anti-Globo H antibodies suppressed tumor progression in PDAC-bearing mice.

Conclusion:

The results indicate Globo H is a potential marker for cancer stem/initiating cells of PDAC.

P830
Studying the Molecular Mechanism Responsible for the Down-regulation of TGFBR3 in Oral Cancer

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Backgrounds:

Transforming growth factor β receptor III (TGFBR3, also known as betaglycan), a co-receptor for TGFβ, is believed to exert tumor suppressor functions due to the decrease of TGFBR3 expression in several cancer types including head and neck cancer. In the same line of observation, we also detected the down-regulation of TGFBR3 in oral cancer patients and the down-regulation is associated with poor clinical outcomes among these patients.

Materials & Methods:

To examine if epigenetic control was involved in the TGFBR3 down regulation, we treated low-TGFBR3 expressing CAL27 cells with 5'azaC (DNA methyltransferase inhibitor) and/or histone deacetylation (HDAC) inhibitor. Methylation-specific PCR was used to confirm the involvement of DNA methylation in the deregulation. Furthermore, we used chromatin immunoprecipitation (ChIP) to measure if methyl CpG binding protein 2(MeCP2) as well as histone modification markers would bind to the putative CpG island spanning between -366 and +1066 in the proximal TGFBR3 promoter. Since DNA methyltransferases(DNMTs) are the key enzymes participating in DNA methylation, the expression level of DNMTs was measured by RT-PCR following the treatment of CAL27 with 5'azaC or HDAC inhibitors. The inverse relation of TGFBR3 and DNMTs mRNA expression will be also examined in oral cancer patients.

Results:

We found a significant induction of TGFBR3 mRNA expression in the 5'azaC or HDAC inhibitor-treated oral cancer cells, suggesting the involvement of epigenetics in regulating TGFBR3 expression. Using luciferase assay driven by serially truncated TGFBR3 promoter constructs, a minimal TGFBR3 promoter for TGFBR3 resides in the putative CpG island between -116 and +174 using transcription start as +1. Using in vitro methylation, we found that the CpG island methylation drastically reduced the promoter activity, suggesting a negative role of DNA methylation in TGFBR3 expression. Methylation-specific PCR further confirmed that the methylation status at the region between -320 and -160 correlated with the induction of TGFBR3 mRNA by 5-azaC treatment in oral cancer cells. In vivo binding of MeCP2 and histone modification makers to the promoter region between -365 and +406bp was detected by ChIP-PCR. Morever, there was inverse relation of TGFBR3 and DNMT1/3B mRNA expression in oral cancer cells treated with 5'azaC or HDAC inhibitors.

Conclusions:

Although more studies are needed to delineate the action mechanism, epigenetic control may be one mechanism responsible for the deregulation of TGFBR3 expression in oral cancer cells. Whether the deregulation of DNMT1/3B is also associated with oral cancer patient clinical outcome remains to be characterized.

P831
The Study of Signal Transduction Pathways Involved in *E.coli* DNA Recognition in Drosophila Hemocyte Cell Line Mbn-2

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Backgrounds:

Oligonucleotides (ODN) containing unmethylated Cytosine phosphorothioate guanine (CpG) motifs can activate innate immune responses in mammals. Unmethylated CpG motifs act as a danger signal in vertebrate. In invertebrate, the effects of unmethylated CpG motif are still not clear.

Drosophila melanogaster cell line malignant blood neoplasm-2 (Mbn-2) was explored as a model system to study insect immune responses *in vitro* and it was used to investigate the stimulation of CpG DNA in invertebrate.

Because NF-κB signal transduction pathways are highly conserved, *Drosophila melanogaster* provides a good model to study these cascades. Previous research has shown that innate immunity against bacteria and fungi is governed largely by two NF-κB signal transduction pathways, Toll and immune deficiency (IMD). In Toll pathway which controls Drosophila resistance to fungal and Gram-positive bacterial infections.

The aim of the study is to investigate whether the stimulation of CpG motif in Drosophila goes through the Toll pathway.

Materials and Methods:

In order to silence Toll, we designed a plasmid with two T7 promoters to product dsRNA (dsTOLL). The expression of Toll in Drosophila cell line Mbn-2 after dsRNA treatment was analyzed by real-time RT-PCR. Furthermore, the expression of antimicrobial peptide *Attacin* and *Drosomycin* were also examined to investigate the possible signal transduction pathway of *E.coli* DNA recognition.

Results:

Our results showed that dsTOLL-treated Mbn-2 cells showed decreased by 23% in *Toll* expression compared with control Drosophila cell line after 48 hours. We could not detect any significant decrease in *Toll* expression in cell transfected with dsTOLL at 24 hours. After 24 and 48 hours of treatment with plasmid contain Toll gene *in vitro*, *Toll* mRNA was strongly increased in Mbn-2 cells. When the dsTOLL treated cells were stimulated with LPS alone or LPS combined with *E.coli* DNA after 24 and 48 hours, there was no significant difference in the expression of *Attacin* when compared with the untreated control.

Conclusion:

Our results indicated that the transfection of dsRNA can be used in Drosophila cell line Mbn-2 to disrupt function of endogenous genes *in vitro*. The expression of *Attacin* and *Drosomycin* in dsRNA treated cells has no significant different after *E.coli* DNA stimulation. These results showed the stimulation signal from *E. coli* DNA seems not get through the Toll pathway in Drosophila.

P832
The Delay Aging and Anti-Photoaging Effects of *Hericium erinaceum* Extracts

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Backgrounds:

Hericium erinaceum contains abundant biological components and various antioxidants. However, the delay aging and anti-photoaging effects of *Hericium erinaceum* have not yet been well studied. The purposes of this study were to examine the antioxidant capacity, the delay aging and anti-photoaging effects of *Hericium erinaceum* extractions.

Materials and Methods:

Hericium erinaceum were extracted by 4°C cold water and hot water, and followed by three functional assays: antioxidant, delay aging and anti-photoaging assays. Firstly, the antioxidant analysis including total polyphenol content, flavonoid content determination, the scavenging ability of DPPH radicals and chelating ferrous ion was evaluated. Secondly, the delay-aging analysis including scavenging nitric oxide, scavenging hydrogen peroxide and promote the growth of fibroblasts was also studied. Finally, in order to determine the anti-photoaging effects, *Hericium erinaceum* extracts were added to the mouse fibroblast cells (NIH3T3), and followed by the exposure of different UVB dosages (10 and 25 mJ/cm^2). Then, the productions of collagen and superoxide dismutase of these cells were detected.

Results:

According to the results, the cold and hot water extracts of *Hericium erinaceum* contented 2.22~2.30mg/g of total polyphenols and 0.12~0.13mg/g of flavonoids respectively. The results of scavenging DPPH radical's ability showed that the hot water extracts of *Hericium erinaceum* (57.1%) was more than cold water extracts (29.6%). Based on the results of chelating ferrous ion analysis indicated that the cold water extracts of *Hericium erinaceum*(98.3%) was more than hot water extracts (96.9%). In addition, the results of delay aging assays showed that the nitric oxide contents in cold water extract of *Hericium erinaceum* (8.7mg/ml) were much higher than hot water (6.1mg/ml). Moreover, the results of scavenging hydrogen peroxide abilities presented that hot water extracts of *Hericium erinaceum* (69%) were higher than the cold water extracts (48%). According to the results of cell survival experiments, both the cold or hot water extracts of *Hericium erinaceum* presented no damage to the NIH3T3 cells, and they were able to promote the cell proliferations up to 110% by 0.5 and 1 mg/ml of extraction treatments after 48hrs. Finally, the results of anit-phtoaging assays showed that the cold and hot water extracts of *Hericium erinaceum*presented the respective abilities to protect the fibroblast cells (NIH3T3) to against 25mJ/cm^2 of UVB and 200uM of H_2O_2 oxidative damages. These cell viabilities were more than 100%, which were significantly higher than the controls (only 45% and 64%).

Conclusion:

Hericium erinaceum extracts have significantly antioxidant activities, delay aging effects and anti-photoaging effects. The extracts of *Hericium erinaceum* have highly potential to develop the functional health food and skin care products in the future.

P833
The Hemorrhage Diseased of Giant Mottled Eel May Cause by Polyomavirus Virus

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Giant mottled eel (*Anguilla marmorata*) is a potential fish in aquaculture in Taiwan. During Jan 2012 heavy mortality occurred in the farming fish in Pingtung. The diseased fish showed reddening of fins, pectoral fins especially and intensive congestion in gills and inoculation of the extracts into normal cells caused the nuclei enlarged. The syndromes similar to viral endothelial cell necrosis of eel (VECNE), yet the primer sets specific for the virus were not amplified the target sequences. Sequencing of the transcriptome of the infected cells, a T-antigen sequence was discovered. PCR detection using the primers designed from the T-antigen sequence showed positive for all the tissues from the diseased fish; however normal cells tissues were negative. The present study shows that the virus isolated from giant mottled eel is different from the virus of VECNE.

Keywords:
Cell culture, Giant mottled eel, Virus, PCR

P834
Downregulation of HeyL promotes tumor progression in hepatocellular carcinoma

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The Hairy/Enhancer of split-related with YRPW-like motif (HEY) family of proteins were first identified as transcriptional repressors and belongs to downstream effectors of Notch signaling pathways. A number of studies reportedd that *HeyL* gene plays an important role in the development of the cardiovascular system and loss of *HeyL* function causes defects in vascular specification, septation and valve formation. In this study, we first screened the BMP4 downstream effectors/genes and found that *HeyL* was down-regulated in HCC cell lines after BMP4 treatment and further identified HeyL was inactivated or downregulated in human primary HCC tissues. Thus, we hypothesized that HeyL could be an important tumor suppressor during HCC progression and which may be a critical event during hepatocarcinogenesis. In addition to identify the underlying mechanisms of HeyL mediated anti-tumor effects, we also proposed to dissect HeyL-associated signaling networks, and further examine its binding factors involved in regulated HCC tumorigensis or/and metastasis. Furthermore, the understanding of the HeyL interaction models and their functional roles in HCC could undoubtedly provide ideal nodal points for therapeutic intervention of this devastating disease.

Reference :
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P835
A single amino acid residue change in the JEV nonstructural protein 2 affects the early stage of viral RNA replication

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Backgrounds:
In Japanese encephalitis virus (JEV)-infected cells, an extra species of nonstructural protein 1 (NS1) related protein known as NS1', produced from a -1 ribosomal frameshift in a NS2A that can dimerize with NS1. It has been implicated in playing roles in viral neuroinvasiveness and interferon (IFN)-β inhibition of Kunjin virus (a subtype of WNV)-infected cells. Nonetheless, the exact biological functions of NS1' in JEV life cycle remain unclear.

Materials and Methods:
To address this question, we used a cytomegalovirus promoter-driven reverse genetics system to generate mutants of JEV RP-9 strain containing single amino acid substitutions within NS2A.

Results:
We found that the presence of a -1 ribosomal frameshift mutation (A30P) or disrupting stem 2 of the pseudoknot (A30A') in the JEV NS2A gene attenuated NS1' production in virus-infected cells. Although both A30P and A30A' mutant viruses apparently diminished viral virulence in mice, in infected IFN-competent (BHK-21 and N18) and IFN-deficient A549 cells A30P mutant showed delayed viral RNA replication in the early stage of infection. Exogenous expression of NS1', but not wild-type NS1-2A or NS1, in BHK-21 cells was shown to be functional for *trans*-complementation in the growth kinetics of the virus, as measured by the viral burst and genomic RNA levels.

Conclusion:
Based on these results, we suggest that residue A30 of the NS2A plays an important role in regulating viral RNA replication during early stage of JEV infection.

P836
The Molecular Mechanisms of CD44 in the Mineralization of Human Dental Pulp Cells

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Backgrounds:
In previous studies, CD44 has been suggested to play an important role during tooth development. However, little emphasis has been placed on whether the CD44 can impact mineralization of dental pulp cells. One major ligand for CD44 is hyaluronan (HA), which plays a predominant role in cell differentiation. We hypothesized that HA with high molecular weight at optimal concentration may induce odontoblastic differentiation.

Materials and Methods:
Primary human dental pulp cells were obtained from 5 healthy patients (age-range 16-25 years) undergoing therapeutic third molar extractions. We added different doses for high molecular weight of HA to the medium at the first plating of cells. After 3 to 14 days, we evaluated effects of hyaluronan on the alkaline phosphatase activity of dental pulp cells. After 3wk in culture, the effects of HA on mineral deposition were examined using Alizarin red staining. Besides, we cultivated dental pulp cell in normal α-MEM andα-MEM with additive HA (2 mg/ml) in 3 days. Then, PCR array was used to identify the upregulated osteogenesis-associated genes. We further confirmed array data using qPCR. The CD44 knockdown cells and control cells were cultured inα-MEM with additive HA (2 mg/ml) for 3 days. The relationship between CD44 and HA will be further confirmed by qPCR and western blot.

Results:
The results showed that treatment with high molecular weight (1500~1800 kDa) HA in early cultures (days 3) increased alkaline phosphatase activity. On the other hand, high molecular weight (1500~1800 kDa) HA with basal medium, significantly increased mineralization in the dental pulp cells cultures. The data of osteogenesis PCR array identifed several up-regulated osteogenesis-associated genes, including BMP7, BMP3 and ALPL .

Conclusion:
Our analysis indicated that high molecular-weight HA maybe a good material to stimulate dental mineralization in early cultures. A series of in vitro and in vivo assays will be further employed to clarify the molecular mechanisms.

P837
Association of ACE2 Regulation with MMP-2 Expression in Human Cardiofibroblasts

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Backgrounds:

Angiotensin converting enzyme II (ACE2) is a newly identified component of renin-angiotensin system (RAS) and plays a negative regulator of angiotensin II (Ang II). Most of published papers reveal that Ang II broke the balance of matrix metalloproteinases (MMPs) expression to induce heart remodeling, and ACE2 administration attenuated the effect that Ang II induced. However, the mechanism of ACE2 regulate MMP-2 expression to attenuate heart remodeling is unclear. The aim of this study is to investigate the relationship between angiotensin peptides, ACE2 and MMP-2.

Materials and Methods:

The lentivirus, TLC-ACE2 and TRCN-46697 were used to infect human cardiofibroblasts (HCFs) to obtain ACE2 overexpression cardiofibroblasts, HCFs/ACE2, and knockdown the ACE2 expression, respectively. The MMP-2 activity, shed ACE2 activity and the signal pathway of MMP-2 and shed ACE2 expression were been assessed in HCFs/ACE2 treated with angiotensin peptides.

Results:

HCFs infected with TLC-ACE2 at different multiplicity of infection (MOI) showed ACE2 activity of HCFs/ACE2 was enhanced with the MOI. The ACE2 activity of HCFs/ACE2 infected at 1, 5, 10 and 20 MOI was 20, 78, 151 and 292-fold compared to non-infected HCFs, respectively. Like as ACE2 activity, MMP-2 activity of HCFs/ACE2 also increased with the MOI and revealed the gentle trend at 5 MOI infected. The MMP-2 activity, shed ACE2 activity, and the expression of ERK1/2 and a disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) were been inhibited when HCFs/ACE2 treated with Ang II. Ang 1-7 treated HCFs/ACE2 also suppressed shed ACE2 activity, and the expression of ERK1/2 and ADAM17, but not include MMP-2 activity.

Conclusion:

In HCFs/ACE2, ACE2 overexpression induced MMP-2 activity. The expression of ADAM17 and shed ACE2 activity were been regulated via Ang II-AT1R-ERK1/2 and Ang 1-7-Mas-ERK1/2 axes. In addition, Ang II inhibited MMP-2 activity that ACE2 induced through AT1R-ERK1/2 signal pathway. These results revealed that ACE2 play a protect role to opposite the effect of Ang II.

P838
Studies of Binding, Immobilization and Molecular Modulation of EGF for Applying on Bone Tissue Engineering

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Backgrounds:

Epidermal growth factor (EGF) is an important target on cancer therapy and regenerative medicine. Currently, one strategies of cancer therapy is to change binding affinity of ligand to EGFR by using small molecules.

Materials and Methods:

In order to understand the correlation between ligand-binding changing and EGFR modulating, we try to get some small molecular agents from traditional Chinese medicines (TCMs) to modulate EGFR activity by using SPR biosensor system.

Results:

Among 60 kinds of TCMs, we find that some of TCM showed different influence on ligand-binding to EGFR. Among these, sample no. 051 showed a more inhibitive property due to decreasing ligand-binding affinity to EGFR. *In-vitro* study, it also existed an anti-EGFR activity to A549 cells. In a different way, sample no. 040 showed opposite function to no. 051. No. 040 is a most powerful enhancer that can increase the affinity and quantity of EGF binding to EGFR and a pro-EGFR activity on cell culture system.

Conclusion:

In our study, we can successfully regulate the EGFR-mediated cell proliferation and anti-apoptosis by using different TCMs. This way will be a useful method to apply on the therapy of human diseases, such as bone reconstruction.

P839
The Potential Role of ARNT in The Regulation of Cisplatin-induced Cancer Cell Death

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Backgrounds:

Aryl hydrocarbon receptor nuclear translocator (ARNT) belongs to bHLH-PAS (basic helix-loop-helix-Per-ARNT-Sim) family, which can form heterodimer with other family member in exposure to xenobiotic compounds. In addition, ARNT is essential to embryonic development and is related to tumor growth. However, the role of ARNT on tumor resistance to cisplatin is unknown. In this study, we investigated the mechanisms involved in ARNT-regulated the sensitivity of cancer cells to anticancer drugs.

Materials & Methods:

To determine the effect of ARNT on cell proliferation, MTT and BrdU incorporation assays were used. Flow cytometry and DNA fragmentation assay were used to detect cisplatin-induced cell death. Calcein-AM was used to determine p-glycoprotein (MDR1) pumping activity. The reporter assay was used to detect MDR1 promoter activity. In addition, DAPA was used to determine the binding affinity of Sp1 and ARNT to DNA. Xenograft analysis of SCID mice was used to study the effect of cisplatin on tumor growth.

Results:

In our study, we found that cancer cell proliferation was decrease in ARNT knockdown condition. Cisplatin dramatically induced the p53 expression and caspase-3 activation, resulting in the inhibition of ARNT expression and induction of cell apoptosis in drug-sensitive cells. Overexpression of ARNT reversed the effect of cisplatin on cell death. In contrast, suppression of ARNT reversed the characteristics of cisplatin-resistant cells, making them more sensitive to cisplatin treatment. In addition, siARNT inhibited MDR1 expression, resulting in the retention of drugs and apoptosis in resistant cells. Overexpression of ARNT enhanced the promoter activity of MDR1 via the Sp1 sites located on DNA. The MDR1 promoter activity was also inhibited in ARNT knockdown cells. In a xenograft analysis of SCID mice, cisplatin also inhibited ARNT-deficient c4 tumors but not ARNT-containing vT2 tumor formations.

Conclusion:

These results indicate that ARNT reduces the effect of anti-cancer drugs on tumor cell death through the regulation of MDR1 expression. This study shows previously unrecognized multifaceted functions of ARNT in the establishment and drug-resistant properties of cancer cells, highlighting it as a potential therapeutic target for an important subset of cancers.

P840
Combined Interaction of PHD and Chromo Domains Directs NuA4 to DNA Double-Strand Breaks

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Backgrounds:

DNA double strand breaks (DSBs) are the most dangerous lesions to the integrity of genome. In yeast *Saccharomyces* cerevisiae, the histone modification complex, NuA4 is recruited to DSBs where it acetylates histone H2A and H4, presumably relaxing the chromatin and allowing the access of repair proteins. Two subunits of NuA4, Eaf3 and Yng2, can interact with dimethyl- K36 of histone H3 (H3K36me2) and trimethyl- K4 of histone H3 (H3K4me3) by their chromodomain and plant homeodomain (PHD) respectively in vitro. Here, we investigate the functional importance of interactions between these domains and the specifically modified histones in the DSB repair.

Materials and Methods:

We have combined the yeast genetics and molecular biology to investigate the function of NuA4 in the DSB repair in the yeast *Saccharomyces cerevisiae*.

Results:

Here, we demonstrated that mutations in either Eaf3 chromodomain or Yng2 PHD domain have no significant effect on cell growth or DNA repair. However, combined mutations in both chromodomain and PHD domain show dramatic defect both in cell growth and DNA repair. In addition, the chromatin immunoprecipitation experiments reveal that high level of phospho-S129 of histone H2A (H2AS129p), acetyl-K12 of histone H4 (H4K12ac), H3K4me2, H3K4me3, H3K36me2, Yng2 are enriched at the DSB site, suggesting NuA4 is efficiently recruited to the DSB site. By contrast, the *chromo Δphd* double mutant and the histone H3 mutant H3K4R (arginine to lysine mutation of K4 of histone H3) dramatically reduced the enrichment of H4K12ac and prolonged activation of H2AS129p, suggesting these mutants impair NuA4 recruitment and repair efficiency.

Conclusion:

Our results suggest that the DSB can induce the specific histone modifications at DSB, which in turns recruits NuA4 complex by the multiple interaction between these modified histones and subunits of NuA4. These multiple domain-histone interactions strengthen the recruitment of NuA4 at DSBs.

P841

The role of NBM-T-L-BMX-OS01 (BMX) in neurite outgrowth

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Backgrounds:

Epigenetic modifications such as acetylation, methylation, and phosphorylation are important in gene regulation. Structural modifications of histones mainly occur in acetylation or deacetylation of the N-terminal tail through histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs modulate both histone and non-histone proteins such as transcription factors, cytoskeleton proteins, and other cellular proteins. It has been proven that enriched environment-activated histone acetylation reverses memory impairment and HDAC inhibitor mimics the effect of an enriched environment in restoring mice memory. NBM-T-L-BMX-OS01 (BMX) was derived from the semi-synthesis of osthole, isolated from *Cnidium monnieri* (L.) Cuss., and was identified to be a inhibitor of HDAC. BMX is able to penetrate the blood-brain barrier and shows little neurotoxicity. In the present study, the role of BMX in neurite outgrowth will be investigated.

Materials and Methods:

BMX is semi-synthesized from osthole. The primary hippocampal tissue from Sprague-Dawley rats (E19) was dissociated with trypsin and plated with Neurobasal medium and B27. For neurite outgrowth analysis, cultured hippocampal neurons were fixed with 4% paraformaldehyde and permeablized with 0.1% Triton X-100. The primary Mouse anti-β III tubulin, neurofilament and anti-actin (Millipore) antibodies and Alexa Fluor 488 goat anti-rabbit or anti-mouse antibodies (Molecular Probes) were incubated. Images were obtained using a Axio Observer D1 microscope (Zeiss). Neuronal process longer than 10 μM is defined as neurites. For western blot, primary hippocampal cells were lysed and resolved by 8% SDS-PAGE. The antibodies anti-β III tubulin, neurofilament and anti-actin, followed by HRP-conjugated secondary antibodies were incubated. The membrane was developed by reacting with HRP substrate (Millipore) and exposure to X-ray film. The protein bands were quantified using the NIH Image J Software. Total RNA from tissue was isolated by using the RNAspin mini kit (GE Healthcare). The cDNA was generated from total RNA with ReverAid premium reverse transcriptase (Thermo Fisher Scientific). Real-time PCR analysis was performed with the Rotor-Gene real-time PCR system (Qiagene) by using the SYBR Green PCR Master Mix (Thermo Fisher Scientific) according to the instruction manual. The PCR parameters that were used are as follows: 95 °C for 10 min for 1 cycle, 95 °C for 15 s followed by 60 °C for 1 min for 40 cycles.

Results:

Treatment of BMX increases neurite outgrowth in primary hippocampal neurons. Real-time PCR and western blot results revealed that BMX increases neurofilament expression, while leaves actin and tubulin unchanged.

Conclusions:

Whether BMX enhances memory formation or neurite outgrowth through the modification of neurofilamet awaits further investigation.

P842

The Functional Role of a Sorting Nexin Family Protein in The Regulation of TLR4-Mediated Immune Response

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Backgrounds:

Toll-like receptors (TLRs), recognize pathogen-associated molecular patterns (PAMPs) or damage- associated molecular patterns (DAMPs) released from pathogens or damaged tissues, and regulate the expression of cytokine, chemokines and type I interferons to regulate inflammation response. Dysregulation of TLR-mediate immune response is associated with many diseases, such as cancer, inflammatory bowel diseases. TBK1 is crucial for type I interferon production upon TLR activation. However, the mechanism by which TBK1 is activated is poorly understood. A sorting nexin family protein was obtained from our yeast two hybrids screening using TBK1 as bait.

Materials and methods:

We knocked down the sorting nexin protein using lentivirus containing shRNA in murine macrophage cells RAW 264.7 and conducted functional studies such as real time qPCR and western blot to analyze signaling and the expression TLR4-mediated genes after LPS stimulation.

Results:

We showed that TBK1 associated with this sorting nexin protein in co-immunoprecipitation assays. We also found that, upon LPS treatment, TBK1 and candidate protein colocalized in immunofluorescence studies. In addition, we demonstrated that the mRNA expression of *Ccl5, Ifnb* and *Il6* decreased; whereas there was no change in the mRNA levels of *Tnf and Nfkbia* in candidate sorting nexin protein knockdown cells in response to LPS. Furthermore, phosphorylation and nuclear translocation of IRF3 were decreased in LPS-stimulated knockdown cells.

Conclusion:

Our results strongly suggest that the candidate sorting nexin protein plays an important role in regulating TLR--mediated immune response. The molecular mechanism by which this sorting nexin protein regulates TLR4 signaling is currently under investigation.

P843

Molecular Mechanism of the Synergistic Interaction of Erlotinib with Pemetrexed in Non-Small-Cell Lung Cancer Cells

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Backgrounds:

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, erlotinib, and the multitargeted antifolate, pemetrexed, are effective agents in treatment of non-small-cell lung cancer (NSCLC) in clinical. In this study, we investigate the molecular mechanism underlying combination with erlotinib and pemetrexed in NSCLC cell lines.

Materials and Methods:

The interaction between erlotinib and pemetrexed is synergistic, and their interaction was assessed by the combination index (CI) method. The cell growth, apoptosis, and phosphorylation are detected by WST-1 assay, FACS and Western blot assay. Phosphatase activity assay was performed to assess whether phosphatase following the erlotinib and pemetrexed influenced Akt up-stream involved in anti-cancer signal and apoptosis induction.

Results:

Erlotinib-pemetrexed combination is highly synergistic and significantly increased apoptosis. Erlotinib or pemetrexed reduced cell growth and induced apoptosis in NSCLC cells. Especially, combined the erlotinib with pemetrexed significantly enhancement of apoptosis in 3 cell lines reaching to 3~5 folds. Moreover, increase amounts of erlotinib enhancement apoptotic effect which diminished by progressive Akt phosphorylation. Besides, combination of erlotinib and pemetrexed increase PP2A activity.

Conclusion:

Based on our findings, the efficacy of erlotinib combination with pemetrexed for enhancement apoptosis of NSCLC cells via Akt phosphorylation reduction resulting from PP2A activation. The combination of pemetrexed and erlotinib is synergistic in NSCLC cells.

P844

Knockdown of CITED2 using short-hairpin RNA impairs DNA repair and sensitizes cisplatin chemotherapy thought down-regulation of DNA repair genes

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Backgrounds:

Cisplatin cause genomic damage is widely used in anticancer therapy. However, the effective use of cisplatin is limited by the development of drug resistance in cancer cells.Although biochemical and pharmacological studies have implied underlying mechanisms for the resistance, the specific genes responsible for the resistance are starting to be identified. Intending to search in a genomewide scale for candidate genes that may participate in chemoresistance, we have initially identified several cisplatin resistance candidate genes, including *CITED2*, and characterized for their role in chemoresistance.CITED2, a transcriptional modulator implicated in human oncogenesis. However, in the previous study, CITED2 not only affects cell growth, transformation, and development but also acts as an anti-apoptotic protein. We previously found that knockdown of CITED2 by short-hairpin RNA causes dramatic sensitization of HEK293 cells to cisplatin dependent on the status of p53.

Materials and Methods:

We analysis to CITED2 regulated DNA repair gene by shRNA knockdown of CITED2. Further, we have identified transactivation of the repair gen by knockdown of CITED2 as characterized by ChIP assay and reporter assay.

Results:

In this study, we characterized that CITED2 regulates DNA repair gene expression in p53-dependent and –independent manner. The expression level of regulated repair gene faithfully explains cell sensitivity to cisplatin, but not mitotic damager such as taxol. Furthermore, p53 binding to the promoter of *ERCC1* (a representative DNA repair gene) and p53 transactivation of the repair gene was impaired by knockdown of CITED2 as characterized by ChIP assay and reporter assay.

Conclusion:

These results support the notion that CITED2 may regulate DNA repair gene expression at transcription through controlling access of p53 to the gene promoter, and subsequently regulate cell sensitivity to cisplatin and drug resistance.

P845
Effects of Promoting Blood Circulation compounds on survival self renewal and multilineage differentiation of mesenchymal stem cells

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Backgrounds:
Human mesenchymal stem cells (hMSC) are self-renewing precursor cells that can be expanded in vitro and differentiated towards osteogenic, chondrogenic or adipogenic lineages. However, the limit number and inefficient hMSCs differentiation are the major barriers in hMSC-based therapy. It would be extremely beneficial if a naturally occurring agent could be identified that could induce hMSCs to undergo specific lineage differentiation. In the present study, we investigated the role of promoting blood circulation compounds on hMSC proliferation and differentiation.
Materials and Methods:
Markers of hMSCs were measured using flow cytometry. hMSCs differentiated into adipocytes were analyzed by r Oil-Red O staining. Osteogenic differentiation was identified by the mineralization of calcium deposits using von Kossa staining. hMSCs induced into endothelial cells were analyzed by tube-formation assay. The proliferations of hMSCs were analyzed based on cell counting.
Results:
hMSCs used in these experiments expressed high levels of CD90, HCAM, MCAM and STRO-1 surface markers when analyzed by flow cytometry, thereby confirming their MSC phenotype. hMSCs undergo osteogenic differentiation displayed calcium deposition was indicated by von Kossa staining. Adipogenic differentiations of hMSCs were positive for Oil Red O staining. Matrigel angiogenesis assay displayed that endothelial differentiated hMSCs were able to form the capillary network. Additionally, the proliferation ability of hMSCs was inhibited by salvianolic acid B.
Conclusion:
The results demonstrate hMSCs has multilineage differentiation potential. The proliferations of hMSCs were inhibited after salvianolic acid B treatment. Future mechanistic studies will demonstrate the effect of compounds on multipotential differentiation of hMSCs.

P846
The Effects of *Magnolia Officinalis* Extract Honokiol on Anti-cancer Proliferation in Human Non-small Cell Lung Cancer

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Backgrounds:
Honokiol is an active component from *Magnolia officinalis*, and it has been demonstrated to have anti-inflammatory, anti-angiogenic and anti-tumor activity in many cancers. However, the anti-cancer mechanism of honokiol in lung cancer cells is still unclear. In this study, we investigated the molecular mechanism of honokiol on anti-cancer proliferation in human non-small cell lung cancer.
Materials and Methods:
We used H23 cell line and treated with various concentrations (0, 5, 10, 15 and 20 μM) of honokiol. The cell viability was measured by MTT assay. Cell was treated with honokiol for 24 h and cell cycle distribution was analyzed by flow cytometry. Western blotting was performed to detect cell cycle regulated protein, such as cyclins, CDKs, CDKIs and histone deacetylases. *In vitro* and *in vivo* HDAC activity was determined by HDAC Activity Assay Kit. The CDKIs mRNA expressions were performed by RT-PCR.
Results:
First, we examined the cytotoxicity effect of honokiol in H23 NSCLC cell lines through MTT assay. The results showed that honokiol significantly inhibited cell viability in H23 cell. The anti-proliferative activity of honokiol was due to G1 cell cycle arrest. Western blotting analysis exhibited that honokiol decrease the expression of cyclins D1, D3, E and cyclin-dependent kinases 2, 4 and 6, but up-regulation of CDK inhibitors, p21 and p27. And the expression of p21 by honokiol was due to transcriptional regulation. Moreover, honokiol also decrease class I histone deacetylases expressions and increase acetylated histone H3 expression. Furthermore, honokiol also inhibited HDAC activity indirectly.
Conclusion:
The anti-proliferative activity of honokiol may through G1 cell cycle arrest induction in H23 lung cancer cells. This G1 arrest was due to up-regulation of p21 gene expression. Furthermore, the p21 up-regulation may through class I HDAC inhibition in honokiol-treated H23 cells. Our data implicated that honokiol may be a chemopreventive agent in lung tumorigenesis.

P847
The Putative Roles of Human METCAM in Modulating the Development and Progression of Nasopharyngeal Carcinoma

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Backgrounds:
HuMETCAM/MUC18, a cell adhesion molecule in the Ig-like gene superfamily, is an integral membrane glycoprotein and promotes metastasis of melanoma, and breast and prostate cancers. We previously found that huMETCAM/MUC18 expressed in all of the normal nasopharynx specimens, not expressed in 73% of and weakly expressed in 27% of clinical specimens of three subtypes of nasopharyngeal carcinoma (NPC). But it was expressed again in all of metastatic lesions. Thus we suggested the hypothesis that huMETCAM/MUC18 suppresses the development, but promotes the metastasis of NPC. In this study we tested this hypothesis by investigating the possible roles of huMETCAM/MUC18 in modulating the development and progression of NPC.
Materials and Methods:
We transfected the huMETCAM/MUC18 cDNA into NPC-TW01 and NPC-TW04 cell lines and isolated G418-resistant clones from each cell line that expressed different levels of the protein. We used three clones that respectively expressed high, medium, and low levels of the protein for testing the effect of different huMETCAM/MUC18 expression level on *in vitro* cell migration, invasion (EMT), and tumorigenesis, and *in vivo* tumorigenesis and metastasis in immunodeficient mice.
Results:
We found that huMETCAM/MUC18 was weakly expressed in the two NPC cell lines. We obtained 48 clones from each cell line that expressed a high, medium, or low level of the protein. We are in the process of using these clones for the above *in vitro* and *in vivo tests*. Results will be presented.
Conclusion:
HuMETCAM/MUC18 may suppress the development, but may promote the metastasis of NPC.

P848
Elucidating Diversity of IgG Glycosyl Pattern and Its Immunomodulatory Effect in HBV-related Liver Diseases

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Backgrounds:
Aberrancy of N-link glycans on immunoglobulin G (IgG) Fc fragment has been mentioned in many autoimmune diseases and viral infections. However, the alternation of IgG glycosyl patterns in HBV-related liver diseases and its immunomodulatory effects remain unclear.
Materials & Methods:
Serum IgG glycoforms were identified by using LC-MS approach. Cross-sectional and longitudinal studies were executed to analyze the correlation between IgG glycosyl aberrancy and the disease status of chronic hepatitis B. Moreover, a time course study was performed to trace the dynamic of IgG glycosyl patterns during the anti-viral treatment. In addition, a macrophage phagocytic assay was applied to investigate the immunomodulatory effect of IgG glycovariants on opsonizing activities. All statistical analyses were performed by using SPSS software, version 17.0.
Results:
Our cross-sectional data revealed that IgG galatosylation decreased in chronic hepatitis B (CHB) and liver cirrhosis (LC) patients in comparison with healthy controls (HC). Moreover, the longitudinal data showed that the IgG galactose-deficiency in CHB and LC patients were restored under anti-HBV treatment, particularly entecavir. The time course study showed that altered IgG galactosylation patterns were correlated with the ALT and AST decline. We also analyzed the correlation between IgG1 and IgG2 subclasses and found a high similarity of their glycosylation pattern dynamics at baseline and week 48 post-treatment. Intriguingly, IgG opsonizing activity was reduced in CHB but partially restored in disease remission, which was correlated to IgG galactosylation profiles.
Conclusion:
Our data revealed that the lack of IgG terminal galactose residues was coincided with the severity and progression of HBV-related liver diseases. Surprisingly, we found a different pharmaceutical effect of variable anti-HBV drugs on IgG glycosyl restoration. The activity of IgG opsonization was highly associated with the level of Fc-galactosylation. These findings clearly demonstrated the clinical significance of IgG glycosyl profiles on the status of HBV-related liver diseases, as well as the impact of IgG-Fc glycans on modulating immune responses.

P849

Resveratrol Protects Retinal Pigment Epithelial Cells From Acrolein-Induced Oxidative Damage and Cigarette Smoke-Induced Choroidal Neovascularization via Increase in Mitochondrial Bioenergetics

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Backgrounds:

Resveratrol (RSV) alleviates the oxidative damage on human adult retinal pigment epithelial (ARPE) cell. Similar protection was observed in the UV irradiation damage model of human RPE cells. The purpose of this study was to study the role of mitochondrial bioenergetics in the cytoprotective effect of RSV. Its role in protection against the adverse effect of cigarette smoke (CS) in experimental choroidal neovascularization (CNV) was also examined.

Materials and Methods:

Cultured ARPE-19 cells were treated with acrolein alone or with additional of RSV. Temporal changes in cell viability, expression of the antioxidant protein, and mitochondrial bioenergetics were evaluated. In animal study, CNV lesions were created in Brown Norway rats by laser-induced photocoagulation. Effects of CS alone or with additional treatment of RSV on CNV lesion were quantified by fluorescein isothiocyanate-dextran labeling.

Results:

In ARPE-19 cells, RSV reduced acrolein-induced cell death. This was accompanied by reversal of acrolein-induced superoxide dismutase expression and the increase in mitochondrial bioenergetics, including basal respiratory rate, ATP turnover, and maximal mitochondrial capacity. In animal experiments, we found that CS-induced CNV following laser injury was appreciably prevented in rats subjected to peripheral infusion of RSV.

Conclusion:

Our results indicated that RSV, a major polyphenol found in red wine, exerts protection against acrolein-induced cytotoxicity in human ARPE-19 cells via increase in the mitochondrial bioenergetics. In addition, the antioxidant effect of RSV may contribute to the protection against the laser-induced CNV in animals exposed to CS. Therefore, RSV might be beneficial for treatment of acrolein-induced or CS-evoked RPE degeneration.

P850

MicroRNA-125b Regulates Poteasome Pthway in Oral Squamous Cell Carcinoma(OSCC)

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Backgrounds:

Aberrant expression of miRNAs has been implicated in the pathogenesis of a variety of diseases including cancer. Recently, proteasome pathway has gathered much attention in cancer studies as it is the main degradation system for oxidatively damaged proteins and also for several proteins involved in the cell cycle regulation and transcription, which are important for cancer initiation and progression. However, the interaction between miRNAs and proteasome system has not been well characterized.

Materials and Methods:

Microarray experiment: oral cancer tissue using Affymetrix Human Genome U133A 2.0 Array ;OSCC cell using Illumina HumanHT-12 v4 Expression BeadChip.

Pathway analyses with the Gene Set Enrichment Analysis (GSEA) algorithm:To identify pathways regulated in oral cancer tissue and OECM1 cell line(knockdown miR-125b).Using gene sets from KEGG pathway 250 gene sets.

Two-layer regulatory Network modeling: To construct the miR-125b-regulated network, we uploaded he proteasome related genes and miR-125b to the target relationship as customized interactions in a MetaCore database (GeneGo, St Joseph, MI, USA). The uploaded dataset was used to construct a separate network consisting of the shortest paths.

miRNA and mRNA expression analysis:The expression levels of miRNAs were determined by stem-loop RT–PCR;For quantitating mRNA expression, the total RNA was reverse transcribed using oligo-dT RT–PCR. Using an ABI Prism 7900 Fast Real-Time PCR system (Foster City,CA, USA).

Western blot analysis: The protein bands were visualized by enhanced chemiluminescence detection system. GAPDH for verification of loading control.

Results:

Previously, our laboratory simultaneously profiled the expression levels of 270 miRNAs by stem-loop RT PCR and the mRNA expression levels by microarray in 49 oral cancer tissues. We found that 11 miRNAs are up-regulated and 38 miRNAs down-regulated in oral cancer tissues. Analyzing the distribution of these differentially expressed genes on individual pathways in the KEGG database revealed that several up-regulated genes are located in proteasome-related pathways. Comparing the proteasome-related genes levels in microarray data and miRNAs expression level across these samples revealed a strong inverse correlation with miR-125b. But most of these proteasome-related genes are not miR-125b direct target. Recent studies show that miRNAs may modulate the expression levels of multiple targets in a pathway by targeting critical transcription factors. Therefore, we used the shortest-path algorithm and the GeneGo MetaCore database to perform network modeling. The resulting model suggests that miR-125b has the ability to regulate multiple proteasome-related genes in a two-layer regulatory network by targeting multiple transcription factors (TFs).

Conclusion:

To test this hypothesis, we established the miR-125b overexpression and knockdown systems in OSCC cell (OECM1). Initial study confirmed that overexpression of miR-125b down-regulated those proteasome-related genes and TFs in mRNA and protein levels; knockdown miR-125b up-regulated the proteasome-related genes and TFs on mRNA and protein levels. Our study confirmed that miR-125b regulated multiple proteasome-related genes mainly through c-Myc. Further studies to confirm the interaction not only between miR-125b and c-Myc but also between c-Myc and candidate proteasome-related genes are currently underway.

P851

Early Administration of Probiotics Attenuates Bacterial-mediated Intestinal Inflammation and Smad 7 Pro-Inflammatory Cell Signaling

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Backgrounds:

Probiotics such as L. acidophilus play an important role to microflora homeostasis in gastric-intestinal duct. To determine the cellular mechanisms by which early administration of probiotics and/or prebiotics in the presence of enteric pathogens altered host response via Smad 7 and NF-κB /I-κBα expression in the human intestinal epithelium in vitro.

Materials and Methods:

Intestinal epithelial cells (Caco-2 or T84 cells) were exposed to Salmonella typhimurium. Culture supernatants (medium) were collected for IL-8 cytokine detection at 1, 2, 3 hours post-pathogen exposure. The cell lysates were used to detect Smad7, NF-κB and I-κBα by Western Blot analysis. Furthermore, Caco-2 cells(or T84 cells) were pre-administered with probiotic (L. acidophilus) and/or prebiotic (inulin supplemented with oligofructose). Subsequently, the cells were infected with S. typhimurium for one hour. Post pathogen exposure, the culture supernatants were used for cytokine determination and cell lysates were used for determination of gene or protein expression with real-time PCR and western blot analysis, respectively.

Results:

Pathogens activated the NF-κB pathway within 30 min to 1 hour in T84 cells, while Smad 7 induction occurred within 1 hour in T84, Caco-2 cells. Smad 7 induction was attenuated by pre-treatment with probiotics, while Salmonella infection alone enhanced Smad 7 intracellular production in Caco-2 cells. Probiotic pre-treatment prevented I-κBα degradation and the activation of the NF-κB pathway, while pre-treatment with prebiotics or Salmonella alone enhanced I-κBα degradation and activation of NF-κB pathway in Caco-2 cells. Additionally, there was approximately a 2-fold reduction in total IL-8 production in Caco-2 cells pre-treated with probiotics prior to Salmonella inoculation 24 hours post infection.

Conclusion:

The NF-κB pathways were activated early in the inflammatory response to enteric pathogens. However, Smad 7 was activated much later in the inflammatory response to enteric pathogens. Smad 7 and NF-κB induction confer to pro-inflammatory cytokine secretion (IL-8). Pro-inflammatory cytokines enhanced Smad 7 accumulation within the cell. Furthermore, probiotics attenuated Smad 7 to induce I-κBα expression while infection in human epithelial cells.

P852

Resveratrol enhances chemosensitivity in mouse melanoma model through connexcin 43 upregulation.

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Backgrounds：

Gap junctions mediate cell communication by allowing the passage of molecules from one cell to another. Gap junctions are formed by two hemichannels, called connexons, each made of six connexin (Cx) prorein. Cx43 is ubiquitous and reduced in a variety of tumor cells. Cx43 may influence the response of tumor cells to treatments by facilitating the passage of antitumor drugs or death signals between neighboring tumor cells. Although current studies indicate that resveratrol exhibits potential antitumor activities, the precise mechanisms of its beneficial effects are not fully understood. This work is warranted to elucidate the underlying mechanism of antitumor effects by the combination therapy of resveratrol and cisplatin. The presence of functional gap junctions is highly relevant for the success of chemotherapy.

Materials and Methods：

The melanoma cancer cell lines were treated with resveratrol and cisplatin. Cell viability was determined by WST-1 assay and the protein expression was determined by Western blot analysis.

Results：

Following resveratrol treatment, dose-dependent upregulation of Cx43 expressions were observed. To study the pathway underlying these resveratrol-induced effects, we found that resveratrol induced a significant increase in mitogen-activated protein kinases (MAPK) signaling pathways.

Conclusion：

That resveratrol cotherapy leads to increase Cx43 gap junction communication and enhances the combination of cisplatin therapeutic effects.

P853
2-Deoxyglucose Increased Cisplatin Chemosensitizing and Cytotoxic Effect in Melanoma

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Backgrounds:

Melanoma is the most aggressive form of skin cancer and is notoriously resistant to all current chemotherapy for cancer. Cisplatin is one of the most potent and frequently used antitumor agents and is clinically effective against a variety of solid tumors, including malignant melanoma. However, the resistance of melanoma against this therapy is a major limitation of cisplatin-based chemotherapy. In our previous study, cisplatin induced chemoresistance in melanoma via elevated cancer stemness marker CD133 and ABCB5, and the chemoresistance mechanisms are involved in ATP synthesis pathway: mitochondria were increased and ATP production were elevated in cisplatin-treated melanoma. The glucose analog 2-deoxyglucose (2-DG), which is a glycolytic inhibitor, causes decrease of cellular ATP.

Results:

2-DG causes decrease of cellular ATP in cisplatin-treated melanoma. Besides, the cellular levels of ABCB5 and CD133 were also reduced by 2-DG after cisplatin treatment. Moreover, 2-DG supplement enhanced the inhibitory effect of cisplatin on anchorage-independent growth of B16-F10 melanoma cells. Invasion assay indicate that 2-DG 2mM treat with cisplatin 3uM could attenuate B16-F10 melanoma cells invasion ability compare to 2-DG or cisplatin treatment alone.

Materials and Methods:

Cellular ABCB5 and CD133 protein level was determined by western blot. B16-F10 melanoma cells were seeding 500 cells per well in 6 well plate to allow colony formation. Cisplatin and 2-DG were treated after 24 hours seeding. Colony was colored by crystal violet after 7 days. B16-F10 Cells were pre-treatment 2-DG 2mM / cisplatin 3uM for 48 hours, and Boyden chamber was used to investigate B16F10 melanoma invasion ability. After treatment with 2-DG / cisplatin, ATP production of melanoma cells was analysis by ATP Bioluminescence Assay Kit CLS II from Roche.

Conclusion:

We demonstrated that 2-DG attenuate expression of ABCB5 and CD133 after cisplatin treatment, 2-DG co-treat with cisplatin effectively reduced melanoma cells invasion and independent-growth ability. 2-DG also has been applied in combination with other therapy in clinical trial.

P854
AIM2 Forms a Inflammasome with ASC and Induces Anti-Cancer Activities in MDA-MB-231 Breast Cancer Cells

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Backgrounds:

It has been identified AIM2 (absent in melanoma 2) as a cytoplasmic DNA sensor for the inflammasome. Inflammasomes may have contradictory roles through enhanced anti-cancer immunity and induced oncogenesis. However, the function of the AIM2 inflammasome in breast cancers and the underlying mechanisms linking inflammation and tumorigenesis remain unclear. Thus, the aim of this study is to investigate the roles of AIM2 inflammasome in breast cancer cells.

Materials and Methods:

Stable 231-ASC or 231-ASC-AIM2 cell lines were generated by transfecting with pcDNA3-ASC plasmid or 231-ASC with dual expression plasmid pCMV-GFP-AIM2 following by selection in G418 and blasticidin-containing medium. The interactions of AIM2 with ASC or caspase-1 were analyzed by immunoprecipitation. The expressions of pro- IL-1β and cleaved IL-1β were analyzed by western blotting and ELISA. RNA interference was used to decline AIM2 expression. XTT assay and Annexin V-FITC staining were performed to measure cell proliferation and apoptosis. Transwell inserts in 24-well plates were used for the migration and invasion assays on stable 231-ASC or 231-ASC-AIM2 cells.

Results:

Here we show that AIM2 interacts with ASC to activate caspase-1 and produce the active IL-1β from MDA-MB-231-ASC-AIM2 cells. Knockdown of AIM2 produced by siRNA impaired maturation of IL-1β. AIM2 infalmmasome also participates in cell proliferation, apoptosis, migration and invasion in stable 231-ASC-AIM2 cells.

Conclusion:

This study indicates that AIM2 inflammasome functions as an attenuator of breast cancer and is a promising target for breast cancer therapy.

P855
Molecular Characterization of Orange-spotted Grouper (Epinephelus coioides) LC3 as Autophagy Marker

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Autophagy is an important cell protective mechanism to sustain cell survival during stress. It performs degradation and recycling cytosolic proteins and organelles via formation of autophagosome. LC3, an important building block for autophagosome formation and currently widely used as autophagy marker. Autophagy also plays as an important role during virus infection. Meanwhile, previous finding shows autophagy of grouper induced during nervous necrosis virus infection. However, currently there is no orange-spotted grouper LC3 cloned and characterized. In this study, grouper LC3 gene which encodes protein with length of 126 amino acids was cloned. Based on previous findings, grouper LC3 also has an important protease cutting site and its function is proven from the protease assay and point-mutation. By transfecting plasmid pDsRed-LC3 into GF1 cell line to observe autophagosome formation. Starvation and rapamycin treatment could induce the autophagy mechanism in GF1 cell line.

Keyword:

Autophagy, Epinephelus coioides, LC3, autophagosome, nervous necrosis virus.

P856
Conditional Activation of Notch Signaling Induces Osteogenesis and Hyperosteogeny in Zebrafish

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Backgrounds:

Notch is one of several highly conserved signal pathways in the embryonic development of organisms. It mediates the cell-to-cell interaction and control the proliferation, differentiation, apoptosis and cell fate decision. Although functions of Notch signaling in mice bone development has been reported, however, the result still controversial and need more experiments to provide solid conclusion. Therefore, we aim to dissect the possible role of Notch signaling on osteogenesis of zebrafish.

Materials and Methods:

We dissect the function of Notch signaling in the bone lineage by creating a stable transgenic fish overexpressing the Notch intracellular domain (NICD) under the control of *osterix* promoter. In order to investigate the osteogenesis activity, we performed Calcein stain at embryonic stage as well as ALP/TRAP stain for *in vitro* scale culture at adult stage. The bone morphology and bone density is evaluated by Alizarin red stain and microCT scan. The bone marker gene expression is determined by real-time RT-PCR for embryos aged 7 dpf and adult.

Results:

Over-activation of Notch signaling induces osteogenesis and hyperosteogeny in zebrafish. In embryo, the relative mineralized vertebrae area is higher comparing with control group. Real-time RT-PCR shows majority of the early osteoblast markers are up-regulating, suggests the Notch signals affect the terminal mineralization activity by increase the mesenchymal to osteoblast differentiation. In adult, the inside neural and hemal arches of vertebrae are thicker and the area of neural and hemal canals is narrower due to acute osteogenesis. The both of ALP and TRAP activity are increased by *in vitro* scale culture indicating the bone remodeling are dramatically activated by Notch signaling.

Conclusion:

Our studies provide first evidence to support Notch signaling play a role on bone growth in fish. It clarifies the contradictory result derived from mice model and reveals notch signaling play a positive role on bone growth. Together, these studies provide a new insight into the effect of Notch on bone growth and also support zebrafish is a good animal model to study bone development and disease.

P857
Selection of potent therapeutic genes for EGFR over-expressed lung cancer cells by bioinformatics

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Backgrounds :
The epidermal growth factor receptor (EGFR) is a kind of tyrosine kinase receptor. Activation of the EGFR stimulates tumor growth, angiogenesis, invasion, metastasis and inhibition of apoptosis. Over-expressions of EGFR are widely found in many kind of cancers. Cetuximab which is a humanized monoclonal antibody is developed against the EGFR over-expressed cancer cells. As clinical results, it has been proved to enhance the therapeutic efficacy by significantly inhibiting tumor growth and metastasis. In addition, liposomal encapsulations of anti-tumor drug have also been proved that they can provide more advanced therapeutic efficacy. Based on such findings, we attempt to combine these two elements together.

Materials and Methods :
Our lab has already developed a cationic liposome complexed with polyethyl- enimine and polyethylene glycol polymer (LPPC) that can absorb various proteins without covalent conjugation. Conjugating with cetuximab, the LPPC- cetuximab complex could directly target to EGFR over-expressed tumor cells both in vitro and in vivo. However, which therapeutic materials have the optimum efficacy is still undefined. To search for the best targets, we developed a system to screen the total gene profiles of EGFR over-expressed tumor cell lines.

Results :
With powerful analysis tools of bioinformatics technology and rich online microarray datasets, we selected those high expressive genes in EGFR over-expressed tumor cells comparing to normal cells. Besides, we further calculated the over- expression frequencies in tumor cells. And if the frequencies are over 80%, the genes would be picked up. Following the criterias, we got 76 genes from 15 EGFR over- expressed lung cancer cell lines and 56 genes from 279 clinical lung cancer samples. Crossing the two gene sets, there were 23 genes identical. The relationships of these 23 genes were analyzed and they were classified into several groups by their tumorigenesis-related signaling pathways.

Conclusion :
In the future, the selected genes would be verified their effects in EGFR over- expressed tumor cells and one of them would be applied to cancer therapy by using the LPPC-cetuximab delivery system.

P858
CXCR4 Association with Epithelial-Mesenchymal Transitions (EMT) in Non-small Cell Lung Carcinoma Cells

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Backgrounds:
Cancer is a leading cause of death worldwide responsible for more than 7.6 million deaths per year. In Taiwan, the lung cancer mortality has been ranked at the 1 in the total cancer patient.
An association between the development of cancer and inflammation has long been appreciated. EMT is morphological and molecular changes that occur when epithelial cells lose their characteristics, gain mesenchymal properties, and become motile, and it plays a critical role in the invasion of cancer cells. However, the mechanisms of how chemokines and their receptors participate in the progression of lung cancer remains unknown. This study was undertaken to investigate the CXCR4 expression induced by LPS in lung adenocarcinoma epithelial cells.

Materials and Methods:
Based on the results obtained, lung adenocarcinoma epithelial cells were stimulated by the LPS, and epithelial-to-mesenchymal transition (EMT) was determined by RT-PCR, Western analyses, wound healing assay. and Gelatin Zymography experiments.

Results:
Stimulation with LPS for 48 hours induced morphological and phenotypic changes with a spindle-like, elongated shape and fibroblast-like appearance. Furthermore, LPS induced the down-regulation of E-cadherin and up-regulation N-cadherin.

Conclusion:
In this study, we demonstrated that LPS–induced EMT, as shown by morphological changes in human alveolar epithelial cells and changes in cell markers.

P859
Pir51 modulates cell cycle distributions and enhances cell migration in HeLa cells

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Backgrounds:
Pir51 interacts with Rad51 and its function involves many pathways. It is crucial to understand how Pir51 is regulated. In addition, this study will investigate the biological functions of Pir51 on cell cycle and cell migration in HeLa cells.

Materials and Methods:
Two Pir51 promoter luciferase constructs including the short fragment (about 420 base pairs, pir51-420) and the long fragment (about 872 base pairs, pir51-872) have been used for evaluating the Pir51 promoter activity. The long fragment contains a P53 putative binding site, but the short not. The P53 putative binding site is located about 825-base pairs upstream from the transcription start site. The miR-23b putative binding site is found in the 3' untranslated region of Pir51. Western blotting analyses and real time polymerase chain reaction assays were performed to examine the expression levels of Pir51 downstream targets. Flow cytometry and wound-healing assays were used to characterize cell cycle distribution and to observe cell migration in Pir51 knockdown HeLa cells.

Results:
In the P53 kncokdown HeLa cells, pir51-420 and pir51-872 promoter activity increases 355% and 230%, respectively. In contrast, when HeLa cells were overexpressed P53, pir51-420 and pir51-872 promoter activity increases 61% and 18%, respectively. miR-23b inhibited Pir51 by using luciferase assays. Furthermore, STAT 1 and P21 increased and cyclin D1 decreased in the Pir51 kncokdown HeLa cells by western blotting. The percentage of the G1 phase cells increases by about 10% in the Pir51 kncokdown HeLa cells. In addition, wound-healing assay showed that cell migration was reducing when Pir51 was inhibited.

Conclusion:
Our data suggested that except P53, other transcription factors might regulate Pir51 expression. Moreover, Pir51 may be involved in modulating cell cycle and cell migration.

P860
The Role of Jab1 in Cigarette Smoke Extract-Induced ICAM-1 Expression

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Backgrounds:
Epidemiologic studies have shown a strong association between cigarette smoking and cardiovascular diseases. Cigarette smoking increases directly or indirectly the production of reactive oxygen species, and induces inflammatory responses, which caused leukocyte recruitment in endothelial cells. Previous studies have shown that CSE (cigarette smoke extract) activated AP-1 (activator protein 1) and up-regulated ICAM-1 (intercellular cell adhesion molecule-1) surface expression in human umbilical vein endothelial cell via an MAPK-independent pathway. The Jab1 (Jun activation domain-binding protein) was first identified as a co-activator of c-Jun. The interaction of Jab1 with c-Jun enhanced the transactivating ability. Therefore, the purpose of this study is to investigate whether CSE-induced Jab1 nuclear translocation in EA.hy926 cells.

Materials and Methods:
The cell viability, Jab1 protein expression and translocation were determined by crystal violet assay, Western blot and immunofluorescence microscopy using anti-Jab1 antibodies, respectively. Quantitative image analysis of nuclear and total Jab1, were measured by ImageJ.

Results:
The results showed that CSE could significantly induce the cytotoxicity in EA.hy926 cells. We have demonstrated that CSE exposure causes Jab1 expression in a dose- and time-dependent manner. In addition, CSE treatments induce accumulation of Jab1 in nuclear and increase its total expression.

Conclusion:
CSE treatment of endothelial cells resulted in increased total amount and nuclear translocation of Jab1 proteins. These results indicate that CSE-induced ICAM-1 up-regulation can be mediated through Jab1.

P861

Leptin Indicates a Poor Prognosis for Nasopharyngeal Carcinoma and Enhances Tumor Cell Motility through AKT Activation

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Backgrounds:

The functions of Leptin in nasopharyngeal carcinogenesis are largely unexplored. In this study, we examined the expression of Leptin at different malignant stages of nasopharyngeal carcinoma (NPC).

Materials and Methods:

Q-RT-PCR and Western blotting were used to evaluate Leptin mRNA and protein expressions, respectively in unpaired NPC patient specimens. To determine the possible correlation between Leptin expression and various clinical characteristics, patients with NPC were evaluated by immunohistochemical staining.

Results:

Strong Leptin expression was a significantly prognostic marker and predictor of aggressive NPC. The overall and disease-specific 5-year survival rates were significantly correlated with strong expression of Leptin (p<0.001). Functionally, overexpressed Leptin could promote NPC cancer cells migration and invasion by Transwell chambers, and wound healing assay. Conversely, the suppression of Leptin expression using Leptin-mediated-siRNA was sufficient to decrease cell motility. Furthermore, our data also illustrated that AKT activation was participated in Leptin-elicited cancer cell motility in NPC cells. Finally, immunohistochemical, and Western blotting analysis of human aggressive NPC specimens showed a significant positively correlation between Leptin and phosphor-AKT expression.

Conclusions:

Taken together, our results suggest that Leptin/AKT pathway is associated with survival, tumor progression and metastasis of NPC patients.

P862

Non-invasive monitoring of *let7i* suppressed metastasis in HNSCC

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Backgrounds:

To investigate the effects of Twist1-let7i signaling pathway on the progression of human HNSCCs in nude mice using the reporter gene imaging system.

Materials and Methods:

For Cell line and cell cultured, Human head and neck squamous FaDu cell line was cultured in RPMI1640 medium supplied 10% FBS (Fetal Bovine Serum), 1% L-glutamine and Penicillin Streptomycin. For Stable transfection, let7i was manipulated in Fadu cell lines by transducing pmCherry–spg-let7i and pmCherry–spg-control are delivered into Fadu. The stable cell was selected by G418 (300 μg/ ml) in selecting FaDu cells. For non-invasive reporter system, the Fadu cell line was transduced with a co-expressed multi-reporter gene system including EGFP, fluc and HSV1-tk using the lentivirus. The function and expression of this triple modality of reporter genes have been verified in vitro. For verification in vivo, bioluminescence and EGFP signals were confirmed by IVIS 50; HSV1-TK images was acquired by Micro Single photon emission computed tomography (μSPECT) after 8 hours post-injection of ^{123}I-FIAU. Immunohistochemistry Staining was used for pathological verification of tumor.

Results:

We established stable cell lines by transfecting pmR-mcherry-sponge-let-7i into HNSCC cell line FaDu which natively expresses high level of let-7i. Subsequently, this cell line was transduced with a co-expressed multi-reporter gene system including EGFP, fluc and HSV1-tk using the lentivirus. The resultant cell line was named FaDu-spg-let-7i-3R cells. The control cell line named FaDu-spg-control-3R was also established by the insert which is not complementary to any known miRNA. Both clones were separately implanted in the tongue tips of nude mice and monitored using the in vivo imaging system (IVIS) and μSPECT/CT periodically. Compared to the spg-control cells, the tumor metastasis was enhanced in FaDu-spg-let-7i-3R cells via the results of animal imaging. Additionally, the role of let-7i on the intrinsic metastatic ability will be determined using tail vein injection. This observation was opposed to the parental cell type. We expect to use this system as preclinical model to predict the favor location invaded by HNSCC during tumor development. Moreover, whether the tumor recurrence or distal metastasis is related to let-7i would be explored as well.

Conclusion:

This study is to provide translational medical evidence of TWIST1-let7i-NEDD9 regulatory axis metastasis in vivo using biomedical imagimg. Our results not only strengthened the original findings of the role of let7i in metastasis in vivo, but also important for the field of molecular imaging that is eager to dissolve the mechanism of metastasis and recurrences after chemotherapy. This translational medical research will also be important for the design of clinical treatment on the head and neck cancer in the future.

P863

Bone Morphogenetic Protein 2 Inhibits Liver Fibrosis By Blocking Transforming growth factor β Signaling

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BACKGROUND:

Liver fibrosis results from damage about chronic liver diseases to conjunction with the accumulation of extracellular matrix proteins. Hepatic stellate cell represents the trademark of liver fibrosis which is activated by fibrogenic cytokines such as Transforming growth factor (TGF)-β1. TGF-β1 is a central regulator in chronic liver disease to all stages. TGF-β exerts its biological functions mainly through its downstream signaling molecules, Smads. Bone Morphogenetic Protein (BMPs) belong to transforming growth factor beta TGF-β superfamily According to previous literature, Several studies have demonstrated found BMP-2 can ameliorate renal tubule fibrosis by suppressing type I TGF- β recepter. The previous study demonstrated that BMP-2 was downregulated in fibrotic liver of mice, and downregulated in hepatocytes. The purpose of this study was to determine whether mutual regulatory mechanisms exist between BMP-2 and TGF-β1 in liver.

METHODS:

Preparation of the human hepatic stellate cell line HSC-T6. TGF-β1 (5ng/ml) was used to induce liver cellular fibrosis in HSC-T6. BMP-2 (1ng/ml, 10ng/ml, 100ng/ml) was used to product liver cellular fibrosis in HSC-T6. Secreted fibronectin was assayed using ELISA. Fibronectin and Smads was elucidated by Western blot.

RESULTS:

TGF-β1 induced HSC-T6 activation of fibronectin expression. BMP-2 inhibits TGF-β1 induce fibronectin and α-SMA expression in HSC-T6 cells. BMP-2 induces Smad7 overexpression in HSC-T6 cells.

CONCLUSION:

BMP-2 might be a novel therapeutic agent for fibrosis in hepatic cells and Smad7 may play roles in the regulation and protection of liver fibrosis.

P864

Epigallocatechin-3-gallate Induces Cell Death and Reduces HHV8 Viral Replication in Primary Effusion Lymphoma Cells.

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Backgrounds:

Epigallocatechin-3-gallate (EGCG), the major constituent of green tea, has been shown to induce cell death in cancer cells. Primary effusion lymphoma (PEL) is an aggressive neoplasm caused by human herpesvirus 8 (HHV8). HHV8 displays two different phases of viral lifecycle, latent and lytic cycles. Most of PEL cells are latently infected. In this study, we tried to examine the role of EGCG on PEL cells in leading cell death and HHV8 replication.

Materials and Methods:

Two PEL cell lines, BCBL-1 and BC-1, were tested. The cell viability and ROS generation in the EGCG treated PEL cells were determined by trypan blue exclusion assay and a fluorogenic 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) probe. The signal pathway involved in EGCG induced PEL cell death was examined by Western Blot analysis. The production of progeny HHV8 virus from EGCG treated PEL cells were determined by PCR.

Results:

EGCG induced both cell death and ROS generation in PEL cells at a dose-dependent manner. N-acetylcysteine (NAC) inhibited the EGCG induced ROS but did not rescue the EGCG induced cell death. Activation of c-jun-N-terminal kinase was observed in the EGCG treated PEL cells. JNK inhibitor, SP600125, did not attenuate but sensitize the EGCG induced cell death. Even though EGCG induced ROS generation in PEL cells, it reduced the production of progeny virus from PEL cells without causing HHV8 reactivation.

Conclusion:

EGCG induced PEL cell death is through ROS-independent pathway and JNK inhibitor can sensitize the EGCG induced PEL cell death. EGCG treatment inhibits the production of HHV8 from PEL cells, suggesting EGCG may represent a novel strategy for the treatment of HHV8 infection and HHV8-associated lymphomas.

P865
Attenuation of Cancer-Initiating Cells Stemness Properties by Abrogating S100A4 Calcium Binding Ability in Head and Neck Cancers

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Background:

Epithelial-mesenchymal transition (EMT) is a process that epithelial cells acquire a migratory mesenchymal phenotype or stem cell (SC) properties. S100A4, a member of calcium-binding proteins competent to induce EMT and directly controlled by Wnt/β-catenin signaling pathway. Calcium binding activity of S100A4 has been highly associated with the regulation of downstream targets and development of a metastatic phenotype. Our previous research indicated that S100A4 play a crucial role in the maintenance of head and neck cancer-initiating cells (HN-CIC) population. Inhibition of S100A4 decreased the HN-CICs stemness and self-renewal property, both in vitro and in vivo. The regulation of S100A4 and its molecular targets to control HN-CICs stemness capability has still unknown.

Methods:

In this study, we establish the mutants of two calcium-binding sites and a deletion of the last 15 amino-acid residues of S100A4 in head and neck squamous cell carcinomas (HNSCC) to investigate the mechanism of S100A4 enhances stemness properties of CICs

Results:

We found that reduced the calcium binding activity of S100A4 results in decreasing of anchorage independent growth ability when it compare to the wild-type. The western blot analysis results showed that S100A4 not only increased the protein level of Vimentin and Nanog but also decreased the expression of E-cadherin. However, mutants of S100A4 reduced the protein of Vimentin and Naong and increased E-cadherin. To further investigate the connection between S100A4 and stemness determinants, we enriched HN-CICs from S100A4 wild-type and mutants by sphere formation. The results showed that S100A4 mutants highly decreased the stemness marker CD133 and GRP78.

Conclusion:

Our study provides a hint that S100A4 Ca^{2+} binding play an important role in the maintenance of self-renewal and stemmness properties of HN-CICs. The study of how modulators of Ca^{2+} dependent processes operate the stem-like properties and the tumorigenicity of HN-CICs need to further elucidation.

P866
Visualizing the Complexes Formed by Protein Phosphatase 2A Catalytic Subunit (PP2Ac) and its interacting protein in cells

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Backgrounds:

Protein phosphatase 2A (PP2A) is a major serine/threonine phosphatase. Canonically, PP2A is composed by a scaffolding subunit (A), a catalytic subunit (PP2Ac), and a variable regulatory subunit (B). In addition, several cellular proteins have been identified to interact with PP2Ac, including α4, TIP, and MID1, and have been shown to play a crucial role in regulating PP2A activity. In our study, we focus on visualizing the known PP2Ac-associated complexes in living cells, and to explore novel multi-subunit complexes formed by PP2Ac and PP2Ac interacting molecules.

Materials and Methods:

Both indirect immunofluorescence and direct fluorescence microscopy were used to investigate the subcellular localization of PP2Ac and its binding partners. Further, bimolecular fluorescence complementation (BiFC) analysis was applied to study complexes formed between PP2Ac and its interacting partners. BiFC and fluorescence resonance energy transfer (FRET) were combined to visualize PP2Ac-associated multi-subunit complexes in cells. Co-immunoprecipitation (Co-IP) and in vitro pull-down analysis were used to verify the findings using BiFC and BiFC-FRET methods.

Results:

The results of immunofluorescence showed that PP2Ac was distributed throughout the entire cells, whereas α4 was majorly expressed in cytoplasm. TIP was located at cytoplasm, and MID1 was predominantly associated with microtubules. Further, BiFC analysis demonstrated that PP2Ac-α4 was either ubiquitous or cytoplasmic, but the PP2Ac-TIP complex was ubiquitous. There was no BiFC signals detected for the complex of PP2Ac-MID1, whereas in the presence of wild-type α4, but not a mutant α4 defective in binding to PP2Ac, PP2Ac-MID1 formed BiFC complex with a microtubule-like distribution pattern. The BiFC signal of the MID1-α4 complex exhibited both microtubule-like threads and cytoplasmic clumps. Intriguingly, we found the BiFC signal of the novel MID1-TIP complex with a distribution pattern similar to the MID1-α4 complex. Moreover, the association of MID1 and TIP was confirmed by Co-IP. BiFC-FRET analysis indicated that the trimeric complex of PP2Ac, MID1, and α4 was successfully observed in cells, whereas the trimeric complex of PP2Ac, MID1, and a mutant α4 defective in binding to PP2Ac can not be detected.

Conclusion:

We established BiFC and BiFC-FRET to visualize interactions between PP2Ac and its binding molecules in cells, and the results were consistent with previous studies using methods such as co-IP and in vitro pull down assay. Interestingly, we identified a novel complex formed by MID1 and TIP. Currently, we are investigating the dynamics of the PP2Ac-associated multi-subunit complexes in living cells.

P867
Study the anti-inflammatory effects of *Alpinia officinarum* and *Vitis amurensis* extracts

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Backgrounds:

Inflammation is the immunoreaction caused by biological tissues' trauma, bleeding or pathogen infection. The inflammatory symptoms include redness, heat and pain, etc.…

Materials and Methods:

In the research, we use lipopolysaccharide (LPS) to activate a mouse macrophage cell line (RAW264.7) as inflammatory cell model. The inhibitory effects of nitric oxide (NO) productions were examined as the initial screening for anti-inflammatory activities of herbal extracts.

We injected a large dose of TAA in rats, causing an acute inflammation of their livers, to establish acute hepatitis model rats.Various herbal extracts were feeded to evaluate their anti-inflammatory activitys of acute hepatitis model rats.

Results:

Current results showed more than 70% NO inhibitory effect and more than 80% cell viability by MTT assay when RAW264.7 cells treated with 37.5μl/ml *Alpinia officinarum* extract Otherwise, treatment of 50μl/ml *Vitis amurensis* extract showed more than 70%, NO inhibitory effect.However, results of MTT assays showed that the cell survival rate is about 40% in this concentration. Thus,the *Alpinia officinarum* extractwere continuely used in acute hepatitis model rats.

Conclusion:

The preliminary results showed that low dose of *Alpinia officinarum* extract has no beneficial effect for the TAA-induced acute hepatitis rats. Treatments with high-dose of *Alpinia officinarum* extracts are in progress.

P868
Antiproliferative Effect of Extract Prepared from Ganoderma neojaponicum on Human Hepatocellular Carcinoma Cells

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Backgrounds:

Ganoderma lucidum (Reishi or Ling-Zhi) has been widely used as a medicinal mushroom for promoting health in China and other eastern Asian countries. A number of pharmacological studies on the aqueous extracts of Reishi have revealed that the mushroom possesses antiproliferative effects on many cancers. In this study, we examine the anti-proliferative effects of extracts from another kind of Reishi, called Ganoderma neojaponicum, on human hepatoma cells.

Materials and Methods:

The fruit bodies of Ganoderma neojaponicum were extracted with boiling water and the supernatant obtained was lyophilized to yield water-soluble (WS) extract. The WS extract was further treated with ethanol to prepare the ethanol–soluble (ES) and –insolube (EI) fractions. The lyophilized ES fraction was used to examine its anti-proliferative effect on human hepatoma cells.

Results:

The ES fraction was found to elicit a concentration-dependent growth impediment through cell cycle arrest at S phase and induce apoptosis in human hepatoma cells, Hep G2 and SK-Hep-1 cells. Western blotting data showed that the up-regulation of cell cycle regulatory protein (p21and p27) and bax/bcl-2 ratio increment may be the culprits for ES-induced cell cycle arrest and apoptosis, respectively. ES-treated HCC cells also displayed transient increase of ROS, followed by the disruption of mitochondria membrane potential. The presence of a ROS scavenger (N-acetyl-L-cysteine) blocked ROS production but it only prevented limited amount of cells from becoming apoptosis-prone.

Conclusion:

Taken together, our data suggested that ES fraction from the fruit bodies of Ganoderma neo- japonicum exhibited direct cytotoxic effects on HCC cells through ROS-dependent and -independent pathway.

P869

Areca Nut Extract induces Pyknotic Necrosis in Serum-starved Oral Cancer Cell Lines: an Implication for Cytopathic Effects in Betel Quid Chewers

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Backgrounds:

Although areca nut was implicated with various pathogenic effects in oral cavity, molecular mechanism for pathogenesis was still illusive.

Materials and Methods:

Oral cancer cell lines: OC2, OCSL and SAS. Effects of areca nut extracts (ANE) on cell viability, morphologically, pyknotic necrosis, and biomarkers of signal transduction related to this novel pyknotic necrosis were evaluated, observed, verified and identified by DAPI staining, ethidium bromide/acridine orange stain, DNA fragmentation assay, and treatments of specific inhibitors for various kinases.

Results:

We discovered that ANE strongly caused swelling in various serum-starved oral cells. Accompanied with swollen cell volume, ANE also induced nucleus shrinkage (pyknosis like), which was observed morphologically in betel chewer's mucosa. The result of propidium iodide (PI) and acridine orange/ethidium bromide staining suggested that ANE indeed induce necrosis in serum-starved cells. Consistently, no laddered DNA was observed in cells after ANE treatment, indicating that ANE induced pyknotic necrosis instead of apoptosis. Surprisingly, LC3-II transition and PARP cleavage were still detected, suggesting that autophagy/apoptosis and pyknotic necrosis were not mutually exclusive. This novel necrosis was proved to be mediated via reactive oxygen species (ROS) and GSK3 pathways.

Conclusion:

Clinical observations demonstrate that betel quid may primarily induce necrosis in vivo. Since oral epithelium cells theoretically exist in an environment short of nutrients relatively, this research provides a model for studying oral carcinogenesis in betel chewer's mucosa. Currently, we focus on using this model to decipher the progress from initiation of ANE-induced chronic inflammation, followed by pathogenesis, tumorigenesis and metastasis.

P870

The Effects of Arecoline on IL-6/IL-6 Receptor Signaling in Hepatoma Cells

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Backgrounds:

Our previous study showed that arecoline induced anoikis of hepatoma cells, HA22T/VGH, by inhibition of STAT3, increase in RhoA/Rock activation, and reduction in interleukin-6 (IL-6), a tumor cell survival factor, levels. Furthermore, we observed that, in hepatoma cells, arecoline induces the expression of IL-6 binding receptor, gp80 but not of its signal transducing receptor, gp130. In this study, we in advance investigated the fate of the arecoline-induced gp80.

Materials and Methods:

Hepatoma cell lines including HepG2, PLC/PRF/7, and HA22T/VGH were treated with arecoline, and then the expressions of IL-6, IL-6 receptors gp80, and gp130 were evaluated by RT-PCR, real-time PCR, or flow cytometric method. In addition, human peripheral blood mononuclear cells (PBMCs) were treated with supernatants of cultured medium from arecoline-treated hepatoma cells, and then the STAT3 activation was detected by Western blotting. The cell viability was evaluated by trypan blue exclusion method.

Results:

The results showed different basal IL-6 receptors expressions were in three hepatoma cell lines (HepG2 > PLC/PRF/7 > HA22T/VGH). Arecoline treatment induced gp80 expression in HA22T/VGH and PLC/PRF/7, but not in HepG2. Combined with arecoline and dexamethasone, a known inducer of IL-6 receptor expression, treatment induced gp80 and gp130 expressions in PLC/PRF/7; and this increased expression was greater than each alone treatment. Dexamethasone alone neither alters the expression of gp80 nor gp130 in HA22T/VGH; but combined with arecoline would result in increased gp80 expression, but not gp130. Interestingly, the cell viability of three hepatoma cell lines was not changed by the combined treatment. Additionally, PBMCs treated with supernatants of cultured medium from arecoline-treated hepatoma cells had increased STAT3 activation, and which was a time-dependent manner.

Conclusion:

This study showed that arecoline would increase gp80 expression of hepatoma cells by which STAT3 of PBMCs would be activated. It suggests the activation of STAT3 of PBMCs may be, even in part, through trans-IL-6/IL-6 receptor signaling pathway. (This work was supported by grant NSC101-2320-B-037-045-MY3 and NSC101-2811-B-037-027 from the National Science Council, Executive Yuan, Taiwan.)

P871

The inhibitory potency of *Polyalthia longifolia* in colorectal cancer cell.

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Colorectal cancer is one of prominent cancer that comes from uncontrolled cell growth in the colon. The mortality rate is the third place of all cancer. Previous study reports the cells transmit messages by Notch ligand and control the cell proliferation by wnt/β-catenin signal pathway. 16-hydroxy-cleroda-3,13-dine-15,16-olide (HCD) isolated from *Polyalthia longifolia* possesses some medicinal values including anti-hepatoma and anti-breast cancer. In this study aimed to investigate the beneficial effect of HCD on colorectal cancer cells (Caco-2). Using various doses of HCD treated colorectal cancer cells for 48 hours by MTT assay, found that the IC50 of HCD was 2 uM/ml. PI stain/Flow cytometry showed that the sub-G1 phase increased with the concentration of HCD. Accordingly, Prodigiosin (PG) can inhibit cancer cell by apoptosis signaling pathway, therefore PG was taken as a positive control. Western blot showed that the Bcl-2 decreased with respect to caspase8 and caspase9 proteins increased in the apoptosis pathway. And p53 and p21 proteins increased in the Notch wnt/β-catenin pathway, and COX-2 and iNOS proteins related to inflammation were decreased after HCD treatment. Moreover, PG had the same effect as HCD on Caco-2. These results imply that HCD and PG potentiate the apoptosis of colorectal cancer cells.

P872

Bimodal epigenetic silencing of miR-193a in ovarian cancer

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Ovarian cancer is one of the most lethal cancers in the female reproductive system. One of the hypotheses suggests that ovarian cancer arises from cancer stem cell with surface antigen CD44 and CD117 (or c-kit).MicroRNAs (miRNAs) are endogenous, noncoding RNA that can inhibit the function of their target mRNAs. MiRNAs contribute to cancer development and progression by acting as oncogenes or tumor suppressor genes. In order to identify miRNAs that can regulate the expression of c-kit, bioinformatic prediction identified miR-193a as one of the regulators. As miR-193a was expressed in immortalized ovarian surface epithelial cells (IOSE) upon treatment with TGF-β but silenced in a panel of ovarian cancer cell lines, it is of interest to examine the mechanisms leading to its down-regulation in ovarian cancer.

Treatment with demethylation agent partially restored miR-193a expression in ovarian cancer thus suggesting that epigenetic mechanism may be responsible for the down-regulation of this miRNA which resides on a CpG island. Further studies using bisulfite pyrosequencing revealed that the CpG island of miR-193a was heavily methylated in ovarian cancer cell lines as well as in 109 ovarian cancer patients samples but unmethylated in IOSE and normal OSE cells. Kaplan-meier analysis found that except for a group patients showing low methylation of miR-193a but also low recurrence interval, methylation of miR-193a is associated with poor survival and recurrence in this patient cohort. We therefore suspected that additional mechanism may exist in controlling the function of miR-193a in ovarian cancer as suggested by the recent competitive endogenous RNA (ceRNA) hypothesis that the function of miRNAs can be perturbed by the expression of their targeted mRNAs by acting as miRNA decoys. Our result demonstrated that over-expressing c-kit 3'UTR resulted in a upregulation of E2F6, another target of miR-193a in a miR-193a expressing ovarian cancer cell line. In conclusion, our result suggested that function of miR-193 can be inhibited by 2 different modes of epigenetic mechanism in ovarian cancer.

P873
Rab GTPases Regulate ADAM9-ITGB4 Complex Endocytosis and Degradation

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Backgrounds:

Studies demonstrated ADAM9 regulate cancer metastasis, which is the major hallmarks of lethal phenotypic cancer progression. Preliminary evidences have showed the ADAM9 promoted ITGB4 degradation to enhance cell migration and invasion. Here we try to access the molecules that process ITGB4 endocytosis and degradation.

Materials and Methods:

Overexpression Rab GTPases including Rab4, Rab5, Rab7 and Rab11 were constructed by TOPO TA Cloning. The expression of Rab GTPases were validated by the in vitro cell imaging and western blot. ADAM9-Rabs and ITGB4-Rabs endocytic vesicles were analyzed by immunofluorescence microscopy after stimulation.

Results:

We observed the colocalization of ADAM9 and Rabs after treating cancer cells with laminin in prostate cancer cell line, PC3. The colocalization of ADAM9 endocytic vesicle with Rab4, Rab5 and Rab11 was observed after treating PC3 with laminin. However, Rab7 did not show to colocalization with ADAM9 during laminin treating. By contrast, ITGB4 was showed to enter into lysosomal degradation vesicle through the colocalization with Rab GTPases. The lysosomal vesicle of ITGB4 can be confirmed by fluorescent imaging analysis of LAMP-1 and ITGB4 as well as degradation studies.

Conclusion:

It has been demonstrated that Rab4 and Rab11 belong to the short loop and long loop recycling pathways, respectively. In adding, Rab5 belong to the early endocytic vesicle, whereas Rab7 belong to lysosomal vesicle. Our evidences demonstrated both ITGB4 and ADAM9 can be endocytosis into Rab GTPases vesicle. However, ADAM9 did not show to enter into lysosomal degradation. By contrast, ITGB4 was leading into lysosomal degradation. Continue studies will be conducted to analyze the molecules that regulate the separation of ADAM9 and ITGB4 during endocytic vesicle trafficking.

P874
Epigallocatechin-3-gallate Suppresses Cell Invasion and Migration of Human Lung Cancer Cells through Inhibiting Transforming Growth Factor-β1-Induced β-catenin Nuclear Translocation and the Resulting Epithelial-to-Mesenchymal Transition

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Backgrounds:

TGF-β1 may induce epithelial-to-mesenchymal transition (EMT) and invasion of human A549 lung cancer cells by activating PI3K/Akt and MEK/Erk1/2 signaling pathways. Epigallocatechin-3-gallate (EGCG), the green tea polyphenol, has been reported to block TGF-β1-elicited EMT and invasion of A549 cells on a Smad-dependent manner. Herein, whether EGCG can inhibit EMT and invasive phenotypes of human small cell lung cancer cells via modulating TGF-β1-induced PI3K/Akt and β-catenin signaling pathways was investigated.

Materials and Methods:

The MTT assay was firstly applied to evaluate the proliferative effect of EGCG along with TGF-β1 on human A549 cells. Then the effects of EGCG on cell invasion and migration in TGF-β1-stimulated cells were examined by Transwell assay and wound-healing assay. Western blot analysis was used to elucidate the effects of EGCG on the expressions of TGF-β1-induced EMT markers and the PI3K/Akt and MEK/Erk1/2 signaling molecules in A549 cells. Moreover, cellular distributions of EMT-related makers were monitored by immunofluorescence assay with cytosolic and nuclear sub-fractions.

Results:

As shown in MTT assay, EGCG together with TGF-β1 had a slight effect on proliferation of human A549 cells. EGCG markedly suppressed cell-based wound-healing and in vitro invasion and migration on a Transwell membrane of TGF-β1-stimulated A549 cells. In parallel, EGCG significant reduced the expression levels of TGF-β1-induced mesenchymal markers N-cadherin, Snail and vimentin, and increased epithelial marker E-cadherin expression, suggesting the decrease in TGF-β1-driven aggressive phenotypes by EGCG via blocking EMT program. Exploring it action mechanism, EGCG was found to inhibit PI3K/Akt signaling induced by TGF-β1 and causally prevent β-catenin nuclear translocation, from which TGF-β1 drives EMT program. Furthermore, β-catenin siRNA or LY294002 (PI3K/Akt inhibiter), similar to EGCG, potently decreased the protein levels of β-catenin and Snail in TGF-β1-induced A549 cells. On the other hand, LiCl (GSK-3β inhibiter) combined with TGF-β1 treatment restored β-catenin expression and nuclear translocation (compared to that of EGCG-co-treated cells). These results led us to know EGCG can inhibit TGF-β1-induced EMT via a Smad-independent pathway in human A549 lung cancer cells.

Conclusion:

Our preliminary results indicate that one possible action mechanism by which EGCG suppressed TGF-β1-induced EMT and invasion of human A549 lung cancer cells is that EGCG restored GSK-3β activity and subsequently inhibited β-catenin signaling through suppressing PI3K/Akt pathway.

P875
LiCl Inhibits GSK3-mediated Bcl2L12 Apoptotic Role through Interplaying Relationships with BclxL and Bax in U87MG Cell

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Bcl2L12 has been reported to be involved in post-mitochondrial apoptotic events through multiple molecular mechanisms including p53, alpha-crystallin, caspase-3, and caspase-7 in glioblastoma (GBM), as well as GSK3-mediated Bcl2L12 phosphorylation at Ser156 is critical for its anti-apoptotic roles. Nevertheless, the underlined molecular mechanisms of how Bcl2L12 triggers anti-apoptotic effect remains unclear. In this study, we showed that truncated Bcl2L12 fragments, $Bcl2L12_{70\text{-}240}$ and $Bcl2L12_{70\text{-}266}$, can interact with full length BclxL and/or themselves, but not Bax using yeast two-hybrid system. In contrast, full length BclxL is as expected, can binds to Bax. These data may indicate that Bcl2L12 possibly interacts and facilitates its binding role with BclxL in competing with Bax-BclxL, which may important to its anti-apoptotic effect in GBM. Furthermore, we identified within amino acid sequence corresponding $Bcl2L12_{70\text{-}240}$, a hydrophobic region, locates in 70-240, and is important to the binding fashion to the BclxL. Site-directed mutagenesis on residue within the hydrophobic region successfully abolished the interaction between Bcl2L12 and BclxL. Ectopic expressed GFP-fused Bcl2L12, but not Bcl2L12(S156A) in U87MG cells robustly leads to a repression of apoptotic markers (cleaved caspase-3, -7, -9 and PARP) as well as Bax under both staurosporine (STS) and hydrogen peroxide insults using real-time PCR and immunoblotting techniques. The Bax/BclxL ratio was observed to be decreased. Interestingly, the anti-apoptotic effect of Bcl2L12 can be reversed by LiCl. Altogether, we established a model to demonstrate that GSK3-mediated phosphorylates Bcl2L12 confers an anti-apoptotic role through interplaying with BclxL and Bax in U87MG cell, which may shed a newly approach of using LiCl to inhibit GSK3 to reverse anti-apoptotic role of Bcl2L12 in GBM.

P876
Directed differentiation of Mouse Embryonic Stem Cells into Retinal Pigment Epithelial Cells

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Backgrounds:

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of blastocyst-stage embryos. They can differentiate into three germ layers, namely ectoderm, endoderm and mesoderm. Previous studies showed that mouse ES cells can differentiate into retinal pigment epithelial (RPE) cells by a stepwise treatment with defined factors. In addition, a co-culture system with RPE cells was also tested for ES cells differentiation. In this study, different differentiation conditions for inducing mouse ES cells in to RPE cells were investigated.

Materials and Methods:

Mouse ES cells were differentiated into RPE cells with one of the follow of different treatments. ES cells were (A) treated with defined factors; (B) co-cultured with RPE cell line; (C) cultured in RPE cell conditioned medium. The efficiency of ES cells differentiation is evaluated morphologically and RPE specific cell markers by RT-PCR or immunocytochemistry.

Results:

Day 9 after induction of differentiation, retinal progenitor genes, MITF and Pax6 were expressed among three groups in mouse ES cells. Morphology of RPE cell was observed in three groups after differentiation for 16 days. The tight junction marker ZO1 of RPE cells was observed on Day 21 of differentiation.

Conclusion:

RPE cells were successfully induced in these three differentiation systems. However, further studies are required to increase the efficiency of RPE differentiation.

P877
Thymoquinone induces autophagy and apoptosis in oral cancer cells.

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Backgrounds:
Thymoquinone (TQ) is a phytochemical compound extracted from the plant *Nigella sative*, one in Islamic medicine commonly used herbs. Recent studies reported that TQ exhibited inhibitory effects on cell proliferation, migration, and tumor angiogenesis in various cancer cell lines. However, the effects of TQ on anti-cancer properties in head and neck squamous cell carcinoma (HNSCC) remain unclear.

Materials and Methods:
Cells were treated with thymoquinone to determine the effect on cell death by MTT assay, DAPI staining, JC-1 staining, AVO staining, MDC staining, colony formation assay, cell cycle analysis, annexin V/propidium iodide apoptosis assay, tumor growth assay, and changing of mitochondrial membrane potential. The molecular mechanisms of thymoquinone mediated autophagy and apoptosis were further investigated by Western blotting analysis.

Results:
In this study, we demonstrated that the TQ induced a strong cytotoxic effect toward a highly malignant HNSCC cell line, SASVO3, from primary tumors using three sequential rounds of xenotransplantation. The mechanisms of the cytotoxic effect were concentration-dependent. TQ induced apoptotic cell death in SASVO3 cells, as evidenced by increases in the expression of Bax and activation of caspase. On the other hand, cells treated with TQ revealed morphological and ultrastructural changes of autophagocytic death under electron microscopic observation. Furthermore, these cells showed increased levels of autophagic vacuoles and LC3-I and LC3-II proteins, specific markers of autophagy. The levels of Bcl-2, Akt, and mTOR, which have been implicated in the down-regulation of autophagy were decreased upon TQ treatment.

Conclusion:
Taken together, these findings indicate that TQ induced cell death in oral cancer cells via two distinct antineoplastic activities, the ability to induce apoptosis and autophagocytosis, and TQ represent promising candidates for future phytochemical-based mechanistic pathway-targeted cancer prevention strategies.

P878
Extracellular Histones Enhanced the Septic Mortality through Apoptosis and Autophagy and Serum Albumin Blockade the Damage

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Backgrounds:
Sepsis progressing to septic shock and multiorgan dysfunction remains the most common causes of death in patients under intensive care. Extracellular histones from neutrophils or inflammatory cells caused systemic inflammation and sepsis, also associated with cytotoxicity of endothelial cells and lead to death of septic mice model especially H3 and H4. We want to investigate the mechanism of histone-induced cytotoxicity toward endothelium, and evaluate the interacted molecules applied to block the damage.

Materials and Methods:
Using cecal ligation and puncture (CLP) to autonomously induce sepsis to evaluate the mechanism of histone-induced cytotoxicity toward endothelium in vivo. To directly bridge the relationships between H3 and septic severity, we used ELISA to detect H3 level in sera of septic mice, and used LC MS/MS to analyze the H3-interacted molecules in septic sera. Further, to validate the histone-induced damage effect in detail, we harvested the histone-treated endothelial cells and detected the expression level of cellular death-associated molecules.

Results:
We found that administration of histone mixtures enhanced the mortality and elevated the pulmonary injury of the septic mice in a dose-dependent manner, and H3 level in serum of CLP mice was dynamic changed after surgery by ELISA detection. We wondered whether there were molecules in sera to modulate the H3 concentration in CLP mice, using LC MS/MS to analyze septic sera and many proteins were identified including albumin, we also confirmed the interaction between albumin and H3used immune-precipitation (IP) assay in vitro. To examine the role of albumin function in sepsis, administration of albumin decreased histone-induced cytotoxicity toward endothelium in vitro, suggesting that albumin is able to reduce the adverse effect of histone to endothelial cells. In addition, histone-induced cellular damage of endothelium is through apoptosis and autophagy.

Conclusion:
Conclusively, we have demonstrated that extracellular histone exacerbates progression and severity of sepsis, also increase the mortality and severe pulmonary injury in a septic mouse model, strongly supporting that histone is a negative indicator for the outcome of sepsis in patients. Finally, we found extracellular histone exhibits cytotoxic effect toward endothelial cells through apoptosis and autophagy, and albumin provided the protection for endothelium from histone-induced cellular damage.

P879
The Studies for Antibacterial Effects of Hyaluronic Acid Silver Nanoparticles and Their Safety Evaluation to Skin Cells

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Backgrounds:
Silver nanoparticles have become more popular in medical applications. However, the functions of hyaluronic acid silver nanoparticles (HA-AgNPs) were very few been reported. The purposes of this research included: (1) to synthesize different size of HA-AgNPs; (2) to examine the antibacterial efficacy of HA-AgNPs; (3) to evaluate the safety of HA-AgNPs to skin cells.

Materials and Methods:
HA-AgNPs were synthesized by direct chemical reduction method, and the particle sizes and the stability were detected by the absorption wavelength of UV−visible spectra. The antibacterial efficiencies of HA-AgNPs were analyzed by different concentrations. In addition, the cytotoxicity of HA-AgNPs was further studied to evaluate the safety of the HA-AgNPs on the skin cells.

Results:
According to the results, two different sizes of HA-AgNPs (15.1nm and 69.9nm) were synthesized. The maximum absorbencies of these two kinds of silver nanoparticles were between 394 to 396nm. In addition, the results of antibacterial efficiencies showed that 130μg/ml of HA-AgNPs in both different sizes were able to inhibit 1×10^7cfu/ml of *Staphylococcus epidermidis* and *Escherichia coli up* to 48hrs. Moreover, the 15.1nm of HA-AgNPs presented the 50% of adsorptive effects to bacteria, whereas the 69.9nm of HA-AgNPs were able to approach to 100%. Based on the results of safety assessment, the 15.1nm of HA-AgNPs were able to promote the proliferation of skin cells (NIH3T3) with different dosages treatments (0.25μg/ml, 0.5μg/ml, 1μg/ml and 2μg/ml) for 48hrs. They also presented the dose-dependent effects that the cell viabilities were more than 110%, whereas the 69.9nm of HA-AgNPs only exhibited 97% of cell viabilities. In summary, both 15.1nm and 69.9nm of HA-AgNPs presented no toxicity to skin cells.

Conclusion:
In this study, HA-AgNPs demonstrated that the significantly antibacterial effects on *E. coli* and *S. epidermidis*. In addition, HA-AgNPs were non-toxicity to skin cells, and were also able to promote the cell proliferation. These results suggested that HA-AgNPs had highly potential to apply on the development of biomaterial products in medical industry in the future.

P880
Silibinin Represses Tumor Initiating Stem-Like Property in Head and Neck Cancer through Activation MiR-494-targeting Bmi1 and ADAM10

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Purpose:
Tumor initiating cells (TICs) possessing stemness properties was shown to be enriched after therapy, resulting in the relapse and metastasis of head and neck squamous cell carcinomas (HNC). An effective therapeutic approach targeting the HNC-TICs would be a potential method to improve the treatments for HNC-related malignancies.

Experimental Design:
The chemo-therapeutic effect and regulatory mechanisms of silibilin, a polyphenolic flavonoid isolated from the milk thistle, on targeting HNC-TICs were determined in vitro and in vivo.

Results:
We first observed that the treatment of silibinin (SB) significantly down-regulated the ALDH1 activity, CD44 positivity, self-renewal property, stemness signatures expression (Oct4, Nanog, and Nestin) of sphere-forming HNC-TICs in a dose dependent manner. Using miRNA -microarray and mechanistic studies, SB significantly increased expression of tumor suppressive miR-494 and identified Bmi1 and ADAM10 as the novel targets of miR494. Importantly, in vivo nude mice model showed that SB treatment by oral gavagev to xenograft tumors reduced tumor growth and prolonged the survival times of tumor-bearing mice by activation of miR-494-targeting Bmi1/ADAM10 regulatory axis. HNC patient survival analysis indicated that a miR494lowBmi1highADAM10high phenotype predicted a poor clinical outcome.

Conclusions:
We conclude that the inhibition of tumor aggressiveness in HNC-TIC in by SB was in part was mediated by up-regulation of miR-494, suggesting that SB would be a valuable therapeutics clinically in treatment modalities for malignant HNC.

P881
The Role of miR-31 in Lipid Metabolism in Oral Squamous Cell Carcinoma

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Backgrounds:

MicroRNAs (miRNAs) are a family of small endogenous RNA molecules that play an important role in a variety of cellular processes, such as growth, differentiation and metabolic homeostasis. Dysregulation of microRNA has been linked to the development of various types of human diseases, including cancer. Our precious study identified several miRNAs whose expression levels were highly elevated in OSCC tissues. Among them, miR-31 was the most significantly up-regulated microRNA in OSCC tissues. However, the role of miR-31 in OSCC metabolic function has not been explored. Computational microRNA target prediction revealed that several genes involved in lipid metabolism are potential targets for miR-31. In this study, we aimed to investigate the potential role of miR-31 in the regulation of lipid metabolism and to establish the relationship between lipid metabolism and tumor pathogenesis in OSCC cells.

Materials and Methods:

LNA-modified miR-31 antisense oligonucleotide was used to suppress the endogenous miR-31 in OSCC cells. The effect of miR-31 on lipid metabolism was analyzed using LC-MS. Real-time PCR and miRNA reporter assays were used to characterize miR-31 targets. In addition, cell proliferation, migration, and invasion assays were used to evaluate the effect of miR-31 on the tumorigenesis of OSCC cells.

Results:

Data analysis using publicly available OSCC microarray and microRNA data sets revealed that the expression levels of miR-31 inversely correlate with expression levels of several genes involving in lipid beta-oxidation, such as ACOX1, HADH, and ACADL. Depletion of miR-31 using antisense oligonucleotide increased the level of ACOX1, the first enzyme involved in lipid beta-oxidation, in cultured OSCC cells. Metabolite profiling study using LC-MS further confirmed that depletion of miR-31 reduced the levels of several lipid metabolites in OSCC cells, including decanoyl-CoA, ganglioside GD3, phosphatidylethanolamide, diacylglycerol, triacylglycerol and 3-O-sulfogalactosylceramide. These data suggested that miR-31 may regulate lipid metabolism by targeting genes involved in lipid beta-oxidation pathway. In cultured OSCC cells, depletion of miR-31 had no effect on their proliferation and colony forming ability. However, the migration and invasion capability were significantly reduced after the treatment of miR-31 antisense oligonucleotide.

Conclusion:

Our findings suggest that elevated miR-31 level may promote tumor migration by targeting critical enzymes involved in lipid metabolism in OSCC cells.

P882
Thrombospondin-1 Modulates VEGF Signaling via CD36 by Recruiting SHP-1 to VEGFR2 Complex in Microvascular Endothelial Cells

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Backgrounds:

Thrombospondin-1 (TSP-1) is an endogenous anti-angiogenic factor. Small peptides derived from TSP-1 are potential therapeutic molecules for cancer by suppressing angiogenesis. TSP-1 inhibits growth factor signaling at the receptor level in microvascular endothelial cells (MVEC) and thus suppresses angiogenesis. CD36 has been suggested to be involved in this inhibition, however, the mechanisms are not known.

Materials and Methods:

Primary human MVEC and cd36$^{-/-}$ mice were stimulated with vascular endothelial growth factor (VEGF) in this study. A recombinant protein containing the CD36 binding domain of TSP-1 (known as the TSR domain) was used to suppress VEGF signaling in MVEC. The involvement of proteins within VEGF receptor 2 (VEGFR2) complex was first test by co-immunoprecipitation and then demonstrated with specific siRNAs in MVEC or in cd36$^{-/-}$ mice. The inhibitory effect of TSR was evaluated by VEGFR2 phosphorylation, cell migration and *in vitro* tube formation.

Results:

TSR induced association of SHP-1 with the VEGFR2/CD36 signaling complex and also promoted SHP-1 phosphatase activity within VEGFR2 complex, thereby suppressed VEGFR2 phosphorylation. CD36 is required for TSR-induced SHP-1/VEGFR2 complex formation *in vitro* and *in vivo*. Silencing SHP-1 expression in MVEC by siRNA abrogated TSR-mediated inhibition of VEGFR2 phosphorylation as well as TSR-mediated inhibition of VEGF-induced endothelial cell migration and tube formation. After all, VEGF stimulated a stronger VEGFR2 phosphorylation in *cd36*$^{-/-}$ mice compared to wild-type mice, which indicates the binding of endogenous TSP-1 to CD36 is sufficient to suppress VEGF signaling *in vivo*.

Conclusion:

These studies reveal a SHP-1 mediated mechanism by which TSP-1 inhibits VEGF signaling at the receptor level, provide new understanding of the role of CD36 in angiogenesis regulation, and point to a novel target to modulate angiogenesis therapeutically.

P883
Characterization of EBV Encoded MicroRNA miR-BART3 in Nasopharyngeal Carcinoma Cells

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Backgrounds:

Nasopharyngeal carcinoma (NPC) is a malignancy of the human nasopharynx epithelial cells. Studies indicated that EBV infection is closely associated with NPC and suggested that products derived from EBV genome are involved in the pathogenesis of NPC. Our laboratory used the next generation sequencing technology to profile the expression patterns of EBV microRNAs and discovered that the EBV-encoded microRNA miR-BART3 was expressed at high level in NPC tissues.

Materials and Methods:

We established lentiviral-based BART3-expression system in EBV-negative NPC cells. We also used locked nucleic acid (LNA)-modified BART3 antisense oligonucleotide to suppress the levels of miR-BART3 in EBV-positive NPC cells to evaluate the effect of BART3 on cell migration and invasion. A spontaneous tumor metastasis model was used to evaluate the role of BART3 in NPC tumor invasion and metastasis in vivo.

Results:

Computational prediction identified several putative targets for miR-BART3-3p clustering in the migration-related pathways and suggested that miR-BART3-3p may be involved in the migration and invasion of NPC. Ectopic expression of miR-BART3-3p significantly increased migration and invasion capability of cultured NPC cells. In contrast, treatment of LNA-modified anti-sense oligo of miR-BART3-3p significantly suppressed the migration and invasion capabilities in EBV-positive NPC cells. In vivo spontaneous tumor metastasis model also showed that miR-BART3 promoted tumor migration and invasion capability of NPC.

Conclusion:

Our data showed that miR-BART3-3p enhanced NPC cell migration and invasion and suggested that miR-BART3-3p may play an important role in NPC metastasis. Therefore, inhibition of miR-BART3-3p expression may provide a potential strategy for NPC treatment.

P884
Systematic Study on G9a Function in Colon Cancer Initiating Cells by A Conditional Activable Mouse Model

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Backgrounds:

Epigenetic alterations such as aberrant chromatin structure changes play important roles in the establishment of tumor suppressor gene silencing during tumorigenesis and metastasis. Recently, the methylation of histone H3 lysine 9 (H3K9) and the corresponding G9a methyltransferase has been shown to play an important role in promotion of the epithelial-mesenchymal transition (EMT), which is a phenotypic conversion linked with metastasis, in some cancer cells . However, the function of the histone H3 lys9 methyltransferases in colorectal cancer initiating cells remains largely unclear. This raises the interesting possibility that the epigenetic factor G9a is able to increase the transformation rate of colon cancer stem cells (CCSCs) or enhance their ability to metastasize.

Materials and Methods:

To understand the global distribution of target genes that are affected by methylation of H3K9 in CCSCs, we established a stem-cell-type-specific activable G9a mouse model to provide *in vivo* phenotypic answers through combined immunohistochemical characterizations and integrative omics analyses. Here, we performed chromatin immunoprecipitation coupled with ultra-high- throughput DNA sequencing (ChIP-seq) to find *G9a* downstream targets, and used the cDNA microarray technology to monitor changes in the differential expression of these candidate genes.

Results:

We successfully used the Cre/loxP technology-mediated conditional overexpression of G9a in specific colonic stem cells and observed G9a overexpression in CCSCs increasing their ability to invade and metastasize. We not only used the Lgr5-EGFP-IRES-CreERT2 allele to perform stem-cell-type-specific G9a overexpression, but also used the Apc mutant mouse and the inflammation-related colorectal carcinogenesis model as the existing colorectal cancer models to dissect the epigenetic regulatory networks in cancer progression *in vivo*. Disease and molecular function analyses revealed that most of these differentially expressed genes were involved in tumorigenesis (*p-value*=4.24x10^{-19}) and in cellular movement (*p-value*=6.77x10^{-17}), suggesting that the majority of *G9a* downstream genes are significantly associated with cancer initiation and metastasis. By transcription factor binding site enrichment analysis, we also found that the putative binding sites of PPAR-γ, AP-1, and NFκB were located in the promoter regions of G9a-downstream targets.

Conclusion:

The objective of this topic is to uncover the epigenetic molecular mechanism underlying colon cancer progression and to lay the knowledge foundation for the development of pharmacological therapies in the future.

P885
The Role of Thrombomodulin in Corneal Epithelial Wound Healing

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Backgrounds:
Thrombomodulin (TM) is a transmembrane glycoprotein that presents in endothelial cells and epithelial cells. TM is participated in regulation of coagulation, inflammation, and cell proliferation. Cornea, a transparent tissue in the front part of the eye, provides barrier function and refraction of light. Generally, cornea is divided into 3 layers: the outmost is corneal epithelium followed by corneal stroma and corneal endothelium. The cornea epithelium consists of 4-5 layers of epithelial cells which are divided into basal cells, wing cells, and superficial cells. Previous study showed that TM is expressed in corneal epithelium of diseases associated with endophthalmitis and herpetic keratitis, suggesting that TM may regulate corneal inflammation. However, the expression pattern of TM in normal cornea and the regulation of TM expression in corneal epithelium are still unknown. Therefore, we hypothesize that TM is expressed in corneal epithelium where it regulates corneal epithelial wound healing and inflammation.
Materials and Methods:
The expression of TM in mouse cornea was examined by using RT-PCR and immunofluorescent stain. In addition, a mouse model of corneal epithelial debridement wound was performed to study the correlation of TM expression and wound healing. The wound healing process within 48 h after debridement was monitored using the fluorescein staining, and the eyes were collected for immunohistochemistry.
Results:
The preliminary result showed that TM was expressed in the corneal epithelium and corneal endothelium in normal adult mice. The TM expression was increased in the early phase of wound healing and it was decreased after wound recovery, suggesting that TM may play a role in corneal epithelium wound healing.
Conclusion:
In conclusion, we successfully characterized the expression of TM in mouse cornea, thus it may provide a useful platform to investigate the biological role of TM in mouse cornea as well as in inflammation.

P886
The functional role of has-miR-181b in colorectal cancer

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Backgrounds:
To identify the prognostic factors of colorectal cancer before patients who take chemotherapy or surgery.
Materials and Methods:
In our studies, sixteen and thirty pairs of the recurrent and non-recurrent colorectal cancer samples from National Cheng Kung University Hospital were analyzed. Total RNAs were isolated and the microRNA and gene expression level was quantified by real time qRT-PCR analysis. Then, we used the reporter assay to identify the possible target gene of miR-181b. Finally, we identified the functional role of the possible prognostic factors in colorectal cancer.
Results:
We found the expression level of hsa-miR-181b in tumor tissues was higher than normal tissues. We also verified the TIMP3 is a direct target of miR-181b by reporter assay and the expression level of TIMP3 was rescued after blocked the miR-181b expression with miR-181b inhibitor. In the functional assay, we found that the colorectal cancer cell lines were more sensitive to 5-FU treatment after blocking miR-181b expression.
Conclusion:
Our analysis indicated that the mRNA expression levels of hsa-miR-181b and TIMP3 show an opposite behavior in colorectal cancer tissues and cell lines. The reason is that miR-181b could reduce TIMP3 expression by targeting to TIMP3 3'-UTR. Moreover, the colorectal cancer cells became more sensitive to 5-FU treatment after blocking miR-181b expression. Therefore, miR-181b may play a critical role in CRC. In the future, these two factors may provide nice prognostic indicators for the better therapeutic efficiency of colorectal cancer.

P887
Screening of Antimitotic Drug Combretastatin A-4 Derivatives That Are Capable of Inducing Autophagy in Neuroblastoma SHSY5Y Cell Line

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Backgrounds:
Antimitotic agents including Combretastatin A-4 (CA-4) are useful anticancer drugs targeting microtubule, however they exhibit high toxicity and low selectivity drawbacks. To improve these drawbacks, efforts are ongoing in synthesizing better CA-4 derivatives as anticancer drug candidates.
Materials and Methods:
A series of antimitotic drug Combretastatin A-4 (CA-4) enediyne derivatives were synthesized, the srutctures of these compounds were determined and confirmed. Drug treated SHSY5Y cells were processed for either confocal microscopic analysis for the microtubule formation, or for western blotting for the autophagic marker protein LC3-I to LC3-II conversion.
Results:
Screening of 11CA-4 enediyne derivatives, we found some CA-4 enediyne derivatives such as LO-OMe exhibited comparable antimitotic activity when compared with prototype drug CA-4 in neuroblastoma cells. Other derivatives such as HYH10F had weaker antimitotic activity, or CPC15a had none. However, all these three derivative compounds could dose-dependently promote LC-3-I to LC3-II form conversion, an indication of autophagy induction.
Conclusion:
Our results indicated that enediyne derivatives of CA-4 were capable of inducing neuroblastoma cellular autophagic process. Although these derivative compounds had lower activity in inhibiting microtubule formation, but they could be useful candidate agents for modulating cellular autophagic process, which could be beneficial in the adjuvant cancer therapy.

P888
Antiproliferative Effect of Extract Prepared from Ganoderma neojaponicum on Human Monocytic Leukemia Cells

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Backgrounds:
Ganoderma lucidum (Reishi or Ling-Zhi) has been widely used as a medicinal mushroom for promoting health in China and other eastern Asian countries. A number of pharmacological studies on the aqueous extracts of Reishi have revealed that the mushroom possesses antiproliferative effects on many cancers. In this study, we examine the anti-proliferative effects of extracts from another kind of Reishi, called Ganoderma neojaponicum, on human monocytic leukemia THP-1 cells.
Materials and Methods:
The fruit bodies of Ganoderma neojaponicum were extracted with boiling water and the supernatant obtained was lyophilized to yield water-soluble (WS) extract. The WS extract was further treated with ethanol to prepare the ethanol-soluble (ES) and -insolube (EI) fractions. The lyophilized ES fraction was used to examine its anti-proliferative effect on THP-1 cells.
Results:
The ES fraction was found to separately inhibit THP-1 cells growth and promote normal peripheral blood mononuclear cells (PBMC) proliferation. Further studies also showed that the anti-proliferative effect of ES fraction on THP-1 cells may be executed via the induction of cell cycle arrest and apoptosis. In addition, ES-treated THP-1 cells also displayed transient increase of ROS, followed by the disruption of mitochondria membrane potential. The presence of a ROS scavenger (N-acetyl-L-cysteine) blocked ROS production but it only partially protected cell death induced by ES fraction.
Conclusion:
Taken together, our data suggested that ES fraction from the fruit bodies of Ganoderma neojaponicum exhibited selective cytotoxicity in THP-1 cells.

P889

Fully-Human Anti-interleukin-6 Antibody (FB704) Suppresses Tumor Metastasis, Growth and Angiogenesis in Murine Model

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FOUNTAIN BIOPHARMA INC

Background:

Human interleukin-6 (IL6) is a multifunctional cytokine which acts on B cells, T cells, fibroblasts, hepatocytes, osteoclasts, neural cells, mesangial cells, epidermal keratinocytes, and hematopoietic progenitor cells. The IL6 signal is regulated through two distinct proteins, the IL6 receptor (IL6R) and glycoprotein 130 (gp130) and the transduction of intracellular signal is induced by the formation of an IL6/IL6R/gp130 complex. The pathological significance of IL6 for various diseases has been indicated in numerous studies.

Materials and Methods:

Phage display technology is a powerful tool that allows the rapid identification of ligands for various target molecules such as proteins, cell surface receptors and small compounds. Previously, we used this technique to select several tumors and tumor vasculature targeting peptides. To broaden its applicability, we generated phage libraries displaying human antibody fragments and used those libraries to identify high-affinity antibody against human IL6.

Results:

We used affinity maturation technology to enhance the binding activity of selected antibodies. We found our high affinity fully-human anti-IL6 antibodies (FB704) could inhibit IL6-dependented cell proliferation and phosphorylated STAT signaling transduction. FB704 decrease IL6 induced angiogenic formation in transplanted matrigel plagues in vivo. In addition, the antibody significantly suppresses human prostate tumor metastasis and shows synergic efficacy with Oxaliplatin in human pancreatic tumor in murine model.

Conclusion:

Phage display technology is a powerful tool that allows the multiple applications including drug discovery. We generated several fully-human antibody libraries and used those libraries to identify high-affinity antibody to hIL6. Moreover, we used affinity maturation to enhance the affinity and bio-activity of FB704. Our data show that FB704 is a potential drug candidate for cancer therapy.

P890

LKB1 binds to APC and modulates Wnt signaling pathway in lung

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Backgrounds:

LKB1 is inactivated in a significant number of Peutz–Jeghers syndrome (PJS) and sporadic cancers, most frequently in adenocarcinoma of the lung. Little is known about how the LKB1 signaling regulates the progression of lung cancer.

Materials and Methods:

In this study, we first confirmed that LKB1 inhibits lung cancer cell proliferation, migration and transformation in vitro after we restored LKB1 expression in LKB1 null A549, A427 and H460 lung cancer cells; similar results were obtained from H441 cells with stable shLKB1 knockdown experiments. Using the Immunoprecipitation-HPLC- Mass Spectrometry (IP-LC-MS) analysis to identify novel proteins interacting with Lkb1 in lung cancer cells, we identified Lkb1 interacts with Apc, a large scaffolding protein that regulates cellular microtubule dynamics, and this physical interaction between Lkb1 and Apc was further confirmed by reverse- immunoprecipitation assays.

Results:

Our study here provides the first evidence that Lkb1 interacts with Apc to suppress WNT/b-actenin signaling pathways, which involve in the regulation of cell proliferation and migration of lung cancer cells, and downstream Wnt associated gene analyses lead to the identification of several Wnt regulated genes, *CD44, COX-2and SURVIVIN*, whose expression levels are controlled by LKB1.

Conclusion:

Altogether, our results informed that *LKB1* suppresses tumorigenic/metastatic potential of lung cancer through the inhibition of WNT/b-actenin signaling pathway.

P891

LL37 and hBD-3 elevate the *β*-1,3-exoglucanase activity of *Candida albicans* Xog1p, resulting in Reduced fungal adhesion to plastic

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Backgrounds:

Candida albicans infections occur with an incidence of 1.1–24 cases per 100000 humans. Over the past three decades, *C. albicans* infections have emerged as a significant cause of human morbidity and mortality. *C. albicans* cell-wall *β*-glucan and chitin, which are associated with mannoproteins, form the main structural microfibrillar polymer and provide the cell wall with structural rigidity. AMPs (antimicrobial peptide) have distinct functions in response to different pathogens. For example, hBD (human *β*-defensin)-1 and hBD-2 have substantial microbicidal activity against Gram-negative bacteria, but not against Gram-positive bacteria. Conversely, hBD-3 is a broad-spectrum AMP that kills many pathogenic bacteria and opportunistic pathogenic yeast, including *C. albicans.* The AMP LL37 is also a broad-spectrum antimicrobial peptide that is active against Gram-positive and Gram-negative bacteria and pathogenic fungi. We found that *C. albicans* cell-wall Xog1p is an LL37 receptor, which suggested that LL37 may prevent *C. albicans*–host cell interactions.

Materials & Methods:

For the present study, Xog1p(41–438)-6H, an N-terminally truncated,active, recombinant construct of Xog1p and Xog1p fragments were produced and used in pull-down assays and ELISA in vitro, which demonstrated that all constructs interacted with both AMPs.

Results:

Our previous study indicated that LL37 might interact with the cell wall *β*-1,3-exoglucanase Xog1p, which is involved in cell-wall *β*-glucan metabolism, and consequently the binding of LL37 or hBD-3 to Xog1p might cause the decrease in adhesion.

Conclusion:

The present study demonstrates that LL37 and hBD-3 elevate Xog1p activity by interacting with the enzyme and that elevated Xog1p activity is key to reduced C. albicans adherence.

P892

Heparan Sulfate Is Required to Migration Phase of Primordial Germ Cells during Early Embryonic Developmen

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Backgrounds:

Heparan sulfate glycosaminoglycan (HS-GAG) is a linear polysaccharide residing on the animal cell surface and the extracellular matrix usually in the form of heparan sulfate proteoglycans. It binds many proteins, such as chemokines, growth factors and morphogens, and participates in intracellular signal transduction and then regulates biological processes. After specified, zebrafish primordial germ cells (PGCs) migrate following specific routes from their sites of origin to gonadal ridge during early embryonic development. Studies have shown that some HS-GAGs can bind to SDF-1 which plays a key role in the guidance of PGCs migration. Therefore, HS-GAGs may play important role in the migration of PGCs into gonadal ridges.

Materials and methods:

Antibodies against two heparan sulfate structures detected the PGCs migration environment of zebrafish embryos from dome stage to 24-hours post fertilization (hpf). To study the potential role of HS-GAG in zebrafish PGCs migration, we overexpressed recombinant Hpse1 specifically in PGCs by injecting Hpse1-*nanos-1* 3'UTR mRNA in I-cell stage embryos of zebrafish. Patterns of PGCs in embryos after microinjection detected by whole-mount *in situ* hybridization.

Results:

Cleaved heparan sulfate was found in PGCs whereas intact heparan sulfate presented around the cells. These findings supported our initial hypothesis. In silico analysis, indicated that zebrafish Hpse1 is highly conservative throughout vertebrates, while enzymatic assay demonstrated the HS-GAGs degrading activity of the Hpse1 we cloned. PGCs in Tg(Kop:EGFP-F-*nanos1*-3'UTR) transgenic line disappeared after injecting Hpse1-*nanos-1* 3'UTR mRNA in 24 hpf embryos. Whole-mount *in situ* hybridization showed that PGCs mismigrated and barely formed four clusters at 6hpf . In addition, the PGCs reduced in number in 8hpf and 10hpf embryos.

Conclusion:

These results suggested that zebrafish Hpse1 is conserved throughout evolution and indicated that PGCs migration and increasing in number requires HS-GAGs. Further experiments are required to elucidate whether HS-GAGs are required for proliferation of PGCs or for the inhibition of apoptosis of PGCs during embryonic development. Furthermore, it is of interests to find out which signals are critical for this phenomenon via HS-GAGs.

P893
Functional studies of arginine methylation of cellular nucleic acid binding protein

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Backgrounds:

Cellular nucleic acid binding protein (CNBP) is a single-stranded nucleic acid binding protein with seven Cys-Cys-his-Cys (CCHC) zinc fingers and an arginine / glycine (RG) rich region between zinc finger 1 and 2. There are five arginines (R25, R27, R30, R32, and R34) in the RG rich area of CNBP. Previous studies showed direct interaction of CNBP with PRMT1and methylation of recombinant CNBP by PRMT1. Full-length but not RG deleted (ΔRG) FLAG-CNBP protein can be recognized by methylarginine-specific antibodies.

Materials and Methods:

1. We analyzed the distribution of endogenous and exogenous CNBP in HeLa cells.
2. We prepared (His)6-tagged CNBP-RK1 (R25K, R27K) and RK2 (R30K, R32K, R34K) mutant protein for in vitro protein methylation.
3. We analyzed CNBP RNA (interaction using RNA affinity chromatography.

Results:

In this study, we analyzed the distribution of endogenous and exogenous CNBP in HeLa cells. Regardless of the treatment of a methylation inhibitor AdOx or the RG deletion, there were no significant changes of CNBP distribution in cells. Therefore, deletion of the RG-rich region and protein arginine methylation modification do not affect the localization of CNBP in cells. Significantly reduced in vitro methylation of RK2 but not RK1 compared with the wildtype CNBP suggest that the major CNBP methylation sites are R25 or R27. We further explored whether arginine methylation can affect the RNA binding capacity of CNBP. AdOx treatment appeared to strengthen the RNA binding of FLAG-CNBP.

Conclusion:

These findings contribute to the understanding the functional regulation of CNBP by protein arginine methylation.

P894
Low dose radiation mediated upregulation cofilin and c-myc induced cellular senescence phenomenon

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Backgrounds:

Cellular senescence prevents unlimited proliferation and may lead to death. This phenomenon is believed to be associated with up-regulation of p53 and $p16^{INK4}$ tumor suppressor. Actin depolymerizing factor (ADF)/cofilin family protein is important for accelerating the pool of G-actin in cells. Recent study also demonstrates that oncogenes (c-myc and Ras) may be a potential characteristic of replicative senescence. And we investigated whether forced expression of cofilin-1 can influence the ratio of senescence.

Materials and Methods:

we choose the lung primary cell, WI-38 and MRC5, and lung cancer cell line, A549 and H1299 (lacking p53 and $p16^{INK4}$ gene), were transfected c-myc plasmid. Redistribution of the cofilin and c-myc assay using Aleax Flour 594 and Aleax Flour 488 fluorescence to confocal fluorescence microscopic examination. Concomitantly, cellular senescence was demonstrated by senescence-associated β-galactosidase (SA-β-gal) assay. Then the protein or mRNA level expression analysis are by western blot or qRT-PCR. And use luciferase assay to detect the gene expresstion.

Results:

Our results provide evidence that ROS may induce cofilin-1, c-myc and replicative senescence via an alternative pathway. And also c-myc can induce cofilin-1 by confocal fluorescence microscopic examination. In addition, SA-β-gal and western blot data also show that c-myc can induce senescence.

Conclusion:

That cofilin-1 may a senescence biomarker to detect the cellular senescence . It may interact with c-myc that provide an important implication for cancer treatment by promoting cell senescence through cofilin regulation.

P895
Functional Studies of CD93 in Endothelial Inflammation

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Backgrounds:

CD93 (C1qRp) is a transmembrane glycoprotein. Expression of CD93 occurs in various cell types including hematopoietic stem cells, NK cells, and monocytes, especially in the endothelial cells express abundantly. Although typically membrane-bound, a soluble form of CD93 (sCD93) has recently been identified. This shed or "soluble" form of CD93 (sCD93) is found in human blood and might indicating that sCD93 might participate in some physiological processes. Recently, CD93 is emerging as a novel regulator of inflammation; however, its molecular function is unknown.

Materials and Methods:

In this study, we constructed the whole extracellular region of CD93 recombinant proteins (rCD93-D123) including N-terminal lectin-like domain (as refer to Domain 1, D1), repetitive epidermal growth factor (EGF)-like domain (D2) and mucin-like domain (D3). In order to realize the physiological role of rCD93 in vascular, first, we used the cell adhesion assay to assess whether human micorvascular endothelial cell (HMEC-1) were stimulated by rCD93. Next, the carboxy-H2DCFDA was used to detected intracellular reactive oxygen species (ROS) formation in HMEC-1. In addition, western blotting was used to analyze the signaling pathway which rCD93 might part in. Finally, the carotid ligation model was used to evaluate neointima formation.

Results:

In our data showed that rCD93 could enhance THP-1 cell binding on treated HMEC-1 with dose-dependent manner (adherent numbers of THP-1 monocytes are 80±2, 94±28, 234±13 cells, indicating 0.1 to 10 μg/ml of treated concentration, respectively). Then, compared with control HMEC-1, CD93 treated HMEC-1 were induced more intracellular reactive oxygen species (ROS) formation. In addition, the activation of ERK, p38, and HIF-1α were also observed. However, the activation of NF-κB, one of the common pathways during inflammatory, was not observed. Finally, we found that rCD93 could significantly increase carotid neointima formation in the carotid ligation mouse model ($p<0.05$).

Conclusion:

These results support the conclusion that rCD93 perhaps participated in the procession of vascular inflammation; however, its mechanism remains to be a future research.

P896
Graptopetalum Paraguayense Ameliorates Chemical-Induced Rat Hepatic Fibrosis In Vivo and Inactivates Stellate Cells and Kupffer Cells In Vitro

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Backgrounds:

Graptopetalum paraguayense (GP) is a folk herbal medicine with hepatoprotective effects that is used in Taiwan. The aim of this study was to evaluate the hepatoprotective and antifibrotic effects of GP on experimental hepatic fibrosis in both dimethylnitrosamine (DMN)- and carbon tetrachloride (CCl_4)-induced liver injury rats.

Materials and Methods:

Hepatic fibrosis-induced rats were fed with the methanolic extract of GP (MGP) by oral administration every day. Immunohistochemistry, biochemical assays, and Western blot analysis were performed. The effects of MGP on the expression of fibrotic markers and cytokines in the primary cultured hepatic stellate cells (HSCs) and Kupffer cells, respectively, were evaluated.

Results:

Oral administration of MGP significantly alleviated DMN- or CCl_4-induced liver inflammation and fibrosis. High levels of alanine transaminase, aspartate transaminase, bilirubin, prothrombin activity and mortality rates also decreased in rats treated with MGP. There were significantly decreased hydroxyproline levels in therapeutic rats compared with those of the liver-damaged rats. Collagen I and alpha smooth muscle actin (α-SMA) expression were all reduced by incubation with MGP in primary cultured rat HSCs. Furthermore, MGP induced apoptotic cell death in activated HSCs. MGP also suppressed lipopolysaccharide-stimulated rat Kupffer cell activation by decreasing nitric oxide, tumor necrosis factor-a and interleukin-6 production, and increasing interleukin-10 expression.

Conclusion:

The results show that the administration of MGP attenuated toxin-induced hepatic damage and fibrosis in vivo and inhibited HSC and Kupffer cell activation in vitro, suggesting that MGP might be a promising complementary or alternative therapeutic agent for liver inflammation and fibrosis.

P897

WABE, A Novel Method for Identifying Differentially Expressed Genes Based on Sample-specific Errors and z-test Provides Superior Reliability Compared to Standard Methods Based on Gene-specific Errors and t-test

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Backgrounds:

The application of high-throughput expression profiling to detect genes differentially expressed between sample groups often leads to discordance in results between similar studies.

Materials and Methods:

We examined 175 data sets and discovered that expansion of variance is an important property of differential expression. Because even with sufficient samples the property invalidates conventional statistical tests, including t-test and F-test, we identified it as the fundamental cause of the discordance and present a method as a solution.

Results:

The method, named WABE, is insensitive to normalization and unsusceptible to outlier samples. It provides reproducible gene lists with high statistical power and complete statistical control. In a demonstration using data from allergic contact dermatitis patients and non-allergic controls, we showed among five methods only WABE extracted reliable biological information. The information contradicted the conclusions of the original study and revealed stronger genomic responses to nickel exposure in the controls than in the patients.

Conclusion:

The finding suggests the disease cause is dysfunction of an innate system and creates a new perspective for alternative prophylactic and therapeutic strategies. Overlooked information detected in the data sets suggests 60-70% of existing data need to be reexamined.

P898

MBNL3 regulates cellular proliferation through cyclin D1

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Backgrounds:

Muscleblind-like 3 (MBNL3) belongs to MBNL splicing regulators and is over-expressed in myotonic dystrophy type 1 (DM1), a neuromuscular disease caused by expanded CUG repeat RNA. It was shown that MBNL3 expression is abundant in proliferative tissues and greatly decreases during differentiation. Using C2C12 cells as a model, we have found that MBNL3 depletion down-regulates cyclin D1 expression and decreases the proliferation rate. On the other hand, recent clinical reports indicated that DM1 patients are at increased risk in developing several types of cancer. These findings lead us to propose that MBNL3 may regulate cellular proliferation and participate in tumorigenesis through cyclin D1.

Materials and Methods:

C2C12 myoblasts and ovarian cancer cell lines, HeyC2, MCP2 and CP70, and control ovarian cells, IOSE, were included in this study. The expression of MBNL3 and cyclinD1 were determined by using quantitative PCR and western blot. Heterogenous cyclin D1 promoter-luciferase plasmid was constructed for the study of transcriptional regulation by MBNL3. MBNL3 knockdown and cyclin D1 expression cells were produced by using lentiviral system and their proliferation rate and tumorigenic features were assessed by MTT assay and soft-agar assay.

Results:

Our results show that over-expression of cyclin D1 may restore the proliferation rate of MBNL3 knockdown C2C12 cells and that cyclin D1 promoter activity is down-regulated by MBNL3 depletion. Meanwhile, we find that MBNL3 expression is up-regulated in all ovarian cancer cell lines examined. However, cyclin D1 expression level is increased in HeyC2 but not in MCP2 and CP70 cells. Further knocking down the expression of MBNL3 in HeyC2 cells significantly decreases the proliferation rate and affects cell survival.

Conclusion:

Our findings provide further experimental evidence to support the notion that MBNL3 may regulate cellular proliferation through cyclin D1. In addition, they raise the possibility that the tumorigenicity of cancer cells can be alleviated by MBNL3 knockdown.

P899

WW domain-containing oxidoreductase in stress responses

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Background:

WW domain-containing oxidoreductase encoded by human fragile *WWOX* gene on chromosome 16 has been suggested to be a tumor suppressor. The expression of WWOX/WOX1 protein is highly associated with cancer progression. It has been reported that WWOX/WOX1 is involved in UV-induced apoptosis. However, it remains largely unclear how WWOX/WOX1 regulates stress responses.

Materials and Methods:

Western blot and real-time PCR were used to investigate the protein and mRNA levels. Cell viability after serum starvation was analyzed by WST-8 colorimetric assay. Cell cycle analysis was performed by PI staining followed by flow cytometry.

Results:

Our results showed that both protein and mRNA levels of WWOX/WOX1 were upregulated upon stress responses, such as serum deprivation and treatment of hydrogen peroxide and anticancer drugs in SCC15 cells. Similar results were observed in wild-type mouse embryonic fibroblasts (MEFs) after starvation and hydrogen peroxide treatment. To further investigate the functional role of WWOX/WOX1 in stress responses, we examined the survival rate in both wild-type and *Wwox*$^{-/-}$ MEFs following serum deprivation. We found that serum deprivation induced high levels of cell death in *Wwox*$^{+/+}$ MEFs, as compared with *Wwox*$^{-/-}$ MEFs. Previous study has suggested that anti-apoptotic proteins in the Bcl-2 family regulate mitochondrial homeostasis and prevent serum deprivation-induced oxidative stress and cell death. Our results showed that protein expression of Bcl-X_L and Mcl-1 was downregulated in *Wwox*$^{+/+}$ but not in *Wwox*$^{-/-}$ MEFs following serum starvation. Conversely, the expression levels of Bcl-X_L and Mcl-1 proteins remained unchanged upon starvation in SCC15 cells transfected with lentiviral shRNA targeting WWOX/WOX1.

Conclusion:

Serum starvation increases WWOX/WOX1 expression, thus downregulating the expression of Bcl-X_L for the induction of cell death.

P900

Interleukin-6 Regulates Stemness Expression and Early Recurrence of HBV-related Hepatocellular Carcinoma via IGF-IR Activation

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Background:

Hepatocellular carcinoma (HCC) is an inflammation-associated cancer. The expression of stemness-related genes in HCC is linked to tumor recurrence.

Methods:

The effects of IL-6 on the expression of stemness-related properties in HCC cells were examined in cell culture and xenograft animal model experiments. RNA interference and molecular inhibitor targeting of the IGF-I receptor (IGF-IR) were used to examine the role of IL-6-induced IGF-IR-mediated downstream signaling in cancer stemness. The expression of the octamer 4 (OCT4) and NANOG transcription factors and the phosphorylation of IGF-IR were examined using western blotting and immunohistochemical staining. The levels of *OCT4, NANOG*, and *IGF-IR* expression were compared with disease-free survival (DFS) in hepatectomized HCC patients (n = 119) using Kaplan-Meier analysis.

Results:

A high positive correlation between IGF-IR and OCT4/NANOG transcriptional expressions in frozen human HCC tissues. The concurrent expression of OCT4, NANOG, and IGF-IR correlated with HBV-related HCC, and was significantly associated with reduced DFS. IL-6 stimulated the expression of autocrine IGF-I and IGF-IR in a STAT dependent manner, and the activation of IGF-I/IGF-IR-mediated signaling produced stemness-related properties in both the cell lines and the xenografted mouse tumors. The inhibition of IGF-IR activation significantly suppressed the IL-6-induced stemness in vitro and in vivo.

Conclusions:

The expression of OCT4 and NANOG is regulated by IL-6-induced IGF/IGF-IR mediated signaling in HBV-related HCC, and is associated with early recurrence of HCC.

P901

Anti-Cancer Activities of Organic Extracts from *Euphorbia hirta* L. on MDA-MB-231 Breast Cancer Cells

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Backgrounds:

Recently, Chinese medicinal herbs have been reported to have various anti-cancer properties, including suppressing cell proliferation and inducing cell apoptosis. Euphorbia hirta L. has been widely used in India and Chinese society and as a traditional medicine to treat numerous diseases such as hypertension, tumors, anti-inflammatory activities. Euphorbia hirta L. has been reported to have the triterpenoids which have shown to have anti-cancer activity. However, the effects of Euphorbia hirta L. on breast cancer cells have not been elucidated. In the present study, we investigate the anti-cancer activities and related mechanisms of extracts of Euphorbia hirta in MDA-MB-231 breast cancer cells.

Materials and Methods:

To determine whether *E. hirta* would affect cell viability in breast cancer cells, the cells were treated with crude extract of *E. hirta* and the amount of viable cells was determined by the XTT assay. Transwell inserts (with 8 μm pores) in 24-well plates (BD Biosciences) were used for the migration and invasion assays on MDA-MB-231 breast cancer cells. We obtained conditioned medium from treated and control cells, and this conditioned medium was used to induce tubulogenesis in human endothelial cells (HUVECs). The level of VEGF in conditioned medium was quantified by VEGF ELISA kit. Protein expression of MMP-2 and MMP-9 was determined by western blotting. Production of MMP-2 and MMP-9 by cancer cells were analyzed by gelatin zymography.

Results:

Organic extracts of *Euphorbia hirta* L. showed cytotoxicity on MDA-MB-231 breast cancer cells. Exposure of MDA-MB-231 breast cancer cells to extracts of *Euphorbia hirta* L. (12.5 and 25 μg/ml) resulted in inhibition of cell migration/invasion. The anti-angiogenesis effect of Euphorbia hirta L. on HUVEC also has been observed from conditioned medium of treated cells. Activities of MMP-2 and MMP-9 were decreased by extracts of *Euphorbia hirta* L. treatment in a dose-dependent manner.

Conclusion:

Our results demonstrate that *Euphorbia hirta* L. effectively inhibits cell viability and suppresses cancer cell motility and invasion by inhibiting MMP-2 and MMP-9 activity in MDA-MB-231 cells. It indicates that *Euphorbia hirta* L. is a potential medicinal herb for human breast cancer treatment.

P902

Protein Expressions of VEGF and VEGFR2 in Patients with Breast Cancer

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Backgrounds:

Previous studies showed that vascular endothelial growth factors (VEGFs), which are secreted from tumor cells, will bind to their receptors to increase cancer risks and enhance cancer growth and aggravate the progression of tumors. We aimed to analyze protein expression of VEGF or VEGFR2 and evaluate their correlations with the clinicopathological features in breast cancer patients.

Materials and Methods:

We collected 278 breast cancer tissues from 283 breast cancer cases and analyzed the protein expressions of VEGF and VEGFR2 in tumor tissues from Kaohsiung Medical University Chung-Ho Memorial Hospital by immunohistochemistry staining (IHC).

Results:

Among the 278 breast cancer patients, 60.9% of their VEGF protein expression was positive, while 39.4% was negative; 68.6% of their VEGFR2 protein expression was positive, while 31.4% was negative. There was a significant positive association between the expression of VEGF and histological stages in our study. We also found that the VEGFR2 protein expression in the patients with HER-2 (positive) or triple-negative breast cancer (TNB) was higher ($P<0.05$).

Conclusion:

In conclusion, we suggest that doctors should observe the protein expression of VEGF and VEGFR2 in patients with breast cancer in order to provide personalized medical care.

P903

Role of Hsa-mir-125b in Hepatoma Cell Line Malignancy

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Backgrounds:

Previously study showed that hsa-mir-125b was an oncomir in several cancer cell types. Accordingly, microRNA of several hepatoma cell lines with different malignant status were analysis by microRNA array. Results showed that those hepatoma cell lines with higher migration ability and/or lower drug sensitivity expressed higher hsa-mir-125b. Therefore, role of hsa-mir-125b in hepatoma malignancy was explored in this study.

Materials and Methods:

Low hsa-mir-125b expression liver cancer cell line, HepG2, was transfected with pLV-hsa-mir-125b plasmid. Upon transfection, the hsa-mir-125b expression level of HepG2 cell was quantified by quantitative RT-PCR (qRT-PCR). Once expression of hsa-mir-125b has been confirmed, malignant properties of cell, such as migration ability, growth and drug sensitivity of cell was evaluated respectively. Target of hsa-mir-125b was also explored through cross matching the miRBase and/or TargetScan data bank with mRNA array and 2D-protein gel electrophoresis. The putative target was further verifying by western or q-RT-PCR.

Results:

In our preliminary study, hepatomas with different differentiation status showed different malignant properties. Through the miRNA array analysis, oncomir hsa-mir-125b is highly expressed in those hepatoma cell lines, such as Hep J5 and SK-Hep1, with higher migration and/or invasion ability. Comparing to HepJ5 and SK-Hep1, HepG2 is low hsa-mir-125b expression cell line. Over-expression of hsa-mir-125b in HepG2 by transfect pLV-hsa-mir-125b, morphology of HepG2 transformed from flat round to spindle-like shape. Further analysis showed that migration ability of HepG2 increased while cell over-expressing hsa-mir-125b. In addition, hsa-mir-125b over-expression also enhance drug resistant ability of HepG2. Through data bank and 2D protein gel electrophoresis, several putative target proteins were identified.

Conclusion:

Hsa-mir-125b was an oncomir at HCC cell lines, which over-expression would enhance HCC's malignant properties.

P904

Association of the DNA Repair Gene hOGG1 Polymorphisms and Acute Myeloid Leukemia (AML)

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Backgrounds:

The DNA repair enzyme OGG1 is a DNA glycosylase/AP lyase that has been hypothesized to play an important role in preventing carcinogenesis by repairing oxidative damage to DNA. Specifically, glycosylase/AP lyase can efficiently repair 8-OH-G a major base lesion produced by ROS, formed as a byproduct of endogenous metabolism or exposure to environmental oxidizing agents, such as ionizing radiation or chemical genotoxic compounds. Ser326Cys polymorphism in the hOGG1 gene is involved in the repair of 8-hydroxyguanine in oxidatively damaged DNA. To investigate the relationship between single nucleotide polymorphisms in the hOGG1 genes and patients suffering from Acute Myeloid Leukemia (AML).

Materials and Methods:

hOGG1 genotyping was performed by PCR-restriction fragment length polymorphism analysis of genomic DNA isolated from 66 Taiwanese acute myeloid leukemia cases and 345 individual healthy donors .

Results:

We found that the frequency of hOGG1 Ser/Ser, Ser/Cys, and Cys/Cys genotypes were 22.7,39.4., and 37.9% in tongue cancer, and 18.6, 46.9, and 34.5% in the controls. ($p>0.05$) . No statistically significant associations between the genotypes and acute myeloid leukemia (AML) were observed.

Conclusion:

Our analysis indicated that the hOGG1 Ser326Cys polymorphism is not a major genetic risk factor for acute myeloid leukemia(AML).

P905

Expression of Oct4 and Sox2 and their impact on tumorigenesis and clinicopathological outcome in buccal mucosal squamous cell carcinoma

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Backgrounds:

Buccal mucosal squamous cell carcinoma (BMSCC) is the most common form and more aggressive than other originating subsites in oral cancer in Taiwan. Cancer stem cells (CSCs) have been implicated in tumorigenesis and prognosis. Reprogramming factors employed to induce iPS (induced pluripotent stem) cells are associated with CSCs formation. The purpose of this study was to investigate the relationship of the protein expression levels of two reprogramming factors, Octamer-binding Protein 4 (Oct4) and Sex-determining Region Y (SRY)-related Box 2 (Sox2), with the tumorigenesis, clinicopathological outcome, and survival.

Materials and Methods:

Expression levels of Sox2 and Oct4 were evaluated by immunohistochemistry in 200 surgically resected BMSCC specimens and 35 normal oral epithelium specimens, using tissue microarray slides.

Results:

The expression levels of Sox2 were decreased when normal tissue progressed to tumor ($p<0.001$). A higher level of Oct4 expression was correlated with early stage of disease (stage I+II vs. III+IV, $p=0.043$) and small size of tumor (T1+T2 vs. T3+T4, $p=0.047$). In addition, a higher level of Sox2 was correlated with lower grad of tumor (I vs. III, $p=0.007$; II vs. III, $p=0.037$, respectively), early stage of disease ($p=0.002$) and absence of lymph node metastasis ($p=0.012$). However, Oct4 and Sox2 expression were not associated with disease-specific and disease-free survival.

Conclusion:

Oct4 and Sox2 were biomarkers for tumorigenesis and clinicopathological outcome for BMSCC.

P906

The Whole Cell Proteomics of Clinical Isolates of *Saccharomyces cerevisiae* by Mass Spectrometric Analysis

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Backgrounds:

Saccharomyces cerevisiae has recently been described as an emerging opportunistic fungal pathogen. We conducted an investigation of possible pathogenic mechanisms in clinical isolates of *S. cerevisiae.*

Materials and Methods:

Stable-isotope labeling by amino acids in cell culture (SILAC) followed by LC-MS/MS was used to relatively quantitate the proteins from different cellular fractionations of the mixture of clinical isolates and laboratory strains. The peptide abundances were determined by MaxQuant and MassMatrix (unpublished) softwares.

Results:

2893 of the proteins totally were determined by MaxQuant in our analysis, and 2175 proteins by MassMatrix, which covered about half of the proteins of yeast genome. Among the proteins detected, 248 and 296 proteins, determined by MaxQuant and MassMatrix respectively, were expressed at significantly higher levels in clinical isolates than in lab strains. The significantly up-regulated proteins were categorized into several biological processes, such as ergosterol biosynthesis, nucleotide metabolism, and fatty acid metabolim. It has been reported that mutation of ergosterol biosynthetic enzyme Erg6 was associated with reduction in cell wall thickness and virulence in *Candida glabrata.* Our results suggested that genes involved in ergosterol biosynthesis pathways might be important in fungal pathogenicity.

Conclusion:

Our analysis has demonstrated comparative proteomics of clinical isolates of *S. cerevisiae,* and also showed that *S.cerevisiae* would be a good model organism to study fungal pathogenicity. In addition, genes involved in ergosterol biosynthesis, such as, *ERG6* and *ERG11,* might be crucial factors in cell wall integrity, which is also highly correlated to fungal virulence.

P907

The Hypoxic Adaptation of Nrf2 is The Key Player in A549 Human Lung Carcinoma Cell

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Backgrounds:

Many types of tumors are characterized by rapid growth, which leads to a long-term hypoxia microenvironment. Under hypoxia, the main sources of energy become glucose and fatty acid for ATP production, resulting in additional oxidative pressure with ROS accumulated. Severe oxidative stress induced by ROS can cause cell death which is usually associated with decrease of antioxidant defense. Nevertheless, tumor cells are able to tolerate the long-term hypoxia. To date, many anti-cancer drugs, those are developed for malignant cancers, aim on the anti-oxidant ability of cancer cells.

Materials and Methods:

In this study, A549 human lung carcinoma cell line is used as a model to observe the ROS level and related gene and protein expression under hypoxia (2% O2). In order to exam the rapid response toward hypoxia in cancer cells, the short-term (less than 24 hour) hypoxia condition is designed. Western blot analysis and PCR were used to screen for RNA and protein expressions. DCFH-DA was used to screen the ROS level in cells with flow cytometry.

Results:

The intracellular ROS level was lower in short-term hypoxia than in normoxia. Also, with the treatment of H2O2, the result showed that cells under hypoxia condition had the ability to lower the ROS level produced by H2O2. It is expected that A549 cells could produce some proteins to lower down the ROS level under short-term hypoxia. In previous researches, Nrf2, a transcription factor, increased under high level of ROS and triggers the production of downstream phase II antioxidant enzymes those can detoxify the ROS to water-soluble metabolites, for example NQO1. However, our results showed that there was no change in both RNA and protein level of NQO1. We wonder which protein in the downstream pathway of Nrf2 is the key enzyme to relieve the oxidative pressure. Under our observation, hypoxia induced an increase in the expression of GSSG, GCLC and GCLM. All of which are the main sources of glutathione production. We suggested that Nrf2 may decrease the ROS level by increasing the production or activity of glutathione and adapt tumor cells to hypoxia microenvironment.

Conclusion:

Nrf2 plays a key role in antioxidant activity on many malignant cancers. According to results showed above, Nrf2 knockdown or block the production or activity of glutathione may give rise to lessen the adaptability of tumor cells to hypoxia, increase the efficiency of anti-cancer drugs, and even find out a new way to defeat the malignant tumor.

P908

Effects of hypertension on adult hippocampal neurogenesis

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Backgrounds:

Hypertension (HT), a common disease in the elder people, is associated with increased cerebrovascular diseases. HT has been linked to neurodegenerative diseases and cognitive impairment in humans. Adult neurogenesis in the hippocampus is known to be involved in the formation of certain types of memories. Recently, the numbers of total neurons and new-born neurons (neurogenesis) in the hippocampi of rats with spontaneously HT were found lower than that of wild-type rats. However, whether the reduced neurogenesis is a result of HT or different genetic background is unclear. The objective of this study is to examine the effect of HT on adult hippocampal neurogenesis in a line of mice with same genetic background.

Materials and Methods:

Coarctation of the left renal artery, known as two kidney one clip (2K1C) method, was used to induce HT in adult B6C3 mice. The blood pressure of the mice was monitored at 7, 14, 21, 28 and 30 d after the 2K1C surgery using tail-cuff method. At 30 d after the 2K1C surgery, the mice were subjected to hippocampus-dependent non-spatial object recognition task for a period of four days. One day after the memory task, mice received four injections of 5-bromo-2'-deoxyuridine (BrdU, 0.5mg/kg BW, i.p.) in a 4 h interval to label proliferating cells. The animals were sacrificed next day. One extra injection of BrdU was given 2 h before sacrifice. Neurogenesis was determined by counting cells positive stained by BrdU and doublecortin (DCX), an immature neuronal marker. The levels of brain-derived neurotrophic factor, a vital neurogenesis-associated neurotrophic factor, and its receptor, TrkB, were also measured by western blotting.

Results:

The blood pressure of the 2K1C mice was higher than that of the sham group since 7 d after the surgery and remained high up to 30 d. The ratio of novel object exploring time over the total exploring time was decreased in the 2K1C mice suggesting an impaired hippocampus-dependent non-spatial memory. 2K1C surgery lowered the numbers of BrdU+ and BrdU+/DCX+ cells, while the numbers of DCX+ cells were unaltered, suggesting that 2K1C reduced the proliferation rate of neural stem cells in the hippocampal subgranular zone. The levels of hippocampal BDNF and TrkB were unchanged.

Conclusion:

This study suggests that HT reduces adult hippocampal neurogenesis and impairs hippocampal dependent memory performance. Such changes are not caused by the reduction of BDNF and TrkB levels in the hippocampus.

P909

The Mechanism of Midazolam Induced Apoptosis on MA-10 Mouse Leydig Tumor Cells

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Backgrounds:

Midazolam, a derivative compound from benzodiazepine, is widely used as sedative anesthetic induction agent. In previous study, we have found that midazolam could induce steroidogenesis in MA-10 mouse Leydig tumor cells via PKA and PKC pathways to accompany the expression of PBR and StAR proteins. In addition, midazolam at higher dosages induced rounding-up, membrane blebbing, and then cell death phenomenon in MA-10 cells. In fact, we have found that midazolam could stimulate caspase and MAPK, but inhibit Akt pathways to induce apoptosis in MA-10 cells.

Materials and Methods:

MA-10 cells were treated without or with different concentrations of midazolam (6, 30 and 150 μM) for 1, 3, 6, 12 and 24 hr, respectively. We then examined the expression of apoptotic proteins by mitochondrial protein isolation method and immunoblotting analysis.

Results:

The preliminary results showed that midazolam induced the expression of Bax and cytochrome C proteins, but decreased the expression of Bid, phosphor-Akt and phosphor-mTOR proteins. Moreover, midazolam treatment induced the expression of cleavage of caspase-12, phosphorylations of PKC, and PKCα/ β II in MA-10 cells.

Conclusion:

Our data illustrated that midazolam could activate intrinsic apoptosis pathway plus autophagy. phenomenon in MA-10 cells, and these results demonstrated that midazolam might activate ER stress and PKC pathways to induce MA-10 cell apoptosis.

P910

Protective effects of (-)-epigallocatechin gallate on free radical-induced retinal pigment epithelium cells disorders

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Background:

Short-wavelength blue-light radiation is believed to cause retinal damage or contribute to the development of age-related macular degeneration (AMD). Light-induced ocular diseases can be caused by a variety of cellular mechanisms that involve oxidative stress. Therefore, the eye depends on the presence of antioxidants to protect it from light-induced damage. Previous investigations have demonstrated that estrogen receptors (ERs) have antioxidant and anti-inflammatory effects on neuronal cells in the brain. However, the ER-mediated effects of the (-)-epigallocatechin gallate (EGCG), which is extracted from green tea, have not been extensively examined in the ocular tissues of the eyeball. The ability of EGCG to elicit ERs and ER-mediated gene expression will be examined in vitro.

Methods:

In vitro study, human retinal pigment epithelium (RPE) cells were exposed with hydrogen peroxide after pretreated with EGCG. Cultured cells were analyzed by morphology, immunocytochemistry, and Western blot analysis.

Results:

Expressions of apoptosis related proteins caspase-3 were significantly increased after free radical damage. After EGCG treatment, increased ER protein production and inhibition the death of cultured cells after free radical exposure. The hypothesis of the current proposal is that EGCG regulates the expression of cellular protective proteins, such as estrogen receptors; hence, ERs also modulate the degenerative responses in ocular tissues.

Conclusions:

The findings from this research program will delineate detailed molecular roles of ERs in cellular physiology and human ocular diseases, and may propose target choices for potential therapeutic regimes in the future.

P911

The Effect of BMP-6 and iPSC Application on Periodontal Tissue Regeneration

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Backgrounds:

The purpose of this study is to evaluate the periodontal tissue regeneration ability of iPS cells. The addition of Bone morphogenetic protein-6 (BMP-6) is expected to foster this process.

Materials and Methods:

Transcription factors Oct3/4, Sox2, c-Myc and Klf-4 were transfected into mouse embryonic fibroblasts to reprogram the cells into induced pluripotent stem cells (iPSCs). Bone morphogenetic proteins (BMPs) are powerful inducers of osteogenesis. BMP-6 has been shown to have the ability to induce periodontal tissue regeneration. Different concentration BMP-6 were added to chitosan thermosensitive hydrogel, a 3D culturing environment for iPSCs, to observe the effect of BMP-6 on the periodontal regeneration ability of iPSCs. Mineralization ability of cells was first checked in different groups. The periodontal defect model rats were sacrificed after 6 weeks. Micro-CT and H&E stain were used to study periodontal tissue regeneration condition. QRT-PCR was used to check the bone marker expression pattern of cells under different environment.

Results:

In the group with BMP-6 addition, bony tissue differentiation ability of iPSCs under differentiation medium was elevated. The chitosan thermosensitive hydrogel demonstrated a steady drug release pattern and high cell survival rate. Periodontal tissue regeneration ability was increased in the BMP-6 group when compared to other groups without BMP-6.

Conclusion:

Our study indicates that this chitosan thermosensitive hydrogel is a good vehicle for stem cells delivery and survival. BMP-6 could increase the periodontal tissue regeneration ability of iPSCs. The combination of iPSCs, BMP-6 and chitosan hydrogel is a good treatment modality for periodontal tissue regeneration.

P912

Melatonin Improves Nerve Regeneration by Promoting Schwann Cell Proliferation via Melatonin Receptor-dependent Pathway.

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Backgrounds:

Activate proliferation of Schwann cells is crucial for axonal guidance and successful nerve regeneration following peripheral nerve injury (PNI). Considering melatonin plays an important role in the proliferative regulation of a variety of cells, the present study is aimed to determine whether melatonin can effectively promote Schwann cell proliferation and improve nerve regeneration after PNI by both *in vitro* and *in vivo* approaches.

Materials & Methods:

Cultured RSC 96 cells were firstly analyzed by quantitative polymerase chain reaction (QPCR) to detect the potential existence of melatonin receptors. The possible melatonin receptor-mediated signaling participated in the proliferative regulation was examined by measuring the phosphorylation of extracellular signal-regulated kinases (ERK1/2) pathway. The end-to-side neurorrhaphy (ESN) was performed to act as the *in vivo* model of PNI, and the quantity of Schwann cells as well as the number of re-innervated motor end plates (MEP) on target muscles were detected to express the functional recovery of injured nerves.

Results:

Results from QPCR indicated that MT1 is the dominant receptor presented in Schwann cells. Immunoblotings revealed an enhanced phosphorylation of ERK1/2 following melatonin administration. Cell counting and proliferative assay also showed increased expressions of RSC 96 cells after melatonin treatment, suggesting that melatonin could exert its proliferative effects on Schwann cells via MT1 receptor-dependent pathway. *In vivo* results corresponded well with *in vitro* findings in which melatonin effectively increased the amount of proliferated Schwann cells and re-innervated MEP on target muscles following PNI.

P913

Investigating the Neuroprotective Effects of Citalopram and Resveratrol in a Hearing Loss Animal Model Using Neuroimaging Techniques.

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Backgrounds:

In recent years, the treatment of noise-induced hearing loss is an important issue in clinical medicine. Excessive noise may induce the impairment of central auditory system. Hearing loss patients under this circumstance may have some psychiatric diseases, such as depression and anxiety. Previous studies showed that depression and anxiety may have abnormal levels of serotonin and serotonin transporters. Serotonin transporter (SERT) is a protein located presynaptically in serotonergic neurons and regulates the serotonin concentration in synaptic cleft. The purpose of this study was to investigate the effect of citalopram and resveratrol against noise-induced serotonergic abnormality in rat brain using 4-[^{18}F]-ADAM (a serotonin transporter imaging agent) and small animal-positron emission tomography (small animal-PET).

Materials and Methods:

In this study, male Sprague Dawley rats were exposed to the noise at intensity of 116 dB SPL and frequency at 8 kHz. The citalopram (20mg/kg) and resveratrol(30mg/kg) was injected intraperitoneally before noise exposure and once per day for 4 successive days. The 4-[^{18}F]ADAM /micro-Positron Emission Tomography (micro-PET) and Auditory Brainstem Response test (ABR test) were performed at 1 day, 1 week, and 4 week after noise exposure. Finally, immunohistochemistry (IHC) staining results will be compared to the *in vivo* nenuroimage findings in hearing loss model.

Results:

The specific uptake ratio(SUR) data show that noise-induced hearing loss could cause the reduction of 4-[^{18}F]-ADAM uptake in various brain regions after noise exposure 1 day and 1 week ($p<0.01$). The SUR of 4-[^{18}F]-ADAM in the rats with citalopram or resveratrol treatment was significantly higher than those of without drug-treated rats in the midbrain, thalamus, hypothalamus, striatum, and frontal cortex at 1 day and 1 week after the noise exposure. As for the result of ABR test, the threshold of hearing loss group was higher than that of normal group ($p<0.05$), and there is no significant difference between the drug-treated and without drug-treated rats.

Conclusion:

These results suggest that the citalopram and resveratrol may provide neuroprotective effects against noise-induced serotonergic abnormality and the 4-[^{18}F]-ADAM coupled with small animal PET may be feasible to monitor the status of SERTs in the therapeutic progress.

P914

The Protective Effect of Aliskiren on Myocardial Infarction

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Backgrounds:

Myocardial infarction (MI) is a major health problem and the leading cause of death and disability in both industrialized and developing nations. According to the report from World Health Organization (WHO), it showed that there were 5.7 million people died from MI in 2011. The efficacy of aliskiren, a direct renin inhibitor (DRI), is first to developed as an antihypertensive drug, but several studies indicated that aliskiren treatment may attenuate cardiac remodeling after MI. However, the mechanism remained unclear. Here we investigate the protective effect of aliskiren and elucidate its related mechanism.

Materials and methods:

Myocardial infarction is induced in 8 to 12-week old C57BL/6 mice by ligating the descending coronary artery. After MI, mice were randomly divided into 3 groups: (1) the control group, (2) the MI group, and (3) the aliskiren treated group (25 mg/kg/day via subcutaneous injection). The transthoracic echo-cardiographic assay was performed before surgery, and at 1, 3, 7, and 14 days after MI with a dynamically focused 40 MHz linear-array transducer (Prospect, S-Sharp, Taipei, Taiwan). M-mode tracings were recorded from the short axis at the level of the papillary muscle in the left ventricle.

Results:

The percentages of fractional shortening (FS%) and the ejection fraction (EF%) were measured. FS% reduced from 33 (control mice) to 9 (14 days after MI) and EF% decreased from 62 to 19. But the aliskiren-treated group showed a significant improvement of cardiac function 14 days after MI. (FS% and EF% are increased to 17 and 35, respectively). Furthermore, fibrosis area and TGF-β1 expression were lowered in aliskiren-treated group compared to those in MI group. The decreased number of apoptotic cells was also observed in aliskiren-treated group by TUNEL assay. In addition, we used neonatal cardiomyocytes as an in vitro model. The number of apoptotic cells were increased in hypoxia-treated neonatal cardiomyocytes when compared to control cells, whereas the decreased number was observed in aliskiren-treated group.

Conclusion:

Based on these findings, aliskiren could be as an effective therpay for the prevention of the progression of myocardial infarction.

P915

TNF-α Induces MMP-9 Expression and Soluble ICAM-1 Release via TRAF2, c-Src, MAPKs and NF-κB in Osteoblast-like MC3T3-E1 Cells

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Backgrounds:

Matrix metalloproteinase-9 (MMP-9) has been shown to be induced by cytokines including TNF-α and may contribute to bone inflammatory diseases. However, the mechanisms underlying MMP-9 expression induced by TNF-α in MC3T3-E1 cells remain unclear.

Materials and Methods:

We applied gelatin zymography, Westen blot, RT-PCR, real-time PCR, selective pharmacological inhibitors including transcription (actinomycin D, Act.D), translation (cycloheximide, CHI), c-Src (PP1), MEK1/2 (U0126), p38 (SB202190), JNK (SP600125), and NF-κB (Bay11-7082), transfection with respective siRNAs, promoter assay, immunofluorescence staining, and ELISA to investigate the mechanisms by which TNF-α induced MMP-9 expression and sICAM-1 release in MC3T3-E1 cells.

Results:

Here we demonstrated that TNF-α induced the expression of MMP-9 protein and mRNA which were attenuated by Act.D, CHI, PP1, U0126, SB202190, SP600125, or Bay11-7082, and transfection with siRNA for ERK2, p38, or JNK2. TNF-α-stimulated TNFR1, TRAF2, and c-Src complex formation was revealed by immunoprecipitation and Western blot. Furthermore, TNF-α-stimulated phosphorylation and translocation of NF-κB (p65) into the nucleus was blocked by Bay11-7082, but not by PP1, U0126, SB202190, and SP600125. TNF-α time-dependently induced MMP-9 promoter activity which was also inhibited by PP1, U0126, SB202190, SP600125, or Bay11-7082. Up-regulation of MMP-9 was associated with the release of sICAM-1 into the cultured medium, which was attenuated by pretreatment with MMP-9 inhibitor.

Conclusion:

In this study, we demonstrated that TNF-α up-regulates MMP-9 expression via c-Src, MAPKs, and NF-κB pathways. In addition, TNF-α-induced MMP-9 expression may contribute to production of sICAM-1 by MC3T3-E1 cells. The interpaly between MMP-9 expression and sICAM-1 release may exert an important role in the regulation of bone inflammatory diseases.

P916

EGCG protects against from blue light-induced retinoblastoma Y-79 cells degeneration

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Background:

Light-induced photoreceptor cell death can be caused by a variety of cellular mechanisms that involve oxidative stress. Therefore, the eye depends on the presence of antioxidants to protect the retina from light-induced damage. Excessive exposure to light would be damaging to the eye. The short-wavelength visible light between 430 nm to 500 nm (blue light) is especially associated with retina damage as evidenced by photoreceptor degeneration. Recent investigations have demonstrated that estrogen receptors (ERs) have been has been shown to have antioxidant and antiinflammatory effects on neuronal cells in brain. However, estrogen receptor (ER)-mediated effects of the (-)-epigallocatechin gallate (EGCG), extracted from green tea, have not been examined extensively in photoreceptors of the eyeball. EGCG were examined for the ability to elicit ERs and ER-mediated gene expression *in vitro.*

Methods:

In vitro study, human retinoblastoma Y-79 cells were treated with EGCG and irradiated with low output power LED light. After exposure to LED lighting, cells were analyzed by morphology, immunocytochemistry, and Western blot analysis.

Results:

Our studies were demonstrated that the cell degeneration of retinoblastoma Y-79 cells was observed after blue light exposure. Apoptosis related proteins, p53 and caspase-3, increased the expression after blue light illumination. After EGCG treatment, increased ER proteins production and inhibited the blue light-induced retinoblastoma Y-79 cells death were investigated.

Conclusions:

These results indicated the short-wavelength visible light, such as white LED exposure, leads to retinoblastoma Y-79 damage. EGCG regulates the expression of neuroprotective proteins, ER, and modulates degeneration responses *in vitro.*

P917

Involvement of Human Papillomavirus Type 16 in the Prognosis of Oral Squamous Cell Carcinoma

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Backgrounds:

This study was carried out to evaluate the frequency of human papillomavirus (HPV) and its E6 protein on the paraffin-embedded tissue sections of oral squamous cell carcinoma (OSCC), and its relationships with the clinicopathological parameters.

Materials and Methods:

The paraffin embedded tissue blocks of surgically removed oral cavity OSCC from 181 patients treated in Chang Gung Memorial Hospital, Linkou during 1998 and 2000 were sectioned. After deparaffinization, HPV in the tumor tissue sections were examined with chromogenic *in situ* hybridization (CISH) method. Biotin-labeled HPV type 16 genomic DNA (PanPath) was used as probe, and the signals were amplified with the GenPoint system (Dako) to reveal the viral load and the distribution of HPV16. The results are graded by the density and amount of signals. The expression of E6 viral protein was detected by immunohistochemistry (IHC) method using anti-HPV16/18 E6 antibody (Santa Cruz). The results were scored under microscope for the intensity of signal and the percentage of E6 expressed in the tumor region. .Only those cases with positive CISH and positive IHC were designated as HPV positive. Statistical analysis was performed using the SPSS statistical package. The correlation between the HPV status and the clinicopathological parameters was examined, and the survival curves were constructed using the Kaplan–Meier method.

Results:

Among 181 cases examined, 74 cases (40.9%) demonstrated HPV (+) by both CISH and IHC. Neither the staging of OSCC, nor the metastasis status is correlated with HPV positivity. All the cases with blood vessel invasion tend to be HPV (+) (p = 0.003). The cases with HPV (+) have shorter median disease free survival time than HPV (-) cases (35 vs. 70 months) and overall survival time (57 vs. > 96).

Conclusion:

HPV may be a factor associated with the prognosis of OSCC. After followed up to 80 months the survival curves demonstrate, though not significantly, that the post surgery outcomes are worse in HPV (+) cases.

P918

Ischemic Postconditioning Attenuate Reperfusion Injury of Small Intestine: Impact of Mitochondrial Permeability Transition.

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Background:

Ischemic postconditioning (IPoC) modulates the reperfusion maneuver to mitigate ischemia- reperfusion (I/R) injury. This study aims to investigate the effects and protective mechanism of IPoC on intestinal I/R injury.

Materials and Methods:

Intestinal I/R was induced by occluding the superior mesenteric artery (SMA) for 30 minutes followed by reperfusion for 60 minutes on male Wistar rats. IPoC was elicited by 3 cycles of 30 seconds reperfusion and re-occlusion of SMA at the initiation of reperfusion.Carboxyatractyloside

(CATR), a mitochondrial permeability transition pore (mPTP) opener, and NIM811, a mPTP inhibitor, were administered separately in selected groups. The serum and intestinal sections were collected for analysis.

Results:

IPoC and the administration of NIM811 significantly diminished the expression of I-FABP and LDH (3427± 236.8 U/L for I/R; 1190.5± 36.7 U/L for IPoC; 1399.3± 295.6 U/L for I/R+NIM811; 2002±370.9 IU/L for IPoC+CATR) in portal blood, the release of cytosolic cytochrome c and cleaved caspase 9 expression in intestinal mucosa after intestinal I/R injury ($p< 0.05$). Histopathologically, IPoC and NIM811 mitigated mucosal damage after IIR as well (Chiu's score: 3.8±0.4 for I/R, 0.2±0.2 for IPoC, 0.4±0.2 for IR + NIM811 and 4.2±0.2 for IPoC+CATR; apoptotic index 59.5± 4.6% for I/R, 15.7±15.7% for I/R+ IPoC; 3.5± 3.5% for I/R + NIM811, and 67.1±9.3 % in IPoC+ CATR). Carboxyatractyloside negated the protection conferred by IPoC.

Conclusion:

IPoC and NIM811 attenuate intestinal I/R injury. The addition of Carboxyatractyloside negated the effects of IPoC, indicating that the protective mechanism of IPoC was associated with the modulation of mPTP opening.

P919

Inactivating epithelial-mesenchymal transition by Eugenol Thwarting Peritoneal Dissemination of Gastric Cancer in nu/nu Mice

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Backgrounds:

Eugenol, a natural phenolic extract, has previously been reported to activate cells apoptosis and suppress the inflammation. Whether Eugenol inhibits the tumor growth and peritoneal dissemination of gastric cancer cells remains unknown.

Materials and Methods:

Xenograft gastric tumor mouse model, PET-CT, Immunohistochemstry, H&E stain, Western blot,.

Results:

In a xenograft gastric tumor mouse model, Eugenol significantly inhibited tumor growth and peritoneal dissemination detected by PET/CT images. Eugenol-treated tumors showed increased apoptosis signature such as increased expression of ER stress markers. Epithelial-to-mesenchymal transition (EMT) is a key process in tumor cell invasiveness and metastasis. Furthermore, Eugenol-treated tumors showed increased epithelial signature such as increased expression of p-ECadherin and cytokeratin-18, decreased expression of Snail, but not Vimentin and p-Cadherin. Similar observations were made when SCM-1, AGS and N87 cells were treated *in vitro*. Importantly, Eugenol effectively reduced aryl hydrocarbon receptor (AhR) expression and activated Calpain-10, which was correlated with the down-regulation of EMT markers, but not Calpain-1 or Calpain-2. Calpain inhibitors and specific siRNA calpain-10 transfection significantly reversed the Eugenol-induced AhR degradation and EMT markers.

Conclusion:

Our results suggest that enhancing Calpain-10/ AhR interaction by Eugenol suppresses both gastric tumor growth and peritoneal dissemination by inducing apoptosis and inhibiting EMT.

P920

Melatonin ameliorates D-galactose-induced aging-like behavioral and CNS changes

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Backgrounds:

Aging and especially the associated deterioration of memory and cognition are prominent social issues. It's associated with decline of melatonin, the antioxidant of the pineal gland that could modulate reactive neurogenesis. In fact, oxidative stress is believed to play critical role in senescence-associated degradation of CNS functions such as poor sensory-motor integration, cognitive performance, attention, memory and physical endurance. Interestingly, D-galactose (D-gal) is known to increase oxidative stress and accelerate aging, thus can be exploited in developing aging animal model for testing the associated syndromes and treatments.

Materials and Methods:

Male SD rats (250-350 g) were studied. D-gal was given intraperitoneally in high dose (500 mg/kg) for 2 weeks (D-gal-H group) or low dose (150 mg/kg) for 6 weeks (D-gal-L group). Control received saline injection. Melatonin (10 mg/kg daily for a week) was given to another group of D-gal-L rats intraperitoneally during the last week of the D-gal treatment. Weight-loaded forced swimming test and Morris Water Maze task were used to evaluate motor endurance and spatial learning and memory before and 6 weeks after the beginning of treatment. Brains were fixed with perfusion and the somatosensory cortex and hippocampus studied. Part of the brain was prepared into serial sections for Neutral Red and nNOS, GFAP and GSA-IB4 labeling, the rest into 350-μm-thick coronal slices for intracellular dye injection to reveal the dendritic arbors of Layer III and V pyramids of the somatosensory cortex and CA1 hippocampal neurons. Dye-filled neurons were reconstructed 3-dimensionally for analysis including the densities of dendritic spines that represent excitatory connection to these neurons.

Results:

Behavioral tests show that either dose of D-gal treatment impaired animals' motor endurance and spatial learning ability. The treatment did not change the cytoarchitecture but increased nNOS, GFAP and GSA-IB4 labeling densities and reduced superoxide dismutase in the cortex. These demonstrate that D-gal causes oxidative stress to impair central functions as in aging. At the cellular level, D-gal treatment did not alter the dendritic arbors of the somatosensory cortical and CA1 hippocampal pyramidal neurons but decreased their dendritic spine densities consistently, consistent with the proposition that oxidative stress reduced excitatory connection to CNS neurons and could underlie aging-associated behavioral impairments. Melatonin effectively reversed the D-gal-induced behavioral impairments and returned dendritic spines on both somatosensory cortical and CA1 hippocampal neurons to normal levels. Melatonin treatment reversed nNOS, GFAP and GSA-IB4 labelings to control levels as well.

Conclusion:

D-gal can induce aging-like behavioral and brain neuronal changes in young adult rats. It induced oxidative stress and inflammatory reaction to the CNS, reduced dendritic spines on cortical and hippocampal output neurons, and compromised animals' motor endurance and spatial learning as happened in aging. Melatonin supplement effectively reversed all D-gal-induced cellular and behavioral changes. Its effectiveness in treating naturally aged animals remains to be explored. The model in addition provides an opportunity to study the mechanisms of aging-related cellular and molecular changes.

P921

The study of *Toona sinensis* leaf extracts-induced cell death in renal cell carcinoma

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Backgrounds:

Renal-cell carcinoma is characterized by a lack of early-warning signs which results in a high proportion of patients with metastases and therapeutic inefficiency. *Toona sinensis*, a traditional herb, was reported to possess various biological functions such as anti-cancer, anti-oxidant, anti-diabetes, anti-angiogenesis, and anti-inflammation. Aqueous leaf extracts of *Toona sinensis* (TSL-1) has been shown to inhibit cancerous cells proliferation including lung, ovarian, prostate, and premyelotic cells.

Materials and methods:

Cell viability was determined by MTT assay. Apoptotic effects were confirmed by morphological analysis, propidium iodide (PI)-annexin V double staining assay, acridine orange/ethidium bromide (AO/EB), and immunoblotting. Cell cycle effects were evaluated by flow cytometry and immunoblotting analysis.

Results:

The antiproliferative effect of TSL-1 on 786-O and A498 cells is prominent after 24 h treatment. TSL-1 arrested RCC cells G2/M phase and increased subG1 phase proportion. PI-annexin V and AO/EB assays further confirmed that TSL-1 induced RCC cells apoptosis in a concentration- and time-dependent manner. Moreover, TSL-1-induced RCC cells apoptosis also examined by immunoblotting analysis (increased expressions of caspase-3 and cleaved PARP). TSL-1 treatment also decreased the expression of cell survival proteins such as B-cell lymphoma-2, cytosolic cytochrome c (accompanied by increased Bax), survivin, and proteins linked to cell proliferation, such as cyclin D1. Following TSL-1 treatment, phosphorylated PI3K, hypoxia inducible factor 2 (HIF2α), and vascular endothelial growth factor (VEGF) all reduced, suggesting that TSL-1- induced cell cycle arrest and apoptotic effects might go through PI3K-HIF2α -VEGF signaling pathway.

Conclusion:

In summary, these results indicate that TSL-1 exerts cell cycle arrest and caspase-dependent cell death in RCC cells, supporting its potential usage in chemotherapeutics choice for kidney cancer.

P922

The Effect of Ginsenosides on Glucose Uptake in HK-2 Cells

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Backgrounds:

Ginseng, an important traditional herb medicine widely used to ease many distresses without adverse effects for long term usage, has been reported to modulate blood glucose level. In spite of a highly valued traditional medicine, its regulatory mechanism is largely unknown. In our previous studies, the purified ginsenoside Compound K (CK) and Rg1 were found to induce or inhibit glucose uptake through modulation of sodium-dependent glucose cotransporter 1 (SGLT1) gene expression, respectively, in differentiated human intestinal Caco-2 cells. In this study, we examined the effect and mechanism of these ginsenosides on glucose uptake and SGLT2 expression in differentiated human kidney HK-2 cells.

Materials and Methods:

The human renal tubular HK-2 cell line was cultured and left to differentiate for 5 days after confluency. To examine the effect of CK and Rg1 on these cells, alpha-methyl-D-glucopyranoside (α-MG) uptake, Western blotting and semi-quantitative RT-PCR were performed. Transient transfection was carried out using a series SGLT2 promoter luciferase reporter constructs. To understand the transcription factors that are involved in the CK- and Rg1-modulated SGLT2 gene expression, EMSA and ChIP assay were also used.

Results:

We found that CK increases and Rg1 inhibits glucose uptake, respectively, in HK cells. The observed effect on the rate of glucose uptake was due primarily to the increased and decreased SGLT2 both protein and mRNA levels, respectively, in a time- and dose-dependent manner. The effect is regulated at the transcriptional level, as the CK and Rg1 had no effect on SGLT2 protein and mRNA stability. Transient transfection analysis also demonstrated that CK and Rg1 induced and inhibited the transcription activity of SGLT2 promoter, respectively. The signaling pathways involved in the action of these compounds were also analyzed.

Conclusion:

In summary, ginsenosides CK and Rg1 were shown to modulate glucose uptake in human renal tubular cells through induction or inhibiton of SGLT2 gene expression, respectively. Our finding indicate that Rg1 has the potential to serve as an SGLT2 inhibitor for diabetic therapy. This study is important in understanding the pharmacological mechanism and potential applications of ginsenosides.

P923

A Nanobiomedical Sensing Platform-Protease Activated Fluorescent Self-assembled Gold Nanoparticles

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Backgrounds:

Fluorescence-based assays and detections are trend for nowadays for their high sensitive and application in biological researches. Nanoparticles are widely used in biosensing field and among all the nanoparticles, gold nanoparticles (AuNPs) have the character of fluorescence resonance energy transfer (FRET) and good chromophore acceptor that AuNPs have outstanding quench ability to wide range fluorescent. Moreover, nontoxic characteristic makes AuNPs becoming a popular material in biotechnology. However, there still is the need of procedure improvement for reducing probe unstable circumstance resulting in mistake signal.

Materials and Methods:

In this study, a biomedical fluorescence-based sensing platform was established for the rapid detection of proteases activity. The target protease is proteinase K used as an efficient enzyme for building model. The 20 nm AuNPs were conjugated with peptide as a protease substrate and the peptide was commercially modified with FITC. With the existence of proteinase K in sample, which can cleavage substrate peptides, the fluorephore will diffuse away from the surface of AuNPs beyond distances of efficient quenching and start to glow.

Results:

This study provided a self-assembled strategy of AuNPs probe and focused on following treatment for enhancing the stability of AuNPs probe in sample condition. Furthermore, tend to enhance the specificity of proteinase K to AuNPs-peptide conjugates by extending the length of peptide according to the idea of decreasing steric barrier by peptide conjugates. The proteinase K activity could be evaluated by emitting fluorescence of FITC at 515 nm. A linear correlation was established with the proteinase K concentration ranging from 10 to 400 ng/mL. Also, this study proved that increase the length of peptide conjugates could efficiently enhance the specificity of proteinase K to AuNPs-peptide conjugates about 3 folds.

Conclusion:

The procedure established in this study could be further applied in other proteases by simply replacing the efficient peptide substrate. The assay by this platform could be applied in clinical or industrial routine detections and also could contribute to the diagnosis of in vivo proteases activity.

P924

Conformational Dynamics of the H+-translocating Pyrophosphatase Probed by Hydrogen-Deuterium Exchange

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Backgrounds:

H^+-translocating pyrophosphatases (H^+-PPase) exists in various endomembranes of plants, bacteria, and some protists. It transports H^+ into lumens at the cost of hydrolyzing pyrophosphate (PPi). Although the crystal structure of mung bean H^+-PPase has been solved recently, the proton translocation reactions of H^+-PPase is unclear. Here, we coupled hydrogen/deuterium exchange (HDX) with mass spectrometry (MS) to study the molecular details of H^+ translocation in H^+-PPase. When proteins were placed in a D_2O solution, the backbone hydrogens, which exchange with deuterium, would be identified by MS. In H^+-PPase, the exchange rate of the labile backbone hydrogens in amino acids involved in proton pumping would elevate when their solvent accessibility was increased in the process of proton transport. After identifying the regions related to proton translocation by MS, a possible model would be constructed to illustrate the dynamics of the H^+-translocating pyrophosphatase.

Materials and Methods:

To obtain the information of the dynamics during proton transport in H^+-PPase, we incubated the protein complex in D_2O solution for different time intervals. After quenching the reaction with HCl, the protein sample was digested into peptides by CNBr and pepsin beads. C18 spin column was then used to remove interfering contaminants and concentrate peptides. The sample was gently dried in a vacuum evaporator and then carefully suspended in 1-2 μl matrix solution. Finally the mass of each deuterated peptide was determined with MALDI-MS.

Results:

The dynamics during proton translocation of H^+-PPase was activated by pyrophosphate hydrolysis. We expect that the m/z ratio of peptides from the predicted proton pathway and catalytic sites would be increased when pyrophosphate was hydrolyzed in D_2O buffer. The conformational dynamics of H^+-PPase during proton transport was thus obtained by analyzing the m/z ratio of peptides involved in proton translocation.

Conclusion:

The results of hydrogen/deuterium exchange indicate that the conformational dynamics of H^+-PPase has occurred during proton transport. This technique could be extended likely to unravel transport phenomena in other transmembrane channels as well.

P925

Mass preparation of a novel phosphate transporter PHT1:1 from Arabidopsis

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Backgrounds:

Phosphate (P_i) is an essential inorganic nutrient for organisms such as plants. To uptake phosphate in to the cell, several phosphate transporters have evolved in a wide range of the organisms. PHO84, the yeast phosphate transporter, was first identified in *Saccharomy cescerevisiae* in 1991 (*Bun-Ya et al., 1991*). In plants, there are several phosphate transporters. PHT1 family, belongs to the major facilitator super (MFS) family, is a group of phosphate transporter found in *Arabidopsis thaliana*. It contains 9 numbers, named as PHT1;1 through PHT1;9, and we are interested in PHT1;1.

Materials and Methods:

First, we generated the wild-type PHT1;1 gene from *Arabidopsis thaliana*, and constructed the sequence in to the plasmid pYES2 adding six histidine. We then transformed the plasmid containing PHT1;1 gene in to E. coli for amplification. The PHT1;1 protein would then heterologously expressed in yeast and purified for further studies.

Results:

We found the optimal induction time for PHT1:1 expression in yeast was least then 48 h. The characterization of PHT1:1 is currently under investigation.

Conclusion:

The appropriated protocol is developing for mass production of PHT1:1 for further studies including 3D X-ray crystallography.

P926

MicroRNA-449a mediated through to inhibit parental and Apicidin resistant HA22T Hepatocellular carcinoma cells correlated with cell proliferation.

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Background:

As of now, chemically synthesized drugs were being used for cancer treatment, and prolonged use of this chemically synthesized drugs make the cancer resistance to treatment and thus result in high mortality rate. MiRNAs, a small group of noncoding RNA has drawn a greater attention in the recent years, but their functional role in chemo-resistant HCC cells is not clearly understood. Thus in this present study, we aim to investigate the molecular mechanism altered by miR-449a in both HA22T and Apicidin resistance HA22T hepatocellular carcinoma cells (HCC).

Materials and Methods:

Quantitaive PCR was performed to analyze for the three specific miRNAs, Cell viability and Trypan blue assay was performed to analyze the inhibitory effect of miR449a, Sanger, TargetScan and MicroCosm database & western blotting to identify the putative targets of miR449a.

Results:

Compared with the HA22T cells, Apicidin resistance HA22T cells expressed lower levels of miR-122a, miR-449a and miR-21a. Overexpression of miR449a decreased the cell viability and cell number in parental and resistance cells. This microRNA was further analyzed for their putative target using miRNA database, we found the Wnt singal pathway, cell cycle protein and c-Met may be targets of miR-449, and then further confirmed by Western blotting assay. Inaddition MiR449a overexpression decreased Cyclin-D1 expression only in HA22T cells but not in the Apicidin resistance - HA22T cells.

Conclusion:

From our experiment we observed that miR449a was down regulated in HCC cells, and by overexpressing this miR449a decreased HA22T cell growth by inhibiting CyclinD1 protein expression. Using human samples and animal models will be performed to further analyze the functional role of miR449a in HCC.

P927

Inhibitory effects of lipid peroxidation stimulated by linoleic acid hydroperoxide on rat lung mitochondria from *Scutellaria baicalensis* root

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Backgrounds:

Recently, for the air quality is getting worse, there are more and more damages associated with respiratory system, which leads to pulmonary diseases are increasing. Most lung function decreased is considered to relate oxidative stress arise by producing free radical and lipid peroxidation. Barbed Skullcap is one kind of heat clearing herbs in traditional Chinese medicine. In this study, we investigate the protective effect of different solvent extracts derived from *Scutellaria baicalensis roots.*

Materials and Methods:

In this study, we extracted *Scutellaria baicalensis* roots by ethyl alcohol, acetone , ethyl acetate and water respectively. And main constituents of baicalein, baicalin, wogonin and oroxylin A were isolated. ,ROS damage protection evaluated the linoleic acid hydroperoxide induced lipid peroxidation on rat lung mitochondria. Antioxidant capabilities of each extract were displayed by DPPH free radical scavenging. And the phytochemical analysis of total phenol, flavonoid content were investigated. Each constituent of extracts was quantitative analyzed by HPLC. .

Results:

The extracts from *Scutellaria baicalensis* root by water, ethanol, acetone, and ethyl acetate were observed 52.5%, 72.5%, 55.00% and 59.17% inhibitory of lipid peroxidation (LPO) on rat lung mitochondria, respectively. And exerted remarkable free radical scavenging activities. Baicalein is the most potential LPO inhibitor.

Conclusion:

Baicalein is the main active constituent of LPO inhibitor from *Scutellaria baicalensis* root. The acetone extract is a high potential extract from the *Scutellaria baicalensis* root, and a lead ompound for lung damage protection phytochemical or functional food.

P928

A Gaze at the Beauty and Fullness of Plant Phosphate Transporters-Biophysical and Dynamic Aspects

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Backgrounds:

Plants develop different strategies against the Pi limiting environment. Indeed, the instinctively Pi transporters play a crucial role that increases Pi uptake from soil. The Pi transporters are phosphate: H+ symporter (PHS, TC 2.A.1.9.1), belonging to the MFS (Major Facilitator Superfamily). Although more than 1,000 MFS proteins are identified, there are only 5 3D crystal structures determined. PHT1 and its members are major Pi transporters in Arabidopsis and have drawn intensive attentions of many workers on their physiological significance. However, studies on molecular level of Pi transporters still require more endeavors to fully understand their structure/function relationship and Pi uptake mechanism.

Materials and Methods:

A mass production and purification strategies of Pi transporters would be set up for heterologous over-expression in yeast. Appropriate site-directed mutagenesis would be used to identify essential amino acids, motifs, termini, signal peptides and domains along transport pathway. Structural mapping and dynamics of Pi transporters would be dissected.

Results:

We have successfully cloned wild-type and 22 mutants the PHT1;1 from Arabidopsis and set up an overexpress system in yeast. Yeast complementary experiments have been currently conducted for observing the function of Pi transporters.

Conclusion:

The P_i transporters play an important role in all organisms. In order to comprehend P_i transporter uptake mechanism, we will study key residues, motifs, and domains in structure and functions of plant Pi transporters.

P929
GSN may interfere with the EGFR-mediated metastasis of human epidermoid carcinoma cell

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Backgrounds:

Cancer metastasis is a significant contributor to the problem to cancer treatment and death in cancer patients. One of key mechanisms in cancer metastasis is linked to epidermal growth factor receptor (EGFR) signaling. EGFR may mediate cancer cell motility and metastasis via changes in the organization of actin cytoskeleton. Gelsolin (GSN) is the most important actin binding protein (ABP) which regulates dynamic actin filament organization. Moreover, many studies have devoted to establish the role of dynamic actin filament formation by GSN in controlling the proliferation and motility of malignant tumors. This study investigates the relationship between EGFR and GSN and the relevant mechanism of metastasis.

Materials and Methods:

We used epidermoid carcinoma A431 cells stably transfected with human cytoplasmic GSN cDNA. Western blotting was used to determine the expression level of GSN and EGFR. In addition, we used the transwell assay to measure the ability of GSN to inhibit A431 cell migration.

Results:

Our results reveal that the endogenous protein expression level of GSN and EGFR are opposite in EGFR-overexpressed epidermoid carcinoma A431 cells. Furthermore, our results also show that GSN may inhibit A431 cell migration. In addition, GSN might interfere with the human epidermoid carcinoma A431 cell metastasis via regulation of EGFR pathway.

Conclusion:

Our findings suggest that GSN might have anti-metastasis effect via modulation of EGFR signaling pathway.

P930
Receptor Guanylate Cyclase-G is a Thermal Senor Involved in Cooling-Induced Ultrasonic Distress Calls in Neonatal Pups

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The GG (Grueneberg ganglion), an olfactory subsystem located in the anterior nasal region, operates as a dual sensory organ detecting both chemical cues and cool temperatures essential for avoidance and survival. Receptor GC (guanylate cyclase)-G-mediated cGMP signaling components expressed in the GG neurons has been implicated in sensing cool ambient temperatures in neonatal pups. However, whether GC-G acts as a temperature sensor remains elusive. Here we showed that GC-G is a direct thermosensory receptor that can be maximally stimulated by cool temperature at 15□ by both in vivo cellular cGMP accumulation assays and in vitro GC assays with a purified recombinant protein. Cells co-expressing GC-G and the cycle nucleotide-gated channel CNGA3 responds to cooling temperature by elicit a rapid influx of calcium. Furthermore, we found a marked coolness-induced expression of the activity-dependent gene c-Fos in the GG neurons in wild-type neonatal pups but not in the GC-G-knockout littermates. Consistent with these findings, cooling-elicited ultrasonic vocalizations were significantly impaired in the GC-G-knockout neonatal pups compared to wild-type littermates. Together, our data demonstrated that GC-G acting as a temperature sensor in the GG is involved in the mother-child interactions under cooling condition when isolated from the nest to emit ultrasonic distress calls for material attentions.

P931
Rapid Screening of Natural Products by Cell-based Assays

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Bioresource Collection and Research Center, Food Industry Research and Development Institute

Backgrounds:

To explore the applicable potential of the microbial resources and natural products, we have been developing a series of target-based screening assays to explore the potential of microbial metabolites that could modulate biological activities. An expanding screening panel has been established to explore the applicable potential of the microbial collection in Bioresource Collection and Research Center (BCRC). The system could facilitate industrializing natural resources and discovering novel bioactive ingredients.

Materials and Methods:

For evaluating nuclear hormone-modulating activities, 9 cell-based reporter screens were established. By transfecting luminescent reporter constructs containing cis-acting elements into human cells expressing corresponding human hormone receptors or transcription factors, the resulting luminescence of transfected cells correlates to the level of activation of the specific hormone receptor. The system provides a mean to assess the activity of nuclear hormone receptors in vivo. A system for analyzing natural products with immuno-regulatory activities by utilizing cultured macrophage was also established for rapid screening application. Anti-cancer activities are accessed by analyzing the activity of Wnt signaling pathway using a reporter gene system.

Results:

The signal/noise ratio (S/N ratio) of the assays is over 5.0 and all assays have positive Z factors. The system enables high-throughput identification of molecules that could stimulate or antagonize the hormone receptor of interest. We has screened over 3,000 microorganism extracts and identified more than a dozen of novel compounds.

Conclusion:

Besides analyzing in-house samples, we offer screening services to researchers and industries that are interested in assessing biological activities. New assays have been continuously added to our screening panels and currently we have over ten assays in three categories including a sex hormone panel, a metabolic hormone panel, an immunomodulatory panel, and a cancer biomarker assay. All of our assays are conducted in microplates and samples in the forms of pure compounds, fractions, or crude extracts could be analyzed.

P932
Amphetamine Causes Apoptosis in Human Lung Cancer A549 Cells.

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Aims:

Amphetamine, with addictive properties, is well known as to have neurotoxicity. Amphetamine is commonly uptaken via nasal route. Amphetamine could entry into respiratory system and might cause damage. We investigate the cytotoxicity of amphetamine on human lung adenocarcinoma A549 cells.

Materials and Methods:

A549 cell line was obtained from ATCC and cultured following its protocol. Cytotoxicity was measured by MTT. The apoptosis was measured via sub G1 observation, the activity of caspase 3, 8 and 9. The mRNA levels of p53 and p21 were measured by PCR and their protein levels were measured by Western blotting.

Results:

In A549 cells, amphetamine causes cytotoxicity in a concentration and time dependent manner. In 6 days treatments, the IC50 of amphetamine was nearly 0.5 mM. Serum did not have protection effects. The present of several nicotinic acetylcholine receptor (nAChR) subunits mRNA had been detected in A549 cells. Either the agonist, epibatidine, or antagonist, hexamethonium, of nAChR unaffected the cytotoxcity of amphetamine. The activity of caspase 9 was raised under the treatment of amphetamine. The subG1 level of A519 was raised. The mRNA level and protein amount of p53 raised under amphetamine treatments via PCR and Western blotting.

Conclusion:

Our data show that amphetamine, at high concentration, caused apoptosis in A549 cells. Amphetamine can entry the cells and intracellular vesicles via membrane transporters. After entrance, amphetamine might entry mitochondria to lead their membrane potential collapse and activate caspase 9 to lead apoptosis. Amphetamine-induced apoptosis might be related with p53 pathway. In past study, amphetamine can cause DNA oxidation and damage that might trigger p53 pathway to lead p21 transcription. The mRNA level and protein level of p53 and p21 was evidenced by PCR and Western. Thus, our study evidenced that amphetamine trigger p53 activation to lead cell cycle arrest via p21 and apoptosis via mitochondria and caspase 9 pathway.

P933
The Effects of Phthalates on Cytosolic Calcium Homeostasis And Proliferation

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Aims:

We investigated the phthalates on the cytosolic Ca2+ homeostasis in excitable and non-excitable cells. Human neuroblastoma SH-SY5Y cells and human osteosarcoma HOS cells are represent for excitable and non-excitable cells, individually. Ca2+ can trigger cell proliferation so we also examine the phthalates on cell proliferation.

Materials and Methods:

All three cell lines were obtained from ATCC and cultured following their protocol. Cytosolic calcium concentration ([Ca2+]c) were measured via Ca2+ fluorescent dye Fura-2. Cell proliferation was measured by MTT.

Results:

In excitable neuroblastoma SH-SY5Y cells, dialkyl phthalates induced [Ca2+]c elevations in a dose dependent manner. The relative levels of [Ca2+]c elevation were observed in the order of di-n-butyl phthalate (DBP) > benzyl butyl phthalate (BBP) > di(2-ehtyl hexyl) phthalate (DEHP) > dicyclohexyl phthalate (DCHP) > di-n-hexyl phthalate (DHP). DBP induced the highest elevation in [Ca2+]c that was suppressed by verapamil and nifedipine, voltage-operated Ca2+ channel (VOCC) inhibitors. DBP enhanced cell proliferation. In non-excitable HOS cells, phthalates induced [Ca2+]c elevation and the relative levels of [Ca2+]c elevation were observed in the order as: DCHP≈ DHP> DBP> DEHP> DPrP> DEP. DCHP induced highest response of [Ca2+]c elevation. DBP could induce [Ca2+]c elevations in the absence of extracellular calcium. Verapamil could not suppress the DBP-induced [Ca2+]c elevation. DBP enhanced cell proliferation.

Conclusion:

Our data show that phthalates have capability to induce [Ca2+]c elevation in both excitable and non-excitable cells but from different sources. In excitable cells, VOCC might be intracellular or extracellular activated by phthalates based on their amphipathic characters. In non-excitable cells, DBP could induce Ca2+ release from intracellular Ca2+ stores. Phthalates enhanced proliferation that might be due to phthalate-induced Ca2+ elevations from extracellular influx or the intracellular release. The possible roles of phthalates to induce or enhance cancer are required further studies.

P934
Inhibition of human bone marrow mesenchymal stem cells- induced cell motility by silencing HOXA9 in human gastric cancer cells

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Background:

Epidemiological studies report that gastric cancer is one of the most common cancers worldwide, and is also the second leading cause of cancer-related mortality. The poor prognosis of gastric cancer may be partly attributed to the complicated molecular networks operating the aggressiveness of gastric cancer. Although a large body of studies has revealed the deregulation of certain genes in gastric carcinogenesis, the molecular mechanisms behind gastric tumor development are not yet fully understood.

Method and Material:

We measured the HOXA9 expression in human gastric tissues including gastric cancer tissue, gastric polyp, gastritis, gastric ulcer, duodenal ulcer, and human gastric cell lines (CSN, AGS, CS12, NCI-N87 and MKN45). We applied the HOXA9 short haipinRNA (shRNA) to successfully knock down the expression of HOXA9 gene and subsequently explore the role of HOXA9 in CS12 gastric cancer cells. We co-culturedCS12 cells (control shRNA-stable CS12 or HoxA9 shRNA-stable clones) and HBM_MSCs, and then respectively measured the motility of CS12 cells and HBM_MSCs in the co-culture system. We also observed the expression of cytokins and chemokines in CS12 cells and HBM_MSCs. IL-6, IL-8, CXCL-1 and CCL-5 were used to identify the effect in the motility of CS12 cells

Result:

In the present study, we found that homeobox A9 (HOXA9) is over-expressed in gastric cancer tissue more than in other tissues including gastric polyp, gastritis, gastric ulcer and duodenal ulcer. We further found that human bone marrow mesenchymal stem cells (HBM_MSCs) induce cell motility in human CS12 gastric cancer cells by secreting cytokins and chemokines. The motility of CS12 cells mainly were promoted by IL-6, IL-8 and/or CXCL-1 form HBM_MSCs. When applying HOXA9 shRNA in CS12 cells, we observed that HOXA9 knockdown inhibits HBM_MSCs-induced CS12 cell motility, and even reduces CS12 cells-enhanced the capacity of motility in HBM_MSCs.

Conclusion:

Homeobox A9 (HOXA9) gene is over-expressed in gastric cancer tissue more than in other tissues including gastric polyp, gastritis, gastric ulcer and duodenal ulcer. Silence of HOXA9 gene reduces HBM_MSCs-mediated cell motility in human CS12 gastric cancer cells. HOXA9 knockdown in human CS12 gastric cancer cells reduces HBM_MSC motility.

P935
Anti-HBV Effect of *Antrodia Camphoratus* Fruit in HBV-Viemia Animal Model

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Backgrounds:

Antrodia Camphoratus (AC), a Taiwan medicinal fungus, was tested for its anti-HBV-activity. The prevention of anti-virus drug is able to be tested in this system.

Materials and Methods:

The established HBV-viremia animals were treated with different treatments, 3TC, AC powder, AC ethanolic extract and GongBo essence product for 15 days. After treatment, the mice were sacrificed and blood was collected. Serum HBsAg and HBV DNA were analyzed for anti-HBV biomarkers.

Result:

The serum HBsAg and HBV DNA were analyzed for 3TC, AC powder, AC ethanolic extract and GongBo essence product efficacy. Results appeared that 3TC and AC ethanolic extract decreased the levels of HBsAg and HBV DNA in serum. The HBV DNA viral load in serum was decreased to 97 % by 3TC and 56.5 % by AC ethanolic extract. The serum HBsAg level was decreased to 41% by 3TC and 24.5% by AC ethanol extract. It is suggested that the replication of HBV was inhibited by 3TC and AC ethanolic extract, whereas the secretion of HBV virion was partially suppressed by 3TC. AC powder and GongBo essence product also decreased the level of HBV DNA in serum up to 28% and 73.6%. However, the levels of HBsAg were not decreased in serum.

Conclusion:

In conclusion, results suggested that AC powder and GongBo essence might interfere the encapsidation of HBV DNA.

P936
The Effect of *Cymbopogon citrates* Extract on Human Keratinocyte HaCat Cultured with *Malassezia furfur*

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Backgrounds:

M. furfur is a lipophilic yeast that causes skin disease. To evaluate the effect of *C. citrates* extract on M. furfur invasiveness in human keratinocyte cell line (HaCat).

Materials and Methods:

HaCat cell line was cultured as monolayer in a standard culture medium DMEM. The cytotoxic effects of *C. citrates* extract on HaCat cell was determined by MTT assay. M. furfur grown in mLNA medium were harvested and used for co-incubation with HaCat in the present or absence *C. citrates* extract. Cells were stained with the May-Grumwald and Giemsa to evaluate the adhesion and invasive capacity.

Results:

Treatment of HaCat cells with *C. citrates* extract for 3 days had no significant effect on cell viability up to 800 μg/ml concentration. *C. citrates* extract had antifungal activity and can reduce M. furfur invasion and adhesion to HaCat cells at sub-MIC concentration. In addition to invasion of cells, *M. furfur* would aggregately adhere on the cell surface. While in the present of *C. citrates* extract, the *M. furfur* aggregates were significant decreased and instead with scattered adhesion to HaCat cells.

Conclusion:

The results demonstrated that *C. citrates* extract can decrease the invasiveness of *M. furfur* to keratinocyte at sub-MIC concentration. It was suggested that C. citrates extract may influence the infection of *M. furfur* through modulate the immune response of keratinocyte instead of directly fungicidal activity and the mechanism need to be further investigation.

P937

Insulin-Like Growth Factor-Binding Protein-3 Mediates High Glucose-Induced Apoptosis by Increasing Oxidative Stress to stabilized HIF1 alpha in H9C2 Cells

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Backgrounds:

High glucose can cause intracellular ROS generation, inactivate insulin-like growth factor-I (IGF-I) cell survival signaling, leading to cell apoptosis. IGF-binding protein-3 (IGFBP-3) is the most concentrated carrier protein for IGF-I in blood. Recently, IGFBP-3 was reported to mediate high glucose-induced cell apoptosis. HIF-1 alpha, a transcription factor, is an upstream protein of IGFBP3. In this study, we investigated the role of IGFBP-3 in high glucose-induced apoptosis in cardiac cells.

Methods and Results:

H9c2 cells were treated with 5.5 mM and 33mM (high glucose, HG) glucose for 36 hr. We found HG resulted in a time-dependent increase in ROS generation, intracellular and extracellular (secreted) IGFBP-3, as well as reduced IGF-I signaling activity. The results of co-immunoprecipitation (Co-IP) assay showed that compared with 5.5 mM glucose, HG enhanced the extracellular association of IGF-I with IGFBP-3, which was also observed in serum sample of STZ-administrated rats. Interestingly, Treatment of IGFBP-3 antibody in medium reversed the decreased IGF-I signaling activity and the apoptosis development in HG-exposed cells. IGFBP-3 siRNA treatment showed the similar results. Additionally, HG time-dependently promoted HIF-1 α nuclear translocation examined by immunofluorecence and western blot. However, the RNA level was not affected. HIF-I α siRNA treatment decreased intra- and extracellular IGFBP-3, apoptosis level and enhanced the reduced IGF-I signaling activity induced by HG. In contrast, the treatment of overexpressed HIF-Iα reversed altered protein levels induced by HG. Using the apocynin, a cytosolic ROS inhibitor, and rotenone, a mitochondria ROS inhibitor, the results showed that increased levels of HIF-Iα, secreted IGFBP-3 and apoptosis as well as the decreased IGF-I survival signaling by HG were significantly reversed by the ROS scavengers, and mitochondria is the major ROS source in cells exposed to HG.

Conclusion:

Our findings suggest that increased IGFBP-3 expression and secretion by oxidative stress mediate high glucose-induced apoptosis in H9c2. The increased oxidative stress from high glucose stabilized HIF1 alpha protein expression to regulate IGFBP-3 expression and extracellular secretion, which further induce cell apoptosis.

Key Words:

High glucose; IGFBP-3; IGF-I; HIF-1 alpha; Apoptosis; ROS.

P938

Necrotic cells induce neutrophil extracellular traps

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Backgrounds:

Neutrophil extracellular traps (NETs), which are formed when neutrophils are in contact with microbes or other stimuli, are extracellular structures composed of DNA, histones, and granular and cytoplasmic proteins that trap and kill pathogens. Neutrophils forming NETs also undergo a form of cell death termed “NETosis.” In addition to the antimicrobial effect, NETs are also associated with tissue damage and certain autoimmune diseases. It is known upon tissue damage, even in the absence of infection, the injured cells can release endogenous damage-associated molecular patterns (DAMPs) that trigger an acute inflammatory response characterized by an influx of neutrophils to the injured site. In this study, we investigated that during sterile inflammation, whether the recruited neutrophils can be stimulated by DAMPs to form NETs.

Material and methods:

Purified human blood neutrophils were treated with necrotic human HL60 cells or recombinant IL-1α, and purified mouse bone marrow neutrophils were treated with necrotic mouse EL4 cells. Necrotic cell lysates depleted of DNA and proteins using DNase I and proteinase K, respectively, were also used to stimulate neutrophils. Neutrophils stimulated with phorbol myristate acetate (PMA) served as positive controls. To observe NET formation, neutrophils were stained for the release of DNA and myeloperoxidase (MPO) with DAPI and anti-MPO antibodies, respectively, and observed under a fluorescence microscope. To quantify NET formation, neutrophils were incubated with DNase I, and the digested and released DNA was stained with Hoechst 33342 and measure for the fluorescent intensity. The production of reactive oxygen species (ROS) in neutrophils was measured by dihydrorhodamine 123.

Results:

Human neutrophils treated with necrotic HL60 cells formed NETs that were characterized by the release of DNA and MPO extracellularly. Necrotic cell-stimulated neutrophils showed increased ROS generation, which could be responsible for causing NET formation. NET induction was markedly attenuated when necrotic cells were depleted of DNA or proteins, suggesting that both cellular DNA and proteins could serve as DAMPs for stimulating NET formation. IL-1α has been shown to be a DAMP, and we found that neutrophils could also be induced by recombinant IL-1α to form NETs. In addition, we also observed NET formation when purified mouse bone marrow neutrophils were treated with necrotic murine EL4 cells, although it required a longer time of induction.

Conclusion:

Taken together, our data demonstrated that neutrophils could be stimulated by necrotic cells to form NETs, indicating that there is a dynamic interplay between the injured cells and the recruited neutrophils during sterile inflammation. Further investigation will be carried out to elucidate how this interaction may affect tissue homeostasis or pathogenesis.

P939

Selection and characterization of EV71 antibody by phage display human scFv antibody library

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Backgrounds:

Enterovirus 71 (EV71), a positive single-stranded RNA virus belongs to the *Picornaviridae* family, may cause hand-foot-and mouth disease (HFMD) and severe neurological disease in infants and young children in Taiwan since 1998. Because of severe epidemic infection occurs periodically, prevention and treatment of EV71 has become an urgent subject.

Materials and Methods:

Here, we use the human scFv-phage display library to select the antibody fragment to against to EV71via immobilized biopanning method. First, purified EV71 virus was coated on microtiter plate at 4 degree overnight. Then, the sc-Fv phage library was added into the microtiter plate to recognize the EV71. After washing unbound phages, the sc-Fv binders of EV71 were eluted by competitor MAB979 antibody which recognizes EV71. The eluted phages were amplified by infecting *E. coli*, TG1 strain. After three times biopanning, the selected sc-Fv phage candidates were further analysis by ELISA and western blot.

Results:

The 385 phage clones were random picked from biopanning and screened by ELISA. Twenty-eight phage clones showed potential ability to recognize EV71 viral particles. In addition, we identified five sequence variant phage clones of twenty-eight clones by RFLP and sequencing. Furthermore, the five unique phage clones were aligned by ImMunoGeneTics (IMGT) database for human variable heavy chain region (V_H), but only phage clone 3 and phage 9 have variable light (V_L) region. On the other hand, the phage ELISA results showed the O.D 450 values were in proportional to the major phage clone when increasing the titer. Besides, the selected phage clones can recognized EV71 by western blot.

Conclusion:

In our study, we selected the sc-Fv phage clones to against EV71 via immobilized biopanning method. After the sequencing and IGMT database analysis, the found five distinct phage clones which have ability to bind EV71. In the future, we will confirm the interaction between these phage clones and EV71. Furthermore, we will try to develop the antibody fragment for applications in diagnosis of EV71 or as the therapeutic agent.

P940

Epirubicin-Induced ATP6V0B Expression in Bladder Cancer Cells

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Backgrounds:

ATP6V0B gene encodes a component of vacuolar ATPase (V-ATPase), a multisubunit enzyme that mediates acidification of eukaryotic intracellular organelles. V-ATPase is crucial for the control of tumor microenvironment by proton extrusion to the extracellular medium. The acid environment favors tissue damage, activation of destructive enzymes in the extracellular matrix, and those are characteristics of metastatic tumor necessary for invasion. Nevertheless, little is known about the role of V-ATPase in cell death, and especially the underlying mechanisms remain mostly unknown. The aim of this study is to investigate the role of ATP6V0B in Epirubicin-induced cell death in T24 cells, human bladder cancer cells.

Materials and Methods:

Human urothelial cell lines, T24, RT4, NTUB1 and J82 were used to determine internal WWOX expression levels by western blot. Results showed that the protein level of WWOX of RT4, NTUB1 and J82 was higher than T24. The T24 cell was used to investigate the effects of tumor suppression and gene expression under WWOX overexpressed situation. ATP6V0B was expressed in T24 cells by transfecting cells with cDNAs encoding ATP6V0B which constructed in pLKO_AS2.puro (a mammalian expression vector). The protein levels of ATP6V0B were determined by western blotting.

Results:

Epirubicin was further evidenced that it can induce ATP6V0B protein expression in dose-dependent manner in ATP6V0B-transfected T24 cells. The expression and localizations of ATP6V0B in T24 cells after Epirubicin treatment were detected by immunofluorescence.

Conclusion:

In conclusion, ATP6V0B may play a role in Epirubicin-induced cell death in human bladder carcinoma cell line.

P941
Activation of WWOX expression reduces cellular growth and migration in rat bladder cancer cells

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Backgrounds:

Urothelial cell carcinoma (UCC) is one of the most common cancers of the urinary tract in the world and may result from mutations or the inadequate expressions of tumor suppressor genes. WW domain-containing oxidoreductase (WWOX) is a tumor suppressor and identified as a putative tumor suppressor in cancer cells because it lies in a genomic region that is frequently altered in pre-neoplastic and neoplastic lesions. To better understand the molecular mechanisms of WWOX action on UCC, WWOX gene was introduced into AY-27 cells, rat bladder cancer cell lines.

Materials and Methods:

The WWOX protein level was determined by western blotting and its localization was identified by immunofluorescence. The cell growth was examined by cell count assay. The effects of WWOX on cell migration were studied by scratch assay and wound healing studies.

Results:

When coupled with lentivirus-mediated transfer of the WWOX -cDNA, we could achieve long-term reconstitution of WWOX expression both in vitro and in vivo. Over-expression of WWOX reduced cells growth, migration, wound healing in AY-27 cells.

Conclusion:

These results indicate that WWOX may play an important role in suppressing bladder tumor growth.

P942
Study on Structure and Function of Human Cyclin D1 and Antizyme

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Backgrounds:

Ornithine decarboxylase (ODC), antizyme (AZ) and antizyme inhibitor (AZI) are polyamine metabolism regulation factor. AZ is the center in the polyamine homeostasis. Cyclin D1 plays a critical role in the G1 to S-phase cell cycle transition. Previous studies of human AZ protein have suggested that the C-terminal segment, AZ_95-228, is important for the binding and inhibition of ODC. In this study, we quantified the biomolecular interactions between AZ and the other proteins to identify AZ-binding affinities. Furthermore, we constructed the AZ and CD1 peptides and determined that the binding regions on each other.

Materials and Methods:

Protein expression & purification: Proteins were cloned into pQE30 vector, overexpressed in *E. coli* JM109 cells, and purified by HIS-selectTM Nickel affinity chromatography. Site-directed mutagenesis: Truncation Mutants were constucted by QuickChange® kit. ***Enzyme assay : continuous spectrophotometric:*** The ODC activity was measured at 37 ℃ by using CARBON DIOXIDE L3K® kit and detected by spectrophotometer at the absorbance 405 nm. ***Size-distribution analysis by analytical ultracentrifugation:*** Sedimentation velocity experiments were carried out using a beckmen optima XL-A analytical ultracentrifuge.

Results:

The K_d value of AZ-Cyclin D1 was 0.81, four-fold and forty-fold larger than that of ODC_WT and AZI_WT, respectively. In addition, the activity of the AZ-inhibited ODC enzyme was hardly rescued by Cyclin D1, and the efficiency of Cyclin D1 in the rescue of the ODC enzyme activity was less than that of AZI. The K_d value of AZ_1-124 peptide was similar to that of AZ_WT. In size-distribution analysis, there is no interaction between the AZ_WT and Cyclin D1_155-295.

Conclusion:

1. AZ-binding affinity: AZI > ODC > CD1. **2**. The N-terminus of AZ may play an important segment in binding to Cyclin D1. **3**. Our data have suggested that the N-terminal segment, Cyclin D1_21-154, is essential factor for AZ binding.

P943
The RNA-Recognition Motif of NIFK Regulates Ribosomal-RNA Processing and Leads to Cell Cycle Progression.

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Backgrounds:

Previously, a human nucleolar protein interacting with the forkhead-associated (FHA) domain of Ki67, NIFK, was identified by yeast two hybrid using the FHA domain of Ki67 as bait. Human NIFK comprises two defined domain: a RNA-recognition motif (RRM) and the Ki67-FHA interaction domain (Ki67FHAID), however its function remains unclear. Given that Ki67 is a proliferation marker widely used in cancer diagnosis, the role of human NIFK in cell proliferation was investigated.

Materials and Methods:

Human osteosarcoma cell line, U2OS, was used as cell model. Genes mentioned in this study were silenced by siRNA. mRNA and protein level were examined by quantitative-PCR and western blot, respectively. For cell proliferation assay, grown cells were harvested every 24 hour for cell number estimation. For cell cycle analysis, nocodazole-synchronized cells were analyzed by flow cytometry. Subcellular localization of NIFK was defined by antibodies of NIFK combined with nucleolus marker and analyzed by confocal immunofluorescence microscopy. 32p-orthophosphate-based pulse chase assay are used to tract the kinetic of nascent rRNA processing. To examine whether the phenotype caused by NIFK-siRNA was specifically resulted from NIFK-knockdown and clarify the functional importance of RRM and Ki67-FHAID, rescue experiments were performed. To these two purposes, cells stably expressing wildtype /RRM and Ki67FHAID deletion (dRRM and dKi67FHAID) /putative RNA-interaction residue mutant (RRM mutant) NIFK-siRNA resistant NIFK were generated by retroviral transduction and analyzed as above mentioned.

Results:

Cells showed reduced proliferation when NIFK was silenced, and this was resulted from G1 cell cycle arrest when analyzed by flow cytometry. Meanwhile, G1 checkpoints, p53 and p21, were concomitantly upregulated. NIFK downregulation-induced G1 arrest was compromised when p53/p21 was co-knocked down. Immunofluorescent staining showed that NIFK colocalized with fibrillarin, the marker of dense fibrillar component in nucleolus. In line with the canonical function of nucleolus, the pulse chase result indicated that silencing NIFK delayed rRNA processing and thus inhibited 28S rRNA generation. This effect was reproducible when p53 was co-knocked down. In addition, rescue experiments showed that both wildtype and dKi67FHAID -NIFK, but not the dRRM/ RRM mutant -NIFK, could restore the G1 progression and 28S rRNA generation following NIFK-knockdown.

Conclusion:

Functionally, silencing NIFK inhibited cell proliferation through p53 pathway dependent G1 arrest, which is the consequence of defects in rRNA processing. 28S rRNA generation was still affected following NIFK and p53 co-knockdown, indicating it is not resulted from p53-mediated inhibition of rRNA transcription. Mechanistically, RRM, but not the Ki67FHAID, is crucial for NIFK to regulate rRNA processing and thus G1 progression. Collectively, we demonstrated the role of human NIFK in cell proliferation, at least in part through regulation of rRNA processing.

P944
Ginkgotoxin-induced seizure increases *leptin b* expression and alters lipid desposition in zebrafish

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Seizure is a transient symptom characterized by physical changes in behavior occurring after an episode of abnormal electrical activity in brain. Recurrent and unprovoked seizures are important symptoms of epilepsy since they affect patient's physiological functions profoundly besides behavioral impact. Studies showed that drug-induced convulsive seizure affected lipid metabolism and the distribution of different lipid metabolites. However, the impact to the health of affected individuals and the mechanism underlying remain elusive.

Previously, we reported that ginkgotoxin (GT), a toxin in Ginkgo biloba, induced a seizure-like behavior in larval zebrafish by inhibiting pyridoxal phosphate (PLP) biosynthesis. In the current study, we found that GT-treated larvae exhibit an increase in leptin b mRNA level, which is reversed by PLP supplementation. However, elevated leptin b mRNA is also observed in Pentylenetetrazol (PTZ)-induced seizure larvae, suggesting that the up-regulation of leptin b is seizure-specific. Leptin has been shown to be crucial in regulating energy intake and expenditure, ie. appetite and metabolism. Using nile red staining, we also show a significant decrease of lipid deposition in GT-treated larvae as compared to untreated control larvae of the same stage.

Our results imply that the seizure-induced leptin b up-regulation might contribute to the altered lipid deposition observed in individuals affected by seizure. Overexpressing and knocking-down leptin b in GT-treated embryos to confirm the role of leptin b in seizure-related pathogenesis is underway.

P945
Crystal Structure of Cell Binding Factor 2 from Helicobacter Pylori

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Backgrounds:

To investigate the peptidyl-prolyl isomerization mechanism of parvulin-type PPIase domain of cell binding factor 2 from Helicobacter pylori, the crystallographic structure was determined.

Materials and Methods:

The cDNA plasmids of *Hp*CBF2 were transformed into *E.coli* BL21 (DE3) as the expression host for protein expression and purified by Ni-NTA His-bind resin column. The isomerization activity of *Hp*CBF2 was performed by a coupling enzyme assay with Suc-AAPF-pNA as a substrate analog. *Hp*CBF2 was crystallized using Jeffamine M-600 as a precipitant by vapor diffusion method.

Results:

*Hp*CBF2 has been overexpressed and isolated with a molecular weight of 31910 Da. *Hp*CBF2 forms a dimer in solution. The isomerization activity of HpCBF2 was performed by a coupling enzyme assay with Suc-AAPF-pNA as a substrate analog. The catalytic efficiency (k_{cat}/K_m) of *Hp*CBF2 was calculated as 238 ± 7.2 $mM^{-1}s^{-1}$. The overall structure of *Hp*CBF2 contains a chaperone domain and a PPIase domain. The PPIase domain is composed of three β strands and α helices. There is a substrate-binding pocket in PPIase domain and it consists of eight conserved residues, H131, L133, D169, L181, M189, F193, F214 and H217. The chaperone domain is composed of four α helices. The chaperone domains of dimer are interlocked together and form a domain-swapped architecture.

Conclusion:

The structure of the catalytic site of *Hp*CBF2 conflicts with the original hypothetical catalysis mechanism of parvulin PPIases. Recent studies also recognize the deficiency of the parvulin catalysis mechanism. The catalysis mechanism remains to be clarified.

P946
Ganoderma Tsugae Increases the Life Span and Motor Function of Drosophila Melanogaster

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Backgrounds:

Aging is a natural process of multicellular organisms involving special pathogenic events, e.g., progressive organ disorder, higher mortality, and susceptibility to degenerative diseases. Ganoderma tsugae (GT) is a traditional Chinese medicine and has been used for centuries. It is widely revered as a valuable healthcare supplement worldwide. This study investigates the healthcare efficacy of GT via evaluating the life span and motor function of Drosophila melanogaster.

Materials and Methods:

GT was kindly provided by the Luo-Kuei-Ying Fungi Agricultural Farm, Taoyuan, Taiwan. For longevity experiment, flies were collected (30 flies per vial) and cultured on fly-food (containing yeast, corn flour, and sugar) at 25℃ . The negative geotaxis assay was used to evaluate the climbing ability of flies. Briefly, the flies were placed in a new vial and the vial was gently tapped to let the flies fallen to the bottom of the vial. This tapping treatment was performed until all flies died.

Results:

Our results demonstrated that the life span of D. melanogaster could be extended after treatment with GT. The effective concentration of GT used in the life span experiment was also responsive to the motor function test, i.e., GT could increase the motor function and thus the muscle strength of D. melanogaster.

Conclusion:

Our findings indicate that GT may extend the life span and increase the motor function of D. melanogaster. The GT-mediated molecular mechanism affecting the life span and motor function of D. melanogaster is currently under investigation.

P947
Investigating The Role of Cdc13 on Pif1 Helicase Activity Stimulation and Telomerase Removal

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Backgrounds:

In Saccharomyces cerevisiae, telomere DNA repeats are ~250-300 bps of TG1-3/C1-3A sequences.

Telomeres are required to prevent chromosome end-to-end fusion, maintain chromosome integrity and regulate telomere elongation. Telomerase is a ribonucleoprotien that utilizes its RNA template to elongate the G-rich strand telomeric DNA. In S. cerevisiae, the telomerase catalytic subunit is Est2 and TLC1 RNA is the template. Telomeres are coated with many telomere-associated proteins to mediate telomere function. Notably, Pif1 is a single-stranded DNA (ssDNA)-dependent 5' to 3' helicase that utilizes ATP hydrolysis to remove telomerase from telomeres. Cdc13 is a single strand telomeric DNA binding protein that was shown to regulate telomerase through cooperating with different telomere-associated proteins. We aim to define the role of Cdc13 and Pif1 on regulating telomerase activity.

Materials and Methods:

Using an in vitro reconstituted system, we designed and synthesized a tailed-duplex DNA with the single-stranded TG1-3 annealed to a 18-mer oligonucleotide with the sequences similar to the template region of TLC1 RNA. The substrate mimics the structure of telomerase RNA-bound telomeres. Purified recombinant proteins were used in this study.

Results:

Using purified proteins, we found Cdc13 stimulated Pif1 helicase activity. The stimulating activity did not appear to require the telomeric DNA binding activity of Cdc13 as Cdc13 stimulated Pif1 activity in DNA substrates lacking single-stranded telomere DNA. Moreover, a Cdc13Y522C mutant that failed to bind to telomeric DNA still activated Pif1 activity.

Conclusion:

We conclude that Cdc13 stimulates Pif1 helicase activity and the DNA binding activity is dispensable for its stimulating activity. Our results also implicate that a direct interaction between Cdc13 and Pif1 is involved in this stimulating activity.

P948
Colchicine derivative as a potential anti-glioma compound

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Glioblastoma is the most primary malignant brain tumor in adults. Common treatments include a combination of surgery, radiotherapy, and chemotherapy. However, the poor survival rate remains. Colchicine, one of the microtubule inhibitor, is a common agent to treat gout, and also used as an antitumor drug with side effects through disrupt the microtubule structure. Accordingly, the development of its derivatives with low dose efficacy and less side effect could bring impact on anti-tumor treatment. In this study, we present the effect of a colchicines derivative so named as AD1 on U373MG and U87MG, the grade human glioblastoma cell lines. Through immunostaining of α-tubulin and F-actin, we found that the treatment of the two glioma cell lines with AD1 for 24 h at a very low concentration, 10 nM, led to severe cell damage. MTT cell viability analysis showed that AD1 caused the cell death of U373 and U87 cells in a time-dependent manner. Further experiments also revealed that the application of AD1 increased the conversion of LC3-I to LC3-II in U373 and U87, indicating that AD1 may induce the autophagy of the two glioma cell lines. Thus, we conclude that AD1 exerts anti-tumor effect on human glioma cells through the induction of the autophagic process and cellular distribution (NSC-99-2628-B-006-030-MY3).

P949

Extracellular Production and Function Analysis of Recombinant Functional Peptides Fused with Ganoderma lucidum Immunomodulatory, Ling Zhi-8 by Bacillus subtilis System

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Backgrounds:

Ling Zhi-8 protein (LZ-8) is an immunomodulatory protein of Ling Zhi (Ganoderma lucidum). The LZ-8 consists of 110 amino acids, forms a homodimer which contributes to the immunomodulatory activity. The antioxidative peptides (Leu-Leu-Pro-His-His, LLPHH) is part of the soybean protein digestion product. The antioxidative activity of LLPHH can impair the peroxidation of linoleic acid and scavering of peroxynitrite activity. Lactostatin is a pentapeptide (Ile-Ile-Ala-Glu-Lys, IIAEK) derived from bovine milk β-lactoglobulin. The IIAEK can induce expression of cholesterol 7α-hydroxylase (CYP7A1) gene and exhibits an efficient hypochocholesteroemic activity.

Materials and Methods:

In this study, we fused one copy or two copies of LLPHH or IIAEK into the C-terminal of LZ-8 and introduced trypsin or pepsin cleavage sites. These fusion proteins are successfully expressed in B. subtilis WB800. The acquisition of bioactivies peptides is proceeded by Ni-NTA purification, protease treatment and HPLC separation.

Results:

The functional activity of LLPHH is confirmed by antioxidation of linoleic acid peroxidation. The IIAEK induced gene expression of CYP7A1 in HepG2 cells which indicate the hypocholesterolemic activity of IIAEK. There is no significantly difference in CYP7A1 gene expression between chemical synthesized peptide and in B. subtilis system produced peptide.

Conclusion:

In our system, we can obtain bioactive peptides and the functional activity of LZ-8 protein, simultaneously. The LZ-8 protein was expressed as homodimer which is an essential functional structure of LZ-8.

P950

Study on the anti-inflammatory effect of EMCDO isolated from momordica charantia

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Backgrounds:

This study aimed to determine the anti-inflammatory effect of EMCDO in LPS-activated RAW 264.7 macrophage and investigate the molecular mechanism of macrophage in regulating inflammation.

Materials and Methods:

Macrophages are key players of the immune system , and can induce inflammatory reaction by releasing various types of cytokine such as IL-1, IL-6 and TNF-α and pro-inflammatory mediator such as NO and PGE2. RAW264.7 is a murine macrophage cell line and induced by LPS.

LPS (lipopolysaccharide)is the component of the outer membrane of Gram-negative bacteria, has been show to induced production of pro-inflammatory cytokines, chemokines, in macrophage.

RAW 264.7 macrophage were pretreated with different concentration (1.25 ,2.5 or 5μg/ml) of EMCDO for 4h, and were then treated with LPS (1 μg/ml), and incubated for 24h.total protein were resolved by SDS-PAGE, transferred to nitrocellulose, and detected protein level of iNOS and COX-2.The level of IL-1, IL-6 and TNF-α in macrophage culture medium were measured using commercially available ELISA kit.

Results:

We tested the effected of EMCDO on inflammatory mediator and cytokine expression in RAW 264.7 macrophage. The protein level of iNOS and COX-2 were found to be significantly up-regulated by LPS, but pretreated with EMCDO inhibited these up-regulations in a dose –dependent manner.

Conclusion:

EMCDO suppressed the production of pro-inflammatory mediator iNOS and COX-2 in LPS-induced RAW 264.7 cell is by NF-κB pathway not MAPKs pathway.The data showed that EMCDO significantly reduce the production of iNOS and COX-2 and these effects are mediated by the inhibition of NF-κB transcription activity and IκBα phosphorylation.

P951

Putative Oncogenes Identified by Genome-wide Study in Oral Squamous Cell Carcinoma

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Backgrounds:

Oral squamous cell carcinoma (OSCC) dominates 90% of oral cavity cancer. After decades of research and clinical study, due to the insufficiency of the understanding of cancer development driven by potential oncogenes, there was not much improvement in postoperative survival and/or recurrence rates. It is widely accepted that cancer development is a process of accumulations of serial mutations in oncogenes and tumor suppressor genes.

Materials and Methods:

To verify the tumorigenic cellular processes or pathways caused by these genetic alterations, a genome-wide study covering copy number variation, differential expression, and transcriptome sequencing has been performed on OSCC specimens.

Results:

Based on minimum region of amplification mapping, unique genomic regions at chromosome 3q26 to 3q29 and 8q24 are frequently amplified in near 60% oral cancer patients. Since oncogenes are activated not only by amplifications but also by somatic mutations, analyses of tumor transcriptome by next generation sequencing is a comprehensive and unbiased approach to detect such alterations. Twenty four up-regulated genes in MRA harbor mutations. Based on empirical evidence, we cited fifteen genes as putative oncogenes and discussed their contribution on cancer development.

Conclusion:

This study identified putative oncogenes in OSCC for comprehensive understanding of the cancer's developmental mechanism and improving future clinical therapeutics.

P952

Glutathione-Mediated Intracellular Release and Au Nanoparticle-Induced Oxidative Stress Using GNP-Capped MSN Nanoshuttles: Synergistic Effect Leads to Better Therapeutic Efficacy of Cancer Treatment

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Backgrounds:

Chemotherapy is a common fighting strategy for treating different known cancers but the undesired side effects on normal tissues or organs and insufficient dosage to kill cancerous cells thus develop targeted drug delivery systems and combined treatments for enhancing therapeutic efficacy against cancer is a promising approach to conquer detrimental side effects.

Materials and Methods:

Gold nanoparticle (GNP)-capped, amino-functionalized mesoporous silica nanoparticles (MSN@GNP) as potential candidates for drug delivery. After uptake of MSN@GNP by cancer cells, the presence of a relatively higher concentration of intracellular glutathione (GSH, 10 mM) results in desorption of GNPs from the surface of MSNs. In addition, during the exposure to the GNP, a state of oxidative stress was induced, which is likely to break down cancer cells' defenses, making them more susceptible to chemotherapy.

Results:

It was first confirmed that GSH serves as an effective trigger to release a payload (FITC dye molecules) from MSN@GNP. Then using the TBARS assay to verify the elevated oxidative stress induced by GCMSN in cancer cells and the extent of ROS formation in A549 was higher by the presence of GCMSN than NMSN. Finally we explored the synergic effect in killing cancer cells by loading a hydrophobic anticancer drug (camptothecin, CPT) in the channels of MSN@GNP. Compared with CPT chemotherapy or oxidative stress therapeutic strategy alone, the combined treatment exerted by one nanoshuttle system demonstrated a synergistic effect, resulting in higher therapeutic efficacy.

Conclusion:

CPT-loaded MSN@GNP had the ability of controlling drug release and combined chemotherapy with GNP-mediated oxidative stress mechanism significantly improved the therapeutic efficacy of cancer treatments, which makes them a highly promising candidate as a new-generation nano-DDS carriers.

P953

Establishment of stable repeated-batch cultures system for bacteria consortia from rumen fluid in vitro

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Backgrounds:

The energy from the sun can be captured through a variety of renewable sources, and more than humans are projected to use. For now, we think the only form of solar energy can contribute substantially to transportation fuel needs at costs competitive with fossil fuel is that captured by photosynthesis and stored in biomass. Have some renewable fuel such as cellulosic ethanol can help, and that is made from the non-food portion of renewable feedstocks. The creation of a new industry for converting plant lignocellulose to fuels, there are many barriers must be overcome to make the process ready for large-scale use. For example, cellulose is a recalcitrant substrate for bioconversion, and behoove different type of enzymes are required to produce sugar. So, we have attention to the bacterial consortia from bovine rumen. Within a relatively short time (up to 48 h), the microbial consortium inside the bovine rumen could hydrolyze 60–65% cellulose. However, for the lignocellulose degradation, we think the bacteria consortia from rumen are maybe have this kind of function.

Materials, Methods and Results:

The goal of this study was to develop a cultures system for enrich the lignocellulose-degrading bacteria consortia from rumen in vitro, and that can keep the bacterial phase to stable. In the rumen, the major activity of cellulytic enzymes was xylanase. The xylanase activity and the profile bacterial microflora will be used to evaluate stability of the cultures. The enrichment culture was constructed by repeated-batch cultures with ruminal microflora and napiergrass at 39 ℃ . The lignocellulose-degrading enzyme activities were measured by DNS method in every two days. As a result, the napiergrass 2% (w/v) in the ratio 1:5 for McDougall buffer with ruminal fluid are appropriate. In addition, hydrolysis of rice straw with our rumen fluid for one hour at 39°C yielded 2.9593 mg/10 mg reducing sugars. The profile microbial populations inhabit different batch culture was analyzed by Denaturing Gradient Gel Electrophoresis (DGGE) of PCR-amplified 16S rRNA gene segments.

Conclusion:

The result showed that a higher xylanase activity by ruminal microflora was formed, and the microflora includes bacterial strains such as Ruminococcaceae sp., Prevotella sp. and Clostridium xylanolyticum in two batch cultures. After the 5-genereation enrichments, the ruminal microflora still accrues to stable bacterial consortia. The consortia constituted with three major ruminal microflora even their bacterial phase just similarly. In the future, we were measure the hydrolysis of different renewable feedstocks with our rumen fluid.

P954

Perilla Seed Oil Ameliorate Osteoporosis in Ovariectomic Rat

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Backgrounds:

Estrogen deficiency can increase oxidative stress and cause osteoporosis. In comparing to other plant oils, perilla seed oil consistently contains higher proportion of omega-3 (ALA) fatty acids at 54–64%, and it metabolized into eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6). The previous studies showed EPA/DHA possessed a good antioxidant activity.

Materials and Methods:

Perilla seed oil is fed to the ovariectomic rat for 8 weeks to examine the effects and potential mechanisms.

Results:

The results show the ratio of adipocyte/osteoblast increase and trabecular bone density decrease dominantly in femur bone of ovariectomic rat, and perilla seed oil ameliorates the condition significantly. Runx2 plays a critical role in osteoblast differentiation and the expression of Heme oxygenase-1 (HO-1) represents the oxidative status in individual. The expression of Runx2 and HO-1 decrease in bone tissue of ovariectomic rat, but they are restored after treating with perilla seed oil. RANK and RANKL are important components to regulate osteoclast proliferation. The expression of RANK/RANKL increase in femur bone of ovariectomic rat, and perilla seed oil can reduce the expression of RANK/RANKL.

Conclusion:

The present results show that perilla seed oil has the potential to prevent women from osteoporosis in postmenopausal syndrome.

P955

HPV is a Risk Factor for Breast Cancer in Taiwanese Patients

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Backgrounds:

The role of viruses in the etiology of breast cancer has been acknowledged for over 100 years. Viruses that are associated with human cancer are known as “tumor viruses” or “oncoviruses” and have played an important role in cancer biology. It is estimated that viruses may be the cause of approximately 15% of all human cancers. In females, close to 20% of all cancers may be linked to viral infections, with cervical cancer being the most notable example. These percentages represent a significant portion of cancer instances that are attributed to viruses. However, the pathway from viral infection to tumorgenesis is slow and inefficient; therefore the instances in which a viral infection progresses into cancer is small. There is usually a latent period between the instance of primary infection and progression into neoplastic transformation. It is also important to note that the presence of virus infections alone is not sufficient enough to induce cancer. Virus infections may initiate cancer pathways due to errors in the cellular repair system, but additional risk factors such as the environment, behavior, or genetics are needed to perpetuate this process.

Materials and Methods:

Initial screening

An initial screening process to determine the basic level of association between viral warts and female breast carcinoma was conducted using Taipei Medical University’s Medical End-User Computing (MEUC) system in which the International Classification of Diseases (ICD-9) codes were used. The codes used were 078.1 for viral warts and 174 and 174.9 for female breast carcinoma. A q-value that represented the association factor between these two diseases was calculated through this system. A q-value of greater than 1.0 denoted a positive correlation.

Cohort Data

Following the initial screening, cohort data comprising of one million patient data from Taiwan’s National Health Insurance (NHI) database was used to further examine the association between viral warts and female breast carcinoma. Patient data was retrieved from 2000-2008. The patients were categorized into three groups: breast cancer only, viral warts only, and breast cancer and viral warts. The group of patients diagnosed with both diseases was further separated by age and order of disease diagnosis.

Statistical Analysis

The odds ratio for each age group was calculated through SPSS as well as the associated p-values. P-values above 0.05 represented no significance and were not further analyzed. Odds ratios at 95% confidence intervals and p-values for all age groups were calculated.

Results:

Three age groups are shown in table in which the p-values are 0.026, 0.23, and 0.645 for age groups 20-29, 30-39, and 50-59 respectively.

Table Calculated Odds Ratio and p-values

	Viral Warts First	Breast Cancer	OR ratio	P value
0-9	0	0		
10-19	1	2	0.34	0.364
20-29	13	2	4.72	0.026
30-39	39	20	1.43	0.23
40-49	81	61	0.88	0.547
50-59	55	35	1.12	0.645
60-69	23	24	0.63	0.155
70-79	15	13	0.79	0.543
80-89	2	2	0.69	0.712
90-100	0	0		
Total	229	159		

Conclusion:

Our studies indicate that women who develop viral warts are at a slightly higher risk of developing breast cancer than women who develop breast cancer first. Furthermore, the p-value for women between the ages 20-29 who develop viral warts before breast cancer showed significance (0.026) over the other age groups. This value could indicate that women within this age group are at a higher risk for developing breast cancer than other women in any other age group. If HPV does have a predominate role in breast cancer development, then these women could reduce their chances of developing breast cancer or increasing their chances of survival if encouraged to participate in more frequent screenings.

P956

Comparison of scFv and Fab Phage Displays for VEGF-A Neutralizing Antibody Selection

賴建勳

Phage display has been one of the most widely applied technologies capable of generating new therapeutic antibody drugs in the last decade. Major effective display formats for antibodies are scFv (single chain fragment variable region) and, Fab (fragment of antibody). Compared to the complete antibody, antibody fragments (scFv and Fab) are smaller in sizes; they are easier engineered and faster to produce recombinant proteins. Because of the elimination of the Fc region, they have lower stability which limits their wide applications, especially scFv format. Because of its smaller size, expression of scFv is more easily with higher diversity displayed on the surface of the phage than that of Fab. Both of them have their own advantages and there are no clear rules for estimating which format is more suitable for screening our target. In this work, we have compared these two formats of phage display for antibody screening using immunized rodents as resources. The sizes of both libraries are similar (1~2x109). Through several rounds of bio-panning (ELISA plate), we found that scFv library was faster than Fab to reach its endpoint (maximal O.D. value = 3), but not in using Dynabeads bio-panning method. Their output sequence results (clones from both libraries) indicated that they are distinctively unrelated in sequence population; and the clone diversity of scFv is higher than Fab. Formats of antibody seem tremendously affect the phage preference for displaying of proteins. The binding affinity of scFv proteins, ranging between 10-7 and 10-8M, are better than Fabs; but there are no differences when they become full antibodies.

After comparison of the bioactivities of those full antibodies, we found that the number of positive clones from the scFv library is much more than that from the Fab library; and the bioactivities of some of them are better than Avastin. According to our results, we found that using scFv phage display systems is more suitable for selected our target protein. However, whether it is the case for every target protein, we believe, it is still case by case.

P957
Functional Roles of Tyrosine Residues in Clostridium tetani H+-PPase

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Background:

Proton-translocating pyrophosphatase (H+-PPase, EC 3.6.1.1) is a crucial enzyme which sustains pH homeostasis of organisms. This enzyme generates and maintains the proton gradient across the vacuolar membrane by hydrolyzing the PPi as energy, thus enabling to transport other important ions and metabolites through the biomembrane. Though the research of H+-PPase in plants has been conducted, H+-PPase of Clostridium tetani (CtH+-PPase) was selected as the model for further studies in this thesis.

Materials and Methods

We thus replaced nineteen tyrosine residues in CtH+-PPase individually by alanine with the site-directed mutagenesis technique and analyzed their protein expression by western blot, pyrophosphate hydrolysis, proton-translocation and coupling ratio.

Results:

The enzymatic activities of mutants on Y175, Y226, Y392, Y414 and Y471 were significantly decreased. These five tyrosine residues are highly-conserved in several species. We then substituted these five tyrosine residues with other amino acids. The enzymatic activities of Y414S and Y414T were restored to approximately 75% of wild-type. From ion effects study, Y414 was also found to be associated with Na+-binding.

Conclusion:

According to the predicted three dimensional structure, the residues of Y175, Y226, Y392, Y414 and Y417 are faced to outer substrate-binding site and proton channel of CtH+-PPase,and therefore we speculate that the phenyl group of these significant tyrosines are play the important role in maintaining the structural stability,and the in Y414 the hydroxyl group is important to CtH+-PPas. The functional role of tyrosine residues in CtH+-PPase was substantially elucidated in our study

P958
Involvement of cyclooxygenase-2 in prostate cancer invasion and regulation of matriptase activation

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Backgrounds:

Chronic inflammation has been revealed to contribute to the early development and progression of cancer. COX-2 is a key rate-limiting enzyme and regulator of inflammation-producing prostaglandins. Several studies have suggested that COX-2 is commonly overexpressed and can modulate the cell migration and invasion of several human cancers such as colon, breast, lung and prostate cancers. High COX-2 expression level has been correlated with prostate cancer metastasis. However, the molecular mechanism how COX-2 promotes prostate cancer cell invasion is not well understood. In this study, I am interested in investigating the role of COX-2 in human prostate cancer cell invasion.

Materials and Methods:

We used cell invasion assays to examine the effects of COX-2 inhibitors (celebrex and sulindac sulfide) on prostate cancer cell invasion. Western blot analysis was utilized to detect the total level of matriptase and activated matriptase. To further address whether COX-2 could regulate the expression and activation of matriptase in prostate cancer, overexpression and shRNA knockdown approaches were used to alter the protein expression levels of COX-2. Moreover, the mRNA level of matriptase was detected by real-time RT-PCR

Results:

Celebrex and sulindac sulfide could suppress prostate cancer cell invasion, with reducing matriptase expression and activation. COX-2 knockdown resulted in decreasing the activated level of matriptase, a membrane-anchored serine protease and prostate cancer cell invasion. Using real-time RT-PCR, the mRNA level of matriptase was decreased in COX-2-knockdown PC-3 cells, compared to control cells. In addition, PC-3 cells with COX-2 overexpression could enhance prostate cancer cell invasion, partly due to up-regulating matriptase. These data indicated that inhibition of COX-2 by specific inhibitors or knockdown approaches could suppress prostate cancer cell invasion that was concurrent with down-regulating matriptase expression and activation.

Conclusion:

COX-2 is involved in promoting prostate cancer cell invasion, at least in part due to up-regulating matriptase expression and activation. Our data suggest that COX-2 is a potential target for the clinical prevention and treatment of prostate cancer.

P959
Tpl2 inhibitors thwart endothelial cells function in angiogenesis and peritoneal dissemination

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Backgrounds:

Angiogenesis is critical in the development of cancer, which involves several angiogenic factors in its peritoneal dissemination. The role of protein tumor progression locus 2 (Tpl2) in angiogenic factor-related endothelial cell angiogenesis is still unclear. To understand the precise mechanism(s) of Tpl2 inhibition in endothelial cells, this study investigated the role of Tpl2 in mediating angiogenic signals using in vitro, in vivo, and ex vivo models.

Materials and Methods:

In vitro, in vivo, and ex vivo model, PET-CT, Immunohistochemstry, Primary HUVEC, Western blot, aortic ring assay, Matrigel plug assay, tube formation.

Results:

showed that inhibition of Tpl2 inhibitor significantly reduced peritoneal dissemination in a mouse model by PET/CT imaging. Simultaneously, inhibiting Tpl2 blocked angiogenesis in tumor nodules and prevented angiogenic factor-induced proliferation and expression of proliferative marker PCNA in endothelial cells. VEGF or CXCL1 increased Tpl2 kinase activity and its phosphorylation in HUVEC in a dose- and time-dependent manner. Furthermore, Tpl2 inhibition or ablation by siRNA prevented the angiogenic signal-induced tube formation of endothelial cells in Matrigel plug assay or aortic ring assay. Inhibiting Tpl2 also prevented the angiogenic factor-induced chemotactic motility and migration of endothelial cells.

Conclusion:

Tpl2 inhibition by CXCL1 or EGF in endothelial cells was associated with inactivation of C/EBPβ, NF-κB and AP-1 and suppression of VEGF expression levels. Thus, Tpl2 inhibitors thwart a pathway of Tpl2-regulated VEGF by inactivating transcription factors involved in angiogenic factor-triggered endothelial cell angiogenesis. These results suggest that the therapeutic inhibition of Tpl2 may extend beyond cancer and include the treatment of other diseases involving pathologic angiogenesis.

P960
Mechanism of Licochalcone A induced-autophagic and apoptotic death in human cervical carcinoma SiHa cells

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Backgrounds:

Natural flavonoids have diverse pharmacological activities, including anti-oxidative, anti-inflammatory, and anti-cancer activities. Licochalcone A is an estrogenic flavonoid that has been shown to have anticancer properties in various types of human cancer cells. The aim of this study was to investigate the function and mechanism of apoptosis and autophagy induced by Licochalcone A on human cervical carcinoma cells.

Materials and Methods:

Human cervical carcinoma SiHa cell lines were treated with Licochalcone A to determine the effect on cell proliferation by MTT assay, colony-formation assay, DAPI staining, Annexin-V and the effect of autophagy by AVO staining and immunofluorescence assay. The molecular mechanisms of Licochalcone A mediated apoptosis and autophagy were further investigated by Western blotting analysis including activation of caspase cascade, autophagy marker, mTOR signaling pathway and PI3K/AKT pathway.

Results:

We found that Licochalcone A inhibits the viability of human cervical carcinoma cells in a dose- and time-dependent manner. Licochalcone A-mediated apoptosis of SiHa cells is accompanied by chromatin condensation and by phosphatidylserine exposure. The induction of autophagy was detected by monitoring the processing of an autophagy marker LC3, the aggregation of LC3 into granular structures and the formation of acidic organelles. Moreover, Licochalcone A inhibited a sustained activation of the phosphorylation of AKT, and the phosphorylation of mTOR pathways. Inhibition of autophagy using 3-methyladenine (3MA) almost enhanced Licochalcone A inhibited LC3 expression and induced caspases activation and apoptosis in SiHa cells.

Conclusion:

Taken together, we conclude that Licochalcone A exhibits autophagy and apoptosis-mediated antitumor activity in vitro. These findings define and support a novel function of autophagy in promoting death of cervical cancer cells.

P961

Anchorage-independent growth of melanoma cells affected malignancy by shift of IL-8/IL-8Rs functional axis.

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Backgrounds

Survival of tumor cells under anchorage-independent growth is one of features for malignant melanoma at initial stage of metastasis. The purpose of this study was to explore the specific gene alterations associated with tumor malignancy, which was contributed by anchorage-independency.

Materials & Methods

We generated the anchorage-independent melanoma cells by suspension culture. Tumor formations by subcutaneous inoculation of suspended and adherent melanoma cells at nude mice were compared. The cDNA microarray and bioinformatics analysis was used to explore the potential genes associated with melanoma malignancy under anchorage-independency. The qPCR, western blot and ELISA methods were adapted to validate the gene expressions.

Results

The animal experiments on subcutaneous tumor formation showed relatively larger tumors by inoculations of suspended melanoma cells than by those of adherent melanoma cells. Microarray analysis combining gene-set enrichment analysis (GSEA) showed the up-regulation of IL-8 was potential and might be responsible for tumor malignancy. However, the IL-8 receptors, CXCR1 and CXCR2, were both downregulated under anchorage-independency but were restored upon reattachment. Interestingly, remained upregulation of IL-8 and elevated expression of CXCR1 after reattachment implied their roles in subcutaneous tumor formation.

Conclusion

We showed the anchorage-independency led to shift of IL-8 function exclusively as paracrine by upregulation of IL-8 and downregulation of IL-8 receptor (CXCR1 and CXCR2). Malignancy derived from anchorage-independent growth would be of interest, and its characterization and understanding in tumor metastasis would be potential.

P962

The Growth Inhibitions Effects of Propolin C on Human Breast Cancer Line MCF-7

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Backgrounds:

Autophagy is a housekeeping survival mechanism with a protective function against apoptosis. Propolin C (PPC), a novel prenylflavanones is isolated and characterized from Taiwanese propolis could inhibit MCF-7 cells growth. The growth inhibitions were not through apoptosis but induce autophagy. 3-Methyladenine (3-MA) is used to inhibit and study the mechanism of autophagy and apoptosis under various conditions.

Materials and Methods:

Propolin C treated MCF-7 cells for 72hr and the cell viability were assessed by MTT assay. The cellular protein change were detected by western blot. Finally, autophagy inhibitor 3-methyladenine (3MA) combined with propolin C were assay to evaluate the effects of the MCF-7 cells.

Results:

The cytotoxicity effect of propolin C was first evaluated in the human breast cancer cells MCF-7. The IC50 values of propolin C on the human breast cancer cells was 20 μM. PPC could not trigger cell induced apoptosis but autophagy. Cell morphology by hematoxylin-eosin stain showed that MCF-7 cells found some vacuoles in protoplasm with propolin C treated after 24hr. Propolin C treatment MCF-7 cells could increase autophagy specific protein LC3 expression and these were time and dose dependent increase. When PPC combined with the autophagy inhibitor 3-methyladenine giving rises to increased the resistance of growth inhibition. The LC3 protein were dramatically increased and the pERK were down-regulation.

Conclusion:

In this study we showed that MCF-7 cell survival rate was inhibited by propolin C in a dose-dependent manner. Furthermore, we found that autophagy was activated after MCF-7 cells were incubated with propolin C,and upregulation of LC3 protein. In this study, autophagy serves as a survival pathway to protect the cell from stress.

P963

Lipocalin-2 suppresses the growth and invasion of human prostate cancer cells

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Backgrounds:

Lipocalin-2 (LCN2) is a member of lipocalin superfamily and has an important role in the regulation of cellular oncogenesis and apoptosis, however, the role for LCN2 in prostate cancer remains poorly understood

Materials and Methods:

Expression of LCN2 has been determined by western blotting, qRT-PCR and immunohistochemistry in the human prostate cell lines PC3, DU145, LNCaP, 22Rv and human prostate cancer tissues array. We identified shRNA-LCN2 functional approaches were subsequently applied to determine the role of LCN2 in prostate cancer cell proliferation, migration, invasion. We studied prostate cell proliferative ability were measured by MTT, colony-forming and cell cycle analysis. We studied the role of LCN2 in prostate cell migration and invasion by cell migration assay and matrigel invasion assay. Effect of LCN2 knockdown on prostate tumor growth was assessed in a subcutaneous xenograft model.

Results:

LCN2 protein and mRNA expression are higher in PC3 and DU145 cells than LNCaP and 22Rv cells, and prostate tissue significantly correlated with tumor differentiation ($P<0.017$) and Gleason grade ($P<0.007$). Knockdown of LCN2 in PC3 and DU145 cells decreased cell proliferation, colony formation, cell cycle arrest, migration and invasion. Conversely, overexpression of LCN2 led to the opposite effect in 22Rv cells. In subcutaneous xenograft tumor mice, decreased tumor growth was observed in the LCN2-knockdown mices.

Conclusion:

Our results suggest that LCN2 might play an important role in regulation of proliferation and invasion in human prostate cancer, and could be a potential value as a marker of prostate cancer progression

P964

Matriptase is involved in EGF-induced colorectal cancer cell invasion.

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Backgrounds:

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death worldwide and in Taiwan. Most CRCs appear in the rectum (38%) or sigmoid colon (29%). Cancer cell invasion and metastasis are main causes for patients' death. However, the molecular mechanisms how cancer cells acquire metastatic potentials are still poorly understood. It has been shown that EGFR is overexpressed in a variety of human tumors including colon cancer, which is concurrent with poor prognosis and high metastasis. Cancer metastasis and cell invasion have been shown with up-regulation of pericellular proteolysis including MMPs or serine proteases. Among them, membrane-anchored serine protease matriptase recently has received more attentions because its expression levels are correlated with prostate cancer progression and EGF can induce matriptase activation. To further delineate if dysregulated EGFR signaling to promote colorectal cancer progression to a metastatic or invasive stage is via up-regulating pericellular proteolysis, we propose that matriptase is involved in EGF signaling-induced colorectal cancer (CRC) cell invasion.

Materials and Methods:

In this study, I used a colon cancer progression model (KM12C, KM12-L4, and KM12-SM). KM12C cells are parental colorectal cancer cells. KM12-L4 and KM12-SM cells are highly invasive and isolated in vivo from the metastatic nodules in the mouse liver tissues after primary injections of KM12C cells into animal spleen and colon tissues, respectively. Cell invasion and migration were analyzed using transwell assays. We detected the protein level by using Western blot. The gene expression levels were analyzed by real-time PCR.

Results:

The results showed that the expression levels of EGFR and matriptase were increased in highly invasive KM12-L4 and KM12-SM cells, compared to parental KM12C cells. The activated levels of matriptase but not MMP-2/-9 were correlated with the capability of the cancer cell invasion. Moreover, EGF could induce KM12C cell invasion and matriptase activation. To further analyze the involvement of matriptase in EGF-induced colorectal cancer cell invasion, I knocked down matriptase in KM12C cells and found that matriptase knockdown reduced the stimulatory effect of EGF on the cell invasion.

Conclusion:

Our data indicate that matriptase is involved in EGF signaling to promote colorectal cancer cell invasion.

P965

Essential role of microRNA-21 for ovarian teratocarcinoma PA1 cell growth through sustaining cancer stem/progenitor populations

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Backgrounds:

Cancer stem/progenitor cells (CSPCs) resist to chemotherapy leads to cancer relapse is one obstacle in cancer therapy. Ovarian teratocarcinoma (OVTC) arises from germ cells and also contains pluripotent characteristics that are excellent tools for study cancer stemness. This study demonstrated that microRNA-21 (miR-21) is essential for OVTC growth through maintaining CSPCs populations.

Materials and Methods:

Ovarian teratocarcinoma cell line PA1 was manipulated to overexpress or knockdown miR-21 by lentiviral deliver system. After analyzing of miR-21 expression in PA1 cells, cell growth assay was assessed at every given time point. CD133 stem cell marker antibody was used to identify CSPCs in PA1 cells, and miR-21 expression level in enriched CSPCs was determined. To assess miR-21 effects on CD133+ population progression, stem cell functional assays (sphere assay, CD133 expression) were used to analyze role of miR-21 in PA1 CSPCs.

Results:

We showed that knockdown of miR-21 in PA1 cells resulted in abolished PA1 cells growth, whereas overexpression of miR-21 promoted it. Moreover, miR-21 was up-regulated in CD133+ enriched PA1 cells. In order to delineate the relation of miR-21 mediated cell growth through CSPC self-renewal, we knockdown miR-21 and found abolished CD133+ population and CSPCs sphere formation. On the contrary, miR-21 overexpression promoted both CD133+ population and CSPCs sphere formation. This is the first report to examine miR-21 in CSPCs of OVTC cells, and found essential role of miR-21 in cancer growth.

Conclusion:

This miR-21 effect is major go through regulating cancer stemness in which might be therapeutic target for the future.

P966

To Investigate Antifibrotic Effect of Propolis and Its Extracts Propolin G on Rat Hepatic Stellate Cells.

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Backgrounds:

According to the Department of Health statistics, chronic and cirrhosis of liver diseases are two of the top ten causes of death in recent years. For all of these diseases there is a common pathologic mechanism that leads to fibrosis. Many studies are being made to find a treatment or amelioration of liver fibrosis drugs. Propolis is a complex mixture of which the main bioactive compound is polyphenolic. Several studies show that propolis has antibacterial, antiviral, antioxidant, anti-inflammatory and anti-tumor activity. Prenylflavanones is main bioactive of Taiwanese green propolis, from which propolins are isolated.

Materials and Methods:

In this study, we use propolin G which is isolated from Taiwanese propolis to investigate the anti liver fibrosis drugs. In liver, active hepatic stellate cells are the generated liver fibrosis reason, and as liver injury gets worse, α-smooth muscle actin will increase and result to extracellular matrix excessive accumulation leading to fibrosis. In this study, we use rat hepatic stellate HSC-T6 cell lines to investigate how propolin G influences cells. By using TGF-β1, we stimulate cell activation and treat propolin G to investigate how possible mechanism of protein presents on α-SMA, collagen I, TGF-β, MMP-2, TIMPs, MAPK, TGFβ/Smad and cell cytotoxic,

Results:

The result showed that TGF-β1 stimulated cell activation and caused inflammation and fibrosis-related protein expression to increase, and propolin G downregulated MAPK, TGFβ/Smad pathway to inhibit HSCs activation and decreased liver fibrosis.

Conclusion:

Therefore, propolin G has a great potential for the prevention or treatment of liver fibrosis drugs.

P967

Produce Acute Acetaminophen Hepatotoxicity in Mice by Injection of Propacetamol and Study the Prevention of Galangal rhizome Extract.

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Backgrounds:

Acetaminophen (APAP)-induced liver injury (AILI) represents the most frequent cause of drug-induced liver failure in the United States. It is also the recommended hepatoprotective evaluated method in Department of Health in Taiwan. This is the first report for successful and stable induction of acute AILI by injection of Propacetamol in mice. Galangal rhizome has been used for its antipyretic and anti-inflammatory qualities, and it is used in treatments of various diseases such as bronchitis, heart diseases, chronic enteritis, diabetes, rheumatism and kidney disorders. Here, we examined whether Galangal rhizome extract can prevent the AILI in mice.

Materials and Methods:

Male BALB/c mice were treated orally with two doses (500 and 1,000 mg/kg) of Galangal rhizome extract and silymarin, respectively, and then 600mg/kg Propacetamol (equivalent 300 mg/kg APAP) was IP injected 1.5hr later to induce AILI. Mice were fasted for 12hr before oral treatment and injection. After injection, mice were given tap water and ad libitum. The levels of AILI were assayed by ALT and AST activities in peripheral blood at 12hr after Propacetamol injection.

Results:

The ALT and AST activities of fasted only mice were 42.6±4.8 and 67.3±3.3. Mice injected with Propacetamol had obviously increased ALT and AST activities for 3942±488 and 3047.4±18 . Mice with 1,000 mg/kg silymarin treatments showed obvious decreases of ALT and AST activities for 827.3±543.1 and 526.1±286.1. Mice with 500mg/kg Galangal rhizome extract treatment showed slightly decreases in ALT (2888.7±138.0) and AST (1236.7±39.0) levels. However, mice with 1000mg/kg Galangal rhizome extract treatment showed obvious increases of ALT (13189.0±994.2) and AST (8156.1±668.9) levels that higher than Propacetamol injected only in mice.

Conclusion:

We establish an acute AILI mouse model by injection of 600mg/kg Propacetamol. The known liver protective extract, silymarin, can obviously reduce liver damage in these AILI model mice. Galangal rhizome extract of 500mg/kg shows a slight but obvious protective effect, but the high dose (1,000 mg/kg) of extract enhances the hepatotoxicity in these AILI model mice. We will try to find the most effective dose of Galangal extract and investigate its liver protection mechanism(s).

P968

Structural Analysis of Catalytic Active Site and Activity of Human Nit2/ωamidase: Kinetic Assay and Molecular Dynamics Simulation

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Backgrounds:

Human nitrilase-like protein 2 (hNit2) is a putative tumor suppressor, recently identified as ω-amidase. hNit2/ω-amidase links sulfur metabolism to the tricarboxylic acid cycle.. hNit2/ω-amidase plays a crucial metabolic role by catalyzing the hydrolysis of α-ketoglutaramate and α-ketosuccinamate, yielding α-ketoglutarate and oxaloacetate, respectively. Transamination between glutamine and α-keto-γ-methiolbutyrate closes the methionine salvage pathway. The aim for the study is to elucidate the catalytic specificity of hNit2/ω-amidase, We performed molecular dynamics (MD) simulations on the wild type enzyme and its mutants to investigate enzyme-substrate interactions. Binding free energies were computed to characterize factors contributing to the substrate specificity.

Materials and Methods:

Docking and molecular dynamics simulation , CD and enzyme kenetic analysis were employed. Wild type and mutant type protein were used in the kinetic studies and CD analysis.

Results:

The MD simulations successfully verified the experimental trends in the binding specificity of hNit2/ω-amidase toward various substrates. Our findings have revealed novel structural insights into the binding of substrates to hNit2/ω-amidase. A catalytic triad and the loop residues 116-128 of hNit2 play an essential role in supporting the stability of the enzyme-substrate complex resulting in the generation of the catalytic products.

Conclusion:

These observations are of benefit in the design of inhibitors of hyperammonemic diseases.

P969

A Novel calcium binding cluster of Leptospira LipL32 elicits inflammatory responses through TLR2 signal pathway

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Backgrounds:

Leptospirosis is the most prevalent zoonosis caused by pathogenic Leptospira worldwide. LipL32, a 32 kDa lipoprotein, is the most abundant protein on pathogenic Leptospira outer membrane with an atypical polyD motif (D161DDDDGDD168). The X-ray crystallographic structure of LipL32 unveiled that the calcium binding cluster of LipL32 is composed of several essential residues including Asp132, Thr133, Asp164, Asp165, and Tyr178, respectively.

Materials & Methods:

Site-directed mutagenesis was employed to individually mutate Ca2+-binding residues of LipL32 to Ala, and their effects subsequently observed by CD, fluorescence spectra. The Enzyme-linked immunosobent assay (ELISA) and atomic force microscopy (AFM) analysis further demonstrated the binding of LipL32 variants to TLR2. The inflammatory responses as determined by real-time PCR were induced by LipL32 variants.

Results:

The purposes of this study were to determine conceivable roles of Ca2+ binding cluster in LipL32 on the interaction of Toll like receptor 2 (TLR2) and succeeding induced inflammatory response of human kidney cell. Site-directed mutagenesis was employed to individually mutate Ca2+-binding residues of LipL32 to Ala, and their effects subsequently observed. The mutation abolished primarily the calcium binding to LipL32. The ELISA and AFM analysis further demonstrated a diminution in the binding of LipL32 variants to TLR2. The inflammatory responses induced by LipL32 variants, as determined by TLR2 signal pathway genes containing hCXCL8/IL-8, hCCL2/MCP-1, hMMP7, and hTNF-α, were also lessened.

Conclusion:

In conclusion, the calcium binding cluster of LipL32 plays essential roles presumably in maintaining the conformation for its appropriate association with TLR2 to elicit inflammatory responses of human renal cells.

P970

Deciphering the Mechanistic Action of Swi5-Sfr1 Complex Facilitating Rad51-mediated Recombination

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Homologous recombination (HR) represents a major error-free pathway to eliminate pre-carcinogenic chromosomal lesions. The DNA strand invasion reaction in HR is mediated by a helical filament of the Rad51 recombinase assembled on single-stranded DNA that is derived from the nucleolytic processing of the primary lesion. Recently genetic and cytological studies in fission yeast and mammal indicate that Swi5 and Sfr1 play a role in recombinational repair and facilitate Rad51-mediated recombination. Here, we provide biophysical evidence that the mouse Swi5–Sfr1 complex has a 1:1 stoichiometry. Importantly, the Swi5–Sfr1 complex, but neither Swi5 nor Sfr1 alone, physically interacts with Rad51 and stimulates Rad51-mediated homologous DNA pairing. This stimulatory effect stems from the stabilization of the Rad51–ssDNA presynaptic filament. Interestingly the Swi5–Sfr1 complex enhances the ATP hydrolysis of Rad51. It implied that the mechanism on the stimulation of Rad51 activity by the Swi5–Sfr1 complex is different compared to other accessory factors. We also provide evidence that the RSfp (rodent Sfr1 proline rich) motif in Sfr1 serves as a negative regulatory element. These results thus reveal an evolutionarily conserved function in the Swi5–Sfr1 complex and furnish valuable information as to the regulatory role of the RSfp motif that is specific to the mammalian Sfr1 orthologs. Our recent progress on deciphering how Swi5-Sfr1 complex regulates Rad51 activity will be presented.

P971

Extract of Morus Australis Roots Inhibits Tumor Promoter 12-O-Tetradecanoylphorbol-13Acetate -Induced Transformation of Epidermal JB6 Cl41 Cells

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Backgrounds:

The roots of Morus australis have been used as a traditional Chinese medicine for the treatment of diabetes, arthritis, and rheumatism. In recent years, it evaluated as skin-whitening cosmetics. Other biological activity is not well known. In our preliminary study, it showed ethanol extract of Morus australis roots (MRE) contained polyphenolic composition which may possess chemoprevention properties. Chemoprevention has been acknowledged as an important and practical strategy for the management of cancer. The intervention of cancer in the promotion stage seems to be most appropriated and practical since tumor promotion is a reversible event. In the present study, whether roots of Morus australis are attributed to antitumor promotion potential are evaluated by JB6 mouse epidermal cell model.

Materials and Methods:

First, ethanol extract of Morus australis roots (MRE) was prepared. Cytotoxicity of MRE in JB6 Cl41 cells was assessed by MTT assay. Noncytotoxic concentrations of MRE were evaluated by anchorage-independent growth assay with 12-O-tetradecanoylphorbol- 13 acetate (TPA) as inducer in JB6 Cl41 cells. Further, effects of MRE on TPA –induced cell adhesion and cell migration in JB6 C141 cells were determined. Effect of MRE on TPA-induced morphology change was observed by TRITC-conjugated phalloidin staining of the actin cytoskeleton. Finally, effects of MRE on TPA-induced molecular expression and signal activation involving cell transformation were evaluated by western blot analysis.

Results:

In JB6 Cl41 cells, 12-O-tetradecanoylphorbol-13 acetate (TPA) induced tumorigenic anchorage-independent colonies in soft agar. In the present study, we found pre-treated MRE inhibited TPA-induced tumorigenic anchorage-independent colonies in soft agar in a dose dependent manner after 42 days culture. In addition, MRE inhibits TPA-induced actin rearrangement, cell adhesion and cell migration in JB6 Cl41 cells. Western blot analysis showed MRE inhibited TPA-induced epithelial-mesenchymal transition (EMT), integrins expression and signal activation in JB6 Cl41 cells.

Conclusion:

These results highlight Morus australis roots possessing antitumor promotion activity.

P972

Mulberry Water Extracts Improve Postmenopausal Osteoporosis via Regulating Osteoblast Differentiation

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Backgrounds:

Menopause is a major cause to induce osteoporosis in women. Osteoporosis is highly relative to oxidative stress. Mulberry was showed to possess good anti-oxidative ability. Previous results show mulberry water extract decelerates osteoporosis in ovariectomic rat and also increases the expression of Runx2. Herein, it is aimed to explore the mechanism of mulberry water extract in regulating osteoblast.

Materials and Methods:

The osteoblast precursor cell MC3T3 was treated with conditional medium from the Chinese Hamster Ovary cell and compared with the groups treated with various concentration of MWE. After completing the differentiation, the alkaline phosphatase activity, Alizarin-red stain, and the expression of proteins related to differentiation are detected.

Results:

The results show that high doses of MWE can improve the activity of alkaline phosphatase, bone mineralization, and alter the expression of proteins in regulating osteoblast differentiation.

Conclusion:

The present results point that MWE have the potential to decelerate the postmenopausal women from osteoporosis via regulating osteoblast differentiation.

P973

Zebrafish Embryos Derived from Transgenic Line huORFZ Serve as a Living Biosensor for Monitoring Environmental Toxicants

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Backgrounds:
A well-controlled aquatic animal model is an invaluable tool for monitoring the extent of pollution in the water environment. A novel transgenic zebrafish line huORFZ, in which a human upstream open reading frame (huORF) of the chop gene (huORFchop) fused with GFP reporter is harbored, has been reported (Lee et al., 2011). When stresses are induced to the huORFZ embryos, translation inhibition directed by huORFchop cassette is blocked, which in turn, GFP is expressed. Based on this finding, we next explored the potential of huORFZ embryos for monitoring pollution in the aquatic environment.

Materials and Methods:
huORFZ embryos at 72 hpf were used for toxicity tests of all chemicals. To set up standards of treatments, lethal concentrations were calculated from a linear regression of log probit transformations of the dose response data. The embryos were observed under the microscope to determine if obvious fluorescent signals were induced. TUNEL assay was performed using Roche in situ cell death detection kit following the manufacture's instruction.

Results:
During stress induction, emergence of GFP positively correlates with the increase of endogenous CHOP expression in the embryos. Control embryos in embryo media showed no GFP signals during the experimental period. When embryos were treated with hazardous substances, such as heavy metals and endocrine-disrupting chemicals at their sublethal concentrations (LC50), huORFZ embryos displayed different tissue-specific GFP expression. For example, GFP was apparent in the brain and muscle of embryos treated with 0.1 mM AlCl3, but it was expressed in the lateral line system of embryos incubated with 0.52 mM As2O3. Copper (Cu2+), cadmium (Cd2+) and Chlorpyrifos were selected for further analysis. Signals of TUNEL assay increased in huORFZ treated with stresses. The intensity of GFP signals in huORFZ embryos was in a dose-dependent manner. Increased GFP responses were also observed in other tissues after Cu2+ and Cd2+ treatments were further increased to 48 hr. Removal of stresses partially rescued the huORFZ embryo population from death. Moreover, GFP signals and TUNEL signals declined when huORFZ embryos were displaced to normal embryo medium. This line of evidence indicated that huORFZ possess the ability to monitor changes in the quality of an aquatic environment. Significant GFP response could be observed in huORFZ embryos at 0.5 ppb of Cd2+ and 50 ppb of Cu2+, which are beneath the guideline values of drinking water. Finally, we used huORFZ system in field monitoring. The GFP expression of huORFZ embryos truthfully reflects the existence of pollution in the river water. The unique GFP expression manner, such as spotted skin pattern can even be an indicator of potential pollutants. In relative, no signals were shown to the treatment of controlled river water.

Conclusion:
Different from other biomonitoring animals, huORFZ eliminate time-consuming and complex analysis in physiological conditions. Taken together, huORFZ embryos can serve as a live biosensor with high sensitivity to monitor potential contaminants in the aquatic environment both under lab conditions and field work. They can even indicate if a given concentration of pollutant reaches the point of subcellular stress.

P974

The flavonoid-rich extract from Nelumbo Nucifera leave inhibited fatty liver of type II diabetes mellitus

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Backgrounds:
Type II diabetes mellitus (type II DM) patients have insulin resistance, which leads glucose fail to transport for energy metabolism and causes hyperglycemia. Hyperglycemia induces the generation of reactive oxygen species (ROS) and enhances oxidative stress. Overproduction of free radicals causes various diabetic complications including hyperglycemia, hypertension, hyperlipidemia and other. The objective of the present study was to estimate the anti-diabetes effect of the flavonoid-rich extract from Nelumbo Nucifera leave (NLE) in a rat model.

Materials and Methods:
Consumption of HFD has generally been considered a significant contributor to the development of obesity and insulin resistance. In this study, low dose streptozocin (STZ) was administrated to induce type II DM in HFD-fed SD rats. After the STZ induced, the effect of flavonoid-rich extract from Nelumbo Nucifera leave (NLE) was observed in type II DM rats for 24 weeks. Further we investigated the mechanism by western blotting, immunohistochemistry and anti-oxidant enzyme activities in the liver of type II DM rats. Moreover, fasting blood glucose, fasting insulin, total cholesterol, and triglyceride were measured to evaluate the dynamic blood sugar and lipid metabolism.

Results:
High-fat diet treated rats combined with a single injection of low doses of STZ (30 mg/kg), exhibited significant increase in body weight, basal plasma glucose, triglycerides and total cholesterol levels, but the plasma insulin level was significantly decreased as compared to ND-fed control rats. We found that NLE could reduce lipid accumulated in liver in type II DM rats. Furthermore, we demonstrated that dietary administration of NLE increased anti-oxidant enzymes and through regulated MAPKs and Akt protein expression to reduce fatty liver formation in type II DM rat. We presented evidence that NLE promoted AMP-activated protein kinase (AMPK) energy-sensing pathway to regulate liver lipid metabolism. These results indicate that the NLE not only ameliorate the complications of type II DM, but also attenuate lipid accumulation in liver in type II DM rats.

Conclusion:
In brief, NLE attenuated diabetic fatty liver of type II DM rats. These data suggested that NLE might provide alleviative effects against diabetic deterioration.

P975

Nelumbo Nucifera leaves Extract Inhibits high glucose-Stimulated Vascular Smooth Muscle Cell Migration Through Inhibition of Akt/Mammalian Target of Rapamycin Pathway.

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Backgrounds:
Diabetic patients are at high risk to develop atherosclerotic cardiovascular disease and have a higher restenotic rate after percutaneous coronary intervention (PCI). High glucose promotes vascular smooth muscle cell (VSMC) mitogenesis and migration. We investigated the inhibitory effect of Nelumbo Nucifera leave extract (NLE) on High glucose-induced migration of vascular smooth muscle cells.

Materials and Methods:
In vascular SMCs, A7r5 cell line, high glucose (25 versus 5.5 mmol/L) induced a rapid and sustained increase in proliferation and migration. The inhibitory effect of NLE on VSMCs mobility was measured by boyden chamber, wound healing and ECIS assay. Western blot assay was used to analyze the mechanism of NLE decimated migration in VSMCs by high glucose-induced.

Results:
High glucose treatment enhanced the migration in VSMCs, and this increase was inhibited significantly by NLE treatment. To explore the intracellular mechanisms of action by which NLE affects this process, we examined the effect of NLE on the migration characteristics of high glucose-induced VSMCs. NLE dose-dependently inhibited VSMC migration, decreased Akt/mTOR pathway activity, reduced RhoA, cdc42 and Rac1 expression, diminished ROCK1, ROCK2 and CTGF expression. In addition, NLE inhibited the formation of DCF-sensitive intracellular reactive oxygen species (ROS) by HG-induced and increased anti-oxidant enzyme expression.

Conclusion:
Our results showed that NLE exerts multiple effects on HG-induced VSMC migration, including the inhibition of AKt/mTOR protein activity, and the downregulation of ROS signaling, thereby suggesting that NLE may be a possible therapeutic approach to the inhibition of diabetic vascular disease.

P976

Glutathione S-transferase Mu2 Suppresses Cancer Cell Metastasis in Non-Small Cell Lung Cancer

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Backgrounds:
Glutathione S-transferase Mu2 (GST-M2) is a phase II detoxification enzyme. Low expression of GST-M2 in lung cancers is due to hypermethylation of its promoter. Lung cancer with the GST mu-null genotype is associated with shorter survival. However, a correlation between GST-M2 and important clinical parameters, as well as the role of GST-M2 defective cells in lung cancer, has not been explored by environmental pollutant such as nickel.

Materials and Methods:
The expression of GST-M2 was detected by RT-PCR and REAL-TIME PCR. The migration ability of overexpressed or silenced GST-M2 cancer cells was performed on wound healing and Boyden chamber assays. The cellular morphology and actin disassembly of GST-M2 overexpressed cancer cells were visualized by confocal microscopy. With or without GST-M2 expression on tumor growth and metastasis were investigated in xenograft tumor model.

Results:
The expression of GST-M2 was decreased under NiCl2 exposure when the down-regulated GST-M2 mRNA level was restored in the CL1-0 cells under 5-Aza-dC treatment. The GST-M2 promoter was hypermethylation under NiCl2 exposure. We found that high expression of both GST-M2 and connective tissue growth factor (CTGF) was correlated with favorable survival of lung cancer patients when compared to similar patients without GST-M2 and CTGF expression. GST-M2 can induce CTGF expression by driving the CTGF proximal promoter. Overexpression of GST-M2 decreased the formation of the filopodia, resulting in a remodeling of the reorganized cytoskeletons. Overexpression of GST-M2 significantly suppressed and reduced tumor growth and metastasis in xenograft mice model.

Conclusion:
These data highlight the potential of GST-M2 as a novel tumor suppressor. GST-M2 increases the expression of CCN2 in lung cancer cells, which inhibits cancer cell migration in lung cancer and animal models.

P977
Glycated Bovine Serum Albumin Shows a Novel Membrane-Damaging Activity

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Backgrounds:
Advanced glycation end products (AGEs) are formed by non-enzymatic modification between reducing sugars and proteins. Previous studies suggest that AGEs bind to specific membrane receptor and activate cellular signal pathway implicated in diabetes related complications. Nevertheless, AGEs show some effects via receptor-independent pathway. Thus, the existence of the direct interaction between AGEs and cell membrane may be considered.
Materials and Methods:
In this study, bovine serum albumin (BSA) was glycated with mannose or glucose, and comparative studies on the physicochemical properties of glycated BSA was conducted using mass analyses, HPLC, fluorescence and CD measurement. The membrane-damaging activity, membrane perturbation effect and fusogenicity of glycated BSA on egg yolk phosphatidylcholine (EYPC)/ egg yolk sphingomyelin (EYSM) (10/9, mol/mol) and EYPC/EYSM/cholesterol (Chol) (37/33/30, mol/mol/mol) vesicles were investigated using fluorescence measurement. Moreover, the conformation of lipid-bound glycated BSA was analyzed by Fourier transform infrared spectroscopy (FTIR).
Results:
Conjugation of mannose notably reduced the hydrophobicity of BSA compared with that of glucose. Unlike that native BSA and glucose-modified BSA (Glu-BSA), mannose-modified BSA (Man-BSA) showed a reduction in intrinsic fluorescence. The structural stability of Man-BSA was higher than that of BSA and Glu-BSA. In contrast to BSA and Glu-BSA, Man-BSA showed notable membrane-damaging activity on EYPC/EYSM and EYPC/EYSM/Chol vesicles. Nevertheless, the binding-affinity of BSA, Glu-BSA and Man-BSA for EYPC/EYSM and EYPC/EYSM/Chol vesicles was similar. Noticeably, Man-BSA induced fusion of EYPE/EYSM/Chol vesicles but not of EYPC/EYSM vesicles. Moreover, EYPC/EYSM-bound and EYPC/EYSM/Chol-bound man-BSA showed distinct structure as evidenced by FTIR.
Conclusion:
Our data indicate that man-BSA shows a novel membrane-damaging activity, and that Chol affects the mode of man-BSA on damaging membrane bilayers.

P978
Evaluation of Triazole Pesticides Mixture Effects on Hepatotoxicity by 28 Days Subacute Oral Repeated Dose Toxicity Studies in Rats

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Backgrounds:
Risk assessment for pesticide usually focuses on single pesticide, but farmers may use different pesticides in the field for plant disease control in order to avoid pesticide resistance. So naturally, when people eat different crops may also be exposed to many different types of pesticides at the same time.
Materials and Methods:
This study addresses human health cumulative risk assessment associated with combination of different pesticides which have the same toxicity action mode. Until now, the triazole pesticides still remain the risk to induce hepatotoxicity. In this study, 28 days subacute oral repeated dose toxicity studies were conducted to evaluate the cumulative risk assessments by combined exposure to hexaconazole, difenoconazole and flutriafol triazole pesticides.
Results:
Results showed that combined with difenoconazole 300mg/kg/day and hexaconazole 150 mg/kg/day may aggravate the acute toxicity, relative liver weight, liver hypertrophy and hepatolipidosis induced by either one. In the meantime, combined with difenoconazole 300 mg/kg/day and flutriafol 500 mg/kg/day aggravated relative liver weight and liver hypertrophy induced by either one at high dose level.
Conclusion:
Finally, we expect these results can supply the information for risk assessment on hepatotoxicity induced by mixed triazole pesticides.

P979
Effects of Geloina eros on hepatoprotective and cytotoxicity

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Backgrounds:
We investigated the effect of Geloina eros extract against carbon tetrachloride (CCl4)-induced hepatotoxicity and cytotoxicity.
Materials and Methods:
The male Wistar rats were randomly divided into seven groups with each consisting of 8 rats. Group A: basal diet, Group B: basal diet containing 6% Geloina eros, Groups C-F the rats with carbon tetrachloride (CCl4)-induced liver damage. Group C served as control CCl4. Groups D-F were administered orally the Geloina eros in diet for 6%, 7%, 8%, Group G served as positive control and was given silymarin in diet for 12%, Geloina eros were tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric and trypan blue dye exclusion assay on the growth of Hep G2.
Results:
Our results showed that treatment with Geloina eros extract for 8 weeks significantly reduced the impact of CCl4 toxicity on the serum markers of liver damage, aspartate aminotransferase (AST), alanine aminotransferase(ALT) and alkaline phosphatase (ALP) Antioxidant system was significantly enhanced in the plasma, andhepatic thiobarbituric acid reactive substance (TBARS) levels were lowered while the hepatic superoxide dismutase(SOD) and catalase (CAT) activities and glutathione peroxidase (GSH-Px) protein level and nonenzymatic antioxidants (vitamin E, vitamin C and GSH) were elevated, Geloina eros showed cytotoxic effects on the Hep G2.
Conclusion:
The results indicated that Galina eros extract has a protective effect against acute hepatotoxicity induced by the administration of CCl4 and was found to be comparable to that of silymarin and have been supported by the evaluation of the liver histopathology in rats. The hepatoprotective effects of Geloina eros extract may be due to both the inhibition of lipid peroxidation and the increase of antioxidant activity, useful in hepatocancer cell line chemotherapy.

P980
Enhancement of 5-HT2A Receptor-mediated Responses After a Binge Regimen Methamphetamine in Mice

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Backgrounds:
Repeated administration of methamphetamine (MA) to animals during a single-day multiple-dose produces long-lasting decreases in markers of dopaminergic and serotonergic terminals as well as persistent behavioral deficits in MA abusers.
Materials & Methods:
As 5-HT2A receptors are associated with hallucinations, the main characteristic of MA psychosis, the present study focused on the effects of binge MA regimen on 5-HT2A receptors. Male ICR mice were received one day drug treatment with four injections of MA (4 x 5 mg/kg) or saline at 2 hours interval.
Results:
The binge regimen of MA produced significant impairment in prefrontal serotoninergic terminals and augmented the 5-HT2A receptor-dependent behavioral and molecular responses, showing higher hallucinogenic 5-HT2A/2C receptor agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI)-induced head-twitch response and c-Fos andEgr-2 expression. Furthermore, the levels of 5-HT2A receptors in the prefrontal cortex and hippocampus were increased by MA treatment, whereas 5-HT2C, 5-HT1A, and mGluR2 receptors were unaffected.
Conclusion:
These data reveal that MA-exposed mice with higher levels of behavioral and molecular responses to hallucinogens might be associated with an up-regulation of 5-HT2A receptors in the prefrontal cortex. The biochemical alterations that parallel the behavioral changes observed in a mouse model of MA associated with psychosis may facilitate targeting therapies for treatment and prevention of MA-related psychiatric disorders.

P981
Betaine Facilitates Memory Consolidation for Extinction of Methamphetamine-induced Conditioned Place Preference

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Backgrounds :

Methamphetamine (MA) is a powerful and highly addictive psychostimulant. Betaine is a glycine N-methylated compound, which potentially activates NMDA receptors.

Materials & Methods:

The present study examined the effect of betaine on distinct phases of MA conditioned place preference (CPP) in rats. Administration of betaine (100 mg/kg) 30 min prior to each MA (2 mg/kg) conditioning trial did not affect MA CPP. When the mice exhibiting MA CPP received betaine (100 mg/kg) immediately after confined extinction, with saline injection in previous MA-paired compartment for 3 days, MA CPP expression was significantly reduced during retest.

Results :

In addition, betaine reduced the MA (1 mg/kg)-induced reinstatement.

Conclusion:

These results demonstrated that betaine facilitates memory consolidation for extinction of approach behavior to environmental stimuli previously paired with MA and suppresses MA-induced reinstatement of CPP behavior, suggesting that betaine is a potential therapeutic agent for MA addiction.

P982
Production of Polyclonal Antibody and Development of ELISA for Melamine

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Backgrounds:

Melamine has been banned on adding to foods and feeds since the 2008 Chinese milk scandal. In many food recalls, melamine could increase the product's protein content but cause kidney failure in infants and pets. In this study, we would like to production of polyclonal antibody against melamine and development of ELISA to detect melamine in foods and feeds.

Materials and Methods:

2-chloro-4,6-diamino-1,3,5-triazine (CAAT), an analogue of melamine, was taken to conjugate with the immunogen for diamino-s-triazine conjugates. CAAT and 3-mercaptopropanoic acid were synthesized 3-[((4,6-Diamino)-1,3,5-triazin-2-yl]propanoic acid potassium salt (CAAT-3PA), which was reacted with γ-globulin in the presence of DCC and NHS. Female New Zealand white rabbit was immunized with CAAT-3PA-γ-globulin. After boost for four times, the rabbit was bled and applied on cdELISA to detect the specificity of the antibody against Melamine.

Results:

After synthesis reaction, CAAT-3PA was successfully prepared and had been monitored by TLC (Rf=0.2). A significant shift in CAAT-3PA-γ-globulin compared with γ-globulin standard was observed in SDS-PAGE. In the cdELISA, melamine polyclonal antibody(Yu64) was coated onto the wells of ELISA plates, the concentration of CAAT and Melamine causing 50% inhibition of CAAT-3PA-HRP binging to the antibody (IC50) was found to be 28 ng/mL and 480 ng/mL, respectively.

Conclusion:

We have successfully produced the polyclonal antibody against melamine. The IC50 of melamine was 480 ng/mL in the polyclonal antibody-based cdELISA. Our results showed that the hapten synthesis was successful and a potential tool for detecting melamine in foods and feeds has been developed.

P983
Histone Methyltransferase G9a Promotes the Oral Cancer Cells Recovery from Drug-Induced DNA Damage

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Backgrounds:

Oral cancer is one of the serious life-threatening diseases in the world. In addition to surgery, the conventional treatment for oral cancer includes radiation and anti-cancer drugs, which usually act by induction of DNA damage. However, approximately one-third of treated patients will experience local or regional recurrence. G9a is a histone methyltransferase responsible for mono- or dimethylation of lysine 9 in histone H3. Recent works had demonstrated that G9a level is highly correlated with aggressiveness and poor prognosis of several cancer patients in Taiwanese population. However the role of G9a in drug-induced DNA damage is unclear.

Materials and Methods:

SAS human oral cancer cells were stably transfected of shRNA-G9a or shLuc-control. These cells were treated with hydroxyurea (5μM) or etoposide (12.5 μg/ml) for 1 hour, and then we used immunoblotting to analysis of phosphorylation of histone H2AX (γ-H2AX) which provides information on unrepaired DNA damage.

Results:

We found that knockdown of G9a resulted in decrease cell survival and cologenic capacity following DNA damage induced independently by hydroxyurea and etoposide. Depletion of G9a caused sustained phosphorylation of γ-H2AX after drug treatments.

Conclusion:

Our data suggest that a novel function of G9a may increase the DNA repair efficiency and promote the oral cancer cells recovery and survival from the DNA damage agents. We suggest that G9a may contribute to oral cancer proliferation by improvement of homologous recombination repair of DNA double break. Our study paves the way for exploring the blockade of G9a overexpression as a novel approach for the prevention and treatment of oral cancer.

P984
Polyphenols Compositions of Green tea (Camellia sinensis, Theaceae) and Prevention Senescence Mediated Redox Imbalance in Aged Mice

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Backgrounds:

The in vivo antioxidant properties of green tea (Camellia sinensis, Theaceae) were investigated with a study of senescence related redox imbalance in aged male ICR mice.

Materials and Methods:

Animals were orally administered (gavage) green tea extract dissolved in distilled water at doses of 625, 1,250 and 2,500 mg/kg, respectively, daily for 4 weeks. Plamsa lipid peroxidation, total thiols and glutathione (GSH) levels, as well as the antioxidant enzyme activities and protein carbonyls in brain, liver, kidney and serum were measured to monitor senescence mediated redox imbalance in mice.

Results:

In aged control mice, there were significantly ($p < 0.05$) decreased GSH and total thiols content in plasma and the activities of catalase, glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Rd) in the brain, liver and kidney whereas increased protein carbonyls and thiobarbituric acid-reactive substances (TBARS) contents in serum as compared with young control group. In contrast, administering green tea via gavage at doses of 625, 1,250 and 2,500 mg/kg for 4 weeks significantly reduced ($p < 0.05$) protein carbonyls and TBARS levels in the serum and increased GSH and total thiols levels in plasma compared with aged control mice. Moreover, green tea administration significantly increased ($p < 0.05$) the activities of superoxide dismutase, catalase, G6PD, GSH-Px and GSH-Rd in the brain, liver and kidney.

Conclusion:

These results indicate that green tea exhibits potent protective effects against senescence mediated redox imbalance in mice by inhibiting oxidative damage and increasing antioxidant enzyme activities.

P985

The immunomodulatory effects of Neolitsea hiiranensis on T cell immunity

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Backgrounds:

Lauraceae plants have been used as traditional medicine to treat a variety of diseases, including dyspepsia, gastritis and allergic diseases. Many studies showed that Lauraceae species possess several bioactivities including antioxidation, anti-inflammation and immunomodulation. However, the immunomodulatory effects of Formosan Lauraceae plants on T cell immunity need to be further evaluated. Neolitsea genus, belonging to the family Lauraceae, has been reported to have various bioactivities, such as antibacterial, anti-inflammatory activities. The secondary metabolites from leaves of Neolitsea hiiranensis have potential anti-inflammatory effects. The objective of this study is to study the immunomodulatory effects of Neolitsea hiiranensis.

Materials and Methods:

(1)BALB/c mice were sensitized with ovalbumin (OVA) to generate OVA-specific T cells. Splenocytes were prepared and treated with extracts of Neolitsea hiiranensis (0.5, 1, 5, 10 μg/ mL) in vitro.

(2)The effects of Neolitsea hiiranensis on OVA-specific immune responses in vivo were determined. BALB/c mice were administered with Neolitsea hiiranensis (5-20 mg/kg) by intraperitoneal (ip) injection from days 1-3 and then sensitized with OVA/alum (100 μg/1 mg). 7 days later, the mice were sacrificed and the serum and splenocytes were collected for further studies.

Results:

Neolitsea hiiranensis (0.5-10 μg/ mL) significantly attenuated OVA-specific IFN-γ production in vitro, while the production of interleukin (IL)-2, and IL-4 was unaltered. Root and leaf extracts of Neolitsea hiiranensis inhibited antigen-specific IFN-γ production in vivo.

Conclusion:

As IFN-γ is the key cytokine secred by T helper-1 (Th1) cells, Neolitsea hiiranensis may possess potential immunomodulatory effects on Th1 immunity.

P986

ATP Mediates NADPH Oxidase/ROS Generation and COX-2/PGE2 Expression in A549 Cells: Role of P2 Receptor-Dependent STAT3 Activation

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Background:

Up-regulation of cyclooxygenase (COX)-2 and its metabolite prostaglandin E2 (PGE2) are frequently implicated in lung inflammation. Extracellular nucleotides, such as ATP have been shown to act via activation of P2 purinoceptors, leading to COX-2 expression in various inflammatory diseases, such as lung inflammation. However, the mechanisms underlying ATP-induced COX-2 expression and PGE2 release remain unclear.

Principal Findings:

Here, we showed that ATPγS induced COX-2 expression in A549 cells revealed by western blot and real-time PCR. Pretreatment with the inhibitors of P2 receptor (PPADS and suramin), PKC (Gö6983, Gö6976, Ro318220, and Rottlerin), ROS (Edaravone), NADPH oxidase [diphenyleneiodonium chloride (DPI) and apocynin], Jak2 (AG490), and STAT3 [cucurbitacin E (CBE)] and transfection with siRNAs of PKCα, PKCι, PKCμ, p47phox, Jak2, STAT3, and cPLA2 markedly reduced ATPγS-induced COX-2 expression and PGE2 production. In addition, pretreatment with the inhibitors of P2 receptor attenuated PKCs translocation from the cytosol to the membrane in response to ATPγS. Moreover, ATPγS-induced ROS generation and p47phox translocation was also reduced by pretreatment with the inhibitors of P2 receptor, PKC, and NADPH oxidase. On the other hand, ATPγS stimulated Jak2 and STAT3 activation which were inhibited by pretreatment with PPADS, suramin, Gö6983, Gö6976, Ro318220, GF109203X, Rottlerin, Edaravone, DPI, and apocynin in A549 cells.

Significance:

Taken together, these results showed that ATPγS induced COX-2 expression and PGE2 production via a P2 receptor/PKC/NADPH oxidase/ROS/Jak2/STAT3/cPLA2 signaling pathway in A549 cells. Increased understanding of signal transduction mechanisms underlying COX-2 gene regulation will create opportunities for the development of anti-inflammation therapeutic strategies.

P987

Long-Term Anti-Tumor Effect of M1 Macrophage Depends on p53 Accumulation and STAT1 Up-regulation in Cancer Cells

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Backgrounds:

Tumor associated macrophages were divided into M1 and M2. Opposite to M2 promoting tumor growth, classical M1 macrophages possess cytotoxicity via NO, ROS and pro-inflammatory cytokines, such as IFN-γ. But the toxic effects often occur in few hours, and some needs cell-cell direct contact. The M1 macrophages long-term tumoricidal activity remains unclear. In previous study, conditioned medium (CM) from M1 macrophages can induce A549 apoptosis and senescence in long-term culture, but detail mechanism is unexplored.

Materials and Methods:

Significant genes from cDNA microarray were validated by Real-time PCR. The Western blot and Real-time PCR represented p53 and STAT1 expression. Apoptosis assay indicated the effect of M1 CM on H1299, A549 and H460. To identify factors in M1 CM, cytokine array and ELISA were used. Statistical analysis was according our published paper.

Results:

Based on microarray data, the selected M1/M2 regulating gene signatures in lung cancer specimens had a significant correlation with cancer progression and patient survival. Several other significant genes were down-stream genes of p53, the important initiator for apoptosis, which was accumulation in wt-p53 cell lines after M1 CM treatment. But apoptosis effect was not observed in H1299, the p53-null cell line, unless exogenous wt-p53 expressed. Another transcription factor STAT1 was also up-expressed after M1 CM stimulation. STAT1 may interact and assist with p53 to cause cell death. IFN-γ is a well known cytokine for STAT1 activation and highly expresses in M1 macrophages, but IFN-γ neutralization can not reverse A549 apoptosis. M1 macrophages from human PBMCs also had tumoricidal activity.

Conclusion:

p53 and STAT1 are the main tumoricidal regulators induced by M1 macrophages in long-term culture and that is though IFNs-independent pathway. That implies M1 macrophage plays an important role in immune surveillance and may be one beneficial target of cancer immune therapy.

P988

Molecular mechanism of alpha-mangostin induces apoptosis in human hepatoma SK-Hep-1 cells.

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Backgrounds:

α-mangostin is a dietary xanthone that has been shown to have anticancer properties in various types of human cancer cells. This study investigates the molecular mechanism of the apoptosis-inducing effects of α-mangostin on human hepatocellular carcinoma cells.

Materials and Methods:

Five HCC cells lines and normal hepatocyte cells were treated with α-mangostin to determine the effect on cell proliferation by MTT assay, DAPI staining, cell cycle analysis, phosphatidylserine exposure and changing of mitochondrial membrane potential. The molecular mechanisms of α-mangostin mediated apoptosis were further investigated by Western blotting analysis including activation of caspase cascade, MAPK pathway, cytochrome c release and mitochondrial membrane-related proteins. Effects of α-mangostin on SK-Hep-1 tumor xenografted nude mice model were examined.

Results:

We found that α-mangostin inhibits the viability of human HCC cells in a dose- and time-dependent manner. α-mangostin-mediated apoptosis of SK-Hep-1 cells is accompanied by chromatin condensation and cell-cycle arrest (sub G1) and by phosphatidylserine exposure. Furthermore, α-mangostin triggered the mitochondrial/caspase apoptotic pathway, as indicated by the loss of mitochondrial membrane potential (MMP), release of cytochrome c, and the regulation of Bcl-2 family member expression. Moreover, α-mangostin inhibited a sustained activation of the phosphorylation of p38 MAPK, and SB2303580 almost enhanced α-mangostin-induced caspases activation and apoptosis in SK-Hep-1 cells. In vivo xenograft mice revealed that α-mangostin significantly reduced tumor growth in mice inoculated with SK-Hep-1 cells.

Conclusion:

Our studies demonstrate that α-mangostin induces apoptosis through a p38 MAPK-mediated activation of the mitochondrial pathway and that α-mangostin inhibits the in vivo tumor growth of SK-Hep-1 xenograft mice.

P989

Effects of Tributyltin on Embryonic and Gonadotropin-Releasing Hormone Neuronal Development in Medaka (Oryzias latipes)

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Background

The endocrine disrupting chemicals (EDCs) are contaminants found in the environment that may interfere with the physiological function and cause diseases in humans and wildlife. The tributyltin (TBT) is an organotin, a class of EDCs commonly found in the aquatic environment. It was widely used as a biocide in paints applied to ships and marine structures. However, it has been banned since 1990 due to its toxic effects on marine organisms.

In this study, we investigated whether TBT had any effect on Gonadotropin-releasing hormone (GnRH) gene expression at different stages of embryonic development in medaka (Oryzias latipes).The medaka fish express three forms of GnRH: GnRH1 regulates reproduction via gonadotropin release, while GnRH2 and GnRH3 are involved in sexual behavior. We used two lines of transgenic medaka, in which the green fluorescent protein (GFP) was placed under the control of the gnrh1 or gnrh3 promoter. As the medaka embryos are transparent, the GnRH neurons expressing GFP can be monitored in vivo during embryonic development. In addition to the GFP fluorescence intensity, we also examined the effects of TBT on other endpoints during development.

Materials & Methods

Medaka embryos were collected within 5 h post-fertilization and individually incubated at 27℃ . They were exposed to 0.1, 1, and 10 ng/L of TBT, starting from 0, 1, 2, 3 or 4 days post-fertilization. All solutions were replaced every 24 h. The embryos were observed and their images recorded daily, and the development of GnRH neurons, along with several endpoints of embryonic development, including the heart rate, eye development, and day to hatch, were quantified and analyzed.

Results

Our results showed that at various stages of development, TBT significantly affected the expression of GnRH neurons, increased the heart rate, and interfered with the eye development. In addition, TBT had a dose-dependent effect on the larval body length.

Conclusion

TBT can affect GnRH neuronal and embryonic development, and various stages of development may have different sensibility to the toxicity of TBT.

P990

Effects of the Extract of Antrodia Cinnamomea on the Alteration of Lipid Peroxidation and Trace Element Level in Brain Cortex of Rats after Cerebral Ischemia

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Backgrounds:

To investigate the effects of the extract of Antrodia Cinnamomea on the alteration of lipid peroxidation status and essential trace element level after cerebral ischemic insult in rats.

Materials and Methods:

Male rats were divided into control, ischemia, and Antrodia Cinnamomea-treated group. Cerebral ischemia was induced by occlusion of the right middle cerebral artery and right common carotid artery for one hour. Level of malondialdehyde and concentration of essential trace element of iron and copper was analyzed in the homogenates of brain cortex in rats.

Results:

Experimental results showed that a significant increase ($P<0.05$) of the level of malondialdehyde, iron, and copper was found on the ischemic brain cortex as compared to the control subject. Conversely, a marked decrease ($P<0.05$) of the concentration of malondialdehyde, iron, and copper was observed in the subject of Antrodia Cinnamomea-treated rats as compared to the control subject in the present experiment.

Conclusion:

Taken all evidences together, it is conceivable to manifest our experimental findings here that cerebral ischemic injury not only may result in an enhanced oxidative stress but also may lead to an increased lipid peroxidation in the ischemic brain. Meanwhile, it is crucial to note that disturbance of essential trace element level seems to be highly responsible for the pathogenesis of cerebral ischemic injury.

P991

The cytotoxic effects of (+)-catechin on human lung adenocarcinoma A549 cells

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Backgrounds:

Catechin, polyphenolics, had been reported to have many pharmacological properties such as the effects of anti-oxidative, anti-inflammatory, anti-ultraviolet, reduction of blood pressure as well as glucose and cholesterol levels, and anti-carcinogenic effect among many cancer cells. Although there are several isomers of catechine in health benefits have been suggested, the effect of (+)-catechin on human lung adenocarcinoma A549 cells is still unknown.

Materials and Methods:

A549 cell line has been used in this study. The cell incubated in F12K medium with or without (+)-catechin at the dosage of 0.5 mM, 0.75 mM, and 1 mM for 24-48 hours incubation at 37℃ . The cell viability and the protein expression were detected by MTT assay, flow cytometry, and Western blot.

Results:

We found that dose-dependent effect on morphological change was observed on A549 cells after 24 hours treatment with (+)-catechin. The A549 cell viability was also decreased while treated with (+)-catechin for 24 hours incubation. For cell cycle analysis, increased the percentages of apoptotic cells were detected in dose-dependent manner.

Conclusion:

In the present study, the cytotoxic effects of (+)-catechin on A549 cells were investigated. Our results suggest that (+)-catechin has time- and dose-dependent effects on the survival of A549 lung cancer cell line. In further study, we will detect the expression of apoptosis related proteins, bcl-2,caspase3,8,9 and cytochrome c in (+)-catechin-treated A549 cells. To understand the exact biological effect of (+)-catechin, the quality of better health can be improved.

P992

The upregulation of proinflammatory mediators on macrophages by Brevetoxin

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Backgrounds:

Brevetoxin, a potent cyclic polyether toxin, accumulate in the flesh of shellfish. However, the mechanism of brevetoxin induced proinflammatory generation in activated microglia is unclear.

Materials and Methods:

Cytotoxicity was measured by tetrazolium bromide reduction assay. interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)α was analysed by enzyme-linked immunosorbent assay. Generation of nitric oxide (NO) were investigated by Griess assay. Statistical analyses were performed using anova followed by the Bonferroni's t-test for multigroup comparisons.

Results:

At present, we found brevetoxin demonstrated the cytotoxic effect on macrophage RAW264.7 cells in a concentration-dependent manner. Brevetoxin induced generation of proinflammatory mediators, such as nitric oxide (NO), interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)α.

Conclusion:

The mechanism of brevetoxin induced macrophages activation near further studies. These results indicated that brevetoxin induced proinflammatory mediators generation and cytotoxicity on macrophages.

P993

Hemoglobin Adducts as Biomarkers of Estrogen Homeostasis: Elevation of Estrogen-3,4-Quinone as a Risk Factor of Developing Breast Cancer

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Both 17β-estradiol-2,3-quinone (E2-2,3-Q) and 17β-estradiol-3,4-quinone (E2-3,4-Q) are reactive metabolites of estrogen and are thought to be responsible for the estrogen-induced genotoxicity. The aim of this study was to establish a methodology to simultaneously analyze estrogen quinone-derived adducts in human hemoglobin (Hb) and to measure the background levels of these adducts in Hb derived from female breast cancer patients (n=143) and controls (n=147) in Taiwan. Results from in vitro experiments confirmed that both E2-2,3-Q- and E2-3,4-Q-derived adducts rapidly reached maximum values at 10 min mark and remained constant thereafter for up to 24 h. The background levels of estrogen quinone-derived adducts, including E2-2,3-Q-4-S-Hb and E2-3,4-Q-2-S-Hb, were detected in breast cancer patients with median levels at 434 (range 215–1472) and 913 (range 559–2384) (pmol/g), respectively. By contrast, median levels of these same estrogen quinone-derived adducts in healthy controls were 71.8 (range 35.7–292) and 139 (range 69.1~453) (pmol/g). Levels of E2-2,3-Q-4-S-Hb correlated significantly with those of E2-3,4-Q-2-S-Hb (correlation coefficient r = 0.622-0.628, p<0.001). Our findings add further support to the theme that cumulative body burden of E2-3,4-Q is an important indicator of developing breast cancer. We hypothesized that combination of genetic events and environmental factors may modulate estrogen homeostasis and enhance the production of E2-3,4-Q and the subsequent generation of pro-mutagenic DNA lesions in breast cancer patients.

P994

Genetic Polymorphisms in APE1 as a Modifier of the Background Levels of Abasic Sites in Human Leukocytes and a Potential Risk Factor of Breast Cancer

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Backgrounds:

The objective of this research was to investigate association of the risk factors of breast cancer, including age, body mass index (BMI), and polymorphisms of apurinic/apyimidinic endonuclease (APE1) with the background levels of apurinic/apyimidinic (abasic/AP) sites in leukocytes derived from 148 Taiwanese women with breast cancer and 74 cancer-free controls.

Materials and Methods:

Results indicated that the median levels of AP sites were estimated to be 18.5 and 26.5 per 106 nucleotides in controls and breast cancer patients, respectively. As distinguished by age and BMI, we noticed that the background levels of AP sites in breast cancer patients were ~2-3 fold greater than those of controls in subjects with age under 50 or with BMI < 27 (Kg/m2) (p<0.05). The frequencies of variant alleles of APE1 Asp148Glu (rs3136820) were estimated to be 38.5% and 46.3% in controls and breast cancer patients, respectively. In subjects with Asp/Glu + Glu/Glu genotypes, the number of AP sites in breast cancer patients were ~2 fold greater than those of controls (p<0.05), and the tendency was more obvious in subjects under 50 and with BMI < 27 (Kg/m2).

Results:

Additionally, results from the AP site cleavage assay indicated that the AP sites detected in both controls and patients were likely to derive from 5'-cleaved AP sites (~61-64%).

Conclusion:

In conclusions, we confirmed that variant allele of APE1 Asp148Glu may serve as a risk factor of breast cancer and that in combination with age and BMI, it can enhance the accumulation of levels of AP sites in breast cancer patients.

※ 依姓名筆劃順序排列 二劃～七劃

※ 依姓名筆劃順序排列 七劃～八劃

※ 依姓名筆劃順序排列 八劃～十一劃

※ 依姓名筆劃順序排列 十一劃

※ 依姓名筆劃順序排列 十一劃～十三劃

※ 依姓名筆劃順序排列 十三劃～十五劃

※ 依姓名筆劃順序排列 十五劃以上