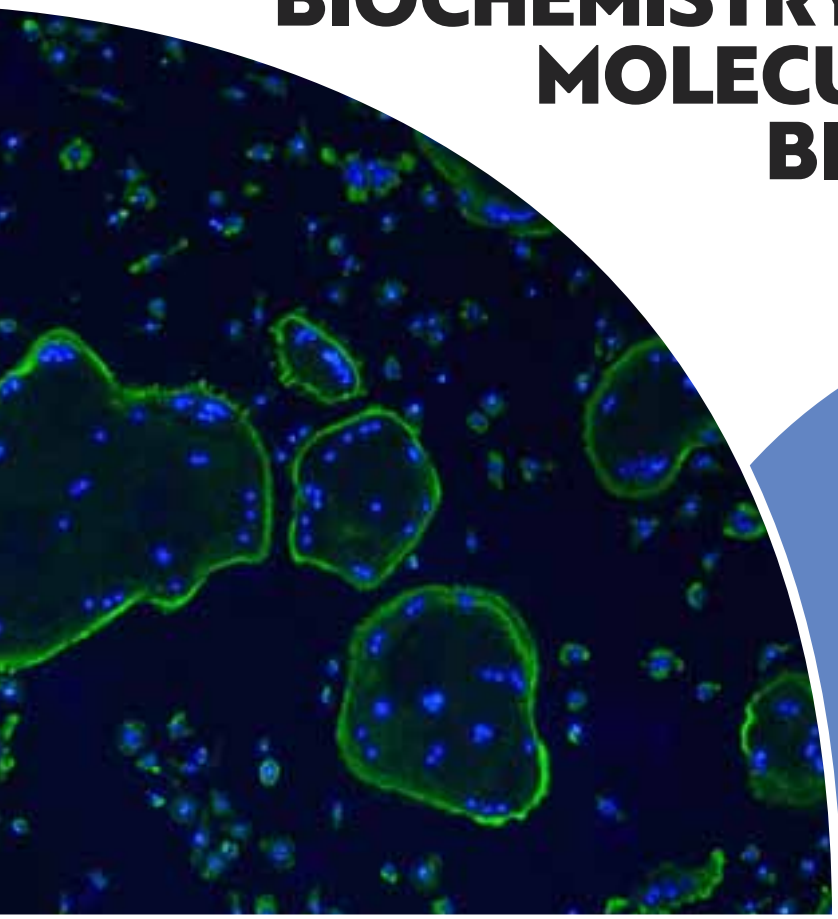
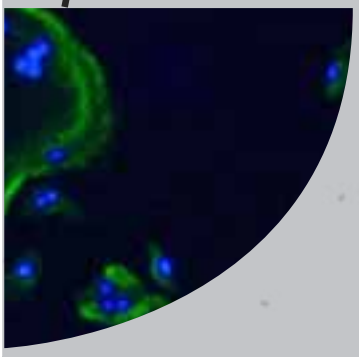
73rd NATIONAL CONFERENCE of the HELLENIC SOCIETY OF BIOCHEMISTRY & MOLECULAR BIOLOGY

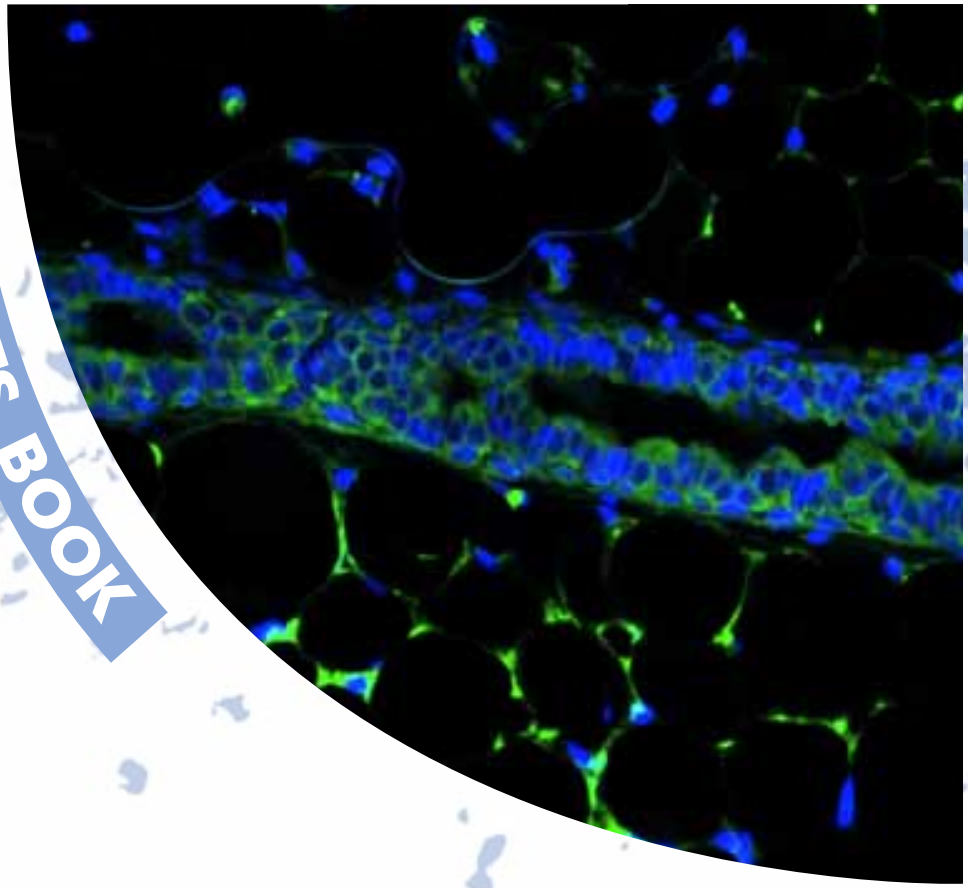


ATHENS 2023

EUGENIDES FOUNDATION

1/3 DEC





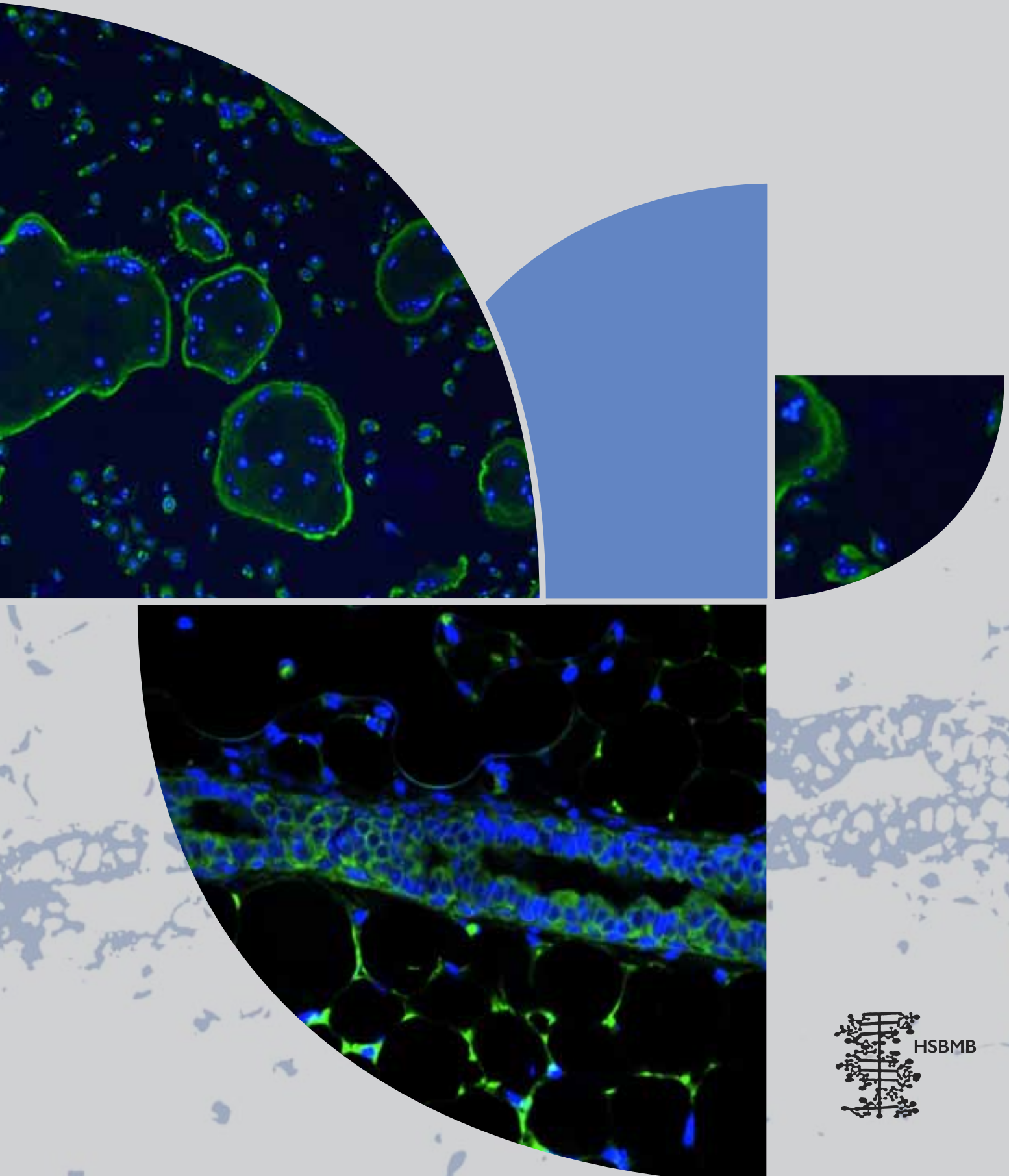
ABSTRACTS BOOK

HSBMB

era congresses and events

www.era.gr

INVITED | PLENARY LECTURES



HSBMB

ER remodelling via ubiquitin and autophagy pathways

Ivan Dikic

Goethe University Frankfurt, Germany

The human body is in a continuous state of repair and renewal, from breaking down and reusing damaged or excess cell parts via process of autophagy. The endoplasmic reticulum (ER) in the cell cytoplasm, critical to the synthesis and transport of cellular components, is no exception. ER-phagy is a major driver of ER remodelling, and ER-phagy receptors play central roles in this process. Loss-of-function mutations in ER-phagy receptors (FAM134b) result in autosomal recessive hereditary sensory and autonomic neuropathy (HSAN) in humans and dogs.

Within the last decades, several other ER-resident membrane-shaping proteins with central reticulon homology domains (RHD) have been associated with hereditary axonal disorders as well, i.e. ATL1, ATL3, REEP1 and REEP2, SPAST, RTN2, and ARL6IP1. They can also cause hereditary spastic paraplegia/HSP, a neurodegenerative disorder characterized by progressive leg spasticity alone or in combination with loss of sensory and pain perception (HSAN). The underlying mechanisms of RHD-containing proteins functions as well as their contribution to pathogenesis of HSP and HSAN neuropathies remain largely elusive. I will discuss the role of ubiquitination in controlling ER-phagy receptor clustering and efficient ER remodelling and renewal, and how defects in these pathways lead to neuronal cell death and neuropathies.

The formation of stem cell organising centres in plants from scratch

Liam Dolan

Gregor Mendel Institute, Dr Bohr Gasse 3, Vienna, Austria

The multicellular bodies of plants develop from single cells; the multicellular diploid phase of the plant life cycle develops from a polarised zygote, and the multicellular haploid phase develops from a spore without polarity. Each of these cell types divide to form masses of cells – enclosed embryos and free-living sporelings respectively. These cell masses form meristems, generative centres from which the body of the plant develops. In bryophytes, meristems develop in both diploid multicellular embryos and haploid sporelings. Our laboratory investigates mechanisms that transform the non-polar spore into a polarised cell in the liverwort Marchantia polymorpha.

The spore of is produced by meiosis and the mature spore lacks any markers of polarity. Upon germination, the spore polarizes de novo. This polarity orients the first cell division, which is asymmetric and produces a larger cell on apical side and a smaller cell on the basal side. The apical cell functions as a regenerative stem cell while the basal cell differentiates as a rhizoid cell. Therefore, the polarity that develops in the germinating spore determines where the first two cell form and their developmental fates. Preliminary data indicate that light polarises the spore cell and orients the cell plate during the first asymmetric cell division. At the two-cell stage, the transcription factor ROOT HAIRLESS SIX-LIKE promotes the differentiation of the basal cell as a rhizoid cell. The apical cell divides to produce a population of cells among which a stem cell niche forms and from which a meristem – the morphogenetic centre that gives rise to the body of the mature plant – emerges.

Recent data on mechanism that operates during the formation of cell polarity in the one and meristem initiation will be presented.

Dissecting and Targeting Cell Death Mechanisms

Evripidis Gavathiotis

Department of Biochemistry; Department of Medicine; Department of Oncology; Montefiore Einstein Comprehensive Cancer Center, Albert Einstein College of Medicine, Bronx, NY, USA

The intricate balance between cell survival and death is a central theme in cellular homeostasis, where dysregulation of cell death mechanisms plays a pivotal role in tumorigenesis and resistance to therapeutic treatments. My laboratory has taken a multidisciplinary approach to interrogate and elucidate the complex landscape of selective cell death mechanisms in mitochondrial apoptosis and autophagy. My talk will focus on the characterization of key effectors' function and regulation and the identification of molecular determinants that govern the switch between survival and death in cancer cells. Furthermore, I will discuss the development of rational small molecule targeting strategies aiming to highlight vulnerabilities in cancer and a path for development of novel targeted therapeutic interventions.

Targeting hyaluronan-CD44 signalling in cancer and infection

Paraskevi Heldin

Department of Medical Biochemistry and microbiology, Uppsala University, Biomedical Center, Box 595, SE-75124 Uppsala, Sweden

We aim to elucidate the molecular mechanisms that contribute to the excessive accumulation of hyaluronan in certain tumors and infections, resulting in over-activity of CD44 signaling. We use 2D and 3D experimental models to evaluate cellular fate, such as cell proliferation, stemness, migration, differentiation, and invasion. We found that increased hyaluronan synthase 2 (HAS2)-synthesized hyaluronan activated CD44 signaling during Dengue virus infection-induced inflammation, disrupting endothelial integrity. Increased serum hyaluronan levels are an early predictor of warning signs for severe dengue virus infection.

Growth factors, such as PDGF-BB and TGFβ are powerful activators of hyaluronan synthesis in mesenchymal cells and epithelial cells, respectively. Our data revealed a correlation between tumor progression and growth-factor stimulation of hyaluronan synthesis, and growth factor-mediated cleavage of CD44 in its transmembrane domain, resulting in the release of a soluble intracellular domain of CD44. The HAS2-synthesised hyaluronan engaged signaling via CD44, as well as via the intracellular domain of cleaved CD44, which cooperate in inducing cell proliferation, stemness, and migration. In certain tumors, such as gliomas, a CD44/hyaluronan feedback circuit drives tumor progression, and is related to the expression of PDGF and PDGF receptor family members.

Based on the knowledge that CD44 is a target for p53 activity and that CD44 physically interacts with the inhibitor of the apoptosis-stimulating protein of p53 (iASPP), we have demonstrated that CD44-iASPP complexes dictate the sub-cellular localization of iASPP-p53 complexes, affecting cell adhesion, migration, and p53-mediated apoptosis.

Currently, we are investigating the possibility of modulating hyaluronan-CD44 signaling by using HAS2 inhibitors and macrocyclic peptides binding to CD44.

Unbiased approaches shed light on mitochondrial contacts with the endoplasmic reticulum

Luca Scorrano

Dept. of Biology, University of Padova, Via U. Bassi 58B, 35121 Padova, Italy
Veneto Institute of Molecular Medicine, Via Orus 2, 35129 Padova Italy

Mitochondria and endoplasmic reticulum (ER) are physically linked and appropriately spaced at specific points known as Mitochondria-Endoplasmic Reticulum (ER) contacts (MERCs) through partially understood protein bridges. To establish a comprehensive molecular atlas of MERCs, we conducted a genome-wide screening using short hairpin RNA (shRNA) and combined it with high-content, ratiometric, quantitative microscopy of a FRET ER-mitochondria proximity probe (FEMP) and iBAQ proteomic analysis of MERCs. Through automated image analysis, statistical evaluations, and iterative screening, we identified 107 gene candidates classified as tethers (genes whose removal increases the distance between ER and mitochondria), which included well-known mammalian tethers like Mfn2. Additionally, we identified 97 Spacer genes (genes whose removal decreases the distance between ER and mitochondria). These gene candidates were found to be enriched in calcium signaling, lipid biosynthesis, and metabolism processes known to concentrate in this interface. By cross-referencing the gene list with the proteome of MERCs determined by iBAQ analysis, we refined our findings to 25 Spacers and 18 Tethers. Orthogonal assays validating mitochondria-ER juxtaposition further highlighted the effectiveness of our screening approach in identifying and functionally characterizing MERCs components.

Zebrafish models of Human Disease

Dimitris Beis

University of Ioannina / Biomedical Research Foundation, Academy of Athens, Greece

Zebrafish has emerged as an invaluable system to model Human Diseases. Cardiovascular development can be monitored non-invasively and zebrafish embryos can survive even without a fully functional cardiovascular system, allowing the study of severe phenotypes at the cellular level in vivo. High-throughput chemical screens allow the identification of molecules that could rescue these phenotypes. Importantly, zebrafish maintains the ability to regenerate their hearts and other organs throughout their lifetime, including adulthood. Evidence from both the zebrafish and small animal models suggest that the innate immunity status critically regulates cardiac regeneration.

We have shown that the secretome of M2 activated mouse macrophages sustains myocardial proliferation and promotes regeneration of zebrafish adult cardiac explants. We now aim to identify the active components of this secretome.

Recent developments in sequencing technologies have enabled Human Genetics studies to address inherited diseases at the single nucleotide level. However, the accumulated genomic data is not currently in line with functional studies. Many gene variants associated with Cardiovascular Disease (CVD) are of unknown significance and thus of limited clinical utility. Progress in CRISPR mediated knockout and knockin technologies now enable the easy, precise, and efficient genetic editing of zebrafish allowing the generation and characterization of human derived variants of unknown significance to study clinically relevant lines in zebrafish.

Finally, identifying new Bioactive Natural Products (BNPs) that may serve as potential drug lead compounds or cosmeceuticals is a constant challenge. We are performing high-throughput screens of BNPs isolated from various sources including plants, algae and venom extracts aiming to identify novel bioactive molecules with potential antiangiogenesis, anti-aging, wound healing and/or cosmeceutical properties.

Emerging roles of SUMOylation in the response to hypoxia

Georgia Chachami

Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Biopolis 41500, Larissa, Greece

Hypoxia is the low oxygen condition that is encountered in cells and tissues during pathological processes, such as tumour development or ischemia. Hypoxia triggers the activation of Hypoxia-Inducible Factors (HIFs) that mediate the physiological response to hypoxia but also regulate multiple steps of carcinogenesis including tumour progression, metastasis and response to therapy. Post-translational modifications (PTMs) diversify cellular proteomes and control protein function. PTM by small ubiquitin-like modifiers (SUMOs) or SUMOylation is the covalent attachment of SUMOs to a vast variety of target proteins. SUMOylation is emerging as essential player in the fine-tuning of several signalling and stress-response processes in eukaryotic cells. Our focus has been on SUMOylation-dependent mechanisms that are activated under hypoxia and the way they influence key players of the hypoxic response pathway. To gain insights into differences of the SUMO proteome of HeLa cells under normoxic and hypoxic conditions, we employed endogenous SUMO-immunoprecipitation in combination with quantitative mass spectrometry (SILAC). We identified proteins whose SUMOylation status changed without concomitant change in their abundance1. We are currently analysing the involvement of these proteins and their SUMOylation in the response to hypoxia and the role of HIFs or other hypoxia-regulated factors in modulating the cellular SUMOylation machinery. Evidence of a SUMO2-dependent transcriptional control of the transcription factor AP-2A (TFAP2A) on HIF-1 regulation will be discussed, as well as recent findings of a novel role for SUMO1 in the regulation of the RNA exosome catalytic subunit EXOSC10 and RNA transcription under hypoxia. In conclusion, our work provides new insights on the fine-tuning of gene expression via SUMOylation. This type of regulation may be important for optimal cellular response and adaptation to low oxygen conditions and could in the long-run provide novel molecular tools for therapeutic interventions.

1. Chachami et al. (2019) Mol Cell Prot 18, 11971209

The research work is supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: 1460 to G.C.).

Dissecting the mechanisms underlying metastasis formation after neoadjuvant chemotherapy

Ioanna Keklikoglou

Assistant Professor, Department of Biology, University of Crete, Greece

Neoadjuvant (pre-operative) chemotherapy is the standard-of-care for the management of breast cancer (TNBC), but despite its successes approximately 30-40% of patients with advanced disease will fail to respond or will eventually relapse, thus increasing the likelihood of metastasis. While research has largely focused on resistance mechanisms at the cancer cell level, it has become apparent that stromal microenvironment alterations are important in modulating tumour responses to therapy and in regulating metastasis formation. Increasing evidence suggests that primary tumours release extracellular vesicles (EVs) that can facilitate the seeding and growth of metastatic cancer cells in distant organs. Using syngeneic and spontaneous models of breast cancer, we found that regimens of neoadjuvant chemotherapy increase spontaneous breast cancer metastasis. Chemotherapy-elicitedEVs (Ch-EVs) are released into the circulation of tumour-bearing mice and interact with pulmonary vascular endothelial cells. Uptake of Ch-EVs promotes upregulation of CCL2 in endothelial cells, a chemoattractant for metastasis-promoting monocytes. Purified Ch-EVs are sufficient to enhance the recruitment of CCR2+ monocytes to the lung of tumour-free mice and to promote metastasis from circulating breast cancer cells. Proteomic analysis of purified EVs identified Annexin a6 as a molecular regulator of Ch-EV-induced metastasis. Notably, we found that neoadjuvant chemotherapy enhanced the abundance of Annexin a6 in circulating EVs from breast cancer patients. These findings suggest that neoadjuvant chemotherapies might also enhance the propensity of breast cancer to metastasize via the induction of Annexin a6+ pro-metastatic EVs.

Spatiotemporal dynamics of early embryonic development

Theodora Koromila

University of Texas, Arlington, TX
Aristotle University of Thessaloniki, Greece

Cell differentiation, which drives embryonic development, is initiated by pioneering transcription factors (PTFs) that prime cells to shift their external roles by transforming their transcriptional landscapes. Neural progenitor specification and differentiation occur early in development with detectable neuroblasts arising a mere hour post gastrulation in the Drosophila embryo. The roles of early PTFs involved in brain development, such as Odd-paired (Opa)/ Zinc finger in the cerebellum (ZIC) and Drosophila's homolog of the Otx family known to regulate neural development in mice, Ocelliless (Oc), are largely conserved across animals. We have previously demonstrated a pregastrulation spatiotemporal niche of co-regulation by Opa and Oc, spatially confined to the early embryonic region of the future brain and occupying distal elements proximal to a panoply of brain genes. Here, we provide insights into Opa's and Oc's mechanisms of action in head-specific gene regulation by developing in vivo optogenetic and real-time transcription observation tools. ChIPseq and multi-omic data were used to identify gene regulatory network relationships between perturbed transcription factors and target genes. Upon mutagenesis of these TFs binding sites gene expression patterns exhibit dynamic changes in the width of reporter-driven expression outputs. To assess these phenotypes systematically, we devised a live imaging quantitative approach to measure the spatiotemporal outputs across the entire embryo. In conclusion, our results demonstrate that Opa and Oc play temporally distinct roles and contribute to dynamic gene expression in the developing embryonic head. This work is vital to understanding the cell fate determinations which beget the coordinated cellular diversity of mature animalia.

The expanding boundaries of ECM remodeling in cancer

Zoi Piperigkou

University of Patras, Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Department of Chemistry, Patras 26504, Greece

The intricate network of interstitial and pericellular non-cellular matrices of macromolecules plays a pivotal role in providing structural support, facilitating cell migration and adhesion, and transmitting crucial signaling cues. Upon interactions, these macromolecules influence the dynamic behavior of the extracellular matrix (ECM) and engage in cell-cell communication via cell surface receptors. The ECM, comprised of a diverse mosaic of components, exhibits a wide range of structural variability, which directly correlates with its capacity to fulfill specific structural and functional roles. These components are finely tuned to engage with other macromolecules, ultimately constructing the dynamic 3D matrix networks that underpin tissue integrity and govern the morphological and behavioral characteristics of cells. The behavior of cells and the overall integrity of tissues are profoundly impacted by proteolytic processes within the matrix. These processes occur both in the context of normal tissue homeostasis and during the development of diseases. Structural modifications, epigenetic changes, and variations in the expression of matrix macromolecules can significantly influence the functionality of the matrix network. Such alterations are closely associated with the onset of prevalent diseases, including cardiovascular diseases and the intricate process of cancer metastasis. Within the complex milieu of the tumor microenvironment, cell-cell interactions orchestrate the reconfiguration of the matrix and facilitating the growth of cancer cells. The extensive turnover of ECMs within the tumor microenvironment directly dictates the characteristics of cancer cells, including their metastatic potential and the establishment of premetastatic niches. This presentation will delve into the multifaceted nature of matrix composition and explore novel approaches for targeting cancer by focusing on groundbreaking discoveries. Ongoing research efforts are centered on unraveling the functions, signaling cascades, and epigenetic signatures governed by matrix proteolytic and glycosidic enzymes, as well as the synergistic relationships among various matrix mediators that collectively govern the dynamics and aggressive traits of cancer cells.

References

1. *Karamanos NK, Piperigkou Z, Passi A, Götte M, Rousselle P, Vlodavsky I (2021) Extracellular matrix-based cancer targeting. Trends Mol Med. 27(10):1000-1013*
2. *Piperigkou Z, Kyriakopoulou K, Koutsakis C, Mastronikolis S, Karamanos NK (2021) Key Matrix Remodeling Enzymes: Functions and Targeting in Cancer. Cancers (Basel). 13(6):1441.*

Tailoring Bacteria for Precision Recombinant Protein Production

Georgios Skretas

Institute for Bio-innovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece
Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece

This presentation will showcase how precise genetic manipulation can transform humble bacteria into highly efficient cell factories for recombinant protein production. We will describe our efforts to generate a series of specialized bacterial strains with the ability to produce dramatically enhanced yields of recombinant membrane proteins, a class of proteins which are notoriously difficult to acquire in satisfactory yields. Through this process we have gained a better understanding about physiological factors that may limit production of this type of proteins in bacteria. Furthermore, we have identified possible ways in which the bacterial protein synthesis machinery can be additionally rewired to enhance membrane protein production yields further. Finally, we will discuss how similar approaches could be applied for the development of new and improved hosts for the recombinant production of soluble proteins and biopharmaceuticals.

Astrocyte reprogramming / activation and brain homeostasis

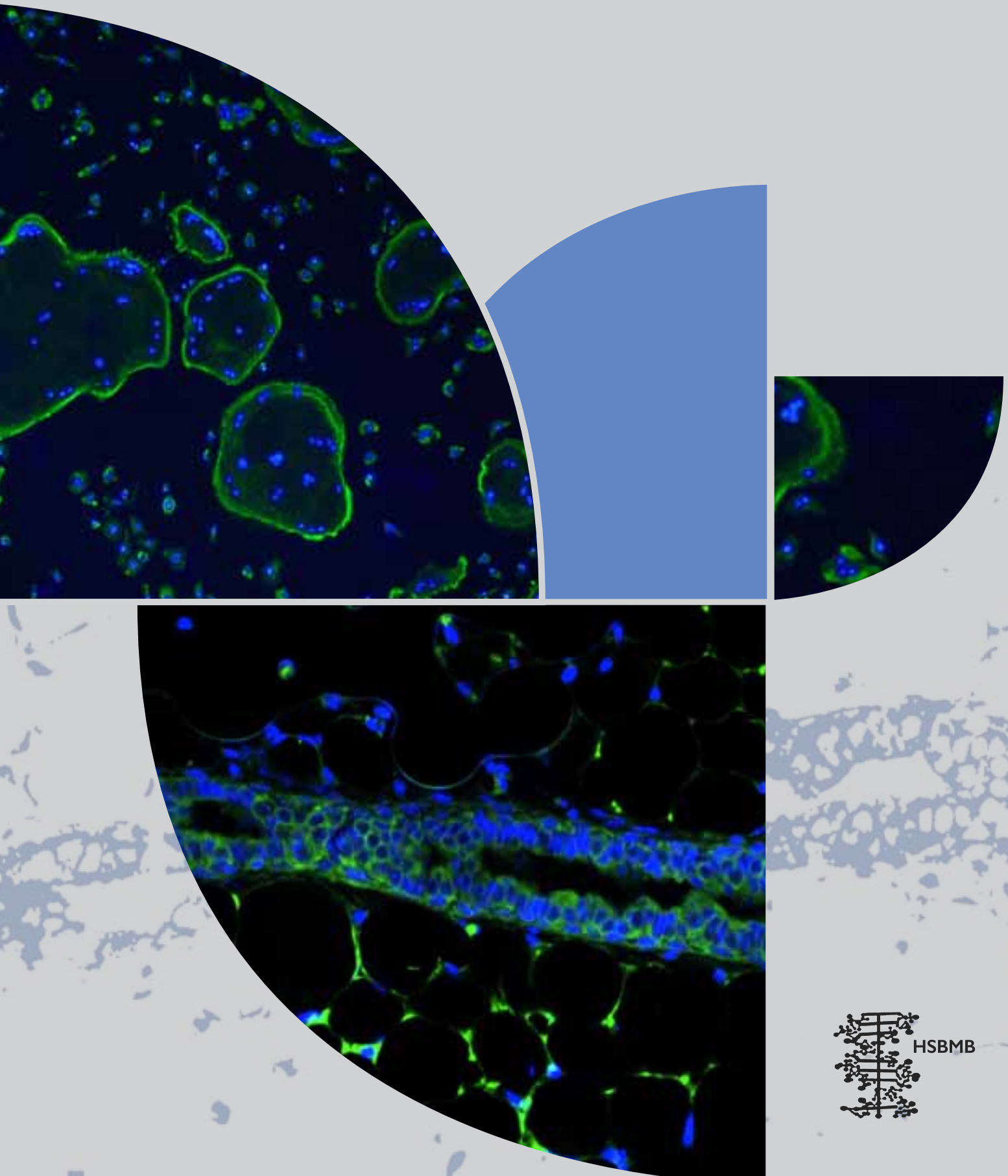
Dimitra Thomaidou

Hellenic Pasteur Institute, Athens, Greece

Astrocytes are multifunctional glial cells, implicated in neurogenesis and synaptogenesis, supporting neuronal activity and maintaining brain homeostasis by controlling blood-brain barrier permeability. Following their activation due to brain trauma or neurodegeneration, astrocytes acquire an intrinsic neural stem cell potential, rendering them prone to neurogenic reprogramming. Based on this property of astrocytes, their reprogramming to induced-neurons (iNs) has been achieved both in vitro and in vivo following force-expression of combinations of transcription factors (TF), miRNAs or chemical cocktails. The neurogenic miRNA miR-124 in particular has been employed in direct reprogramming protocols supplementary to neurogenic TFs and other miRNAs to enhance direct neurogenic conversion, however its independent reprogramming capacity has not been investigated. Here we explored the 'master reprogramming' potential of miR-124, isolated from other reprogramming co-factors and show that miR-124 is a potent driver of the reprogramming switch of astrocytes towards an immature neuronal fate, by directly targeting the RNA-binding protein Zfp36l1 implicated in ARE-mediated mRNA decay and subsequently de-repressing Zfp36l1 neurogenic interactome. Importantly, miR-124 is also potent to guide direct conversion of reactive astrocytes to immature iNs of cortical identity in vivo following cortical trauma, confirming its 'master reprogramming' capacity within the injured cortical microenvironment.

In parallel to their neurogenic reprogramming capacity, astrocytes also participate in the maintenance of blood-brain barrier integrity, which ensures the physiological functioning of the central nervous system and gets affected contributing to the pathology of several neurodegenerative diseases. To study in real time the dynamic physical interactions of astrocytes with brain vasculature under homeostatic and pathological conditions, we performed 2-photon brain intravital imaging in a mouse model of systemic neuroinflammation, which triggers astrogliosis and microgliosis and evokes changes in astrocytic contact with brain vasculature. Our findings indicate that following neuroinflammation, the endfeet of activated perivascular astrocytes lose their close proximity and physiological cross-talk with vasculature, however this event is partially compensated by the cross-talk of astrocytes with activated microglia, safeguarding blood vessel coverage and maintenance of blood-brain integrity.

SHORT TALKS



HSBMB

ST1

A mechanism for activation of the abscission checkpoint in response to chromatin bridges in human cells

Eleni Petsalaki[1], Sofia Balafouti[1], Athina Kyriazi[1], George Zachos[1]*

[1]Department of Biology, University of Crete, Heraklion, Greece
**Email: gzachos@uoc.gr*

Chromatin bridges are strings of chromatin connecting the anaphase poles or daughter nuclei and have been linked to tumourigenesis. Chromatin bridges can arise from segregation of interlinked chromosomes after improper resolution of double strand DNA catenates, or from dicentric chromosomes generated by end-to-end chromosome fusions. In response to chromatin bridges, the abscission checkpoint delays completion of cytokinesis (abscission) to prevent chromosome breakage or tetraploidization; however, how chromatin bridges are detected by the abscission checkpoint has not been previously reported. Here, we show that spontaneous or replication stress-induced chromatin bridges exhibit "knots" of catenated and overtwisted DNA next to the midbody. Topoisomerase IIα (Top2α), an enzyme that can relax DNA supercoils and untangle catenated DNA molecules by catalyzing passage of one double-stranded DNA molecule through a Top2-linked double-stranded break in another DNA molecule, forms abortive Top2-DNA cleavage complexes (Top2ccs) on DNA knots. Furthermore, impaired Top2α-DNA cleavage activity correlates with chromatin bridge breakage in cytokinesis. Proteasomal degradation of Top2ccs is required for localization of the DNA damage sensor protein Rad17 to Top2α-generated double strand DNA ends on DNA knots. In turn, Rad17 promotes local recruitment of the MRN (Mre11-Rad50-Nbs1) protein complex and downstream ATM-Chk2-INCENP signaling to delay abscission and prevent chromatin breakage. In contrast, dicentric chromosomes that do not exhibit knotted DNA fail to recruit Top2α next to the midbody and to activate the abscission checkpoint in human cells. These findings are the first to describe a mechanism by which the abscission checkpoint senses chromatin bridges, through generation of abortive Top2ccs on DNA knots, to preserve genome integrity.

Acknowledgements
This work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members and Researchers" (Project Number: 2486).
E. Petsalaki and S. Balafouti were supported by the H.F.R.I. under the "2nd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers" (Project Number: 629).
Work in our laboratory was also supported by Fondation Santé.

Reference
J Cell Biol (2023) 222: e202303123.

ST2

Unlocking PTEN's potential: Carbon nanotube-mediated delivery in breast cancer cells.

Konstantinos S. Tasioulis[1], Petros V. Kehagioglou[1,2], Maria A. Papaioannou[3], Dimitrios A. Kyriakidis[1] , Rigini M. Papi[1]*

[1]Laboratory of Biochemistry, School of Chemistry, Aristotle University of Thessaloniki, 54124 Greece
[2]Cell Biology Unit, University Medical Center Mainz, 55128 Germany (current address)
[3]Laboratory of Biological Chemistry, School of Medicine, Aristotle University of Thessaloniki, 54124 Greece

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a potent tumor suppressor with frequent expression and function disruption in cancer predisposition syndromes or cancer. Recent scientific evidence highlights the therapeutic potential of PTEN protein administration, however, it still poses hurdles owing to degradation and low membrane permeability. To overcome such issues, functionalized multi-walled carbon nanotubes (MWCNTs) enable efficient penetration and delivery of their cargo. Expression vector containing the human PTEN open reading frame (pORF9-hPTEN v21) served as a template and sequence-specific primers with NdeI and XhoI restriction sites amplified the PTEN ORF through PCR reaction. The amplicon was ligated in pET29c(+) expression vector that have been previously digested with the same restriction enzymes and the recombinant pET29c(+)-hPTEN1 transformed E. coli BL21 (DE3) competent cells. Induction with IPTG in the middle of the exponential bacterial growth phase resulted in recombinant PTEN overexpression and inclusion bodies formation. The subsequent purification of PTEN was performed with immobilized metal ion affinity chromatography (IMAC). At the same time, purity verification of the eluents was achieved through SDS-PAGE and silver staining, confirming the success of the purification process.

Oxidized MWCNTs were modified with bis(3-aminopropyl)polyethylene glycol, and PTEN bioconjugation was achieved through EDC/NHS coupling. Z-potential and FTIR analysis confirmed successful PTEN immobilization. Different concentrations (0, 10, 20, 50, 75, and 100 μg/ml) of PEGylated MWCNTs and PTEN-biofunctionalized MWCNTs were used to assess cell viability. The biological effect in ZR-75-1 and MCF-7 breast cancer cell lines was estimated by RT-PCR and PathScan analysis. Our results indicated noteworthy cell morphological alterations, as well as significant inhibition of cell proliferation and apoptosis induction in ZR-75-1 and MCF-7 cells, transfected with PTEN-bioconjugated MWCNTs even in lower concentrations compared with PEGylated MWCNTs and untreated cells.

ST3

Molecular players and impact of the modulation of the endothelial RhoA-ROCK pathway on metastatic potential

Md Sanaullah Sajib[1], Fatema Tuz Zahra[1], Margarita Lamprou[2], Jee Hyun Park[1], Manuel Osorio3, Paul Tullar4, Racheal Grace Akwii[1], Antonia Marazioti[5], Yi Zheng[6], J. Silvio Gutkind[7], Ulrich Bickel[1], Scott Trasti[8], Constantinos M. Mikelis[1],[2]*

[1]Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, Texas, 79106, USA.
[2] Department of Pharmacy, University of Patras, Patras, 26504, Greece.
[3]Center for Biologics Evaluation and Research, US Food and Drug Administration, Silver Spring, MD, 20993, USA.
[4]Department of Obstetrics and Gynecology, School of Medicine, Texas Tech University Health Sciences Center, Amarillo, Texas, 79106, USA.
[5]Department of Physiotherapy, School of Health Sciences, University of Peloponnese, 23100, Greece
[6]Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, Ohio, 45229, USA.
[7]Department of Pharmacology, UCSD, San Diego, California, 92093, USA.
[8]Laboratory Animal Resources Center, Texas Tech University Health Sciences Center, Lubbock, TX, 79430, USA.
**email: kmikelis@upatras.gr*

Tumor metastasis, the process through which tumor cells colonize in distant parts of the body, is responsible for the majority of cancer-related deaths, and the endothelial monolayer plays an important role during the entrance and exit of the disseminating cancer cells from the vasculature. However, the endothelial regulatory elements of this step remain obscure. The RhoA-Rho kinase (ROCK) pathway is a known mediator of endothelial permeability via cytoskeletal remodeling. Here we demonstrate that endothelial RhoA activation is a determining factor for transendothelial migration and metastasis. Specifically, we show that endothelial RhoA is activated by tumor-derived paracrine mediators and independent cell-to-cell contact mechanisms. RhoA knockdown in vitro, endothelial-specific RhoA deficiency in vivo or pharmacological inhibition of the RhoA-ROCK pathway abrogated the transendothelial migration and metastasis of triple-negative human breast tumor cells and syngeneic murine breast tumor and melanoma cells. The in vitro and in vivo findings demonstrate the alternative mechanisms and the impact of endothelial RhoA activation during tumor cell transendothelial cell migration, highlighting the RhoA-ROCK pathway it as an important target for anti-metastatic treatment.

Acknowledgements
This work was supported by Hellenic Foundation for Research and Innovation (00376).

ST4

Ribosomal Proteins, Splicing and Cell Competition. Connecting the dots.

Eleni Tsakiri[1,#], Myrto Potiri[1,#], Kyriaki Kanakousaki[1], Martina Samiotaki[1], , Panagiota Kafasla[1], Marianthi Kiparaki[1,*]

[1]Biomedical Sciences Research Centre, "Alexander Fleming", Vari, Greece
[#]Equal contribution
Equal contribution

Elucidating the homeostatic mechanisms that safeguard tissue quality and organ size remains a major challenge in biology. Cell competition phenomenon is a quality control mechanism, where cells are eliminated via a non-autonomous mechanism in genetic mosaics, but not in a homogeneous environment. Cell competition was first documented in Drosophila between wild-type cells and cells with heterozygous dominant mutations in ribosomal protein (Rp) genes (known as Minute or Rp+/- genes). During my postdoctoral studies in Nicholas Baker lab, we found that in cells with Rp+/- mutations, RpS12 protein (a non-Minute Rp gene), via an uncharacterized mechanism activates the Xrp1 transcription factor. Xrp1 is responsible for most of the Rp+/- responses, including reduced cell competitiveness, slow growth, reduced translation, and even for the developmental delay of the Minute flies. Interestingly, the RpS12-Xrp1 pathway is responsible for the competitive elimination of some aneuploid cells, since the chromosomal loss can lead to Rp gene haploinsufficiency. Recently, Xrp1-dependent competition was found to result from multiple other genetic insults, highlighting the significance of understanding Xrp1 activation. Here, I will present unpublished work from my group, revealing that RpS12 regulates Xrp1 through alternative splicing, preferentially increasing the Xrp1 short isoform. We are using proteomics, together with genetic and alternative splicing analysis, to investigate the mechanism of splicing regulation of Xrp1 by RpS12 and the role of the two Xrp1 isoforms in $Rp^{+/-}$ responses. Our findings will contribute to the elucidation of the mechanisms of cell competition, and additionally to the understanding of the underlying causes responsible for the $Rp^{+/-}$ dependent phenotypes.

ST5

ERβ1 Sensitizes and ERβ2 Desensitizes ERα-Positive Breast Cancer Cells to the Inhibitory Effects of Antiestrogens and Their Combination with Retinoids

Aggeliki K. Meligova[1], Dimitra Siakouli[1], Sotiria Stasinopoulou[1], Despoina S. Xenopoulou[1], Maria Zoumpouli1, Vassiliki Ganou[1], Eleni-Fani Gkotsi[1], Aristotelis Chatziioannou[2], Olga Papadodima[1], Eleftherios Pilalis[3], Michael N. Alexis[1], Dimitra J. Mitsiou[1]*

[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]Center of Systems Biology, Biomedical Research Foundation of the Academy of Athens, Greece
[3]e-NIOS Applications PC, 25 Alexandros Pantou str., 17671 Kallithea, Greece
**e-mail: dmitsiou@eie.gr*

Breast cancer (BC) is the most common cancer among women worldwide. Almost 70% of all BC cases categorized as estrogen receptor alpha (ERα)-positive. Adjuvant endocrine therapy (AET) comprising antiestrogens (e.g. tamoxifen) and aromatase inhibitors (e.g. anastrazole) is the treatment of choice for early-stage ERα-positive breast cancer. However, almost 40% of tamoxifen-treated cases display no response or a partial response to AET and disease recurrence 20 years after onset of AET following primary surgery is a fairly common outcome.1,2 Therefore, new treatment options and relevant strong predictors of therapeutic response of those patients at intermediate or high risk of relapse are badly needed.

In addition to ERα, BC research has focused on ERβ1 and ERβ2, two isoforms of ERβ (the second ER isotype). At present, the impact of ERβ isoforms on ERα-positive BC prognosis and treatment remains elusive. We have previously shown that ERβ1 is associated with lower risk of early relapse while ERβ2 is associated with higher risk of late relapse of AET-treated early-stage ERα-positive BC.3 In the present study, we established clones of MCF7 cells constitutively expressing human ERβ1 or ERβ2 and investigated their role in the response of MCF7 cells to antiestrogens [4-hydroxytamoxifen (OHT) and fulvestrant (ICI182,780)] and retinoids [all-trans retinoic acid (ATRA)]. We showed that, compared to MCF7 cells, MCF7-ERβ1 and MCF7-ERβ2 cells were sensitized and desensitized, respectively, to the antiproliferative effect of antiestrogens, ATRA and their combination and to the cytocidal effect of the combination of OHT and ATRA. Analysis of the global transcriptional changes upon OHT–ATRA combinatorial treatment revealed uniquely regulated genes associated with anticancer effects in MCF7-ERβ1 cells and cancer-promoting effects in MCF7-ERβ2 cells. Our data are favorable to ERβ1 being a marker of responsiveness and ERβ2 being a marker of resistance of MCF7 cells to antiestrogens alone and in combination with ATRA.

1 *N Engl J Med 2017; 377:1836-1846. DOI: 10.1056/NEJMoa1701830*
2 *Nat Rev Clin Oncol 16, 296–311 (2019). https://doi.org/10.1038/s41571-018-0145-5*
3 *Cancer Lett 2015;358:37-42. doi:10.1016/j.canlet.2014.12.022.*

"This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning 2014-2020» in the context of the project "Validation – Reinforcement of the Prognostic Significance of Estrogen Receptor beta in Early Breast Cancer" (MIS 5050141)."

ST6

Neuron-specific proteasome activation extends the lifespan of nematodes and depends on neuron-muscle communication

Anna Gioran[1,†], Eleni Panagiotidou[1,2,†], Niki Chondrogianni[1*]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, 11635, Athens, Greece.
[2]Department of Biochemistry and Biotechnology, University of Thessaly, 41334 Larissa, Greece.
[†]These authors contributed equally to this work

Proteasome activation is an attractive strategy against ageing and aggregate-related neurodegenerative diseases. Using Caenorhabditis elegans as an animal model, we and others have shown that ubiquitous proteasome activation can extend the lifespan of nematodes and enhance resistance against aggregate-related proteotoxicity. More importantly, we recently revealed that neuron-specific proteasome activation in nematodes can induce proteasome activation in a distal tissue, i.e. the muscle (1). We now sought to investigate whether neuron-specific proteasome activation can also induce lifespan extension of nematodes, just like ubiquitous proteasome activation does. Indeed, we found that nematodes with proteasome activation only in their nervous system exhibit a prolonged lifespan. We also revealed that several of the components required for the inter-tissular communication between neurons and muscle are also necessary for the observed lifespan extension. Interestingly, we found that when small clear synaptic vesicles and the neuromuscular junction are impaired, the lifespan of these nematodes is not simply abolished but is significantly reduced compared to the respective controls. While trying to elucidate this outcome, our preliminary data suggest that neuron-specific proteasome activation causes a type of stress. We are currently focusing our efforts on understanding the type of stress these nematodes undergo and the role of the neuromuscular communication in compensating for its negative consequences.

References

1. *Panagiotidou E, Gioran A, Bano D, Chondrogianni N. Neuron-specific proteasome activation exerts cell non-autonomous protection against amyloid-beta (Aβ) proteotoxicity in Caenorhabditis elegans. Redox Biol. 2023 Jul;65:102817.*

ST7

Sulfated hyaluronan: in vitro and in vivo approaches to its anticancer potential in breast cancer

Christos Koutsakis[1], Zoi Piperigkou[1,2], Marco Franchi[3], Dimitrios Kletsas[4], Nikos K. Karamanos[1,2]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Foundation for Research and Technology-Hellas (FORTH) / Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece
[3]Department for Life Quality Studies, University of Bologna, Rimini, Italy
[4]Laboratory of Cell Proliferation and Ageing, Institute of Biology, National Centre for Scientific Research "Demokritos", Athens, Greece

Breast cancer is one of the most prevalent malignancies worldwide, with the expression of estrogen receptors (ERs) being crucial for disease progression. Hyaluronan (HA) is an extracellular matrix glycosaminoglycan (GAG) that plays a pivotal role in many biological processes. Typically, HA is the only GAG lacking sulfate groups, however chemically sulfated hyaluronan (sHA) has emerged as a promising biomolecule with anticancer potential. The present study seeks to evaluate the effects of sHA on breast cancer cells in vitro in 2D and 3D cell cultures, as well as in vivo. To this end, breast cancer cell lines with different ER expression (MCF-7, MDA-MB-231, shERβ MDA-MB-231, Hs578T) were treated with either HA or sHA fragments of 50 kDa. Proliferation, wound healing, adhesion, and invasion assays were performed to assess the HA fragments effect on the cells' functional properties. Scanning electron microscopy was used to closely observe cell morphology. Gene expression levels of EMT markers, extracellular matrix effectors, and HA partners were analysed with real-time PCR, and their respective protein levels were further corroborated with western blot and immunofluorescence. Cell cycle phase and stemness potential were determined with flow cytometry. Moreover, 3D cell cultures were generated, characterised, and their spheroid-forming capacity, proliferation, and invasion were measured post-treatment. Finally, in vivo tumorigenicity assays were performed by inoculating HA- or sHA-treated MDA-MB-231 cells into SCID mice. The obtained results indicate an anticancer effect for sHA, stronger than the one by the non-sulfated HA of the same molecular size. This action is mediated through the main HA receptor CD44 and can in part be attributed to competition with endogenous HA, as well as the ER status of the breast cancer cells. Overall, this study highlights the anticancer potential of sHA, and comprehensive understanding of the underlying mechanisms could contribute to the development of novel therapeutic strategies.

ST8

TGF-β induces cholesterol accumulation to regulate the fate of tumor-derived extracellular vesicles

Dorival Mendes Rodrigues-Junior[1], Chrysoula Tsirigoti[1], Konstantina Psatha[2], Dimitris Kletsas[3], Michalis Aivaliotis[2], Carl-Henrik Heldin[1], Aristidis Moustakas[1*]

[1]Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Box 582, Biomedical Center, Uppsala University, SE-75123 Uppsala, Sweden.
[2]Laboratory of Biochemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece.
[3]Laboratory of Cell Proliferation & Ageing, Institute of Biosciences and Applications, National Centre for Scientific Research 'Demokritos', GR-153 10 Athens, Greece.

Extracellular vesicles (EVs) can transport transforming growth factor-β (TGF-β) and promote tumor progression and metastasis. Whether TGF-β signaling regulates EV biogenesis, secretion or cargo remains underexplored. Here, TGF-β increased human breast and lung cancer EV release by activating MEK-ERK1/2, the latter phosphorylating sterol regulatory element-binding protein-2 that transcriptionally induces 7-dehydrocholesterol reductase (DHCR7) expression, thus raising intracellular cholesterol abundance. Proteomic profiling revealed that EVs contained TGF-β pathway-related molecules, including matrix metalloproteinase (MMP)-9. Additionally, EVs activated TGF-β signaling, even when EV uptake was blocked by heparin, while MMP inhibitor (MMPi) or proteinase treatment blocked EV-mediated TGF-β signaling, suggesting that EVs induced signaling from cell surface TGF-β receptors. Pro-migratory potential transferred by EVs also utilized cargo MMPs, since MMPi abrogated EVs induced invasion and motility. Finally, EVs transferred chemoresistance to recipient cells and inhibition of MEK or cholesterol synthesis, which reduced EV secretion, sensitized cancer cells to chemotherapeutic drugs. Hence, EV secretion orchestrated by TGF-β effectors, provides useful means in treatment or assessment of cancer outcome.

ST9

PTN enhances c-Met, VEGFR2 and mTORC1 activation and protein synthesis in endothelial cells

Eleni Mourkogianni*, Michaela-Karina Enake, Effrosyni Choleva, Athanasios Xanthopoulos, Evangelia Papadimitriou

*Laboratory of Molecular Pharmacology, Department of Pharmacy, School of Health Sciences, University of Patras, Greece; *e-mail: eleni9119@yahoo.gr*

Pleiotrophin (PTN) is a secreted factor that induces endothelial cell migration through its receptor PTPRZ1 and $\alpha_V\beta_3$ integrin. We have previously shown that genetic deletion of Ptprz1 or inhibition of PTPRZ1 tyrosine phosphatase activity leads to enhanced endothelial cell functions, an effect abolished by the c-Met inhibitor, crizotinib. In the present work, we investigate the activation of mTORC1 downstream of PTN, the implicated receptors upstream and the downstream signaling pathway and activities in endothelial cells. For this purpose, we used human umbilical vein endothelial cells (HUVEC) and lung microvascular endothelial cells (LMVEC) isolated from PTPRZ1 wild type ($Ptprz1^{+/+}$) and knock out ($Ptprz1^{-/-}$) mice. Treatment of HUVEC with PTN induces translation through mTORC1 activation, a phenomenon inhibited by the mTORC1 inhibitor rapamycin. Rapamycin also abolished the PTN-induced HUVEC migration and the increased proliferation and migration of Ptprz1-/- compared to $Ptprz1^{+/+}$ LMVEC. mTORC1 activity and translation are increased in Ptprz1-/- compared to $Ptprz1^{+/+}$ LMVEC. Given the inhibitory effect of crizotinib on the $Ptprz1^{-/-}$ LMVEC phenotype, we tested the involvement of c-Met in PTN activities. PTN enhances c-Met tyrosine phosphorylation through inhibition of the PTPRZ1 tyrosine phosphatase activity. Genetic deletion of Ptprz1 also leads to activation of c-Met. Interestingly, VEGFR2 is also activated by both PTN and genetic deletion of Ptprz1. Inhibition of either c-Met or VEGFR2 tyrosine kinase activities abolishes mTORC1 activation and translation, by both PTN and Ptprz1 genetic deletion, suggesting the involvement of both receptors in this pathway. Finally, the use of the selective $\alpha_V\beta_3$ integrin antibody LM609 or a PTN peptide that inhibits the PTN-$\alpha_V\beta_3$ interaction led to c-Met and mTORC1 activation and increased translation, implicating αvβ3 integrin in the mTORC1 activation. In summary, we suggest a novel signaling pathway of PTN, downstream of PTPRZ1 and $\alpha_V\beta_3$, involving c-Met, VEGFR2 and mTORC1 activation, that results in increased protein synthesis.

Acknowledgements
This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY) (scholarship to EM, EC), and by Andreas Mentzelopoulos scholarship (to MKE). The authors thank the Advanced Light Microscopy facility of the Medical School, University of Patras for using the Leica SP5 confocal microscope.

ST10

Development of decoy receptors and monoclonal antibodies for targeting remodeling of tumor microenvironment

Nikolaos Afratis[1,2], Blake Riley[3], Peter Chandler[3], Shivang Parikh[4], Idan Adir[2], Roma Parikh[4], Inna Solomonov[2], Orit Kollet[2], Carmit Levy[4], Ashley Buckle[3], Irit Sagi[2]

[1]Department of Agricultural Development, Agrofood & Management of Natural Resources, National and Kapodistrian University of Athens, Evripos Campus, 34400 Psachna, Evia, Greece
[2]Department of Immunology and Regenerative Biology, Weizmann Institute of Science, 234 Herzl Street, Rehovot 7610001, Israel
[3]Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia
[4] Department of Human Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Cancer is characterized by excessive extracellular matrix (ECM) turnover, driving disease progression, immune suppression, and treatment resistance. Proteases enable tumor cells to degrade the ECM, while cross-linking enzymes stiffen it. Modulating ECM remodeling is linked to metastasis and chemoresistance, impacting patient outcomes. Despite its significance in solid malignancies, ECM targeting for cancer treatment remains limited. This study introduces two protein-based inhibitors for kallikrein-related metallo-peptidase-4 (KLK4) protease and lysyl-oxidase (LOX) cross-linking enzyme.

Regarding the first case, developing precise inhibitors for metal-binding proteases like KLKs is challenging due to limited specificity understanding. To address this, a novel immunization approach for generation of monoclonal antibodies targeting a specific KLK4 sequence was developed. Analyzing KLK4's crystal structure identified a unique, less conserved region known as KLK4-loop 3, which shows potential for allosteric enzyme inhibition. A highly selective antibody interacting with KLK4 and KLK4-loop 3 hindered ovarian cancer cell proliferation and migration.

As for the second inhibitor it is well-known that targeting collagen cross-linking in cancer progression can hinder propagation when combined with an inflammation/cancer biomarker. In this study, we harnessed the inhibitory activity of LOX propeptide and engineered cross-reactive decoys to target both LOX and heat shock protein 70 (HSP70). These decoys effectively reduced circulating melanoma cells, inhibiting their proliferation and lung metastasis. The metastatic-supporting collagen assembly and ECM-remodeling enzymes were diminished, leading to alterations in melanoma's immune cell composition.

In summary, this research emphasizes the potential of innovative approaches to inhibit ECM enzymes in malignancies. Cancer's complexity demands solutions which address its various aspects, from ECM dynamics to immune responses. The development of selective inhibitors and decoys to target specific ECM enzymes has promising outcomes for cancer progression.

ST11

Small-molecule Structure Correctors Target Abnormal Apolipoprotein A-I Structure and Function Related to Cardiovascular Risk

Christina Gkolfinopoulou[1], Angeliki Bourtsala[1], Daphne Georgiadou[1], Anastasia-Georgia Dedemadi[1], Angeliki Chroni[1]*

[1]Institute of Biosciences and Applications, National Center for Scientific Research "Demokritos", Agia Paraskevi, Athens, Greece

Apolipoprotein A-I (apoA-I), the major protein component of high-density lipoprotein (HDL), plays a key role in HDL biogenesis and is critical for many of the atheroprotective properties that HDL displays. Naturally-occurring mutations in human apoA-I have been shown to disturb protein conformation and induce functional defects that impair the levels and atheroprotective properties of HDL. One such apoA-I mutation, the L178P, was shown to induce major defects in structural integrity and functions of the protein that may underlie the reduced HDL-cholesterol levels and increased cardiovascular risk observed in carriers of the mutation. Here, a library of marketed drugs (~1000 compounds) was screened against apoA-I[L178P] to identify molecules that can prevent mutant apoA-I from adopting its pathological conformation. Screening was performed by the thermal stability shift assay in the presence of fluorescent dye SYPRO Orange. As an orthogonal assay the monitoring of the change of fluorescence intensity of 1-anilinonaphthalene-8-sulfonic acid (ANS) upon its binding on hydrophobic sites on apoA-I was used. Screening analyses identified four potential structural correctors. Subsequent cellular assays (measurement of cytotoxicity) and biophysical analyses (measurement of protein α-helical content and thermodynamic stability by circular dichroism spectroscopy) narrowed the potential structure correctors to two. Functional analyses showed that these two compounds can restore the defective capacity of apoA-I[L178P] to promote cholesterol removal from macrophages, an important step for atheroprotection. Overall, our findings indicate that small molecules can correct defective apoA-I structure and function and may lead to novel therapeutic approaches for apoA-I-related dyslipidemias and increased cardiovascular risk.

Acknowledgment

This research has been co financed by Greece and the European Union through the Operational Program "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020), under the call RESEARCH – CREATE – INNOVATE. Project code: T2EDK-02361 (MIS 5067590). Title: "New therapies aiming to improve the atheroprotective and immunomodulatory properties of high density lipoprotein (HDL) for the treatment of autoimmune and cardiovascular diseases".

ST12

Gemcitabine chemoresistance in pancreatic ductal adenocarcinoma: implications of microbiome bacterial transporters

Nikoleta Iosifidou[1], Eleni Anagnostopoulou[1], Maria Botou[1], Eirini Kalfa[1], Stathis Frillingos[1,2]*

[1]Laboratory of Biological Chemistry, Department of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece
[2]Institute of Biosciences, University Research Center of Ioannina (URCI), Ioannina, Greece

Gemcitabine (dFdC), a widely used anticancer drug, is considered as the «gold-standard» therapy in treating aggressive pancreatic cancers. Recent research [1] has unveiled that γ-Proteobacteria, specifically Pseudomonas putida, Klebsiella pneumoniae and Citrobacter freundii, colonize cancer tumors and contribute to the emergence of drug resistance. These bacteria possess multiple candidate transporters for gemcitabine uptake and metabolize dFdC into a less active form (dFdU), ultimately diminishing its effectiveness. To date, there have been no reports regarding the transmembrane uptake of dFdC, or nucleosides in general, by these species. Our knowledge of dFdC uptake by bacteria relies solely on indirect evidence associated with E. coli NupC nucleoside transporter, without direct bacterial cell-based uptake assays. In this research, we systematically identified and functionally characterized gemcitabine transporters in two chemoresistance-related bacterial strains (K. pneumoniae ATCC 25955 and C. freundii ATCC 8090), marking a significant first. Prior results of our lab indicated NupC and NupG as the main gemcitabine transporters in E. coli K-12. Leveraging this knowledge, we conducted a phylogenetic analysis of putative nucleoside transporters belonging to the CNT (NupC-like) and NHS (NupG-like) families, across all Proteobacteria. Among the homologous transporters identified in K. pneumoniae and C. freundii, we focused on the ones that closely resembled their homologs in E. coli. These selected transporters underwent further functional characterization via heterologous expression in E. coli JW2389 (ΔnupC). We identified a total of five high-affinity gemcitabine transporters (K_M < 12.5 μM and > 2.5 μM), namely KpNupC, KpvcCNT and KpNupG from K. pneumoniae strain ATCC 25955 and CfNupC and CfNupG from C. freundi strain ATCC 8090. Overall, this study lays the groundwork for further investigation into the role of specific bacteria in drug availability within tumors and contributes to pinpointing novel therapeutic targets to address this gemcitabine resistance phenomenon, in pancreatic cancer cells.

[1] Geller et al. (2017) Science 357, 1156-1160.

We acknowledge support by project BIOMED-20 (MIS 5047236) implemented under the action "Reinforcement of Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) co-financed by Greece and EU (European Regional Development Fund)

ST13

AlphaFold Prediction of Structural Ensembles of Disordered Proteins

Faidon Brotzakis[1,2], Shengyu Zhang[1], Michele Vendruscolo[1],*

[1]Centre for Misfolding Diseases, Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge, UK
[2]BSRC Fleming, Vari, Greece

Deep learning methods of predicting protein structures have reached an accuracy comparable to that of high-resolution experimental methods. It is thus possible to generate accurate models of the native states of hundreds of millions of proteins. An open question, however, concerns whether these advances can be translated to disordered proteins, which should be represented as structural ensembles because of their heterogeneous and dynamical nature. Here we show that the inter-residue distances predicted by AlphaFold for disordered proteins are accurate, and describe how they can be used to construct structural ensembles. These results illustrate the possibility of making structural predictions for disordered proteins using deep learning methods trained on the large structural databases available for folded proteins.

Brotzakis, Z. F.; Zhang, S.; Vendruscolo, M. AlphaFold Prediction of Structural Ensembles of Disordered Proteins. bioRxiv 2023, DOI: 10.1101/2023.01.19.524720

ST14

The Structure Based Design of Potent and Selective Antagonists of Human Galectin-3

Anastasia S. Tsagkarakou[1], George Kontopidis[2], Hakon Leffler[3], Ulf J. Nilsson[4], László Somsák[5], Demetres D. Leonidas[1, *]

[1]Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis Campus, 41500, Larissa, Greece
[2]Department of Biochemistry, Veterinary School, University of Thessaly, 43131 Karditsa, Greece
[3]Department of Laboratory Medicine, Lund University, SE-2210 Lund, Sweden
[4]Department of Chemistry, Lund University, SE-2210 Lund, Sweden
[5]Department of Organic Chemistry, University of Debrecen, H-4002 Debrecen, Hungary
**Correspondence to: ddleonidas@bio.uth.gr*

Galectins are soluble β-galactosyl binding proteins that share structural homology within their carbohydrate recognition domain (CRD). They represent the most widely expressed lectins in all organisms and 12 members have been identified in humans [1]. These proteins are further classified according to the CRD distribution by proto-type, chimera-type, and tandem-repeat- type galectins [2]. Galectin-3, the sole member of chimera-type galectins, is so far unique in the family in having an extra long and flexible N-terminal domain consisting of 100–150 residues, made up of repetitive sequence of nine residues rich in proline, glycine, tyrosine, and glutamine, while it lacks any charged or large side-chain hydrophobic residues. The C- terminus is composed of 135 residues forming a globular structure, where it accommodates the CRD [3, 4]. Galectin-3 is widely expressed in immune cells with a role in several pathophysiological processes such as immune and inflammatory responses, tumor development and progression, atherosclerosis, diabetes, and wound healing [1]. The CRD scaffold is conserved but it allows subtle changes which result in different affinities for different carbohydrates. This yields a possibly unique selectivity, which combined with particular domain architecture and environmental modulation, produces a potential variety of pathophysiological responses [5]. We studied the binding of a series of novel carbohydrate- based derivatives to human galectin-3 by fluorescence polarization and isothermal titration calorimetry to show low micromolar Kd values. The best inhibitor displayed a Kd value of 8.0 μM. An analysis of the thermodynamic binding parameters revealed that the binding Gibbs free energy (ΔG) of the new inhibitors was dominated by enthalpy (ΔH). X-ray crystallography uncovered the unique role of water-mediated hydrogen-bonds in conferring enthalpy-driven affinity enhancement for the new antagonists. Our study offers new leads for galectin-3 inhibitor discovery and offers several possibilities for further development.

Acknowledgements
This research is co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme "Human Resources Development, Education and Lifelong Learning" in the context of the Act "Enhancing Human Resources Research Potential by undertaking a Doctoral Research" Sub-action 2: IKY Scholarship Programme for PhD candidates in the Greek Universities implemented by the State Scholarships Foundation (IKY).

References
1. *Tsagkarakou, A.S., et al. (2023), J Med Chem. 66, 12420–12431*
2. *Yang, R.Y., et al. (2008), Expert Rev Mol Med. 10*
3. *Dumic, J., et al. (2006), Biochimica et Biophysica Acta 1760, 616–635*
4. *Slack, R.J., et al. (2021), Int J Biochem Cell Biol. 130, 105881*
5. *Modenutti, C.P., et al. (2019), Frontiers in Chemistry, Vol 7, 823*

ST15

In vitro and in cellulo structural insights on the molecular chaperone Hsp90 via EPR

Angeliki Giannoulis[1], Maria Oranges[1], Arina Dalaloyan[1], Daniella Goldfarb[1]*

[1]Weizmann Institute of Science, Rehovot, Israel

Heat shock protein 90 (Hsp90) is an important molecular chaperone responsible for cellular proteostasis by stabilizing and regulating the activity of numerous substrates many of which are oncogenic proteins1. Therefore, Hsp90 has been proposed as target for cancer therapy. Hsp90 uses ATP and Mg(II) as essential co-factor undergoing conformational changes during function. Hsp90 consists of three domains, a highly conserved amino-terminal domain (NTD), a middle domain (MD) and a carboxyl-terminal domain (CTD). The NTDs comprise the ATPase site, the MDs are binding site for some co-chaperones and the CTDs are responsible for protein dimerization and co-chaperone binding. Using spin labeling and electron paramagnetic resonance (EPR) distance measurements2 we tracked the NTDs and CTDs conformations. Specifically, we resolved three different NTD conformations dependent of nucleotide3 and co-chaperone binding4. Additionally, we tracked the dissociation constant of the CTDs using a combination of biophysical techniques (native mass spectrometry, microscale thermophoresis, MST, and EPR) that allowed resolve allosteric effects of the NTDs and MDs on the CTD dimerization. After introducing labeled Hsp90 in Hela cells we also resolved CTD conformations in cellulo which were found to exhibit compactness compared to in vitro conditions. Our experimental approaches can be extended to a plethora of proteins to characterize structural elements that are difficult to resolve by other techniques due to inherent flexibility (eg. X-ray or cryo-EM) or due to the large size (eg. NMR) of the protein under study. To address the sensitivity issue of NMR spectroscopy in protein samples we showcase an approach that can enhance NMR signals orders of magnitude.

1. *Prodromou, C.; Pearl, L. H., Structure and functional relationships of Hsp90. Curr Cancer Drug Targets 2003, 3, 301-23.*
2. *Giannoulis, A.; Ben-Ishay, Y.; Goldfarb, D., Characteristics of Gd(III) spin labels for the study of protein conformations. Methods Enzymol 2021, 651, 235-290.*
3. *Giannoulis, A.; Feintuch, A.; Barak, Y.; Mazal, H.; Albeck, S.; Unger, T.; Yang, F.; Su, X. C.; Goldfarb, D., Two closed ATP- and ADP-dependent conformations in yeast Hsp90 chaperone detected by Mn(II) EPR spectroscopic techniques. Proc. Natl. Acad. Sci. U. S. A. 2020, 117, 395-404.*
4. *Giannoulis, A.; Feintuch, A.; Unger, T.; Amir, S.; Goldfarb, D., Monitoring the Conformation of the Sba1/Hsp90 Complex in the Presence of Nucleotides with Mn(II)-Based Double Electron-Electron Resonance. J. Phys. Chem. Lett. 2021, 12, 12235-12241.*

ST16

Hepatitis E virus: an investigation of the structural and biochemical properties of ORF1, unexplored, domains.

Maria D. Politi[1], Angelopoulou Aikaterini[1], Angelo Gallo[2], Georgios Bouras[1], Maria Birkou[1], Elvira Koutsouki[1], Bruno Canard[3], Bruno Coutard[3], Georgios A. Spyroulias[1]*

[1]Department of Pharmacy, University of Patras, GR-26504 Patras, Greece
[2]Department of Chemistry, University of Torino IT-10126 Torino, Italy.3
[3]Unité des Virus Émergents (UVE : Aix-Marseille Univ-IRD 190-Inserm 1207), Marseille, France.

Hepatitis E virus (HEV) is an emerging pathogen causing 20 million infections worldwide, leading to an estimated 3.3 million symptomatic cases with ~56,600/year being lethal. HEV belongs to the Hepeviridae family among the most broadly known types of Hepatitis such us A, B, C and D. In developing countries, HEV is spread by the fecal–oral route, while in developed countries the routes of transmission include the ingestion of undercooked meat or meat products derived from infected animals' transfusion of infected blood products and vertical transmission from a pregnant woman to the embryo. The genome of HEV is ~7.2 kb ss(+)RNA with a 5 7-methylguanosine cap structure followed by a short 5' untranslated region (UTR), three major open reading frames ([ORFs]: ORF1, ORF2, and ORF3), and a 3' UTR[1].

This study focusses on the structural and biophysical characterization of HEV ORF1 macro domain and methyltransferase. In general, viral MDs (vMD) can bind both ADP-ribose and its derivatives and possess a key-role in the recognition and removal of ADP-ribosylation. Specifically, vMDs have been identified as erasers of a single or several ADP-ribose moieties (de-MARylation/de-PARylation, respectively) and therefore may be implicated in mechanisms developed by the viruses to escape the early stages of host-immune response[2]. On the other hand, HEV methyltransferase (vMeT), catalyzes the transfer of the methyl group from S-adenosyl methionine to GTP, resulting in m7GTP, a procedure known as mRNA capping[3].

The current investigation encompassed a diverse array of experimental factors in order to optimize the expression, folding and stability of recombinant polypeptides of varying amino acid sequence lengths of vMD and vMeT. In order to acquire a deeper understanding of the biophysical characteristics of the vMD, we performed interaction studies using heteronuclear NMR Spectroscopy, Isothermal Titration Calorimetry (ITC), and biological assays employing western blot analysis.

References

1. *Debing, Yannick, et al. "Update on hepatitis E virology: Implications for clinical practice." Journal of hepatology 65.1 (2016): 200-212.*
2. *Wißing, Michael H., et al. "Virus-Host Cell Interplay during Hepatitis E Virus Infection." Trends in Microbiology (2020).*
3. *S. Anang et al., "Identification of critical residues in Hepatitis E virus macro domain involved in its interaction with viral methyltransferase and ORF3 proteins," Sci Rep, vol. 6, no. 1, p. 25133, Jul. 2016*

Acknowledgments

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant (Fellowship Number: 663).
We also acknowledge support of this work by the project "European Union's HE program (2021-2027), "ESPERANCE" ERA Chair (GA 101087215).

ST17

Crystal structures of the catalytic domain of human angiotensin-converting enzyme 2 (ACE2) in two distinct conformations reveal putative allosteric binding sites for the discovery of novel activators. Complementary studies shed light on the impact of these conformations on SARS-CoV2 binding to ACE2.

Marios Zouridakis[1]*, Oleksii Koupreienko[1,2], Nikolaos Alevizopoulos[3], Myrto-Athina Balachouti[3], Dimitris Georgiadis[3], Aikaterini I. Argyriou[4], Georgios A. Spyroulias[4], Petros Giastas[1,2]*

Structural Neurobiology Research Group, Department of Neurobiology, Hellenic Pasteur Institute, Athens, Greece
Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Athens, Greece
Laboratory of Organic Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Athens, Greece
Department of Pharmacy, University of Patras, GR-26504 Patras, Greece
**Correspondence to: petrosgiastas@aua.gr : mzouridakis@pasteur.gr*

The angiotensin-converting enzyme 2 (ACE2) is a member of the renin-angiotensin-aldosterone system (RAAS) that catalyzes mainly the conversion of the vasoconstrictor angiotensin II into angiotensin 1-7, a peptide contributing to the vascular and renal protection. In addition to its enzymatically important role, ACE2 is the main viral entry site in mammalian cells for the SARS-CoV2 virus, eventually emerging as a multipurpose therapeutic target. In the current study, we obtained the crystal structures of its open conformation in complex with the receptor binding domain (RBD) of SARS-CoV2 spike protein and of its closed, active-like conformation, with a non-hydrolysable substrate, phosphinic angiotensin II, bound to its active site, at resolutions of 2.3- and 1.8-Å, respectively. The comparison of these structures provides key insights on the mechanism of the transition of ACE2 from its resting to the active-like conformation. Moreover, the closed structure with the bound non-hydrolysable substrate allowed for the identification of the available, potential druggable allosteric binding sites of the enzyme's internal cavity, paving the way for the discovery of novel activators towards treatment of RAAS-related diseases. In addition, complementary biochemical and biophysical data elucidate the dispute that has arisen recently, regarding the impact of the conformational state of ACE2 on its binding affinity with SARS-CoV2 spike protein. We provide experimental evidence supporting that the closed conformation of ACE2 upon binding of a competitive inhibitor does not affect its recognition by SARS-CoV2; thus, such molecules (orthosteric enzyme inhibitors) could not have a protective role towards the related infection, based on our results.

ST18

McIdas dysregulation in the cell cycle threatens genome integrity

Spyridoula Bournaka[1], Stavroula Tsaridou[1], Marina Arbi[1], Vasiliki Asteria Lymperdopoulou[1], Stavros Taraviras[2], Zoi Lygerou[1]*

[1]Laboratory of Biology, School of Medicine, University of Patras, Rio, Patras, Greece
[2]Laboratory of Physiology, School of Medicine, University of Patras, Rio, Patras, Greece
**e-mail: z_lygerou@yahoo.com*

Maintaining a balance between proliferation and differentiation is critical for multicellular organisms, and this is accomplished by orchestrating the factors that govern both processes. Geminin superfamily members – Geminin, McIdas, and GemC1 – are a group of distantly related coiled-coil proteins known for their involvement in DNA replication and multiciliated cell differentiation1. We demonstrate how McIdas is regulated throughout the cell cycle and the consequences of its dysregulation on genomic integrity. McIdas levels decline after anaphase and then rise again before S phase. McIdas is an APC/C substrate that is identified by two destruction sequences, DBox and ABBA motif. We performed a mutational investigation on McIdas destruction sequences and discovered that the cell cycle profile of the cells was unaffected. Non-degradable McIdas, on the other hand, resulted in genomic instability phenotypes such as DNA bridges, micronuclei, multinuclear cells, and 53BP1 nuclear bodies. These phenotypes correspond to DNA replication disruption, indicating that McIdas plays a crucial function in genome duplication2. Because members of the Geminin superfamily are engaged in a wide range of cellular activities, studying their roles will help us to better comprehend their involvement in the balance of proliferation and differentiation.

1. *Arbi, M., Pefani, D.-E., Taraviras, S. & Lygerou, Z. Controlling centriole numbers: Geminin family members as master regulators of centriole amplification and multiciliogenesis. Chromosoma 127, 151–174 (2018).*
2. *Bertolin, A. P., Hoffmann, J. S. & Gottifredi, V. Under-replicated DNA: The byproduct of large genomes? Cancers 12, 1–20 (2020).*

ST19

Endoplasmic reticulum homeostasis modulates nanoarchitectural dynamics and activity of de novo sphingolipid synthesis holocomplex.

Chandris P[1,2,3,5,9†], Giannouli C[3,4], Samiotaki M[2], Stamatakis G[2], Coleman RA[5], Singer RH[5], Baltoumas F[2], Pavlopoulos G[2], Dunn T[6], Gupta S[6], Wymann MP[7], Malide D[8], Lavis L[10], Proia RL[3], Kumar A[9], Mautino A[9], Panayotou G[2], Shroff H[1,10]

[1]Section on High Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD, USA
[2]BSRC Al. Fleming, Institute of Bioinnovation, Vari, Attiki, Greece
[3]Genetics of Development and Disease Branch, NIDDK, National Institutes of Health, Bethesda, MD, USA
[4]Center for Autoimmune, Musculoskeletal and Hematopoietic Diseases, the Feinstein Institute for Medical Research, Manhasset, NY, USA
[5]Albert Einstein College of Medicine, Department of Anatomy & Structural Biology, Bronx, NY, USA
[6]Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA
[7]Department of Biomedicine, University of Basel, Switzerland.
[8]Light Microscopy Core, National Heart Lung and Blood Institute, NIH, Bethesda, MD, USA
[9]Advanced Imaging Lab, Marine Biological Laboratory, Woods Hole, MA, USA
[10]HHMI Janelia Research Campus, Ashburn, VA, USA
[†]Corresponding author

Sphingolipids comprise a large family of lipids that share a common aliphatic amino alcohol termed “sphingosine” in their backbone. Their metabolism is organized around two axes: the de novo synthesis and the recycling or salvage pathway. Disrupted sphingolipid metabolism is directly linked to obesity, neurodegenerative disorders and diabetes. Although their recycling pathway has been dissected to a great extent, the regulation of the mammalian holocomplex that initiates de novo synthesis of sphingolipids, named Serine Palmitoyl Transferase (SPT) is now a subject of increasing interest but its precise regulation is still obscured. In this study, we apply high end imaging approaches in live and fixed cells, implementing single molecule live cell imaging along with live and fixed cell nanoscopy and fluorescence anisotropy and provide evidence of a nanoclustered organization of the holocomplex. We further use Fluorescence Lifetime Imaging Microscopy-FRET (FLIM-FRET) and specific bimolecular cross-linking approaches to further dissect these interactions and find that ORMDL proteins which regulate the function of the enzyme exhibit a stimulus-dependent, domain-specific preference in their association with SPT. By implementing proteomics and lipidomics comparative approaches between de novo competent and deficient cells we present evidence that inhibition of ER associated degradation (ERAD) pathway, impacts upon the organization and the activity of the enzyme, unveiling a specific link between sphingolipid synthesis and protein quality control in endoplasmic reticulum.

ST20

Large-scale investigation of orphan genes in the human gut microbiome elucidates their evolutionary origins

Nikolaos Vakirlis[1,2]*, Anne Kupczok[3]*

[1]Institute For Fundamental Biomedical Research, B.S.R.C. "Alexander Fleming", Vari, Greece.
[2]Institute for General Microbiology, Kiel University, Kiel, Germany.
[3]Bioinformatics Group, Wageningen University, Wageningen, The Netherlands.
**Correspondence to vakirlis@fleming.gr , anne.kupczok@wur.nl*

Orphan genes, i.e., genes that lack homologs outside a given species, are ubiquitous in all domains of life. In prokaryotes, orphans can originate (i) in the native genome via de novo evolution from non-genic regions or alternative frames of existing genes, or by rapid divergence and remodeling, or (ii) in a foreign genome, including viruses, followed by horizontal transfer. However, strong quantitative evidence supporting either scenario is lacking. Here we performed a systematic, large-scale analysis of orphan genes from human gut prokaryotes. After exhaustive filtering, we identified more than 3 million orphans in 4,644 species, which lack similarity to other prokaryotes and have no known functional domains. We find that a given species pangenome contains on average 2.6% orphan genes, which are mostly rare within a species. Overall, orphan genes use optimal codons less frequently, and their proteins are more disordered than those of conserved (i.e., non-orphan) genes. Importantly, the GC content of orphan genes in a given genome closely matches that of conserved ones. In contrast, the 5% of orphans that share similarity to known viral sequences have distinct characteristics that set them apart from the rest of the orphans, including lower GC content. By identifying the genomic region from which they evolved in closely related species, we provide evidence for native origination for a small subset of orphan genes and find that these orphans also differ in their properties from the remaining orphans. Finally, predicting orphan function by examining functional annotations in operon-like arrangements suggests that some orphan genes are membrane-related and involved in spore germination. Our results support that orphans emerge due to multiple routes, challenging the notion that external elements such as phages and plasmids are the primary source of prokaryotic genetic novelty. Importantly, origination in the native genome might provide a constant influx of mostly transient genes into the cloud genome of prokaryotic pangenomes, where some orphans may prove adaptive, facilitating evolutionary innovation.

ST21

Identification of genes and transcription factors involved in EGFR inhibitor resistance in Non-Small Cell Lung Cancer cells

John Balanos[1]*, Maria F. Chatziaslani[1]*, Maria Tsagiopoulou[3], Spiros Papakostas[2], Maria Georgiadou[4]

[1]Aristotle University of Thessaloniki (AUTh), Greece
[2]International Hellenic University, Thessaloniki, Greece
[3]Centro Nacional de Analisis Genomico (CNAG), Barcelona, Spain
[4]Biomedical Research Institute, Foundation for Research and Technology, Ioannina, Greece

Background

Lung cancer is a leading cause of mortality in the Western World. Epidermal Growth Factor Receptor (EGFR) inhibitors have revolutionized the treatment of EGFR-mutant non-small cell lung cancer (NSCLC), but resistance often develops, leading to relapse. Recent studies have used single-cell RNA sequencing (scRNA-seq) in EGFR-mutant NSCLC cell lines treated with EGFR inhibitors, revealing multiple cancer cell subpopulations exhibiting distinct transcriptional and metabolic changes (Aissa et al 2021; Oren et al 2021). Our research focuses on re-analyzing these data with emphasis on transcription factors since they play a critical role in several cancer cell processes.

Methods

We compared scRNA-seq data from PC9 cells treated with the EGFR inhibitors erlotinib or osimertinib for 1, 2, 4, 9, and 11 days (erlotinib) or 3, 7 and 14 days (osimertinib). We analyzed a total number of 1,604 cells (drop-seq) for erlotinib resistance and 53,533 cells (10X Genomics) for osimertinib resistance. We analyzed the data using the Seurat package in R, Scanpy, scvi, and pySCENIC packages in Python.

Results

We confirmed the existence of distinct subpopulations, each with distinctive gene expression profiles. The analysis of transcription factor activity revealed TFs that were enriched in these clusters, thereby identifying essential regulatory elements orchestrating cellular responses to erlotinib and osimertinib.

Conclusions

The observed subpopulations and temporal changes in gene expression provide insights into potential resistance mechanisms and therapeutic interventions. We anticipate that our findings will serve as a stepping stone for further scientific research in the identification of novel drug targets, particularly in the realm of understanding transcription factor activities and their role in therapy resistance. This study also underscores the reusability of open-access data for driving new biological discoveries.

ST22

The impact of dietary restriction on the human gut microbiome

Christina Emmanouil[1]*, Konstantinos Rouskas[1,2], Maria Anezaki[1], Antigone Dimas[1,3]*

[1]Institute for Bioinnovation BSRC "Alexander Fleming", Athens, Greece
[2]Institute of Applied Biosciences, Centre for Research & Technology Hellas, Thessaloniki, Greece
[3]Institute of Translational Genomics, Helmholtz Munich, Germany
Correspondence to: dimas@fleming.gr or emmanouil@fleming.gr

Research has highlighted substantial effects of the gut microbiome (GM) on health, including contribution to homeostasis and immune system regulation, but also association with a range of diseases [1, 2]. Dietary restriction (DR, a reduction of nutrient intake without malnutrition) affects the GM [3]. Experiments involving DR in model organisms have reported mostly beneficial effects on health [3]. To explore the effects on humans, we established the FastBio multi-omics study (www.fastbio.gr). We profiled 200 Greek individuals who practice DR in the form of abstinence from animal products for 180-200 days annually for religious reasons. These periodically abstaining (PA) individuals alternate between omnivory and abstinence in a highly consistent manner. Participants were profiled at two timepoints to cover both a period of omnivory (T1) and a period of abstinence (T2). We also profiled 211 continuously omnivorous individuals (non-abstaining, NA) for comparison purposes.

To characterize participant GM, we performed 16S rRNA-Seq on DNA isolated from fecal samples. We constructed the Amplicon Sequence Variant (ASV) table and applied taxonomy. Alpha and beta diversity measures were calculated. Finally, we used MaAsLin2 [4] to call differentially abundant (DA) taxa.

We report a decrease in α-diversity across timepoints for both dietary groups, more prominent in the PA group (PA Observed at T1=379, T2=355, Wilcoxon paired $p<0.0001$, NA Observed T1=397, T2=385, $p<0.05$). Upon DR, we report ~16% DA ASVs in the PA group (q-value<0.05). Notably, 49 ASVs were found at lower abundance, with members of these genera having been linked with gut barrier integrity and neurological diseases [5, 6, 7]. Furthermore, 29 ASVs increased in abundance, including a butyrate-producing taxon with probiotic effects [8]. In the NA group, only ~1% of ASVs were DA.

Shedding light on the DR-driven effects of the GM on health will bring us closer to using dietary interventions as a therapeutic means.

[1] Y. J. Zhang, S. Li, R. Y. Gan, T. Zhou, D. P. Xu, and H. B. Li, "Impacts of gut bacteria on human health and diseases," Int J Mol Sci, vol. 16, no. 4, pp. 7493-519, Apr 2 2015.
[2] K. Hou et al., "Microbiota in health and diseases," Signal Transduction and Targeted Therapy, vol. 7, no. 1, p. 135, 2022/04/23 2022.
[3] L. Fontana and L. Partridge, "Promoting health and longevity through diet: from model organisms to humans," Cell, vol. 161, no. 1, pp. 106-118, Mar 26 2015.
[4] H. Mallick et al., "Multivariable association discovery in population-scale meta-omics studies," PLOS Computational Biology, vol. 17, no. 11, p. e1009442, 2021.
[5] K. N. Iversen et al., "The Effects of High Fiber Rye, Compared to Refined Wheat, on Gut Microbiota Composition, Plasma Short Chain Fatty Acids, and Implications for Weight Loss and Metabolic Risk Factors (the RyeWeight Study)," Nutrients, vol. 14, no. 8, p. 1669, 2022.
[6] D. Radjabzadeh et al., "Gut microbiome-wide association study of depressive symptoms," Nature Communications, vol. 13, no. 1, p. 7128, 2022/12/06 2022.
[7] R. A. Manfready, C. G. Goetz, and A. Keshavarzian, "Chapter Four - Intestinal microbiota and neuroinflammation in Parkinson's disease: At the helm of the gut-brain axis," in International Review of Neurobiology, vol. 167, T. R. Sampson, Ed.: Academic Press, 2022, pp. 81-99.
[8] A. Geirnaert et al., "Butyricicoccus pullicaecorum, a butyrate producer with probiotic potential, is intrinsically tolerant to stomach and small intestine conditions," Anaerobe, vol. 30, pp. 70-74, 2014/12/01/ 2014.

ST23

A large-scale analysis of genetic novelty in budding yeast

Emilios Tassios[1,3], David Rinker[2], Antonis Rokas[2], Christoforos Nikolaou[1], Nikolaos Vakirlis[1]

[1]Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece
[2]Department of Biological Sciences, Vanderbilt University, Nashville TN USA
[3]University of Ioannina, Ioannina, Greece

De novo gene emergence, although a as improbable for decades, is an important source of novelty for organisms, linked with phenotypic innovations and species-specific characteristics. Here, we conducted the largest scale computational investigation of de novo gene emergence to date, exploiting a rich dataset comprised from 332 budding yeast genomes, spanning the entire biodiversity of the Saccharomycotina subphylum. We were able to identify over 400,000 taxonomically restricted genes (TRGs) at different phylogenetic levels, from species-specific ones to conserved across the Saccharomycotina. This enabled us to reveal macro-evolutionary trends of gene and protein properties that hold across yeast lineages, including that GC% of genes do not change with age while intrinsic protein disorder consistently decreases. By employing synteny analysis, we isolated more than 10,000 de novo genes. Additionally, we found thousands of TRGs that have diverged beyond recognition and have properties contrasting those of de novo genes such as longer length and lower biosynthetic cost. Furthermore, we investigated the cryptic property of intergenic regions to encode transmembrane (TM) domains, if theoretically translated, more frequently than expected by chance, a finding previously reported in baker's yeast. This TM-forming enrichment is present genome wide and is not explained by the hydrophobic content of the sequences nor their size and composition. Finally, we found a correlation, across species, between this intergenic enrichment and the number of TM domains in evolutionarily young genes hinting towards a link to de novo emergence.

ST24

JOINTPROMISE: Metabolic Insights into Organoids Development in Automated Bioreactor Platforms

Paraskevi Zagana[1], Alexandra Paxinou[1], Maria Klapa[1*]

[1]Institute of Chemical Engineering Sciences, Foundation for Research and Technology Hellas, Patras, Greece
**Corresponding author: mklapa@iceht.forth.gr*

Addressing the challenge of microtissues and organoids formation within automated bioreactor platforms, the EU-funded JOINTPROMISE project seeks to revolutionize the landscape of joint health by developing complex joint implants. These advanced implants are produced using an automated, GMP-grade platform, which integrate cutting-edge technologies such as organoid culture, 3D-bioprinting, and bioreactors, will hopefully carry the essential biological information required for regeneration, towards osteoarthritis (OA), that affects a quarter of the global adult population.

The Metabolic Engineering and Systems Biology Laboratory (MESBL) of the Foundation for Research and Technology, Hellas (FORTH), Institute of Chemical Engineering Sciences (ICE-HT), focuses on unveiling the metabolic behavior of chondrocytes, foundational building blocks for the organoids.

Combining endometabolomics and exometabolomics, we adhered to standardized sample collection and handling protocols to ensure data accuracy and reproducibility. This dual perspective offers comprehensive insights into how these cells function and interact within the context of automated bioreactor platforms. Advanced chromatography-mass spectrometry techniques, including LC-MS and GC-MS, allow us to precisely characterize the metabolites within these cellular structures. Experimental data will herein be presented to illustrate how these metabolites respond to the automated bioreactor environment, offering a deeper understanding of their behavior.

The integration of our experimental data with the JOINTPROMISE project's broader goals holds the potential to enhance the design and functionality of tissue-engineered joint implants. Our findings may pave the way for more effective regenerative solutions in the battle against OA, ultimately improving the quality of life for those affected by joint-related disorders.

ST25

Blood transcriptome discriminates individuals at high risk for progression from preclinical to clinical lupus

Sofia Papanikolaou[1,2*], Evgenia Emmanouilidou[3], Christina Adamichou[3], Dionysios Nikolopoulos[4], Myrto Nikoloudaki[3], Noemin Kapsala[4], Antigoni Pieta[4], Argiro Repa[3], Eleni Kalogiannaki[3], Nikos Malissovas[5], Nestor Augoustidis[3], Nikolaos Kougkas[3], Dimitra Nikoleri[1], Despoina Kosmara[1], Aggelos Banos[5], Anastasios Eskitzis[3], Giannis Vatsellas[5], Prodromos Sidiropoulos[1,3,6], Antonios Fanouriakis[4], Dimitrios Boumpas[4], Dimitrios Konstantopoulos[2], Christoforos Nikolaou[2], George Bertsias[1,3,6]

[1]Laboratory of Rheumatology, Autoimmunity and Inflammation,Medical School, University of Crete, Greece.
[2]Institute for Bioinnovation-IBI, Biomedical Sciences Research Center "Alexander Fleming", Athens, Greece.
[3]Clinic of Rheumatology, Clinical Immunology, University Hospital of Heraklion, Crete, Greece
[4]Attikon General University Hospital of Athens, Greece.
[5]Greek Genome Center, BRFAA, Athens, Greece.
[6]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology - Hellas (FORTH), Heraklion, Greece.

Systemic Lupus erythematosus (SLE) is an autoimmune disorder characterized by self-reactivity leading to production of autoantibodies (autoAbs) and multisystem inflammation. SLE onset is preceded by a preclinical phase evidenced by the presence of anti-nuclear and other autoAbs, which however, have low predictive value for development of clinical SLE.

The main objective of this work is to define the subgroup of autoAbs-positive individuals who are at high risk for progression into SLE by integrating environmental, clinical/serological, genetic and transcriptome data.

A multicenter, across three European countries, inception cohort of autoAbs-positive individuals or first-degree relatives (FDRs) of SLE patients were monitored prospectively over five years for possible transition to SLE according to the classification criteria. Structured data were collected based on demographics, family and medical history, clinical (criteria and selected non-criteria manifestations) and serological parameters, use of medications, hydroxyvitamin D levels and lifestyle. Blood samples were stored for RNA-sequencing at the first assessment and analysed by integrating the structured data after 2 yeas follow-up. We implemented unsupervised and supervised learning algorithms and performed differential gene expression analysis in order to identify specific genes and molecular pathways that could discriminate the subgroup of individuals who are at high risk for progression into SLE. Additionally, the transcriptomic data were combined with clinical, serological and environmental information.

Differential expression analysis demonstrated increased levels of IFI27, OTOF, IFI44L expression in individuals that progressed to SLE. Co-expression analysis revealed a module that exhibited a significant negative correlation with progression to SLE, particularly enriched with genes associated with the activated TLR4 signaling pathway. Feature selection algorithms identified a set of the most important genes for SLE classification, including collagen related genes.

Our results pointed out several genes that contribute to the progression to established SLE and could be exploited as novel diagnostic biomarkers.

ST26

Peri-weaning cholera toxin consumption suppresses chemically-induced carcinogenesis in mice

Hara Afaloniati[1], Georgios Aindelis[2], Katerina Spyridopoulou[2], Maria K. Lagou[3], Anastasia Tsingotjidou[4], Katerina Chlichlia[2], Suzan E. Erdman[5], Theofilos Poutahidis[3], Katerina Angelopoulou[1]*

[1]Laboratory of Biochemistry and Toxicology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece,
[2]Department of Molecular Biology and Genetics, Democritus University of Thrace, University Campus Dragana, Alexandroupolis, Greece,
[3]Laboratory of Pathology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece,
[4]Laboratory of Anatomy, Histology and Embryology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece,
[5]Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, USA,
**e-mail: kangelop@vet.auth.gr*

Gastrointestinal bacteria have been shown to have an impact on both local and systemic immunity, and as a consequence either promote or suppress cancer development in the gut, as well as in epithelia distally located from it. Particular interest has lately been focused on the potential ability of cholera-toxin (CT), a protein exotoxin of the small intestine pathogenic bacterium Vibrio cholerae, to modulate cancer promoting events, through its well-established immunomodulatory and anti-inflammatory properties. Following the notion that perinatal bacterial exposure might confer immune system competency for life, in the present study we investigated whether early-life administration of CT may shape local and systemic immunity to impart a protective effect against tumor development in epithelia distantly located from the gut. For that, newborn mice were orally treated with low non-pathogenic doses of CT and later challenged with the carcinogen 7,12-dimethylbenzanthracene (DMBA), known to cause mainly mammary, but also skin, lung and stomach cancer. Tumor development outcome was followed throughout and the possible mechanisms through which CT exerts its action were examined. CT administration was found to suppress carcinogenesis and increase overall survival. This long-term effect was shown to be mediated through significant alterations at the preneoplastic stages. CT modified the systemic, local, and gut mucosal immunity by increasing regulatory T-cells and cytotoxic T-lymphocytes, and by decreasing neutrophils. Moreover, CT affected gene expression of several cytokines (e.g. IL-1b, IL10, IFN-γ), classical cancer-related genes (e.g., p21, Her2, c-Myc), as well as cancer/inflammation-related genes (e.g. Runx2, Id2). In addition, mice treated with CT had significantly altered gut bacteria composition with increased abundance of Bacilli class and Lactobacillus genus, and decreased levels of Clostridia class. Overall, these results support the notion that early-life CT consumption is able to affect host's immune, microbiome and gene expression profiles towards the prevention of cancer.

ST27

Lysophosphatidic acid induces invadosome formation in fibroblasts and stimulate ECM invasion

Dimitris Nastos, Paraskevi Kanellopoulou, Ilianna Barbayianni, Vassilis Aidinis*

Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center Alexander Fleming, Athens, Greece.

Lysophosphatidic acid (LPA) is a growth factor-like bioactive (lyso)phospholipid, that is largely produced in biological fluids and inflamed sites by the lysopholipase D Autotaxin (ATX)1. LPA signals through at least six cognate GPCR receptors (LPAR1-6) that exhibit widespread, but differential, cell and tissue distribution, as well as overlapping specificities1. Increased ATX/LPA expression has been reported in different chronic inflammatory diseases1, including Idiopathic Pulmonary Fibrosis (IPF)2 and Rheumatoid Arthritis (RA)3. The pathogenesis of both IPF and RA are characterized by the activation and accumulation of the respective, pulmonary or synovial, fibroblasts that share many similarities with cancer cells, including the activation of invasion and migration. Invasion critically relies on the proteolysis of the underlying ECM via invadosomes (invadopodia in cancer cells and podosomes in other cell types), while the formation of podosomes in lung fibroblasts was recently shown to be an inherent property of IPF fibroblasts, promoting ECM invasion and pulmonary fibrosis4. In this report, LPA was found to stimulate, in both fibroblast types, proliferation, to regulate metabolic status and to rearrange the actin cytoskeleton. More importantly, LPA was found to induce the formation of invadosomes, podosome rosettes in pulmonary fibroblasts and invadopodia in synovial fibroblasts, while preliminary results suggest that invadosome formation stimulates ECM invasion. Moreover, ongoing expression profiling studies coupled with pharmacologic pathway dissection are expected to dissect the involved cellular pathways and to possibly explain the differential invadosome formation in fibroblasts from different anatomical sites.

1 *Magkrioti, C. et al. Autotaxin and chronic inflammatory diseases. J Autoimmun 104, 102327, (2019).*
2 *Oikonomou, N. et al. Pulmonary autotaxin expression contributes to the pathogenesis of pulmonary fibrosis. Am J Respir Cell Mol Biol 47, 566-574, (2012).*
3 *Nikitopoulou, I. et al. Autotaxin expression from synovial fibroblasts is essential for the pathogenesis of modeled arthritis. J Exp Med 209, 925-933, (2012).*
4 *Barbayianni, I. et al. SRC and TKS5 mediated podosome formation in fibroblasts promotes extracellular matrix invasion and pulmonary fibrosis. Nat Commun 14, 5882, (2023).*

ST28

Transcriptomic meta-analysis characterizes shared molecular mechanisms between psoriasis and obesity

Charis Antonatos[1], Georgios K. Georgakilas[1,2], Evangelos Evangelou[3,4,5], Yiannis Vasilopoulos[1,*]

[1]Laboratory of Genetics, Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, 26504 Patras, Greece,
[2]Laboratory of Hygiene and Epidemiology, Department of Clinical and Laboratory Research, Faculty of Medicine, University of Thessaly, 41222 Larissa, Greece,
[3]Department of Hygiene and Epidemiology, Medical School, University of Ioannina, 45110 Ioannina, Greece,
[4]Department of Biomedical Research, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, 45510 Ioannina, Greece,
*[5]Department of Epidemiology and Biostatistics, MRC Centre for Environment and Health, Imperial College London, London W2 1PG, UK, *email: iovasilop@upatras.gr*

Despite the abundance of epidemiological evidence for the high comorbid rate between psoriasis and obesity, systematic approaches on common inflammatory mechanisms have not been adequately explored. Here, we performed a transcriptomic meta-analysis of publicly available RNA sequencing datasets to unveil putative mechanisms that are postulated to exacerbate both diseases and establish the inflammatory circuit. We considered all publicly available total RNA-sequencing datasets between adult psoriasis and obesity patients in comparison to healthy controls. We performed two late stage, disease-specific meta-analyses and explored their commonalities via gene set enrichment analysis. We further investigated shared co-expression patterns through consensus co-expression network analysis on paired-end datasets to unravel conserved co-expression modules derived from the expression matrices of both comorbid diseases. Our systematic search identified 4 psoriatic (cases=76, controls=75) and 5 metabolically healthy obese (cases=76, controls=55) datasets. Single-gene meta-analyses revealed significant overlaps between up- (n=170, $P=6.07 \times 10^{-65}$) and down-regulated (n=49, $P=7.1 \times 10^{-7}$) transcripts, associated with the increased T cell response as well as their polarization to the pathogenic T helper (Th) 17 subtype. Our consensus co-expression clustering approach disentangled eleven consensus correlated modules that incorporated the majority of shared deregulated genes, associated with either the differentiation of leukocytes or metabolic pathways with a similar direction pattern in both comorbid diseases. Notably, all our enrichment analyses highlighted the association between Th17 cells and both comorbid diseases. Our novel findings through whole transcriptomic analyses characterize the inflammatory commonalities between psoriasis and obesity implying the assessment of several expression profiles that could serve as putative comorbid disease progression biomarkers, as well as elucidate the clinical efficacy of Th17-inhibiting therapies in obese psoriatic patients.

ST29

Detection of mitochondrial transfer RNA mutations in patients with Idiopathic Pulmonary Fibrosis, Sarcoidosis, Asthma and Chronic Obstructive Pulmonary Disease

Michaela Kafida[1], Maria Karela[1]*, Stefania Stefanidou[1], Vasileios Chronopoulos[1], Maria Tasiou[1], Zoi Daniil[2], Emily Zifa[1]

[1]Department of Biochemistry and Biotechnology, University of Thessaly, 26 Ploutonos St., 41221, Larissa, Greece.
[2]Department of Medicine, University of Thessaly, 26 Ploutonos St., 41221, Larissa, Greece.

IPF, sarcoidosis, asthma, and COPD are all pulmonary diseases characterized by reactive oxygen species overproduction leading to mitochondrial dysfunction and to tissue injury. To investigate the involvement of mitochondrial genetic background, we performed a systematic mutational analysis of all 22 mt-tRNA-encoding genes and part of their flanking genes of large cohorts of patients compared to a group of matched healthy controls. A wide array of novel as well as already known mitochondrial tRNA mutations, along with specific rRNA mutations, are significantly more frequent in patients than in the controls. Mutations tend to accumulate in the mt-DNA of patients who suffer from severe pulmonary diseases, as 95% of IPF, 84% of COPD (yet only 15/22 mt-tRNA genes have been sequenced), 81% of sarcoidosis, and 73% of asthma patients present one or more mutations. Some of the detected mutations are polymorphic, whereas others have been recognized as pathogenic for both pulmonary and non-pulmonary diseases. For example, the A14696G mutation in mt-tRNA^{Glu} correlated to MELAS, was detected in a COPD patient. Furthermore, unique mutational combinations were uncovered exclusively in patients. For instance, the combination A10398G in mt-ND3 / A12308G in mt-tRNA$^{Leu\ (CUN)}$ which increases breast cancer probability was detected exclusively in women COPD patients. The increased number of mutations seems to be associated with pulmonary disorders with multifactorial inheritance, since both genetic and environmental factors trigger their onset. Although further studies in larger cohorts are needed to confirm this observation, sequencing of the entire mitochondrial genome might lead to the identification of other mutations that contribute to the diseases' phenotype.

- *Zifa, E., Giannouli, S., Theotokis, P., Stamatis, C., Mamuris, Z., & Stathopoulos, C. (2007). Mitochondrial tRNA mutations: clinical and functional perturbations. RNA biology, 4(1), 38–66. https://doi.org/10.4161/rna.4.1.4548*
- *Zifa, E., Daniil, Z., Skoumi, E., Stavrou, M., Papadimitriou, K., Terzenidou, M., Kostikas, K., Bagiatis, V., Gourgoulianis, K. I., & Mamuris, Z. (2012). Mitochondrial genetic background plays a role in increasing risk to asthma. Molecular biology reports, 39(4), 4697–4708. https://doi.org/10.1007/s11033-011-1262-8*
- *Daniil, Z., Kotsiou, O. S., Grammatikopoulos, A., Peletidou, S., Gkika, H., Malli, F., Antoniou, K., Vasarmidi, E., Mamuris, Z., Gourgoulianis, K., & Zifa, E. (2018). Detection of mitochondrial transfer RNA (mt-tRNA) gene mutations in patients with idiopathic pulmonary fibrosis and sarcoidosis. Mitochondrion, 43, 43–52. https://doi.org/10.1016/j.mito.2018.10.004*

ST30

Activation of the complement and kynurenine molecular pathways in schizophrenia

Alex Hatzimanolis[1]*, Stefania Foteli[1], Nikos Stefanis[1], Maria Gazouli[2]

[1]Molecular Genetics Laboratory, Department of Psychiatry, National and Kapodistrian University of Athens Medical School, Eginition Hospital, Athens, Greece
[2]Laboratory of Biology, Department of Basic Medical Sciences, National and Kapodistrian University of Athens Medical School, Athens, Greece

Findings from large-scale genomic and biochemical studies in humans have provided evidence for the involvement of complement system factors in the pathophysiology of schizophrenia (SZ). Further, higher levels of inflammatory markers have been consistently observed in patients with SZ, which may trigger the induction of tryptophan metabolism through the activation of the kynurenine pathway (KP). In the current study, we aimed to investigate alterations in gene expression and protein levels of key complement components (Complement-4A (C4A), Cub and Sushi Multiple Domain 1 (CSMD1)), inflammatory markers (IL-1beta, TNFa, IL-10), and KP pathway factors (IDO/TDO metabolic enzymes, kynurenic acid (KYNA)) in drug-naïve young patients with SZ (n=112) and matched healthy volunteers (n=80). Associations with symptom severity and treatment efficacy were also examined. We observed a positive correlation between C4A and CSMD1 mRNA levels in healthy volunteers, but not among SZ patients. In addition, C4 copy number gene variants previously associated with SZ risk correlated with higher C4A mRNA levels in SZ, which confirms the regulatory effect of C4 locus structural variants on gene expression. Markedly increased serum protein levels of C4A, IL-1beta, TNFa and KYNA were noted in SZ patients, suggesting the up-regulation of the KP pathway in SZ likely through the enhancement of pro-inflammatory processes. Moreover, it is of interest that higher C4A mRNA levels and elevated KYNA predicted more severe psychopathology symptoms and inadequate response to antipsychotic medication. Overall, these findings imply that the over-activation of complement pathway and pro-inflammatory reaction may result to the deregulation of KP and potentially to unfavorable clinical and treatment outcomes in the early course of SZ.

References

- *Sekar A, Bialas AR, de Rivera H, Davis A, Hammond TR, Kamitaki N, Tooley K, Presumey J, Baum M, Van Doren V, Genovese G, Rose SA, Handsaker RE; Schizophrenia Working Group of the Psychiatric Genomics Consortium; Daly MJ, Carroll MC, Stevens B, McCarroll SA. Schizophrenia risk from complex variation of complement component 4. Nature. 2016 Feb 11;530(7589):177-83.*
- *Hatzimanolis A, Foteli S, Stefanatou P, Ntigrintaki AA, Ralli I, Kollias K, Nikolaou C, Gazouli M, Stefanis NC. Deregulation of complement components C4A and CSMD1 peripheral expression in first-episode psychosis and links to cognitive ability. Eur Arch Psychiatry Clin Neurosci. 2022 Oct;272(7):1219-1228.*
- *Gracias J, Orhan F, Hörbeck E, Holmén-Larsson J, Khanlarkani N, Malwade S, Goparaju SK, Schwieler L, Demirel , Fu T, Fatourus-Bergman H, Pelanis A, Goold CP, Goulding A, Annerbrink K, Isgren A, Sparding T, Schalling M, Yañez VAC, Göpfert JC, Nilsson J, Brinkmalm A, Blennow K, Zetterberg H, Engberg G, Piehl F, Sheridan SD, Perlis RH, Cervenka S, Erhardt S, Landen M, Sellgren CM. Cerebrospinal fluid concentration of complement component 4A is increased in first episode schizophrenia. Nat Commun. 2022 Nov 3;13(1):6427.*
- *Erhardt S, Schwieler L, Imbeault S, Engberg G. The kynurenine pathway in schizophrenia and bipolar disorder. Neuropharmacology. 2017 Jan;112(Pt B):297-306.*

ST31

Childhood Asthma Exacerbation: The immune-fibrotic face of NETs.

Maria Ntinopoulou[1], Dimitrios Cassimos[2], Eugenia Roupakia[3,4], Evangelos Kolettas[3,4], Maria Panopoulou[5], Elpis Mantadakis[2], Theocharis Konstantinidis[1], Akrivi Chrysanthopoulou[1],*

[1]Laboratory of Molecular Immunology, Department of Biological Applications and Technology, School of Health Sciences, University of Ioannina, Ioannina, Greece
[2]Department of Pediatrics, University General Hospital of Alexandroupolis, Department of Medicine, School of Health Sciences, Democritus University of Thrace, Alexandroupolis, Greece
[3]Laboratory of Biology, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece
[4]Biomedical Research Institute, Foundation for Research and Technology-Hellas, Ioannina, Greece
[5]Department of Microbiology, University General Hospital of Alexandroupolis, Department of Medicine, School of Health Sciences, Democritus University of Thrace, Alexandroupolis, Greece
**Correspondence: achrysan@uoi.gr*

Childhood asthma is one of the most prevalent pediatric chronic disorders, characterized by recurrent exacerbation-remission cycles. Inflammation and airway tissue remodeling are hallmarks of the disease and seem to be driven by various cell populations, including both immune and structural cells. Neutrophils have been implicated in asthma pathophysiology; however, their precise role remains a scientific puzzle. Our study tried to investigate the contribution of neutrophils in asthma exacerbation, and it mainly focused on the mechanism of neutrophil extracellular traps (NETs). Hence, pediatric patients during disease exacerbation were recruited and serum samples were collected. Subsequently, neutrophils from healthy donors were stimulated with the asthma serum, and the NETotic dynamic of neutrophils as well as the protein profile of the released NETs were explored by RNA and protein methods. Since tissue remodeling is regulated by fibroblasts, the potential crosstalk between asthma-NETs and lung fibroblasts was further examined. An in-vitro co-culture system was deployed, and the functional changes of fibroblasts were analyzed. Particularly, conducted experiments demonstrated that both NETs and interleukin (IL)-17A are present in asthma exacerbation serum. Moreover, the inflammatory disease milieu i.e., serum, can induce the formation of IL-17A-enriched NETs. Disease NETs represent a contributor to lung remodeling since they can influence the behavior of lung fibroblasts. Co-culture system, using NETs and lung fibroblasts, indicated that asthma-NETs induce fibroblast activation and increase their migratory/healing capacity and potential to produce collagen. On the other hand, these functional traits are not acquired by fibroblasts when performing dismantling of asthma-NETs scaffold or IL-17A neutralization on asthma-NETs. Together, this study provides evidence linking neutrophils/NETs to the immune-fibrotic aspects of childhood asthma. NETs appear as potential biomarkers of disease severity as well as targets for new therapeutic interventions.

ST32

A novel imputation strategy for structural variants and its application in schizophrenia

Maria Papavasileiou[1]*, Paraskevi-Maria Moysi[2], Christina Pertsali[3], Fotios Tsetsos[2]

[1]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupoli, Greece
[2]Department of Food Science and Nutrition, University of the Aegean, Myrina, Greece
[3]Department of Medicine, University of Ioannina, Ioannina, Greece

Schizophrenia is a highly heritable and polygenic neuropsychiatric disorder often characterized by poor quality of life and decreased life expectancy, with an estimated heritability of 60-80%. Structural variants (SVs) are large-scale genomic alterations that play a pivotal role in human genetic variation and disease susceptibility. Imputing SVs from Genome-Wide Association Study (GWAS) summary statistics presents a novel challenge in the field of human genetics. In this study, we introduce a pioneering approach to address this challenge by utilizing missingness patterns and the Human Genome Structural Variation Consortium (HGSVC) panel as reference. By examining patterns of missing data, we aim to infer the presence of SVs in regions that have previously not been extensively studied. This innovative strategy allows us to cast a broader net in our search for SVs, potentially revealing previously undiscovered variants associated with complex traits. Leveraging the HGSVC data enhances the accuracy and robustness of our imputation method. To demonstrate the utility and applicability of our approach, we apply it to the 2022 Genome-Wide Association Study (GWAS) conducted by the Psychiatric Genomics Consortium. Schizophrenia is a complex psychiatric disorder with a strong genetic component, and SVs have been implicated in its etiology. By employing our method on this dataset, we aim to uncover previously unexplored SV associations with schizophrenia, potentially shedding light on novel genetic mechanisms underlying this complex condition. Our study introduces an innovative method for imputing structural variants from GWAS summary statistics, utilizing missingness patterns and external reference data from the HGSVC panel. By applying this approach to the schizophrenia GWAS dataset, we aim to advance our understanding of the genetic basis of complex diseases and pave the way for future discoveries in the field of human genetics.

ST33

Combined analysis of circulating tumor cells and extracellular vesicles derived from small cell lung cancer patients

Dimitrios Papakonstantinou[1], Argyro Roumeliotou[1], Evangelia Pantazaka[1], Athanasios-Nasir Shaukat[2], Athina Christopoulou[3], Angelos Koutras[4], Foteinos-Ioannis Dimitrakopoulos[4], Vassilis Georgoulias[5], Athanasios Kotsakis[6], Constantinos Stathopoulos[2], Galatea Kallergi[1]*.

[1]Laboratory of Biochemistry/Metastatic Signaling, Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, Patras, Greece.
[2]Department of Biochemistry, School of Medicine, University of Patras, Patras, Greece
[3]Oncology Unit, ST Andrews General Hospital of Patras, Patras, Greece
[4]Clinical and Molecular Oncology Laboratory, Division of Oncology, Department of Medicine, Medical School, University of Patras, Patras, Greece.
[5]Hellenic Oncology Research Group (HORG), Athens, Greece.
[6]Department of Medical Oncology, General University Hospital of Larissa, Larissa, Greece.
**email: gkallergi@upatras.gr*

The metastatic potential of small-cell lung cancer (SCLC) necessitates the identification of prognostic biomarkers for clinical decisions regarding therapeutic approaches. Our previous research highlighted JUNB and CXCR4 significance in breast and non-small cell lung cancer (NSCLC) patients' circulating tumor cells (CTCs). In the current study, we investigated the expression of these biomarkers in CTCs and plasma-derived exosomes from 100 treatment-naïve SCLC patients. Exosome analysis was conducted on 58 of them. Cytospins were analyzed with VyCAP, while exosomes were characterized molecularly and transcriptomically. CTCs were present in 70% of patients, with CTCs being characterized as follows: 23% as ($CK^+CXCR4^+JUNB^+$), 22% as ($CK^+CXCR4^-JUNB^+$), 17% as ($CK^+CXCR4^+JUNB^-$) and 38% as ($CK^+CXCR4^-JUNB^-$). JUNB and CXCR4 were highly prevalent in CTCs, with 80% of the CK^+ patients expressing JUNB and 76% CXCR4 respectively. Patients exhibited significantly different protein exosomal expression of both biomarkers compared to healthy individuals ($p = 0.003$, $p = 0.027$). High discriminative ability was observed in patients with exosomal overexpression of JUNB and CXCR4 (c-statistic = 0.949, c-statistic = 0.903). The presence of JUNB and/or CXCR4 in CTCs correlated with poorer overall survival ($p = 0.025$). CXCR4 overexpression in EVs was correlated with the presence of CTCs (rho = 0.442, $p = 0.001$), $CXCR4^+$ CTCs (rho = 0.424, $p = 0.002$), and with the $CK^+/JUNB^+/CXCR4^+$ phenotype (rho = 0.366, $p = 0.008$). MicroRNA profiling of exosomes from 4 patients revealed various biological processes and signaling pathways implicated in SCLC pathogenesis. In Conclusion, this study combines, for the first time, two different components of liquid biopsy providing significant information regarding SCLC patients' prognosis and early diagnosis.

ST34

Astrocyte-neuron interactions: game changers in Parkinson's disease?

Olympia Apokotou[1], Christina Paschou[2], Anastasios Kollias[2], Konstantina Charmpi[1], Sofia Dede[2], Martina Samiotaki[3], George Panayotou[3], Era Taoufik[2], Rebecca Matsas[2], Florentia Papastefanaki[1, 2]*

[1]Human Embryonic and Induced Pluripotent Stem Cell Unit, Hellenic Pasteur Institute, Athens, Greece
[2]Laboratory of Cellular and Molecular Neurobiology-Stem Cells, Hellenic Pasteur Institute, Athens, Greece
[3]Institute of Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, 16672, Greece

The accumulation of alpha-Synuclein (αSyn) inclusions, termed Lewy bodies and Lewy neurites, is typical in the brains of Parkinson's disease (PD) patients(1) and the prion-like spreading hypothesis is gaining ground. However, the astrocytic contribution in PD pathology is understudied, although PD-related mechanisms, including aggregate resolution pathways, involve non-cell autonomous components(2). 5-10% of PD cases are linked with mutations, such as the p.A53T-αSyn (G209A in SNCA gene) causing severe, early-onset familial PD(3). To investigate the impact of p.A53T-αSyn on astrocytes and their contribution in PD, we differentiated patient-derived induced pluripotent stem cells (iPSC) to ventral midbrain astrocytes. Interestingly, the p.A53T-αSyn astrocytes displayed pathological traits, including αSyn upregulation, accumulation of protein aggregates, also positive for toxic phosphorylated αSyn, disturbed autophagic flux, and inefficient phagocytic processing, also supported by their proteome analysis. Given that the compromised astrocytic ability to uptake and clear neuronal waste has been proposed as culprit for pathological accumulation of αSyn aggregates in neurons(4), we treated control and mutant astrocytes with conditioned medium from p.A53T-αSyn iPSC-derived neurons. Our results indicate that healthy astrocytes efficiently uptake released neuronal toxic αSyn species, unlike p.A53T-αSyn astrocytes. In a mixed astrocyte-neuron direct co-culture system we observed that p.A53T-αSyn astrocytes introduced neuropathology in healthy neurons, including compromised neuronal viability, impaired neuritic outgrowth, and neurodegeneration features, accompanied by defective synaptic connectivity. Notably, the viability, neurite outgrowth, and synaptic connectivity of p.A53T-αSyn neurons were improved by healthy astrocytes and their neurodegenerative phenotypes, including Lewy-like pathology, were alleviated. Similar phenotypes were recapitulated in a non contact-mediated setup, in which neurons were treated with conditioned medium from healthy or p.A53T-αSyn astrocytes. Our data support a critical role of mutant astrocytes in the neurodegeneration process, attributed to defective clearance mechanisms, and a remarkable ability of healthy astrocytes in rescuing neurodegeneration of mutant neurons, at least partially in a paracrine manner.

Funding
Supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment" (Project 1019-DiseasePhenoTarget; PI: R. Matsas).

References

1. *Desplats, Paula et al. "Inclusion formation and neuronal cell death through neuron-to- neuron transmission of alpha-synuclein." Proc Natl Acad Sci U S A. (2009) doi:10.1073/pnas.0903691106*
2. *di Domenico A, Carola G, Calatayud C, et al. Patient-Specific iPSC-Derived Astrocytes Contribute to Non-Cell-Autonomous Neurodegeneration in Parkinson's Disease. Stem Cell Reports. 2019;12(2):213-229. doi:10.1016/j.stemcr.2018.12.011*
3. *Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., & Nussbaum, R. L. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science (New York, N.Y.), 276(5321), 2045–2047. https://doi.org/10.1126/science.276.5321.2045.*
4. *Loria, F., Vargas, J. Y., Bousset, L., Syan, S., Salles, A., Melki, R., & Zurzolo, C. (2017). α-Synuclein transfer between neurons and astrocytes indicates that astrocytes play a role in degradation rather than in spreading. Acta neuropathologica, 134(5), 789–808. https://doi.org/10.1007/s00401-017-1746-2.*

ST35

A two-pronged approach to tackle ADOA using the nematode C. elegans

Fivos Borbolis[1,2], **Konstantinos Palikaras**[1]*

[1]*Department of Physiology, School of Medicine, National and Kapodistrian University of Athens, Greece*
[2]*Department of Biology, University of Padova, Italy*

Autosomal Dominant Optic Atrophy (ADOA) is a rare, currently untreatable neurodegenerative disorder, with a prevalence that reaches 1/12000 in specific populations. It is clinically characterized by the bilateral loss of vision in early childhood, due to the progressive degeneration of retinal ganglion cells and often involves secondary multi-systemic issues, manifested in approximately 1/5 of the patients. ADOA is primarily caused by mutations in OPA1, a nuclear-encoded inner mitochondrial membrane protein with a critical role during the fusion of the inner mitochondrial compartment, which is also involved in cristae arrangement and mitochondrial Ca2+ uptake. Congruently, mitochondrial structural and functional defects are key factors in ADOA pathogenesis. Given the limited availability of retinal material form patients, our knowledge on the pathogenesis of the disease largely depends on the study of animal models. Among those, C. elegans models offer a powerful system for the mechanistical study of ADOA and a unique platform for the screening of potentially therapeutic compounds. The study of such models has revealed the central role of excessive autophagy/mitophagy and the involvement of Ca^{2+} and AMPK signaling in the induction of pathological events. Here we apply a humanized nematode ADOA model in a two-pronged approach that aims to promote the development of novel therapeutic approaches. First, we present the pipeline and the results of a targeted pharmacological screen for compounds that can ameliorate pathological features of ADOA model animals. Moreover, we delve into the molecular and cellular procedures that drive disease pathogenesis and provide evidence that the inhibition of Ca2+ import inside mitochondria through the MCU-1 uniporter can impede downstream pathological events and prevent the development of disease-related phenotypes. In summary, our study provides promising insights into potential therapeutic approaches for this rare neurodegenerative disorder, emphasizing the importance of targeting mitochondrial Ca^{2+} regulation to mitigate disease onset and progression.

ST36

PERK kinase: A "master tactician" for the emergence of apoptotic and inflammatory nature of SARS-CoV-2 ORF3a protein

Panagiotis Keramidas[1]*, Eleni Papachristou[1]*, Rigini M. Papi[1], Aglaia Mantsou[1], Theodora Choli-Papadopoulou[1]**

[1]Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Greece
**Equal contribution*
***Email: tcholi@chem.auth.gr*

ORF3a is a SARS-CoV-2 accessory protein, which plays a significant role in virus–host interactions and the modulation of host immune responses, contributing to coronaviral pathogenicity through various mechanisms. Our study unraveled ORF3a's dynamic involvement as "stress factor" for the endoplasmic reticulum and the manipulation of activated PERK kinase to initiate and propagate the mechanisms of apoptosis and inflammatory response in lung epithelial cells (A549). Thus, to elucidate these ORF3a's functions, A549 cells were transfected with in vitro synthesized ORF3a mRNA. ORF3a transfected cells showed significant upregulation of all three UPR branches (ATF6, XBP-1 and PERK) -especially the PERK one. Similarly, we observed elevated expression of PERK executioners (CHOP, PUMA) as well as markers of intrinsic apoptosis (Bcl-2 and caspase family proteins, PARP-1) and inflammation (IL-1b, IL-18, IL-6, IL-18, p65 and IκBα). In order to find an interconnection between PERK overexpression and ORF3a complications, transfected A549 cells were treated with either a PERK inhibitor (GSK2606414) or a pan-caspase inhibitor (z-VAD). Indeed, our results showed that although treatment with 50 μM z-VAD could only terminate caspase-dependent apoptosis, treatment with 1000 μM GSK2606414 ameliorates both apoptosis and nFκB activation, thus inflammatory response. Taken together, PERK activation acts as an orchestrator of ORF3a provoked apoptosis and inflammatory response in lung epithelial cells, indicating that PERK inhibitors could be potentially used as "anti-COVID-19" agents to block respiratory tissue damage during SARS-CoV-2 infections.

ST37

The differential role of STAT3 and STAT5 mediated regulation of transcription in leukemic transformation

Eirini Sofia Fasouli[1], Konstantina Giavi[1], Chara Makri[1], Leonid Bystrykh[2], Anthi Bouchla[3], Emmanouil Athanasiadis[1], Giannis Vatsellas[1], Vasiliki Pappa[3], Eleni Katsantoni[1]

[1]Basic Research Center, Biomedical Research Foundation, Academy of Athens, Athens, Greece
[2]European Research Institute for the Biology of Ageing, University Medical Center Groningen, University of Groningen, Groningen, Netherlands
[3]Department of Internal Medicine Propaedeutic and Research Unit, National and Kapodistrian University of Athens and Medical School, University General Hospital "Attikon", Athens, Greece

Accumulation of mutations in hematopoietic stem cells leads to malignancies such as Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukemia (AML), which are characterized by hematopoietic dysfunctions and impaired differentiation. MDS commonly progresses to AML, an aggressive type of leukemia. STAT3 and STAT5 are important cellular regulators and their deregulation has been linked to MDS and AML. To elucidate the role of STAT3 and STAT5 in leukemic transformation, MDS and AML have been utilized to model pre-leukemia and leukemia respectively. RNA-sequencing following STAT3, STAT5A or STAT5B knockdowns collectively with STAT3, STAT5A and STAT5B CUT&Tag binding profiles in MDS and AML cells have determined the direct target gene networks of each factor in both conditions, revealing their distinct functions including pathways involved in the cell cycle, apoptosis and differentiation. Also, an interplay between STAT3 and STAT5, through phosphorylation changes, in AML has been uncovered, showing a functional overlap for the regulation of shared targets essential for important cellular functions, such as leukemic metabolism. Increased chromatin accessibility has been detected through ATAC-sequencing in MDS compared to AML, unveiling potential changes in accessibility for STAT3 and STAT5 binding sites between MDS and AML, indicating plausible leukemia-promoting changes in AML. Furthermore, STAT5A/STAT5B-mediated control of IKZF2 has been determined, concomitant with IKZF2 reduction after STAT5A and STAT5B knockdown in AML through an MSI2-independent mechanism. IKZF2 has been reported to be indispensable for leukemic stem cells in AML. The results are currently validated in hematopoietic cells of MDS and AML patients. Our findings identify a novel STAT5/IKZF2 axis providing an alternative regulatory pathway in leukemia linked to AML maintenance. The role of STAT3, STAT5A, and STAT5B and their target gene networks in leukemic transformation has been delineated, providing novel biomarkers and therapeutic targets for optimized patient stratification protocols and more efficient treatments, respectively, for MDS and AML.

ST38

Fecal proteomics in investigation of the gut-kidney axis in patients with chronic kidney disease

Sonnal Lohia[1,2], Vasiliki Lygirou[1], Sophie Valkenburg[3], Manousos Makridakis[1], Jerome Zoidakis[1] Wim Van Biesen[3], Griet Glorieux[3]*, Antonia Vlahou[1]*

[1]Center of Systems Biology, Biomedical Research Foundation of the Academy of Athens, 11527 Athens, Greece
[2]Institute for Molecular Cardiovascular Research, RWTH Aachen University Hospital, 52074 Aachen, Germany
[3]Department of Internal Medicine and Pediatrics, Nephrology Division, Ghent University Hospital, 9000 Gent, Belgium
**Authors to whom correspondence should be addressed.*

Chronic kidney disease (CKD) exemplarily represents the impact of gut microbiota on the renal system. In CKD, an increase in a myriad of plasma compounds resulting from the reduced kidney function is widely reported. Increase in urea concentrations in the circulation and gut, may lead to an increased bacterial urease activity, potentially contributing to increased gut permeability. In parallel, leakage of uremic cardiotoxins and their precursors from the intestine could further contribute to their increased plasma levels in CKD. Knowledge on the bidirectional interaction in the gut-kidney axis is nevertheless still limited and further molecular analyses are required for its better understanding. In this exploratory study, fecal proteomics analysis was performed. Specifically, fecal suspension samples from patients with CKD stage 1 (CKD1, n=12) and CKD stage 4 (CKD4, n=17) were processed using an optimized GeLC-MS protocol, following analysis by high resolution LC-MS/MS. Protein identification was performed using the entire UniProt RefSeq reviewed Human and Bacterial databases utilizing stringent criteria; following differential expression analysis using the Mann-Whitney U test in CKD4 versus CKD1 samples. In total, changes in the abundance of eight (out of 701) unique human proteins were identified, most of which were previously associated with CKD, such as Intestinal-type alkaline phosphatase and Galectin-3-binding protein. In parallel, the analysis indicated difference in abundance of nine (out of 1036) unique fecal bacterial proteins including Glyceraldehyde-3-phosphate dehydrogenase and NAD-specific glutamate dehydrogenase (all $p < 0.05$). Further bioinformatics and literature analysis indicated that some of the differentially abundant bacterial proteins could indirectly be linked to the butyrate pathway, where butyrate reportedly acts as a beneficial compound for intestinal barrier integrity. Validation of the observed changes in parallel to data integration with gut microbiome data is planned. In conclusion, fecal proteomics analysis can contribute to our existing knowledge on the molecular changes associated with CKD, in context of the gut-kidney axis.

ST39

Amyloidosis: how proteomics can make a difference in clinical practice

Julie Courraud[1,2*], Panagiota E. Nikolaou[3], Diana Canetti[4], Guillaume Médard[2], Janet Gilbertson[4], Ashutosh Wechalekar[4], Jerome Zoidakis[5], Ioanna Andreadou[3], Nikolaos Thomaidis[2], Efstathios Kastritis[1]

[1]Dep. of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Alexandra Hospital, Leof. Vasilissis Sofias 80, Athens 11528, Greece
[2]Laboratory of Analytical Chemistry, Dep. of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, 15771, Athens, Greece
[3]Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupolis, Zografou, 15771, Athens, Greece
[4]Centre for Amyloidosis University College London, and Royal Free London NHS Foundation Trust, Rowland Hill Street, London NW3 2PF, UK
[5]Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, Zografou, 15771, Athens, Greece

Amyloidoses are a group of diseases caused by accumulation, in various tissues, of amyloid fibrils derived from different misfolded proteins. Because several organs are often affected, symptoms are non-specific, delaying accurate diagnosis and treatment. Correct typing of amyloid deposits is imperative to distinguish the various types of amyloidosis, for which different therapies are available. Alongside immuno-histochemistry, mass spectrometry (MS)-based methods are considered the gold standard, but they are only routinely used in a few places in the world.

Here we present how we are implementing a proteomic analysis [1] of laser-microdissected amyloid depots using a nano LC coupled with a timsTOF flex mass spectrometer. While learning from the experience of other centers, we are testing several alternatives and ideas to improve sensitivity and decrease analysis time. Exploiting the capacities of the Parallel Accumulation–Serial Fragmentation (PASEF) technology, we generate high-quality data, which are then processed using MaxQuant, DIA-NN, and R for detecting and inspecting proteins relevant to the diagnosis (Swiss-Prot database for identification). As a preliminary step to validate our method, we have analyzed 16 fat tissue biopsies of patients with various immunoglobulin light chain (kappa or lambda) amyloidosis. Our results are in agreement with the findings of the UK National Amyloidosis Centre in London. We are also testing our method on kidney biopsies. Although proteomics is not (yet) used widely in clinical practice, here is an example where it makes a difference for the patients. Local – and therefore timely and affordable – processing of samples can have a real impact on the choice of appropriate treatment, and ultimately on prognosis.

Bibliography

1. *D. Canetti, et al.; Clin. Chem. Lab. Med., 58, 948-957 (2020)*

ST40

The interactome of the UapA transporter reveals putative new players in anterograde membrane cargo trafficking

Xenia Georgiou[1], Sofia Dimou[1], George Diallinas[1,2]*, Martina Samiotaki[3]*

[1]Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, Athens, 15784, Greece.
[2]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion, 70013, Greece.
[3]Biomedical Sciences Research Center "Alexander Fleming", Institute for Bioinnovation, Vari, 16672, Greece.
**Corresponding authors, HSBMB members.*

Neosynthesized plasma membrane (PM) proteins co-translationally translocate to the ER, concentrate at regions called ER-exit sites (ERes) and pack into COPII secretory vesicles which are sorted to the early-Golgi through membrane fusion. Following Golgi maturation, membrane cargoes reach the late-Golgi, from where they exit in clathrin-coated vesicles destined to the PM, directly or through endosomes. Post-Golgi membrane cargo trafficking also involves the cytoskeleton and the exocyst. The Golgi-dependent secretory pathway is thought to be responsible for the trafficking of all major membrane proteins. However, our recent findings in Aspergillus nidulans showed that several plasma membrane cargoes, such as transporters and receptors, follow a sorting route that seems to bypass Golgi functioning. To gain insight on membrane trafficking and specifically Golgi-bypass, here we used proximity dependent biotinylation (PDB) coupled with data-independent acquisition mass spectrometry (DIA-MS) for identifying transient interactors of the UapA transporter. Our assays, which included proteomes of wild-type and mutant strains affecting ER-exit or endocytosis, identified both expected and novel interactions that might be physiologically relevant to UapA trafficking. Among those, we validated, using reverse genetics and fluorescence microscopy, that COPI coatomer is essential for ER-exit and anterograde trafficking of UapA and other membrane cargoes. We also showed that ArfAArf1 GTPase activating protein (GAP) Glo3 contributes to UapA trafficking at increased temperature. This is the first report addressing the identification of transient interactions during membrane cargo biogenesis using PDB and proteomics coupled with fungal genetics. Our work provides a basis for dissecting dynamic membrane cargo trafficking via PDB assays.

- *Georgiou, X., Dimou, S., Diallinas, G., & Samiotaki, M. (2023). The Interactome of the UAPA transporter reveals putative new players in anterograde membrane cargo trafficking. Fungal Genetics and Biology, 169, 103840. https://doi.org/10.1016/j.fgb.2023.103840*
- *Dimou S, Martzoukou O, Dionysopoulou M, Bouris V, Amillis S, Diallinas G. Translocation of nutrient transporters to cell membrane via Golgi bypass in Aspergillus nidulans. EMBO Rep. 2020 Jul 3;21(7):e49929. doi: 10.15252/embr.201949929.*

ST41

The expanding chemoproteomics toolbox

Guillaume Médard

Proteomics Core Facility, National and Kapodistrian University of Athens
Chair of Proteomics and Bioanalytics, TUM School of Life Sciences, Technical University of Munich

Mass-spectrometry based proteomics is an ideal readout to agnostically study the biochemical effect of small molecules. It is analogous to performing hundreds or thousands of western blots at once. Hence it allows for a system biology, rather than hypothesis-driven, approach when exploring small molecule interactions with the proteome.

We have developed assays for target deconvolution or systematic profiling of molecules. For such assays, tailored affinity matrices or affinity matrices dedicated to particular families of proteins (e.g. Kinobeads for the kinome) need to be engineered. The proteins, which the matrices specifically bind to, constitute the native protein "panel" of the assay. They remain on the bead after washing and can be identified and quantified by LC-MS/MS after digestion (i.e. using a bottom-up proteomics readout). When a lysate is incubated with an inhibitor of interest, the inhibitor binds its targets in accordance with its affinity for each and every of them, and henceforth reduce the enrichment of the targets by the affinity matrix. The dose-dependent reduction of the quantity of a protein remaining of the affinity matrix across the lysate aliquots incubated with increasing doses of an inhibitor, designates this particular protein as a target of the inhibitor and provides an affinity measure. Henceforth, for each drug, a selectivity profile is obtained.

Our recent efforts to expand our chemoproteomics technology have notably revealed that HDACs are targets of lipoic acid [1], that many clinical HDAC inhibitors do not achieve claimed selectivities [2] and that many also inhibit MBLAC2 [3], with effect on extracellular vesicles biology. Within collaborative works, the technology is an excellent asset to investigate the selectivity of new inhibitors made by medicinal chemists [4], or put PTM-proteomics data into context [5].

Publications

[1] *Target Deconvolution Reveals Histone Deacetylases as Targets of (R)-Lipoic Acid. Nat. Commun. 2023, 14:3548.*
[2] *Ricolinostat is not a highly selective HDAC6 inhibitor Nat. Cancer 2023, 4:807.*
[3] *Target Deconvolution of HDAC Pharmacopoeia Reveals MBLAC2 as Common Off-Target. Nat. Chem. Biol. 2022, 18:812.*
[4] *Aza-SAHA Derivatives Are Selective Histone Deacetylase 10 Chemical Probes That Inhibit Polyamine Deacetylation and Phenocopy HDAC10 Knockout JACS 2022, 144:18861.*
[5] *Decrypting drug actions and protein modifications by dose- and time-resolved proteomics Science 2023, 380:93.*

ST42

Egg: A Nutritional Treasure's Proteins Unveiled

Eleana Sarantidi[1, 2]*, Alexandra Ainatzoglou[1], Christine Papadimitriou[1], Eleni Stamoula[1], Katerina Maghiorou[1], Argyro Miflidi[1], Antonia Trichopoulou[3], Konstantinos C. Mountzouris[2], Athanasios K. Anagnostopoulos[1]

[1]Department of Biotechnology, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[2]Laboratory of Nutritional Physiology and Feeding, Department of Animal Science, Agricultural University of Athens; Athens, Greece
[3]Academy of Athens, Athens, Greece

The chicken egg is an animal product of great agronomic interest. As a nourishment, egg is a source of high-quality protein, rich in bioactive components. The aim of the study was to exhaustively analyze the proteome of egg white and yolk by applying proteomics and bioinformatic systems to unravel its full protein potential. A total of 45 freshly laid, unfertilized, chicken eggs were subjected to nanoLC-MS/MS Orbitrap analysis. In parallel to egg white and yolk proteins (EWAYP) identification experiments of the present study, EWAYP reported in the literature, were collected and altogether used to create a united dataset of all known EWAYP. The bioactive role of all EWAYP was determined showing that key egg proteins, such as ovalbumin and lysozyme C, bear antioxidant and anti-hypertensive properties. In addition to already known proteins, our experiments delivered 371 and 428 EWAYP, unckown to date. Beclin-1, as well as alpha-actin-2 and Hes-1, have antioxidant activity, while LIM-domain binding protein 2 and zing finger protein possess an anti-hypertensive role. The molecular functions and biochemical pathways in which EWAYP are involved, were detected, as well. The most prevalent molecular function was that of transport and signal transduction and many newly identified proteins, like ROS kinase and ATPase subunit alpha, were found to manifest such a function. Regarding the pathways that proteins were involved in, the highest enrichment for the egg white proteins was the "apoptosis pathway" and for the egg yolk was the "focal adhesion pathway". Our study denotes that a total of 1,392 proteins are present in the chicken egg white and yolk and are characterized by key roles such as antioxidant, anti-inflammatory, antimicrobial and anti-hypertensive properties, highlighting the biological significance of this landmark food.

References

- *Sarantidi, E.; Ainatzoglou, A.; Papadimitriou, C.; Stamoula, E.; Maghiorou, K.; Miflidi, A.; Trichopoulou, A.; Mountzouris, K.C.; Anagnostopoulos, A.K. Egg White and Yolk Protein Atlas: New Protein Insights of a Global Landmark Food. Foods 2023, 12, 3470. https://doi.org/10.3390/foods12183470*

ST43

Examining the association between CYP2C19 variants and the response to immunosuppressive drugs in kidney transplant recipients from Greece

Aikaterini Kotzamouratoglou[1], John Lakoumentas[1], Marios Papasotiriou[4], George P Patrinos[1,2,3], Stavroula Siamoglou[1]

[1]Laboratory of Pharmacogenomics and Individualized Therapy, Department of Pharmacy, University of Patras, School of Health Sciences, University Campus, 265 04, Rion, Patras, Greece.
[2]Department of Genetics and Genomics, United Arab Emirates University, College of Medicine and Health Sciences, Al-Ain, Abu Dhabi, United Arab Emirates.
[3]Zayed Center for Health Sciences, United Arab Emirates University, Al-Ain, Abu Dhabi, United Arab Emirates.
[4] Department of Nephrology and Kidney Transplantation, University Hospital of Patras, Patras, Greece

Tacrolimus is a drug commonly used in immunosuppressive therapy to prevent graft rejection in solid organ transplants. It is a calcineurin antagonist with pharmacokinetic properties that exhibit a high degree of variability among individuals. A careful monitoring and dose adjustments are necessary to achieve the desired level of immunosuppression while avoiding harmful side effects. Tacrolimus is metabolized by CYP3A enzymes in the liver. Numerous studies have emphasized the significance of polymorphisms in the CYP3A4 and CYP3A5 genes in the metabolism and blood concentration of tacrolimus. However, these studies cannot fully explain the heterogeneity observed in the response to tacrolimus. Therefore, it is necessary to investigate other factors that may affect the response to tacrolimus. Our research examines two polymorphisms in the CYP2C19 gene - the CYP2C19*2 allele associated with reduced enzyme function, and the CYP2C19*17 allele associated with increased function. These single nucleotide polymorphisms were studied in 96 Greek kidney transplant patients, who were treated with tacrolimus for a year following transplantation. The genotyping method used was Restriction fragment length polymorphism (RFLP). A Hardy-Weinberg equilibrium test and a two-sided Fisher's exact test were performed as well as a statistical comparison of the genotypes followed by Therapeutic Drug Monitoring/Dose (TDM/D) with Kruskal-Wallis's test. For most of the studied samples, no association of TDM/D with patients' genotypes was observed. There was only one case in which there was a correlation between genotype and drug concentration seen at 3 months post-transplantation, with Poor Metabolizers (*2/*2) showing lower tacrolimus TDM/D (p=0.047), compared to those with at least one wild-type allele. In conclusion, to personalize and optimize immunosuppressive therapy and minimize the risk of toxicity, further studies on the CYP2C19*2 and CYP2C19*17 alleles should be conducted, in combination with other factors such as co-administered drugs and SNPs in sequences coding metabolic enzymes and transporter proteins.

ST44

A meta-analysis of brain microarray data for Alzheimer and Parkinson neurodegenerative diseases

Eleni Dermitzaki[1,2], Vasileios L. Zogopoulos[1,2], Apostolos Malatras[3], Vassiliki A. Iconomidou[2], Ioannis Michalopoulos[1]*

[1]Centre of Systems Biology, Biomedical Research Foundation, Academy of Athens, Athens, Greece
[2]Section of Cell Biology and Biophysics, Department of Biology, National and Kapodistrian University of Athens, Athens, Greece
[3]CY-Biobank, Centre of Excellence in Biobanking and Biomedical Research, University of Cyprus, Nicosia, Cyprus

Alzheimer's disease (AD) is the most frequent neurogenerative disease and it is highly connected with dementia [1]. Typical symptoms of this disease concern memory loss, progressive cognitive decline and hindering of the daily activities leading to the loss of individuals' autonomy [2]. AD is characterized by the creation of senile plaques (SPs) consist of Aβ amyloids and neurofibrillary tangles (NTFs) consist of tau protein [3, 4].

Parkinson's disease (PD) affects 1% of the total population and 4% over 80 [5]. The symptoms are divided into two categories, motor and no-motor ones. The former include bradykinesia and trembling and the latter include dementia, shuffles and instability [6]. PD is characterized by the accumulation of α-synuclein, Lewie bodies and dopamine neuron degeneration in substantia nigra.

Our analysis was conducted to identify differentially expressed genes (DEGs) related to Parkinson's and Alzheimer's neurodegenerative diseases. Primary Affymetrix cDNA microarray data, freely available from public repositories, were used. Data were selected based on disease and tissue type. Quality control of the samples was then performed to remove defective samples. This was followed by normalization of high-quality samples, batch effect correction and generation of a list of differentially expressed genes for each study. Finally, meta-analysis was performed by generating the final list of differentially expressed genes for the two diseases from all studies. In addition, a biological term enrichment analysis was performed separately for under- and over-expressed genes.

We show that the two pathological conditions involve many common molecular and cellular paths: The under-expressed genes are involved in processes such as chemical neuronal signaling, vesicle-transportation, neuronal differentiation and development, exocytosis, learning capacity, proteasome, etc. Genes that are over-expressed are involved in processes such as apoptosis, mitochondrial stress, immune response, phagocytosis, histone modifications etc. Although, APP, MAPT and SNCA are not differentially expressed, some DEGs influence their accumulation.

References

1. *Prince, M., et al., The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement, 2013. 9(1): p. 63-75 e2.*
2. *DeTure, M.A. and D.W. Dickson, The neuropathological diagnosis of Alzheimer's disease. Mol Neurodegener, 2019. 14(1): p. 32.*
3. *Willem, M., et al., eta-Secretase processing of APP inhibits neuronal activity in the hippocampus. Nature, 2015. 526(7573): p. 443-7.*
4. *Gomez-Isla, T., et al., Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol, 1997. 41(1): p. 17-24.*
5. *Tysnes, O.B. and A. Storstein, Epidemiology of Parkinson's disease. J Neural Transm (Vienna), 2017. 124(8): p. 901-905.*
6. *Jankovic, J., Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry, 2008. 79(4): p. 368-76.*

ST45

Oleanolic acid pathway characterization in Pistachia lentiscus var. Chia

Konstantina Leontaridou[1], Anthi Katsiarimpa[1], Eirini Sarrou[2], Angelos Kanellis[1] *

[1]Aristotle University of Thessaloniki, Greece
[2]ELGO-Dimitra Research Center, Thessaloniki, Greece

Oleanolic acid (OA) is a pentacyclic triterpene, found in plants with many therapeutic activities that have been recently explored with main action against dyslipidemia and diabetes (Castellano et al., 2022). Previous work examined its antiviral, anticarcinogenic, anti-inflammatory and gastroprotective bioactivities (Tian et al., 2002). OA is a constituent of the mastic gum (mastiha) secreted upon wounding by the bark of Pistachia lentiscus var Chia. This resin is known for its action against coughs, sore throats, eczema, stomachaches, kidney stones, pain, and rheumatism. Recently, the European Medicines Agency (EMA) published a monograph describing the use of Chios mastic gum for the treatment of mild dyspeptic disorders and for skin inflammations and healing of minor wounds (EMA, 2015). OA biosynthetic pathway has not yet been elucidated in Pistachia lentiscus var. Chia. This molecule is synthesized by the cytoplasmic mevalonate pathway, which through oxidosqualene leads to sterols (Abe et al., 1993). To approach this issue, RNA-seq followed by a transcriptomic analysis was applied to wounded vs unwounded phloem tissues that has led to the discovery of two genes responsible for the biosynthesis of OA in Pistachia lentiscus var. Chia. The genes were cloned into yeast expression vectors, and expressed in Saccharomyces cerevisiae, that upon extraction with ethyl acetate and LC-MS analysis first revealed a β-amyrin synthase (BAS) activity. Further, a cytochrome P450 (CYP716) enzyme oxidizing β-amyrin at the C-28 position, and eventually producing OA was functionally characterized following the above approach.

Bibliography

- *Abe, I.; Rohmer, M.; Prestwich, G.D. Enzymatic cyclization of squalene and oxidosqualene to sterols and triterpenes. Chem. Rev. 1993, 93, 2189–2206*
- *Castellano JM, Ramos-Romero S, Perona JS. Oleanolic Acid: Extraction, Characterization and Biological Activity. Nutrients. 2022; 14(3):623. https://doi.org/10.3390/nu14030623*
- *Ema (European Medicines Agency), 2015. Assessment Report on Pistacia Lentiscus L., Resin (Mastix).*
- *Pachi,V.K.et al.,Traditional uses, phytochemistry and pharmacology of Chios mastic gum(Pistacia lentiscus var. Chia, Anacardiaceae): A review. Journal of Ethnopharmacology 254 (2020) 112485*
- *Tian, L.-T.; Ma, L.; Du, N.-S. Survey of pharmacology of oleanolic acid. China J. Chin. Mater. Med. 2002, 27, 884–901*

Acknowledgment

This research has been co financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: GR-m4t- T1EΔK-01133)

ST46

Analyzing Artisanal Fermented Foods From The Peloponnese Region: A Multi-Omics Approach

Konstantinos Papadimitriou[1,*], Marina Papadelli[2], John Kapolos[2]

[1]Agricultural University Of Athens, Athens, Greece
[2]University Of The Peloponnese, Antikalamos, Greece

The Peloponnese region is renowned for its rich culinary heritage and boasts several protected designation of origin (PDO) products such as Kalamata olives, Sfela cheese, Agiorgitiko Nemea wine, Moschofilero Mantinia wine, and other artisanal dairy, meat, and vegetable fermented foods. To delve deeper into the intricacies of these products, three different omics tools including metagenomics, in silico metabolomics, and GC-IMS/GC-MS volatilomics were applied. Metagenomics produced a detailed picture of the microbial communities inherent to each product, spotlighting key microbial dynamics that underpin distinct products' attributes. In parallel, in silico metabolomics revealed the unique metabolic blueprints characterizing each fermentation, elucidating specific bioactive compounds and their contribution to the organoleptic properties of the products. Lastly, GC-IMS/GC-MS volatilomics provided a comprehensive profile of volatile compounds, offering a molecular window into the aroma that distinguishes each product. The confluence of data derived from these state-of-the-art techniques has not only increased our understanding of the fermentation processes but also underscored the distinctiveness of Peloponnese artisanal foods and wines. With these insights, local producers are now better poised to synergize traditional practices with contemporary knowledge, ensuring the consistent production of high-quality products.

Acknowledgment
This research has been co-financed by the European Regional Development Fund (ERDF) and Greek National Funds through the Operational Program "Peloponnese 2021-2027" (MIS: 6001408).

ST47

HSP90 molecular chaperone as a key regulator of brassinosteroid mediated signaling processes

Despina Samakovli[1,2], Loukia Roka[1], Panagiota Konstantinia Plitsi[1], Rafael Gkritzas[1], Konstantinos Panagiotopoulos[1], Dimitra Milioni[1], Polydefkis Hatzopoulos[1]

[1]Agricultural University of Athens, School of Applied Biology and Biotechnology, Department of Biotechnology, Iera Odos 75, Athens 11855.
[2]National and Kapodistrian University of Athens, Biology Department, Section of Botany, 15772 Athens, Greece.

HEAT SHOCK PROTEIN 90 (HSP90) through the mediation of the proper protein folding and stability of a cohort of client proteins acts as a master molecular hub of multiple cellular and developmental signaling processes. Its function is considered indispensable for the maintenance of cellular homeostasis under natural and stress growth conditions. Brassinosteroids (BRs), plant steroids sharing structural similarities with animal steroid hormones, play diverse roles in plant physiology, maintain homeostasis and cellular communication and shape development. Thorough analysis of the BR signaling pathway has revealed a signaling cascade that initiates with the activation of cell surface-anchored receptor kinases and leads to the activation of transcription factors to control the transcriptional outcome in the nucleus.

Our research over the last decay has advanced our understanding of the functional regulation of BR-mediated developmental processes by HSP90 in the model plant Arabidopsis. Our findings revealed the multifaceted control of the HSP90 over the BR signaling cascade suggesting a conserved role for the molecular chaperone between plant and animal kingdoms. The HSP90 is involved in the organization and functional activation of the cell surface receptors to the nucleo-cytoplasmic partitioning of downstream key nuclear components including negative regulators and core transcription factors of the BR signaling cascade. In addition, we showed that HSP90 facilitate the crosstalk of the BR signaling cascade with other developmental and hormonal signaling pathways to control plant developmental networks under both normal and stress conditions.

ST48

Unraveling unconventional biogenesis of human circular RNAs (circRNAs)

Christos K. Kontos, Paraskevi Karousi, Maria Papatsirou, Christina D. Sotiropoulou, Katerina Katsaraki, Aspasia Dimitriadou, Diamantis C. Sideris, Andreas Scorilas

Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Panepistimiopolis, Athens, Greece.

Circular RNAs (circRNAs) have emerged as a class of intriguing RNA molecules with diverse cellular functions, far beyond their initial classification as mere splicing byproducts. In this study, we investigated the existence of circRNAs deriving from three human genes: BAX, BCL2L12, and PRMT1. Utilizing total RNA extracts from various cancer and hematological malignancy cell lines (colorectal cancer, breast cancer, acute myeloid and chronic lymphocytic leukemia, as well as myelodysplastic syndrome cell lines), we employed reverse transcription with random hexamers, followed by multiple nested PCR assays using divergent primers designed on each exon of BAX, BCL2L12, and PRMT1. After the clean-up of the PCR products, library construction was carried out, and third-generation sequencing, facilitated by the MinION Mk1C platform coupled with the Flongle adapter, was employed to capture the intricate circRNA landscape. Subsequent analysis of the sequencing data was accomplished through a combination of publicly available tools and in-house-developed Perl-based algorithms. Our findings shed light on a repertoire of previously undiscovered circRNAs originating from these genes. Most of the novel circRNAs are likely to form via back-splicing of non-canonical back-splice sites residing in highly similar regions of exons or introns of the primary transcripts. Importantly, we observed that within the circRNA sequence, only one of these two similar regions was retained, while the other was spliced out during circRNA biogenesis. Notably, we also highlighted the emergence of novel exons and extensions of currently annotated exons within these circRNAs, further supporting the existence of an undiscovered biogenesis mechanism. In summary, the notion of novel biogenesis mechanisms observed in BAX, BCL2L12, and PRMT1 circRNAs represents a significant contribution to our understanding of these RNA molecules.

Acknowledgement
Part of this original research study was funded through a research grant "Asklepios" by Gilead Sciences Hellas M.EPE.

ST49

Coupling tRNAGly redundancy with Staphylococcus aureus pathogenicity: Insights from genome editing and transcriptomics

Adamantia Kouvela[1], Nikoleta Giarimoglou[1], Jinwei Zhang[2], Constantinos Stathopoulos[1]*, Vassiliki Stamatopoulou[1]*

[1]Department of Biochemistry, School of Medicine, University of Patras, 26504 Patras, Greece
[2]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, 50 South Drive, Bethesda, MD 20892, USA
** v.stam@upatras.gr; cstath@med.upatras.gr*

Beyond its canonical role as a decoder in ribosomal translation, tRNA serves multifaceted functions, encompassing gene expression regulation and cell wall reinforcement, contingent upon its aminoacylated state. Staphylococcus aureus harbors five distinct $tRNA^{Gly}$ isoacceptors, all of which are aminoacylated by the same glycyl-tRNA synthetase (GlyRS). Although all five $tRNAs^{Gly}$ interact with the glyS T-box riboswitch to regulate glyS transcription, two of them, denoted as proteinogenic (P1, P2), form tight complexes with EF-Tu to supply glycine for protein synthesis, while the remaining three isoacceptors (NP1, NP2, and NEW) are non-proteinogenic and supply glycine for bacterial peptidoglycan synthesis in the cell wall. To elucidate the role of each of these $tRNA^{Gly}$ isoacceptors in pathogen fitness, we employed the CRISPR/Cas9 genome editing tool to knock out P1 and NP tRNAs. Notably, depletion of the NP tRNA cluster was not sustainable. Conversely, the depletion of P1 $tRNA^{Gly}$ had no discernible impact on the growth and translational activity of S. aureus. However, the edited strain exhibited increased susceptibility to antibiotics targeting the cell wall compared to the wild type. Consistent with these observations, despite the upregulation of NP $tRNAs^{Gly}$ in the edited strain, several crucial genes involved in cell wall formation and antibiotic resistance were significantly downregulated. In line with these findings, the edited strain displayed reduced ability to form biofilms, indicating diminished infectivity and underscoring the pivotal role of P1 tRNA in S. aureus pathogenicity. Furthermore, transcriptomic analysis of the edited strain unveiled the downregulation of genes implicated in amino acid and vitamin biosynthesis, along with differential expression of genes essential for iron homeostasis, a critical process in bacterial infectivity and pathogenicity. These results highlight the intricate interplay of diverse cellular processes orchestrated by tRNAs and emphasize the significance of investigating the roles of distinct tRNA species in combatting bacterial infections.

Acknowledgements
This research was supported by Grant "81344" from the Research Committee of the University of Patras via "C. CARATHEODORI" program.

ST50

Hybrid-seq decodes the transcriptional landscape of the human breast cancer gene 1 (BRCA1) in human malignancies

Konstantina Athanasopoulou, Panagiotis G. Adamopoulos, Michaela A. Boti, Glykeria N. Daneva, Panagiotis Tsiakanikas, Andreas Scorilas*

Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece.

The Breast Cancer Type 1 susceptibility protein is a well characterized 207 kDa protein that is encoded by BRCA1 gene and consists of 1863 amino acids. BRCA1 functions as a tumor suppressor protein that is implicated in multiple physiological processes including the regulation of cell cycle, the maintenance of genomic integrity, and DNA damage repair. While several alternative splicing events characterizing BRCA1 mRNAs have been reported and various proteomics studies have identified different BRCA1 isoforms, limitations in sequencing of mRNAs that exceed 1Kb have hindered the identification of full-length BRCA1 transcripts. Our study aims to decipher the BRCA1 mRNA splice variants in a wide range of malignant and non-cancerous human cell lines. To achieve this, we designed and employed a hybrid sequencing approach (Hybrid-seq), that utilizes sequencing reads from both nanopore and NGS platforms to accurately detect alternative BRCA1 mRNAs. The implementation of the Hybrid-seq method as described resulted in the production of highly precise full-length sequencing reads that facilitated the detection of a broad range of BRCA1 splice variants (BRCA1 sv.7 – sv.52). Moreover, by demultiplexing the barcoded nanopore sequencing data, we uncovered the expression patterns of the described BRCA1 mRNAs in the six investigated malignancies (breast, ovarian, prostate, colorectal, lung, and brain) as well as in the non-cancerous human cell lines. Finally, the implemented in silico analysis strongly suggests that most of the alternative BRCA1 mRNAs contain open reading frames, thus represent coding transcripts that are highly expected to produce protein isoforms that harbor the conserved domains of BRCA1. This, in turn, provides new perspectives on BRCA1's intricate roles in modulating genomic stability and repairing DNA damage under normal and/or pathological states.

Acknowledgements
Part of this work was supported by Empirikeion Foundation, Athens, Greece.

ST51

CRISPR Artificial Splicing Factor mediated splicing modulation of the lncRNA PVT-1 and its effect on MYC expression.

Christos Katsioulas[1] #, Sofia Perdikari[1] #, Evgenia Ntini[1]*

[1]Institute of Molecular Biology and Biotechnology of the Foundation for Research and Technology Hellas, Heraklion, Greece
#Contributed equally
**Corresponding author evgenia.ntini@imbb.forth.gr*

Long non-coding RNAs (lncRNAs) are a large group of non-coding RNAs, involved in fundamental processes, such as genome organization, chromatin remodeling and gene expression regulation. lncRNAs can function in cis, while attached to their transcription site or near to it, or by interacting with other molecules such as RNA-binding proteins. In either case, lncRNAs are responsible for regulating the expression of target genes through various mechanisms. Preliminary experiments aiming to identify parameters that affect chromatin dissociation of lncRNAs, support that splicing efficiency per transcript plays an important role in this process[1]. In this study, we utilize the recent technology of CRISPR Artificial Splicing Factors (CASFx)[2] to examine the importance of co-transcriptional splicing efficiency on the release of lncRNAs from chromatin, by modulating the splicing of the lncRNA plasmacytoma variant translocation 1 (PVT1). PVT1 is being chosen because it is a co-transcriptionally poorly spliced and chromatin retained lncRNA, that has oncogenic role in breast cancer[3], and its dysregulation has been associated with several other types of cancer, classifying it as a lncRNA of high functional importance. Furthermore, PVT1 has always been linked to the regulation of MYC expression since its genetic locus resides around

60kb downstream of the MYC gene. Here, we report the design and first attempts of CASFx-mediated splicing modulation of PVT1, as well as how this modulation might affect its chromatin dissociation and possibly the regulation of MYC expression.

[1] Ntini, Evgenia and Budach, Stefan and Ørom, Ulf Andersson Vang and Marsico, Annalisa, Modeling the Nascent RNA Transcriptome With chrTT-Seq Identifies Distinctive Features of Long Non-Coding RNA Chromatin (Dis-)association. Available at SSRN: http://dx.doi.org/10.2139/ssrn.4284066
[2] Du M, Jillette N, Zhu JJ, Li S, Cheng AW. CRISPR artificial splicing factors. Nat Commun. 2020 Jun 12;11(1):2973.
[3] Wang H, Huang Y, Yang Y. LncRNA PVT1 Regulates TRPS1 Expression in Breast Cancer by Sponging miR-543. Cancer Manag Res. 2020 Sep 4;12:7993-8004.

We acknowledge funding for this work by Fondation Sante and Hellenic Foundation for Research and Innovation.

ST52

3'U-tRNA-derived fragments (3'U-tRFs) in multiple myeloma: 3'U-tRFSerTGA upregulation leads to poor treatment outcome and unfavorable prognosis of the patients

Konstantinos Soureas[1,2], Maria-Alexandra Papadimitriou[1], Aristea-Maria Papanota[3], Panagiotis Malandrakis[3], Ioannis Ntanasis-Stathopoulos[3], Maria Gavriatopoulou[3], Diamantis C. Sideris[1], Efstathios Kastritis[3], Meletios-Athanasios Dimopoulos[3], Andreas Scorilas[1], Evangelos Terpos[3], Margaritis Avgeris[1,2]*

[1]Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece
[2]Laboratory of Clinical Biochemistry – Molecular Diagnostics, Second Department of Pediatrics, School of Medicine, National and Kapodistrian University of Athens, "P. & A. Kyriakou" Children's Hospital, Athens, Greece.
[3]Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Alexandra General Hospital, Athens, Greece

Despite significant advancements in multiple myeloma (MM) therapy, the persistence of refractory cases represents a substantial clinical challenge, underscoring the critical need to urgently develop novel tools for ameliorating tailored patients' management. The 3'U-tRNA-derived fragments (3'U-tRFs), derived from pre-mature tRNAs, have emerged as a novel class of small non-coding RNAs, exhibiting pivotal roles in gene expression regulation and clinical value in cancer patients' prognosis and risk-stratification. The present study aims to investigate the clinical impact of 3'U-tRFs in MM. Bone marrow samples were collected from 130 MM patients at diagnosis (screening cohort). Mononuclear cells were isolated using Ficoll-Paque, while $CD138^+$ plasma cells were positively selected using anti-CD138 mAbs magnetic beads. Total RNA extraction, 3'-end polyadenylation and reverse transcription were followed, while 3'U-tRF^{SerTGA} levels were quantified by qPCR. Patients' mortality and disease progression (relapse and/or death) were assessed as clinical endpoint events, while internal validation was accomplished by bootstrap Cox proportional regression analysis. tRFdb database was accelerated to investigate potential changes of 3'U-tRFs levels among plasma cells, revealing 3'U-tRF^{SerTGA} as the most abundant 3'U-tRF and the most upregulated comparing malignant vs. normal plasma cells (FC=14.03). The analysis of our screening cohort (n=130) documented that elevated levels of 3'U-tRF^{SerTGA} is significantly associated with poor survival (p=0.039) and with higher risk for short-term disease progression (p=0.041) of MM patients. Moreover, 3'U-tRF^{SerTGA}-fitted multivariate models demonstrated the ability of 3'U-tRF^{SerTG}A to ameliorate the clinical routine of the established MM prognostic markers, including "R-ISS stage" and "response to 1st line therapy" (IMWG guidelines). Notably, the evaluation of 3'U-tRF^{SerTGA} levels significantly improved the risk-stratification of MM patients within R-ISS II (p=0.018), optimal (sCR/CR/VGPR) and poor (PR/SD/PD) responders' subgroups (p=0.001). Ultimately, we have identified 3'U-tRF^{SerTGA} as a robust indicator of poor treatment outcome, ameliorating patients' prognosis and risk-stratification in MM.

ST53

Deregulation of alternative splicing in Pancreatic Neuroendocrine Tumors alters neuroendocrine cell function and calcium signaling

Myrto Potiri[1,2], Cleo Moschou[1], Zoi Erpapazoglou[1], Malgorzata Rogalska[3], Panagiota Kafasla[1]*

[1]BSRC "Alexander Fleming", Biomedical Sciences Research Center, Vari, Greece
[2]Department of Biochemistry & Biotechnology, University of Thessaly, Larissa, Greece
[3]Gene Regulation, Stem Cells and cancer, Centre for Genomic Regulation, Barcelona, Spain
**kafasla@fleming.gr*

Pancreatic Neuroendocrine Tumors (PNETs) are rare, diverse neuroendocrine neoplasms, rated as the second most common pancreatic malignancy (<10% of all pancreatic tumors) with rapidly increasing incidence rate over the last two decades. Deregulation of Alternative Splicing (AS) has recently been identified as a hallmark of cancer, yet it remains a relatively unexplored area in the majority of cancer types. AS, the process responsible for producing multiple transcripts from a single gene, adds complexity to the understanding of cancer pathogenesis.

Here we present a novel AS analysis of 101 human RNA-seq datasets derived from patients with PNET and also, pancreatic tissue from healthy donors. Our findings imply a substantial difference of AS profiles between tumor and normal pancreatic samples. GO term enrichment analysis reveals vesicle trafficking and calcium signaling pathways as highly deregulated at the level of AS in PNET.

We present here the results of the validations using the murine model RIP1-Tag2, a worldwide established model for PNET study, with increasing levels of aggressiveness as PNET progresses. We share insights from three different timepoints (8, 12 and 17 weeks of age) that clearly support our analysis outcomes, as we observe substantial shifts in transcript percentages as the disease advances. Using CACNA1D, CADPS2, DCTN1 and ERGIC3 genes as illustrative examples -identified in our splicing analysis and genuinely involved in calcium signaling and vesicle trafficking- we depict changes in their AS-derived transcript percentages as PNET progresses. We explored further the function of these transcripts in neuroendocrine cell function and in PNET development.

We suggest a model for splicing alterations in favor of PNET advance, revealing calcium signaling, vesicle transferring, exo- and endocytosis as significantly deregulated pathways that play a crucial role in PNET progression.

ST54

Integrin-Linked Kinase is a key mechanosensitive switch at muscle attachment sites in the Drosophila embryo

Demosthenes Mitrossilis[1], Efsevia Neonaki[1], Katerina Vakaloglou[1], Vincent Loreau[2], Frank Schnorrer[2], Christos Zervas[1]

[1]BRFAA,
[2]Institute for Developmental Biology Marseille

Cells in our bodies constantly experience mechanical forces from their microenvironment. When cells experienced elevated tension, they hold tight together and allow tissues to function healthily as a group. Integrin-based adhesions to the extracellular matrix (ECM) are emerging as key networks of mechanotransmission. Previously, our lab showed that Integrin-linked kinase (ILK)- a core component of the integrin adhesome- reinforces integrin- ECM adhesion by modulating integrin endocytosis in the developing Drosophila embryo (Cell Rep. 2016, Vakaloglou et al). Here, we aim to elucidate how ILK integrates the cytoskeletal-elicited forces and whether other components of the integrin adhesome synergize with ILK to stabilize integrin-mediated adhesion. We firstly developed an algorithm to track the individual muscle attachment sites and the length of each muscle segment in imaged embryos. By fitting every passive muscle elongation with a Kelvin-Voigt model, we quantified the ratio viscosity/Elastic Modulus. Our quantitative analysis shows that the viscoelastic properties in the Ilk mutant are altered in comparison to the wild type, before the manifestation of the Ilk phenotype. We secondly examine whether and how ILK alters the molecular forces transmitted across Talin, which is a major mechanosensor at integrin junctions, utilizing suitable FRET-based tension sensors. Collectively, our data allowed us to generate a novel mechanical framework on how cells integrate forces and maintain tissue integrity in the living organism.

Acknowledgements
This work was funded by EU (Marie Curie post-doctoral fellowship, #846470), AFMTelethon (#23004), Hellenic Foundation Research & Innovation (#01281).

ST55

The Wnt/TCF7L1 transcriptional repressor axis drives primitive endoderm formation by antagonizing naive and formative pluripotency

Paraskevi Athanasouli [1,#,*], Martina Balli [1,#], Anchel De Jaime-Soguero [1], Annekatrien Boel[2], Sofia Papanikolaou[3,4], Bernard K. van der Veer[1], Adrian Janiszewski [1], Tijs Vanhessche [1], Annick Francis [5], Youssef El Laithy [1], Antonio Lo Nigro[1], Francesco Aulicino [6], Kian Peng Koh [1], Vincent Pasque [1,7], Maria Pia Cosma [6,8,9], Catherine Verfaillie [1], An Zwijsen [5], Björn Heindryckx [2], Christoforos Nikolaou [4], Frederic Lluis[1]

[1]KU Leuven, Department of Development and Regeneration, Stem Cell Institute, B-3000, Leuven, Belgium
[2]Ghent-Fertility And Stem cell Team (G-FaST), Department for Reproductive Medicine, Department for Human Structure and Repair, Ghent University Hospital, 9000, Ghent, Belgium
[3]Department of Rheumatology, Clinical Immunology, Medical School, University of Crete, 70013, Heraklion, Greece
[4]Computational Genomics Group, Institute of Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", 16672, Athens, Greece
[5]Department of Cardiovascular Sciences, KU Leuven, 3000, Leuven, Belgium
[6]Centre for Genomic Regulation (CRG), Dr Aiguader 88, 08003, Barcelona, Spain
[7]KU Leuven Institute for Single-Cell Omics (LISCO), Leuven, Belgium
[8]ICREA, Pg. Lluis Companys 23, Barcelona 08010, Spain
[9]Universitat Pompeu Fabra (UPF), Barcelona, Spain
#These authors contributed equally
**email: paraskevi.athanasouli@kuleuven.be*

Early during preimplantation development and in heterogeneous mouse embryonic stem cells (mESC) culture, pluripotent cells are specified towards either the primed epiblast or the primitive endoderm (PE) lineage. Canonical Wnt signaling is crucial for safeguarding naive pluripotency and embryo implantation, yet the role and relevance of canonical Wnt inhibition during early mammalian development remains unknown. Here, we demonstrate that transcriptional repression exerted by Wnt/TCF7L1 promotes PE differentiation of mESCs and in preimplantation inner cell mass. Time-series RNA sequencing and promoter occupancy data reveal that TCF7L1 binds and represses genes encoding essential naive pluripotency factors and indispensable regulators of the formative pluripotency program, including Otx2 and Lef1. Consequently, TCF7L1 promotes pluripotency exit and suppresses epiblast lineage formation, thereby driving cells into PE specification. Conversely, TCF7L1 is required for PE specification as deletion of Tcf7l1 abrogates PE differentiation without restraining epiblast priming. Taken together, our study underscores the importance of transcriptional Wnt inhibition in regulating lineage specification in ESCs and preimplantation embryo development as well as identifies TCF7L1 as key regulator of this process.

ST56

Characterization of the fibroblast growth factor receptor 4 (FGFR4) gene in P. leptodactylus by investigating its expression at different embryonic developmental stages

Maria V. Alvanou[1], Athanasios Lattos[2], Konstantinos Feidantsis[3], Apostolos P. Apostolidis, Basile Michaelidis[2], Ioannis A. Giantsis[1]*

[1]Department of Animal Science, Faculty of Agricultural Sciences, University of Western Macedonia, 53100 Florina, Greece
[2]Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[3]Department of Fisheries & Aquaculture, University of Patras, Nea Ktiria, 26504 Mesolonghi, Greece
[4]Laboratory of Ichthyology and Fisheries, Faculty of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, 54124 Thessaloniki, Greece

The narrow-clawed freshwater crayfish, Pontastacus leptodactylus, apart from its substantial role as ecosystem "scavenger", represents an export-oriented dietary product of high economic significance. Aquaculture of the species remains, however, underdeveloped. One of the first steps towards this direction is the artificial egg hatching. The fibroblast growth factor receptor (FGFR4), belonging in the tyrosine kinases family, enacts a significant role in embryonic development. In the present work, the FGFR4 gene was characterized for the first time in P. leptodactylus by investigating its expression in embryos of different developmental stages. Two primer pairs were designed based on crustacean corresponding conserved regions that successfully amplified two segments of the FGFR4 gene. FGFR4 expression was substantially increased at the developmental stages closer to hatching. By estimating the expression pattern of FGFR4, which was increased in advanced developmental stages, it is possible to identify environmental factors that contribute to expediting the time of artificial hatching. In addition, the induction of FGFR4 expression apart from the effect on embryos development has been found to be involved in the induction of innate immunity in crustaceans, through the regulation of the NF-κB pathway. In conclusion, the two designed primer pairs targeting FGFR4 amplification are expected to represent useful markers for differential developmental stages evaluation in P. leptodactylus. The above conclusions can operate towards better understanding of artificial eggs incubation in freshwater crayfish.

This work is part of a research project supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects" to support Faculty Members & Researchers (Project Number: 3245).

ST57

Pharmacological targeting of SOS1-KRAS interaction triggers pancreatic β-cell proliferation and sustainably reverses diabetic hyperglycaemia

Adriana Papadimitropoulou[1], Chrysanthi Charalampous[1], Paraskevi Kogionou[1], Diana Reinhardt[2], Johanna Sonntag[2], Anthony Gavalas[3], Marco H. Hofmann[4], Patrik Erlmann[5], Michael Franti[5], Jonas Doerr[2], Thomas Klein[2], Gareth R. Willis[5], Ioannis Serafimidis[1]*

[1]Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[2]Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim am Rhein, Germany
[3]Paul Langerhans Institute Dresden (PLID) of Helmholtz Center Munich at the University Clinic Carl Gustav Carus of TU Dresden, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany.
[4]Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria
[5]Regenerative Medicine, Boehringer Ingelheim Pharma Inc, Ridgefield, CT, USA
**email: iseraf@bioacademy.gr*

A core pathological hallmark of both type-1 and type-2 diabetes is the decreased production of insulin, which leads to detrimental elevation of blood sugar levels and often a lifelong dependence on subcutaneous administration of insulin. Clinical studies have shown that insulin dependence and its side effects can be permanently bypassed by restoring a sufficient mass of functional β cells. Therefore, inducing islet regeneration through in vivo stimulation of β-cell proliferation has been proposed as an attractive strategy to restore β-cell mass and insulin production. Intracellular signaling through KRAS and MAPK has been shown to regulate pancreatic cell proliferation in a cell type and context-dependent manner. We demonstrate here that ectopic expression of a constitutively active form of Kras (KrasG12D) exclusively in endocrine cells suppresses β-cell proliferation, resulting in a dramatic reduction in β-cell numbers and islet size. This finding complements previous evidence that Kras haploinsufficiency leads to a marked increase in β-cell neogenesis. Based on these observations, we hypothesized that inhibition of KRAS signaling could stimulate β-cell expansion and increase β-cell mass. Our findings demonstrate that the potent and selective SOS1-KRAS interaction inhibitor BI-3406 promotes unprecedented levels of β-cell proliferation in primary human islets, both in culture and following transplantation in immunocompromised diabetic mice. Importantly, using murine models of streptozotocin-induced diabetes, we show that BI3406 treatment restores β-cell mass, leading to a gradual normalization of blood glucose and insulin levels, as well as sustainable improvement in glucose tolerance. A BI3406-based lead compound with improved pharmacodynamic and pharmacokinetic properties, currently under clinical development as a drug against different types of KRAS-driven cancers, is equally effective in correcting diabetic hyperglycemia. Our data provide the first preclinical evidence of an orally bioavailable and clinically relevant compound that can directly induce β-cell regeneration and contribute to the reversal of the diabetic phenotype.

ST58

CNS Immunoprofiling: A Comparative Study of Peripheral Blood and Cerebrospinal Fluid in a Hellenic Cohort of Multiple Sclerosis Patients

Vasileios Gouzouasis[1,2], Spyros Tastoglou[3,4], Lila Dimitrakopoulou[5], Anastasia Dagkonaki[1], Maria Eleftheria Evangelopoulos[6], Maria Anagnostouli[6], Nikos Markoglou[6], Dimitris Karathanasis[6], Eleni Tsoukalas[1], Konstantinos Kambas[1], Antonis Giannakakis[2], Lesley Probert[1]

[1]Laboratory of Molecular Genetics, Hellenic Pasteur Institute, Athens, Greece.
[2]Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, Greece.
[3]DIANA-Lab, Department of Computer Science and Biomedical Informatics, University of Thessaly, 35131 Lamia, Greece
[4]DIANA-Lab, Hellenic Pasteur Institute, 11521 Athens, Greece
[5]Department of Immunology, Laiko Hospital, National and Kapodistriakon University of Athens Medical School, Athens, Greece.
[6]Department of Neurology, Eginitio Hospital, National and Kapodistriakon University of Athens Medical School, Athens, Greece.

Objective: This study investigates the potential of comparative immunoprofiling between peripheral blood and cerebrospinal fluid in aiding early prognosis of multiple sclerosis (MS).

Materials and Methods: Paired peripheral blood and cerebrospinal fluid samples were collected from 38 patients presenting with an untreated neurological clinical episode and 7 non-inflammatory controls. The patient cohort consisted of relapsing-remitting multiple sclerosis (RRMS, n=24), clinically isolated syndrome (CIS, n=5), primary progressive MS (PPMS, n=4) and non-inflammatory controls (n=6). Samples were collected into Transfix tubes and immunostained using the following antibody markers CD45, CD66b, CD14, CD19, CD56, CD3, CD4 and CD8. Flow cytometry was performed in a FACS Canto II flow cytometer within three days.

Results: Our findings revealed distinctive immunological variations between the intrathecal and systemic immunity in MS patients. The population of CD3+/CD4+ T lymphocytes was significantly enriched in the CSF compared to the PB (1.39-fold increase), which was also observed in the NICs. RRMS/CIS patients exhibited a higher percentage of monocytes in the PB compared to PPMS patients (1.19-fold increase). The neutrophil-to-lymphocyte ratio was higher in the PB of relapse patients compared to asymptomatic patients (1.71-fold increase). Additionally, patients at their first clinical episode displayed a higher $CD56^{bright}/CD56^{dim}$ ratio compared to asymptomatic and relapse patients. The $CD56^{bright}/CD56^{dim}$ ratio was also higher in the CSF compared to the PB in all patients (5.95-fold increase).

Conclusions: The comparative analysis of immunophenotyping between PB and CSF provides valuable insights into immune cell populations relevant to MS pathology. Different disease stages (asymptomatic, first clinical episode, relapse, progressive) show distinct immune cell subtype enrichment properties in the PB and CSF. These findings highlight the potential of immunoprofiling in aiding early prognosis and personalized treatment approaches in MS.

ST59

Neuronal Nicotinic Acetylcholine Receptor Antibodies in Autoimmune Encephalitis Syndromes

Maria Pechlivanidou[1], Katerina Karagiorgou[1,2], Aigli G Vakrakou[3], Eleni Karachaliou[1], Elisabeth Chroni[4], Elpinickie Ninou[1], Theodora Afrantou[5], Nikolaos Grigoriadis[5], Christina Argyropoulou[6], Nikolaos Paschalidis[7], Maria Dandoulaki[1], Aikaterini Tsantila[1], Renato Mantegazza[8], Andreetta Francesca[8], Leon Dudeck[9], Johann Steiner[9], Erdem Tüzün[10], Jon Lindstrom[11], Dimitrios Tzanetakos[12], Sotirios Giannopoulos[12], Georgios Tsivgoulis[12], Socrates Tzartos[1,13,14], John Tzartos[12]

[1]Tzartos NeuroDiagnostics, Athens, Greece,
[2]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece,
[3]First Department of Neurology, School of Medicine, Aeginition Hospital, National and Kapodistrian University of Athens, Athens, Greece,
[4]Department of Neurology, School of Medicine, University of Patras, Rio, Patras, Greece,
[5]Second Department of Neurology, 'AHEPA' University Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece,
[6]Department of Neurology, Nicosia General Hospital, Nicosia, Cyprus,
[7]Center for Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece,
[8]Neuroimmunology and Neuromuscular Diseases Unit, Fondazione I.R.C.C.S. Istituto Neurologico Carlo Besta, Milan, Italy,
[9]Department of Psychiatry and Psychotherapy, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany,
[10]Department of Neuroscience, Aziz Sancar Institute for Experimental Medical Research, Istanbul University, , Istanbul,
[11]Department of Neuroscience, Medical School, University of Pennsylvania, , USA,
[12]Second Department of Neurology "Attikon" University Hospital, School of Medicine, NKUA, Athens, Greece,
[13]Department of Neurobiology, Hellenic Pasteur Institute, Athens, Greece,
[14]Department of Pharmacy, University of Patras, Patras, Greece

Aims: Autoimmune encephalitis syndromes (AES) comprise a group of disorders in which the host immune system attacks self-antigens expressed in the central nervous system (CNS). Antibodies to several membrane receptors, like NMDAR[1], have been identified in AES, but many AES patients have yet unidentified autoantibodies. Neuronal nicotinic acetylcholine receptors (nAChRs) are abundant in the CNS playing critical roles in brain function, thus making them candidate autoantigens in AES. This study aimed at the improvement of a cell-based assay (CBA) that selectively detects the potentially pathogenic antibodies to the major nAChR subtype (α4β2-nAChR), based on our α3β2-nAChR CBA[2], and its use for identification of such antibodies in "orphan" AES cases.

Methods: The study involved the screening of sera from 1752 patients from Greece, Turkey and Italy, who requested testing for AES antibodies, and from 1203 "control" patients with other neuropsychiatric diseases from the same countries or from Germany. A sensitive live CBA with α4β2-nAChR–transfected cells was developed to detect antibodies against the cell-exposed α4β2-nAChR domain and positive samples were confirmed quantitatively with flow cytometry analysis.

Results: Three patients were found α4β2-nAChR antibody positive by both CBA and FACS. No serum bound to control-transfected cells, and no control serum was positive by the transfected cells. The specific clinical characteristics of the 3 nAChR-antibody-positive patients fall into the AES spectrum. Specifically, one patient had Rasmussen encephalitis while another one had meningoencephalomyelitis.

Conclusions: Using live-CBA we detected serum antibodies against the α4β2-nAChR in patients with AES. Future studies will focus on the identification of more antibody-positive AES patients to characterize the clinical phenotype, on the functional characterization of the α4β2-nAChR antibodies and on their likely pathogenic role in cell cultures.

1. *Dalmau, et al., Lancet Neurol. 2008. 7:1091–1098, https://doi.org/10.1016/S1474-4422(08)70224-2*
2. *Karagiorgou, et al. Neurol. Neuroimmunol. Neuroinflamm. 2022, 9, https://doi.org/10.1212/NXI.0000000000001162.*

This work was supported by an ERA-NET NEURON JTC 2019 grant (NicAb; Novel biomarkers in neurological and psychiatric disorders: autoantibodies to neuronal nicotinic acetylcholine receptors), Greek national funds through the EPANEK (project code: MIS 5075033).

ST60

φ-eye: high-sensitive imaging of fluorescent and bioluminescent probes in vivo

Sofia Lagoumtzi[1]*, George Loudos[1], Maria Georgiou[1], Efthimios Lamprou[1], Isaure de Kernier[2], Eleftherios Fysikopoulos[1]*

[1]BIOEMTECH, Lefkippos Attica Technology Park - NCSR Demokritos, Athens, Greece
[2]First Light Imaging S.A.S., Europarc Ste Victoire Bât 5, Route de Valbrillant, Le Canet 13590 Meyreuil, France

Background Molecular imaging in animal models speeds up the mean time from synthesis to market, in drug development process. Tissue observation with light (i.e. Fluorescence, Bioluminescence) is probably the most common practice, as alternative techniques are often very costly and time consuming to implement [1], [2]. In this work, we present "φ-eye", a highly sensitive, low noise in vivo preclinical optical imaging system.

Materials and Methods In vitro fluorescence sensitivity has been determined using well known fluorescent probes (fluorescein, Sulphorhodamine 101, nile blue).

In vivo fluorescence evaluation has been performed with OsteoSense 680 and TdTomato fluorescent probes in a spondyloarthritis and a cutaneous neurofibromas mouse model respectively [3].

In vitro and in vivo bioluminescence evaluation has been performed using different concentration of U87MG-Luc2 cells. Finally, ICG and IRDye 800 CW have been used for fluorescence evaluation in the SWIR window.

Results The minimum detection limit was found to be 200 nM for fluorescence studies and 100 cells for bioluminescence. Minimum detectability and linearity were determined for a range of 0.5uM-512uM for ICG and 0.1 nM to 4 nM for IRDye 800 CW. In vivo fluorescence and bioluminescence imaging prove that performed studies can shed light on the prompt understanding of the accumulation of the relevant tracing molecules highlighting essential features of the examined diseases such as associated inflammation or tumor growth.

Conclusions In vitro and in vivo experimental results show that "φ-eye" platform may constitute a valuable tool in preclinical research as it combines high performance characteristics, high throughput and small footprint.

Acknowledgement
This research has been co financed by the European Regional Development Fund of the European Union and Greek national funds through the NextGeneration EU, project code: ΤΑΣΦΡ-06387 with acronym EYES.

[1]. Keyaerts M. et al.,Trends in Molecular Medicine, 2012.
[2]. Ntziachristos V. et al., Eur Radiol., 2003.
[3]. Karampelas T. et al., EMIM 2022.

ST61

GemC1 and McIdas mediate ependymal cell reprogramming contributing to the repair of the ependymal layer in hydrocephalic models.

Konstantina Kaplani[1],*, Maria-Eleni Lalioti[1], Stella Vassalou[1], Georgia Lokka[1], Evangelia Parlapani[1], Georgios Kritikos[1], Zoi Lygerou[2], Stavros Taraviras[1]

[1]Department of Physiology, Medical School, University of Patras
[2]Department of General Biology, Medical School, University of Patras

Hydrocephalus is a prevalent neurological disorder marked by the abnormal buildup of cerebrospinal fluid (CSF) within the cerebral ventricles. Malfunction in the multiciliated ependymal cells of the brain is a leading cause of the pathophysiological mechanism of hydrocephalus, as these cells have a key role in CSF circulation and composition, while they support the neural stem cells of the ventricular/subventricular neurogenic niche. Disruption of the neurogenic niche's cytoarchitecture also plays a crucial and persistent role in hydrocephalus. Currently, primary treatments for hydrocephalus mainly involve neurosurgical cerebrospinal fluid diversion, which hold high morbidity and failure rates, highlighting the necessity for the discovery of novel therapeutic approaches.

Ependymal cell differentiation is a multi-step process orchestrated by a tightly regulated transcriptional program. We have previously provided evidence that the Geminin family proteins, GEMC1 and MCIDAS, are the earliest regulators for the cell fate commitment to the ependymal lineage. Additionally, our team and others have revealed that GemC1 initiates the activation of well-established regulators of multiciliogenesis in various organisms. Our objective is to assess the reprogramming potential of GemC1 and McIdas and investigate whether reprogramming cells to ependyma could be beneficial for hydrocephalus.

Ectopic expression of GemC1 or McIdas revealed that both can reprogram cortical astrocytes into ependymal cells. Notably, McIdas is more efficient on establishing functional motile cilia in reprogrammed astrocytes. Additionally, our research demonstrates that McIdas' ectopic expression facilitates regeneration of ependymal cells in two distinct hydrocephalic mouse models: one induced by intracranial hemorrhage and a genetic form of hydrocephalus. Importantly, reprogrammed ependymal cells form pinwheel structures together with neural stem cells in hydrocephalic mice, revealing their potential to regenerate the architecture of the neurogenic niche. Collectively, our data suggest that GEMC1 and MCIDAS are potent reprogramming factors towards ependyma, proposing a novel approach for generating functional multiciliated cells in hydrocephalic models.

H.F.R.I. Hellenic Foundation for Research & Innovation

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: 2735).

ST62

Cold atmospheric plasma regulates breast cancer cells' microenvironment

Varvara-Christina Siagka[1], Aggeliki Kanellaki[1], Maria-Elpida Christopoulou[1,2], Stavros Meropoulis[3], Christos Aggelopoulos[3], Spyros S. Skandalis[1]*

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece
[2]Department of Pneumology, Medical Center-University of Freiburg, Faculty of Medicine-University of Freiburg, Freiburg, Germany
[3]Laboratory of Cold Plasma and Advanced Techniques for Improving Environmental Systems, Institute of Chemical Engineering Sciences, Foundation for Research and Technology Hellas (FORTH/ICE-HT), Patras, Greece
**skandalis@upatras.gr*

The existence of various breast cancer subtypes constitutes an obstacle to the development of targeted and effective therapeutic approaches. Cold Atmospheric Plasma (CAP) is an emerging and innovative technology with multiple applications including anti-cancer treatment. CAP is an ionized gas where the ions are close to room temperature and contains electrons, charged particles, reactive oxygen, and nitrogen species (ROS/RNS). The objective of this project is to inquire into a CAP-based therapy for breast cancer. To this aim, the effect of CAP on the viability of breast cancer cells of different estrogen receptor (ER) status and metastatic potential was examined by conducting three experimental set ups; direct treatment (where CAP was directly applied to cell cultures), indirect treatment (where CAP-treated media were transferred to cells) and medium change treatment (where CAP was directly applied to cells followed by immediate replacement of the treated medium by fresh). CAP treatment in both ER^+ and ER^- cells, induced drastic morphological changes and apoptosis (involving the mitochondrial pathway). The effect of CAP treatment on breast cancer cell viability in the absence or presence of ROS scavengers with different specificity revealed the prominent role of ROS, in particular H2O2, in the observed cytotoxicity of CAP. Recently, we detected significant alterations in the expression of specific matrix effectors such as CD44 (a major cancer stem cell marker and receptor of hyaluronan), proteases and inflammatory mediators [1]. We currently extend these studies by investigating hyaluronan network (hyaluronan, HASes, HYALs, CD44s/v). Moreover, we explore the role of NRF2 transcriptional factor in the oxidative stress induced by CAP in breast cancer cells. Overall, our data strongly support that CAP suppresses breast cancer cell growth through regulation of the tumor microenvironment.

[1] Aggelopoulos et al., Frontiers in Oncology, 2022 (doi: 10.3389/fonc.2021.826865)

ST63

Serglycin is a novel regulator of unfolded protein response pathway in glioblastoma cells

Eleftherios N. Athanasopoulos[1], Theodora Stamatogiannopoulou[1], Dimitra Manou[2], Dimitra Bainantzou[1], Achilleas D. Theocharis[1]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Brain Tumor Biology, Division of Translational Cancer Research, Lund University, Sweden

Glioblastoma (GBM) is the most aggressive tumor of the central nervous system. Tumor microenvironment, especially the surrounding extracellular matrix, plays a central role in oncogenesis and cancer progression in multiple levels. Serglycin (SRGN) is the sole proteoglycan that is located and acts both intracellularly and extracellularly. SRGN is involved in the maturation of secretory granules and regulates their protein cargo in various cell types, while simultaneously enhancing GBM aggressiveness. A variety of factors, such as high translation rates especially in tumor cells, can lead to the accumulation of misfolded proteins and endoplasmic reticulum (ER) Stress. ER stress triggers the activation of unfolded protein response pathway (UPR), which aims at adaptation and cell survival. Aberrant activation of UPR induces cell apoptosis. In this study we assessed the differences between control LN-18 GBM cells that express high levels of SRGN and LN-18 SRGN-suppressed cells, to respond to ER Stress, by investigating the activation of UPR pathways. By using tunicamycin, a N-glycosylation inhibitor, to induce ER Stress, we concluded that control LN-18 cells were more responsive to ER-stress induction than SRGN-suppressed LN-18 cells. More specifically, control LN-18 cells are characterized by increased expression levels of UPR genes compared to SRGN-suppressed LN-18 cells. Upon treatment with tunicamycin control LN-18 cells enhanced the expression of UPR molecules and activated the respective pathways in a dose-dependent manner. On the other hand, SRGN-suppressed LN-18 cells were unable to activate UPR and adapt to the induction of ER stress. Cell properties, such as proliferation and migration capacity were evaluated, suggesting a more effective mechanism and improved viability of control LN-18 cells under prolonged ER stress compared to SRGN-suppressed LN-18 cells. Current results highlight a novel function of SRGN as a mediator for UPR activation and cell survival under prolonged ER stress.

ST64

Comparative targeted metabolomics and proteomics analysis in human lymphoma model cell lines for the study of metabolic rewiring and differential diagnosis

Eleni Stolaki[1,2], Thomai Mouskeftara[3,4], Konstantina Psatha[1,2,5], Georgia Orfanoudaki[2], Aristeidis Kritis[6,7], Helen Gika[3,4], Michalis Aivaliotis[1,2,8]

[1]Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece
[2]Functional Proteomics and Systems Biology (FunPATh), Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Balkan Center, Thessaloniki, Greece
[3]Laboratory of Forensic Medicine and Toxicology, Department of Medicine, Aristotle University of Thessaloniki, Greece
[4]Biomic_AUTh, Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Balkan Center, Thessaloniki, Greece
[5]Laboratory of Medical Biology and Medical Genetics, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece
[6]Laboratory of Physiology, Department of Physiology and Pharmacology, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece
[7]cGMP Regenerative Medicine Facility, Department of Physiology and Pharmacology, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece
[8]Basic and Translational Research Unit, Special Unit for Biomedical Research and Education, School of Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

Objective: Metabolic rewiring is one of the hallmarks of cancer. Metabolism reprogramming is crucial to ensuring cancer cell nutrition, growth, and proliferation. The contribution of metabolomics in cancer research may better define the disease. Early detected metabolic reprogramming is a key point in studying, understanding, and diagnosing cancers characterized by extremely high clinical and histological diversity, such as lymphomas. In the present work, intracellular metabolomic profiles of human lymphoma cell lines are investigated and combined with proteomics data, to study lymphoma metabolism and support differential diagnosis.

Materials and Methods: Three model cell lines were used, corresponding to human lymphoma subtypes: MCL, ALCL, cHL. Gas chromatography-tandem mass spectrometry was conducted in cell extract samples. The applied MRM method targeted 54 organic acids (OAs), which are involved in key metabolic pathways. Metabolomics data were combined with proteomics data, obtained by our laboratory. Databases and bioinformatics tools (MetaboAnalyst, Perseus, Rstudio) were used for data integration, analysis and visualisation.

Results: 20 OAs were identified and quantified, the majority of which are significantly differentiated between lymphoma subtypes. Quantification of characterized oncometabolites, such as lactic acid, succinic acid and GABA was allowed. Metabolomics and proteomics data integration significantly strengthened and enriched our findings. The integration highlighted that those 20 OAs and 20 proteins, found differentiated in proteomics, participate in dysregulated metabolic and signaling pathways in cancer cells (e.g., Warburg-effect, glutamate metabolism, citric-acid cycle, HIF-1 signaling pathway).

Conclusion: The applied targeted GC-MS/MS method seems to be able to differentiate the investigated lymphoma subtypes and provide important information about lymphoma metabolic rewiring. Our results highlight that metabolomics is a promising research approach for the differential diagnosis and study of metabolic and signaling pathways in lymphomas. The integration of metabolomics and proteomics data significantly enhances studying and understanding lymphoma, as well as potential biomarkers' and new drug targets' identification.

Indicative bibliography

- *Mouskeftara T, Virgiliou C, Theodoridis G, Gika H. Analysis of urinary organic acids by gas chromatography tandem mass spectrometry method for metabolic profiling applications. J Chromatogr A [Internet]. 2021 Nov 22 [cited 2023 Jan 24];1658.*
- *Wishart D. Metabolomics and the Multi-Omics View of Cancer. Metabolites [Internet]. 2022 Feb 1 [cited 2023 Sep 7];12(2).*
- *Beyo lu D, Idle JR. Metabolic Rewiring and the Characterization of Oncometabolites. Cancers (Basel) [Internet]. 2021 Jun 2 [cited 2023 May 2];13(12).*

ST65

LPS-induced expression of phospholipase A2 isoforms in paclitaxel resistant and sensitive human triple negative breast cancer cells (TNBC-C) and the relevant extracellular vesicles (TNBC-EVs).

Margarita Tenopoulou[1], Eirini Kitsiouli[1], Dimitra Kolatsi[1], Stylianos Papadopoulos[1], Martina Samiotaki[2], Dimitrios Kordias[3], Angeliki Magklara[3], Marilena E. Lekka[1]*

[1]University of Ioannina, Department of Chemistry,
[2]Biomedical Sciences Research Center "Alexander Fleming" ,
[3]University of Ioannina, Department of Medicine & Biomedical Research Institute Foundation for Research & Technology-Hellas

TNBC is an aggressive type of breast cancer with poor prognosis. Development of chemoresistance in TNBC might involve extracellular vesicles (EVs), "organelles" accomplishing intercellular communication. The current study characterized EVs, their cargo proteins and the mRNA expression of phospholipases in cellular models of TNBC. EVs were collected by differential ultracentrifugations (1) from TNBC sensitive (parental) or resistant to paclitaxel (ptx) cells (2). Using high resolution mass-spectrometry we identified and quantified relative changes in protein expression in cell lysates and EVs under normal conditions and after LPS treatment. Over 4000 proteins were identified in EVs, of which, 506, from ptx cells were not present in EVs from parental cells. LPS-treatment increased the number of EVs-associated unique proteins (593 and 212 in parental and ptx cells, respectively). Targeted analysis of secreted phospholipases A2 (sPLA2s) revealed a 1.5-fold decrease of sPLA2GXV and sPLA2GIIA expression levels in EVs from ptx cells as compared to EVs from parental cells. Moreover, a 0.7-fold increase of sPLA2GIIA expression was recorded in EVs from LPS-treated ptx cells, compared to EVs from untreated cells. qRT-PCR analysis revealed the expression of PLA2G2A, PLA2G3 and PRDX6 in all the TNBC cell lysates, whereas LPS caused a decrease in PLA2G2A and an increase in PRDX6, which were also identified on EVs. Interestingly, LPS increased the PLA2G2A mRNA expression in ptx resistant but not in sensitive TNBC. Finally, the robust proteomic data provided additional insight for ABCB1 and CROT gene products as potential biomarkers in paclitaxel-induced resistance in TNBC. The findings above demonstrate the existence of an effective delivery system in EVs of differentially expressed proteins in distal sites with high permeability through body barriers under inflammatory conditions. Identification of EVs-derived protein signatures in drug-resistant cells may indicate potential targets to overcome chemoresistance and re-establish sensitivity to paclitaxel in TNBC.

References

1. *Papadopoulos S, et al. Secretory Phospholipase A2-IIA Protein and mRNA Pools in Extracellular Vesicles of Bronchoalveolar Lavage Fluid from Patients with Early Acute Respiratory Distress Syndrome: A New Perception in the Dissemination of Inflammation?. Pharmaceuticals (Basel). 2020;13(11):415. doi:10.3390/ph13110415*
2. *Kordias D, Kostara CE, Papadaki S, Verigos J, Bairaktari E, Magklara A. Omics Analysis of Chemoresistant Triple Negative Breast Cancer Cells Reveals Novel Metabolic Vulnerabilities. Cells. 2022;11(17):2719., doi:10.3390/cells11172719*

Acknowledgements

*1. This work was supported by the project **"Establishment of "capacity building" infrastructures in Biomedical Research (BIOMED-20)**" (MIS 5047236) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).*
2. We acknowledge support of this work by the project "An Open-Access Research Infrastructure of Chemical Biology and Target-Based Screening Technologies for Human and Animal Health, Agriculture and the Environment (OPENSCREEN-GR)" (MIS 5002691) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

ST66

Unravelling cannabidiol´s therapeutic potential in stress and Alzheimer´s disease brain pathologies

Anastasia Vamvaka-Iakovou[1,2,3,4], Joana Silva[3,4], Patricia Gomes[3,4], Carlos Campos-Marques[3,4], Martina Samiotaki[5], George Panayotou[5], Anastasia Megalokonomou[1,3,4], Charalampos Brakatselos[6] , Filippos Katsaitis[1], Kalliopi Skourti[1], Georgia Papadimitriou[1,3,4], Beatriz Barros-Santos[3,4], Katerina Antoniou[6], Ioannis Sotiropoulos[1,3,4]

[1] Institute of Biosciences and Applications, NCSR Demokritos, Agia Paraskevi, Greece
[2] Department of Biological Applications & Technology, University of Ioannina, Ioannina, Greece
[3] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal
[4] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimaraes, Portugal
[5] Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Attica, Greece
[6] Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Ioannina, 45110 Ioannina, Greece

Over the last decade, the pharmaceutical industry has been showing a profound interest in Cannabidiol (CBD) while an increasing number of studies have been focused on unraveling the biological and molecular underpinnings of CBD therapeutic potential in the context of neurological and neuropsychiatric disorders such as stress-driven depression and Alzheimer´s disease (AD). However, it remains unknown whether CBD can modulate Tau pathology and related neuronal malfunction/neurodegeneration. Thus, we exposed 4-5-month-old Tau transgenic mice and their wild-type littermates to chronic unpredictable stress with simultaneous CBD treatment for a duration of six weeks. Our findings indicate that chronic CBD treatment effectively ameliorated stress-induced cognitive impairment and mood deficits in Tau Tg mice and their wild-type littermates. Furthermore, our ongoing molecular, neurostructural and proteomic analysis offers novel insight into the molecular mechanisms underlying for the beneficial impact of CBD treatment. These results support the potential therapeutic use of CBD treatment against AD-related Tau pathology and precipitating factors of the disease, contributing to our limited knowledge of how cannabinoid signaling can modulate brain pathology.Top of Form

Funding source

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship Number: 83145/114219/β6.ε).

ST67

Generation of a BRAFV600E human isogenic disease model using CRISPR-Cas12a

Euripides Diamantopoulos[1], Lefki-Pavlina N Giassafaki[1, 2], Ioannis S Vizirianakis[2], Georgios Tzimagiorgis[1, 3] *

[1]Laboratory of Biological Chemistry, Department of Biological Sciences and Preventive Medicine, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[2]Laboratory of Pharmacology, Department of Pharmaceutical Sciences, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]Genetic and Epigenetic Translational Research Group (GENeTres), KEDEK, Balkan Center, Building A nd B, Thermi, Thessaloniki, Greece

Genome editing techniques utilizing CRISPR biotechnology have come to focus in recent years and became a powerful new tool for making precise additions, deletions and substitutions in the genome. Isogenic disease models are proven a useful tool for the in vitro study of human disease and drug testing. BRAFV600E a mutated kinase -responsible for various types of cancer- exhibits resistance to its inhibitors yet lacks such a disease model.

We used CRISPR-Cas12a-huLbCpf1 plasmid and a single stranded DNA oligonucleotide that target the Braf gene to co-transfect HEK293T cells and replace thymine at position 1799 by an adenine, the nucleotide substitution responsible for the oncogenic BRAFV600E driver mutation.

The isogenic HEK293T clones that were generated and carry the BRAFV600E mutation or its knock out cover all possible chromosomal combinations of a DNA knock in experiment and comprise a panel of isogenic clones each of which can be used differentially in the study of BRAFV600E mutation. Interestingly the homozygous BRAFV600E clone spontaneously forms adherent spheroids upon serum deprivation without need of hydrogels or scaffolds or ultra low attachment cell culture dishes or supplementation with growth factors but only DMEM as growth media, a characteristic that is up to our knowledge for the first time reported.

In this study we explored the technical advantages of CRISPR-Cas12a (huLbCpf1) enzyme for editing single point mutations in human cells by generating a human isogenic disease model of BRAFV600E mutation. The strategy used requires basic laboratory equipment, minimum experimental capacity and low cost consumables to generate human isogenic cells engineered to carry cancer driving point mutations of interest like BRAFV600E.

ST68

Significance of Catecholamine Biosynthetic/Metabolic Pathway in SARS-CoV-2 Infection and COVID-19 Severity

George Mpekoulis[1], Evangelos Korakidis[1], Katerina I. Kalliampakou[1], Raphaela S. Milona[1], Despoina Lagou[1], Anastasios Ioannidis[2], Edison Jahaj[3], Emmanouil Vourakis[4], Christos T. Chasapis[5], Dionysis Kefallinos[6], Ioannis Karakasiliotis[7], Anastasia Kotanidou[3], Stylianos Chatzipanagiotou[8], Emmanouil Angelakis[9,10], Dido Vassilacopoulou[11], Alice G. Vassiliou[3], Niki Vassilaki[1,*]

[1]Laboratory of Molecular Virology, Hellenic Pasteur Institute, 11521 Athens, Greece
[2]Department of Nursing, University of Peloponnese, 23100 Sparti, Greece
[3]GP Livanos and M Simou Laboratories, First Department of Critical Care Medicine & Pulmonary Services, National and Kapodistrian University of Athens Medical School, Evangelismos Hospital, 10676 Athens, Greece
[4]Department of Nursing, Faculty of Health Sciences, University of Peloponnese, Sehi Area, 22100 Tripoli, Greece
[5]Institute of Chemical Biology, National Hellenic Research Foundation, 11635 Athens, Greece
[6]School of Electrical Engineering and Computer Science, National Technical University of Athens, 9 Iroon Polytechniou Street, Zografou, 15773 Athens, Greece
[7]Laboratory of Biology, Department of Medicine, Democritus University of Thrace, 68100 Alexandroupolis, Greece
[8]Department of Medical Biopathology, Medical School, University of Athens, Eginition Hospital, 11528 Athens, Greece
[9]Department of Diagnostics, Hellenic Pasteur Institute, Athens 11521, Greece
[10]Aix Marseille University, IRD, IHU Méditerranée Infection, VITROME, 13005 Marseille, France
[11]Section of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, 15772 Athens, Greece
[*]Corresponding author: nikiv@pasteur.gr, +306974174747

SARS-CoV-2 infection has been previously associated with the expression of the dopamine biosynthetic enzyme L-Dopa decarboxylase (DDC). Specifically, despite the increased expression of DDC in nasopharyngeal swab samples of patients infected with SARS-CoV-2 (Wuhan strain) compared to non-infected individuals, a negative correlation was detected between DDC mRNA and SARS-CoV-2 RNA levels in the same samples as well as in in vitro infected epithelial cells. Herein, by comparing DDC expression in the nasopharyngeal tissue of severe/critical to mild COVID-19 cases, we highlighted the importance of DDC as a potential marker of COVID-19 severity. This was further confirmed by comparative studies including patients infected with the Omicron variant of the virus, which cause less severe illness. Moreover, we identified an association of SARS-CoV-2 infection with the expression of key catecholamine biosynthesis/metabolism-related genes, in whole blood samples from hospitalized patients and in cultured cells. Specifically, viral infection downregulated the biosynthetic part of the dopamine pathway (reduction in DDC expression up to 7.5 mean-fold), while enhanced the catabolizing part (increase in monoamine oxidases A and B expression up to 15 and 10 mean-fold, respectively) in vivo, irrespectively of the presence of comorbidities. In accordance, dopamine levels in the sera of severe cases were reduced (up to 3.8 mean-fold). Additionally, a moderate positive correlation between DDC and MAOA mRNA levels ($r = 0.527$, $p < 0.0001$) in the blood was identified upon SARS-CoV-2-infection. Furthermore, L-Dopa or dopamine treatment of infected cells attenuated the virus-derived cytopathic effect by 55% and 59%, respectively. The SARS-CoV-2 mediated suppression of dopamine biosynthesis in cell culture was, at least in part, attributed to hypoxia-like conditions triggered by viral infection. These findings suggest that L-Dopa/dopamine intake may have a preventive or therapeutic value for COVID-19 patients.

ST69

Semi-synthetic analogues of oleuropein with improved anticancer activity in vitro and in vivo

Nikolaos Angelis[1]*, Panagiota Papakotsi[2], Efthimios Paronis[1], Georgia Sarikaki[2], Ioannis Kostopoulos[1], Alexandros-Leandros Skaltsounis[2], Ioannis Kostakis[3], Ourania Tsitsilonis[1]

[1]Section of Animal and Human Physiology, Department of Biology;
[2]Section of Pharmacognosy & Natural Products Chemistry, Department of Pharmacy;
[3]Section of Pharmaceutical Chemistry, Department of Pharmacy, National and Kapodistrian University of Athens, Greece

Oleuropein-rich products of Olea europaea are well-known for their anticancer activity. Herein, a series of novel semisynthetic analogues of oleuropein were designed, synthesized and evaluated preclinically as for their antitumor properties. Twenty-two analogues were tested against various human cancer cell lines (by MTT assay) and their half-maximal inhibitory concentration (IC50) was determined. To evaluate their cytotoxicity against normal cells, healthy donor-derived peripheral-blood mononuclear cells (PBMCs) were used. The mode of action of the most active compounds was investigated by flow cytometry (FC), as for the type of cell death induced, their cytostatic effects and cell cycle alterations, using Annexin V/PI, CFSE and PI staining, respectively. Immunocompetent BALB/c and C57BL/6J mice bearing CT26 colon cancer and B16.F1 melanoma tumors, respectively, were administered 8 doses (1-100 μg/dose) of the most active analogue (GS32) every other day for 15 days. Tumor growth was monitored for 29 days. Immunohistochemistry was performed on tumor sections, using antibodies against immune-cell markers (CD3/CD4/CD8/Mac-3). Spleen cells from treated mice were isolated, co-incubated with the syngeneic CT26 or B16.F1, YAC-1 (NK-sensitive) and WEHI-164 (LAK-sensitive) mouse cell lines, and the expression of CD107 (degranulation marker) was quantified ex vivo via FC. Overall, the oleuropein analogues GS32 and GS36 demonstrated potent and consistent cytotoxicity across all cancer lines tested in vitro. Neither analogue was toxic against PBMCs. Their anticancer activity can be, at least in part, attributed to their cytostatic properties. In vivo administration of GS32 retarded both colon and melanoma tumor growth and prolonged mouse survival, likely through the induction of specific (T cell-mediated) and non-specific (NK and LAK cell-mediated) antitumor immune responses and increased tumor infiltration by T cells. This dual effect of GS32 on tumor progression and immune-cell stimulation, shows promise for the further design of improved small molecules with potent anticancer activity and minimum toxic side effects.

Funding
This research has been co financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T2ΕΔΚ-02423).

ST70

Effect of daily consumption of a novel biscuit enriched with Pleurotus eryngii mushroom on fecal microbiota and immune system

Marigoula Vlassopoulou[1,2,*], Ahina Boulaka[1,*], Aleka Rapti[1,3], Evangelia N. Kerezoudi[2,4], Evdokia K. Mitsou[2], Theodora Brikou[2], Adamantini Kyriacou[2], Mary Yannakoulia[2], Georgios Koutrotsios[5], Georgios I. Zervakis[5], Aggeliki Saridaki[6], Penny Zoumpouli[6], Eleftherios Makras[6], Nikolaos Paschalidis[7], Panagiotis Georgiadis[1], Vasiliki Pletsa[1,]**

[1]National Hellenic Research Foundation, Institute of Chemical Biology, Athens, Greece
[2]Harokopio University, Department of Nutrition and Dietetics, Athens, Greece
[3]University of Crete, School of Medicine, MSc Programme Oncology, Heraklion, Crete
[4]Örebro University, School of Medical Sciences, Örebro, Sweden
[5]Agricultural University of Athens, Laboratory of General and Agricultural Microbiology, Athens, Greece
[6]Department of Research & Development, E.J..PAPADOPOULOS S.A., Athens, Greece
[7]Biomedical Research Foundation of the Academy of Athens (BRFAA), CyTOF Lab, Athens, Greece
**equal contribution*
***corresponding author*

The effect of nutrition on human health through the modulation of Gut Microbiome (GM) has been the subject of intensive research during the last two decades, thanks to advancements in sequencing tools, high-throughput technologies and Big Data processing analysis and integration. This study aims to assess the effect of the consumption of a novel biscuit (enriched with Pleurotus eryngii mushrooms rich in β-glucans) on fecal microbiome and immune system of apparently healthy older adults. Participants (n=31, 60-80 years old), meeting the eligibility criteria, provided biological samples and were randomly assigned in a double-blind manner to one of the intervention groups, i.e., daily consumption of the novel biscuit (biscuit enriched with mushroom powder containing 3g of β-glucans) or the placebo biscuit for 3 months. After a 2 months washout period, the subjects consumed the novel or the placebo biscuit in a crossover design for further 3 months.

Metataxonomic analysis (16S rRNA) of fecal microbiota was performed via Next Generation Sequencing while Cytometry by Time of Flight (CyTOF) was applied to assess immunophenotypic changes in isolated peripheral blood mononuclear cells (PBMCs) of participants. Mean changes in bacterial family and genus levels were comparable between groups, independently of type of the biscuit. However, preliminary results of the CyToF analysis (n=10) indicate that the mushroom biscuit consumption results in the mobilization of the immune system leading to the reduction of inflammatory cell numbers and enhancement of cytotoxicity as manifested by an upward trend in cytotoxic T8 and NKT cells numbers and increased numbers of peripheral T regulatory cells (iTreg) associated with effective immunity against tumors.

A thorough study, also addressing a more focused panel of cellular markers, is ongoing to validate these encouraging results supporting the production of novel functional foods for strengthening the immune system against aging and preventing pathology, cancer included.

Keywords: *nutritional intervention, Pleurotus eryngii, gut microbiota modulation, immunomodulation.*

Aknowledgements
This research was co-funded by the EU and Greek national funds, through the Operational Program Competitiveness, Entrepreneurship and Innovation under the call RESEARCH-CREATE-INNOVATE (T1EDK-03404).

ST71

RESPONSIVE SELF-ASSEMBLED POLYMER DRUG CONJUGATES FOR PRECISION THERAPEUTICS

D. Toumpa, A. Angelopoulou, G. Pasparakis*

Department of Chemical Engineering, University of Patras, 26504 Patras, Greece, gpasp@chemeng.upatras.gr

Cancer remains a major cause of mortality in the Western world, despite significant advancements in treatment options, including innovative surgical techniques, targeted therapies, and more recently, immunotherapies. This study aimed to synthesize polymer-drug conjugates (PDCs) using block copolymers as targeted nanomedicines for cancer treatment. PDCs allow for precise control of drug delivery at desired sites owing to their responsiveness to external cues such as temperature/pH gradients and the presence of biomarkers of disease-related analytes. Starting from precursor monomers, we synthesized both single and dual drug loaded PDCs using living polymerization techniques. Furthermore, we developed responsive PDCs that can be triggered remotely by light or ultrasound, demonstrating significantly increased cytotoxicity compared to the parent drugs. This improvement is attributed to enhanced cellular uptake and the synergy between the drug and the stimuli cues. Our findings highlight the critical roles of polymer architecture, drug combinations, activation methods, and linker chemistry in enhancing in vitro effectiveness. In conclusion, our results provide new insights into the development of targeted nanomedicines to address unmet clinical needs in precision oncology.

Keywords: *polymer drug conjugates, gemcitabine, camptothecin, combinational delivery, thermoresponsive polymers, nanomedicines, responsive polymers*

References

1. *Pasparakis, G. et al., Harnessing photochemical internalization with dual degradable nanoparticles for combinatorial photo-chemotherapy. Nature Communications 5, 3623 (2014)*
2. *Barnett C. et al., Photochemical Internalization Using Natural Anticancer Drugs, Antimetabolites, and Nanoformulations: A Systematic Study against Breast and Pancreatic Cancer Cell Lines, Molecular Pharmaceutics 20 (3), 1818-1841 (2023)*
2. *M. Journal of Materials Chemistry B 6, 2230-2239 (2018)*
2. *ACS Macro Letters 6, 535-540 (2017)*

ST72

Smart design of extracellular vesicles for bioengineering applications

Vivi Bafiti[1], Sotiris Ouzounis[1], Vasiliki Zolota[2], Dimitrios Kardamakis[3], Theodora Katsila[1,*]

National Hellenic Research Foundation, Athens, Greece
University of Patras, Patras, Greece

Extracellular vesicles (EVs) facilitate cell-to-cell communication from short to long distances, carrying a series of biomolecules including nucleic acids, proteins, and metabolites. Thus, they exhibit a wide range of bioengineering applications. The latter are well-supported by key advantages that EVs share as a. they are devoid of cell-related complications, b. their size allow sterilization by filter membranes in large quantities during production, c. EVs remain active following repeated freeze-thaw and freeze-drying processes lowering their threshold for large-scale production, d. they have stable lipid bilayers protecting their contents when in blood circulation and e. EVs represent an evolutionary conserved cellular communication mode with readily available carrier characteristics and mechanisms.

Herein, we showcase various designs and modification strategies of EVs per bioengineering application, alone or in combination with selected biomaterials. Emphasis is put on the surface modification of EVs and their cargo of interest, as they are essential for tailoring their content, enhancing their targeting abilities and ensuring their biocompatibility. Glioblastoma multiforme (GBM) serves as a paradigm. EVs were isolated from a panel of established and characterized GBM cell lines derived from GBM patient surgical samples: U3005, U3024 and U3028, cultured in 3D spheroids. An in-house fully automated framework was employed for the segmentation and quantification of EVs in augmented microscopy images. Our integrated miRNAome and metabolome analysis led to the validation of EV-derived miRNAs using qRT-PCR and key metabolomic signatures.

The proposed roadmap addresses the full potential of current strategies and technologies utilized in the design and engineering of EVs, and discusses the future prospects and hurdles in the translation of engineered EVs into practical solutions. By harnessing the natural capabilities of EVs and refining their properties through innovative design approaches, these vesicles hold great promise for revolutionizing the field of bioengineering and transforming the landscape of modern medicine.

Acknowledgements
The work is supported by the Key Digital Technologies Joint Undertaking and co-funded by the European Union under Grant Agreement nº 101112347 (NerveRepack).

ST73

Multi-targeted apoptotic mechanism against adenocarcinoma cells, caused by organometallic chemotherapeutics

Christina N. Banti[1], Sotiris K. Hadjikakou[1,*]

[1] University of Ioannina, Laboratory of Biological Inorganic Chemistry, 45110, Ioannina Greece
Email: shadjika@uoi.gr

The conjugation of organotin(IV) and organoantimony(III/V) moieties with natural products ingredients (NPI= carvacrol, acetic acid, salicylic acid, cholic acid) is reported [1]. The new formulations were characterized in solid state by melting point, X-ray Fluorescence (XRF), Attenuated Total Reflection Furrier Transform Infra-Red (ATR-FT-IR) spectroscopies, while UV-Vis and NMR spectroscopies were used for the characterization in solution.

The in vitro anti-proliferative activity of the new compounds were evaluated against human breast adenocarcinoma cancer cell lines: MCF-7 (positive to hormones receptor (HR+)), MDA-MB-231 (negative to hormones receptor (HR-)). The in vitro toxicity was checked against normal human fetal lung fibroblast cells (MRC-5). The in vitro genotoxicity was tested with the micronucleus (MN) assay using fluorescence microscopy. Moreover, Artemia salina assay and Allium cepa assays were used for the in vivo toxicity. The MCF-7 cells morphology suggests apoptotic pathway, especially through the mitochondrion damage, which was confirmed by DNA fragmentation, Acridine Orange/Ethidium Bromide (AO/EB) Staining and permeabilization of the mitochondrial membrane tests. Their binding affinity toward the calf thymus CT-DNA was ex vivo investigated by Uv-Vis, Fluorescence spectroscopies and viscosity measurements.

[1] C.N. Banti, S.K. Hadjikakou et.al, Organotin derivatives of cholic acid induce apoptosis into breast cancer cells and interfere with mitochondrion; Synthesis, characterization and biological evaluation, Steroids, 2021, 167, 108798
[2] C.N. Banti, S.K. Hadjikakou et.al, Conjugation of triphenylantimony(V) with carvacrol against human breast cancer cells, Journal Biological Inorganic Chemistry, 2022, 27, 373-389.

Acknowledgments
This program is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Program «Human Resources Development, Education and Lifelong Learning 2014-2020» in the context of the project "Sub-project 6 ("Biological Inorganic Chemistry (BIC)" (MIS 5162213)."

ST74

Design and Synthesis of Novel 2-Substituted-5,7,8-Trimethyl-1,4-Benzoxazine Hybrids against Ageing

Theano Fotopoulou[1], Adamantia Papadopoulou[2], Andromachi Tzani[1], Michail Mamais[1], Harris Pratsinis[2], Maria Koufaki[1], Dimitris Kletsas[2]*, Theodora Calogeropoulou[1]*

[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Athens, Greece

Ageing is an inevitable natural biological process that is linked to the gradual deterioration of organismal homeostasis and the accumulation of damaged macromolecules. The progression of ageing has been highly correlated with increased levels of reactive oxygen species (ROS), thus the discovery of agents with antioxidant activity at cellular level could delay the deleterious effects of ageing.

The 1,4-benzoxazine ring system is as a privileged structure encountered in a wide variety of biologically interesting natural and synthetic agents. In particular, the 5,7,8-trimethyl-1,4-benzoxazine moiety can be considered as a bioisostere of the 5,7,8-trimethyl-1,4-benzopyran nucleus of the well-known antioxidant vitamin E. Moreover, compounds bearing polyphenols (catechol, resorcinol) possess a wide spectrum of biological activities. Capitalizing on our experience on bioactive 1,4-benzoxazine derivatives[1-5] we designed and synthesized six hybrid compounds combining the 5,7,8-trimethyl-1,4-benzoxazine scaffold and catechol or resorcinol moieties. The two pharmacophores are connected through the amide bond bioisostere 1,2,3-triazole ring. The new compounds were evaluated in vitro for their antioxidant properties, both in a cell-free assay (DPPH free radical scavenging) and in cultures of human skin fibroblasts. Two analogues were shown to act coordinately as free radical scavengers, intracellular ROS inhibitors, ho-1 gene expression inducers, and GSH enhancers. In addition, the most potent compound was found to enhance skin fibroblast viability. Interestingly, both senescent and early passage fibroblasts were found to respond similarly to the compounds. All these properties make these derivatives promising candidates in dermocosmetics, especially in products targeting skin ageing.

References
1. *Koini, E. N. et al. Tetrahedron 2012, 68, 10302-10309.*
2. *Koukouli, F. et al. Prion 2012, 6, 470-476.*
3. *Filippou, P. S. et al Bioorg. Med. Chem. 2011, 19, 5061-5070.*
4. *Koini, E. N. Et al. SYNLETT 2011 (11), 1537.*
5. *Koini, E.N. et. al J. Med. Chem. 2009, 52, 2328.*

Acknowledgements
This work was supported by the project "OPENSCREEN-GR (MIS 5002691) implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

ST75

Inactivation of tumor suppressor CYLD inhibits fibroblast re-programming to pluripotency

Nikolaos Bekas[1], Martina Samiotaki[2], Maria Papathanasiou[3], Panagiotis Mokos[1], Athanasios Pseftogas[4], Konstantinos Xanthopoulos[5], Dimitris Thanos[3], George Mosialos[1], Dimitra Dafou[1]

[1]School of Biology, Aristotle University Of Thessaloniki, Thessaloniki Greece
[2]Biomedical Sciences Research Center "Alexander Fleming", Athens Greece
[3]Biomedical Research Foundation Academy of Athens, Athens Greece
[4]Vita-Salute San Raffaele University Division of Experimental Oncology, Milan Italy
[5]Laboratory of Pharmacology, Department of Pharmacy, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki Greece

CYLD is a tumor suppressor gene coding for a deubiquitinating enzyme that has a critical regulatory function in a variety of signaling pathways and biological processes involved in cancer development and progression, many of which are also key modulators of somatic cell reprogramming. Nevertheless, the potential role of CYLD in this process has not been studied. With the dual aim of investigating the involvement of CYLD in reprogramming and a better under-standing of the intricate regulatory system governing this process, we reprogrammed control (CYLDWT/WT) and CYLD DUB deficient (CYLDΔ9/Δ9) Mouse Embryonic Fibroblasts (MEFs) into in-duced Pluripotent Stem Cells (iPSCs) through ectopic overexpression of the Yamanaka factors (Oct3/4, Sox2, Klf4, c-myc). CYLD DUB deficiency led to significantly reduced reprogramming efficiency and slower early reprogramming kinetics. The introduction of WT CYLD to CYLDΔ9/Δ9 MEFs rescued the phenotype. Nevertheless, CYLD DUB deficient cells were capable of establish-ing induced pluripotent colonies with full spontaneous differentiation potential of the three germ layers. Whole proteome analysis revealed that the Mesenchymal to Epithelial transition (MET) during the early reprogramming stages was disrupted in CYLDΔ9/Δ9 MEFs. Interestingly, differentially enriched pathways revealed that the primary processes affected by CYLD DUB de-ficiency were associated with the organization of extracellular matrix and several metabolic pathways. Our findings, not only establish for the first time CYLD's significance as a regulatory component of early reprogramming but also highlight its role as an extracellular matrix regu-lator, which has profound implications in cancer research.

ST76

Haematopoietic stem cells efficiently transfected by a non-viral, episomal vector, maintain physiological β-globin transgene expression.

Emmanouil Simantirakis* [1], **George Vassilopoulos**[1] **and Aglaia Athanassiadou**[2]*

[1]*Gene Therapy Laboratory, Centre of Basic Research, Biomedical Research Foundation of the Academy of Athens (BRFAA),11527 Athens, Greece.*
[2]*Department of General Biology, Medical School, University of Patras, 26504 Patras, Greece.*

Episomes in eucaryotes are essentially plasmids of bigger size and longer retention in the nucleus than common plasmids and bear great vector potential for gene transfer. Current development of non-viral episomal vectors(EV), non-integrating, non-coding for viral proteins, based on Scaffold or Matrix Attachment Regions (S/MAR) for binding the nuclear matrix, ensuring mitotic stability in successive mitoses, may generate valid alternatives to viral vectors for gene therapy.

Lately, lentiviral vector based gene therapy has been successfully applied for treatment of β-Thalassemia, a group of inherited blood disorders with limited conventional therapy options, deriving from mutations mainly in the β-globin gene. However, data from monitoring transduced patients, such as the development of a) clonal expansions in clinical trial with β- thalassaemic patients, (Boulad, F.2022), and b) acute myeloid leukemia in clinical trial with sickle cell disease patients (Jones, R.J, 2021) raised concerns. Additionally, high cost and demanding technology are limiting its wide-world application.

We constructed a novel, non-viral episomal vector, pEPβ-globin (16kb) , for the physiological β-globin gene, based on eGFP reporter gene, the S/MAR chromosomal element and a second chromosomal element, the "β-globin replication Initiation Region (IR)", for the enhancement of replication and establishment of episomal vectors onto the nuclear matrix of the host nucleus. Transfections into CD34+ cells demonstrate an average efficiency of 15.57-11.64%. In colony-forming cell (CFC) assay, fluorescent colonies are at a level of 92.21%, which is directly comparable to that from cells transfected with control vector pEP-IR, (92.68%). Importantly, β-globin mRNA is overexpressed in all cases of fluorescent colonies and it is detected at a 3-fold the level from CFC colonies of the control, non-transfected cord-blood cells, reaching the physiological, adult level. Vector pEP β-globin promotes our understanding of the interplay between plasmid establishment, transgene expression and nuclear architecture, leading to efficient episomal vectors for gene therapy.

ST77

Cardiomyocyte differentiation of highly purified and expanded human umbilical cord blood (hUCB)-derived very small embryonic like stem cells (VSELs)

Aliki Iliadou[1,2]*, Electra Papameletiou[3], Eleni Gounari[2], George Koliakos[1,2]

[1]Department of Biochemistry, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece
[2]Biohellenika Biotechnology Company, Thessaloniki, Greece
[3]University of Vienna

Backround: The use of stem cells isolated from hUCB is a promising therapy of heart failure after myocardial infarction. We here aim to expand hUCB-derived VSELs as isolated by our group and confirm their in vitro capacity to differentiate into cardiac cells.

Methods: hUCB-VSELs were isolated upon gradually increased centrifugation spins, as we have previously described (1). We modified a previously described protocol (2) using a pyramidoindole derivative UM729 to achieve the in vitro expansion of freshly, highly purified VSELs. The absolute number of the isolated VSELs was calculated in a Neubauer chamber, upon staining with Turk's solution. VSELs were treated with 5-azacytidine and TGFβ1 for 24h. The induction medium was switched to the complete medium without 5-AZA for 20 days. On day 7, VSEL-Embryoid bodies (EBs) were characterized by PLAP staining. Gene expression was assessed by RT-qPCR: a. Expansion; pluripotency markers (Oct4, SOX2, NANOG) on days 1, 7 and b. During and after differentiation; cardiac markers (NKX2.5, GATA4, MESP1).

Results: We succeeded a 4-fold expansion of purified VSELs on culture days 7-10. Gene analysis on VSELs on days 1 and 7 of the expansion, verified the maintenance of their pluripotency during proliferation. Expanded cells formed spheres that resemble EBs on day 7 of differentiation. PLAP expression on VSELs-EBs confirmed the embryonic phenotype of these structures. After a 20-day induction, we observed cells with a cardiomyocyte-like morphology. Gene expression analysis performed on VSELs compared to WJ-MSCs before the differentiation revealed a greater presence of markers related to cell proliferation and survival in VSELs. However, they expressed early lineage markers. Finally, the expression of cardiac transcription factors gradually increased during the induction, whereas pluripotency markers gradually decreased.

Conclusion: The present data provide further evidence that hUCB-VSELs could be successfully in vitro guided towards a cardiac cell fate.

Bibliography

1. *Gounari E, Daniilidis A, Tsagias N, Michopoulou A, Kouzi K, Koliakos G. Isolation of a novel embryonic stem cell cord blood–derived population with in vitro hematopoietic capacity in the presence of Wharton's jelly–derived mesenchymal stromal cells. Cytotherapy. 2019 Feb;21(2):246–59.*
2. *Lahlil R, Scrofani M, Barbet R, Tancredi C, Aries A, Hénon P. VSELs Maintain their Pluripotency and Competence to Differentiate after Enhanced Ex Vivo Expansion. Stem Cell Rev and Rep. 2018 Aug;14(4):510–24.*

ST78

HIF-1α phosphorylation by ERK1/2 controls the formation of diverse chromatin-associated protein complexes and the expression of distinct HIF-1 target genes.

Christina Arseni[1], Ioanna-Maria Gkotinakou[3], Martina Samiotaki[2], George Panayotou[2], Ioannis Sanidas[3], George Simos[1], Ilias Mylonis1

[1]Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Larissa, Greece
[2]Institute for Bio-innovation, BSRC "Alexander Fleming", Vari, 16672, Greece.
[3]Massachusetts General Hospital Cancer Center, Harvard Medical School, MA 02114, USA

Hypoxia inducible factor-1 (HIF-1) controls oxygen homeostasis and enables cancer cells to adapt to and survive in the hypoxic tumor microenvironment. In addition to oxygen-dependent regulation, HIF-1 activity is also controlled by phosphorylation of its HIF-1α subunit. We have previously reported that HIF-1α modification by ERK1/2 at Ser641/643 promotes HIF-1α nuclear accumulation, via masking a CRM1-dependent NES, and stimulates its transcriptional activity, via stabilizing its association with NPM1 and chromatin. To further examine the role of HIF-1α phosphorylation in hypoxic gene expression, different GFP-HIF-1α forms carrying mutations at the phosphorylation sites (SE or SA) and/or the NES (IA) were stably expressed in a HeLa HIF1A-/- cell line constructed by CRISPR/Cas9. Cells expressing the wild-type or the mutant phosphomimetic SE form of HIF-1α could thrive under hypoxia, while cells expressing the mutant phosphodeficient but nuclear IA/SA form grew very poorly under low oxygen conditions. Proteomic analysis of these cells under hypoxia revealed different protein expression patterns associated with the phosphorylation status of HIF-1α. Furthermore, analysis of chromatin complexes by RIME (Rapid Immunoprecipitation Mass spectrometry of Endogenous proteins) also identified distinct and phosphorylation-dependent HIF-1α binding partners on chromatin. Our results shine further light on the mechanism through which ERK1/2-mediated phosphorylation controls transcriptional reprogramming and HIF-1 gene target selection under hypoxia by shaping the HIF-1α/chromatin interactome.

This work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: HFRI-FM17-2132 to I.M.).

ST80

Transcriptomic signatures of coding and non-coding RNAs in TgRANKL osteoporosis mouse model

Elisavet Ioannidou[1,2], Vagelis Rinotas[1], Panagiotis Theofilidis[1,2], Marili Skalioti[1,2], Dimitra Andrikopoulou[1,2], Trias Thireou[2], Maria Yavropoulou[3], Eleni Douni[1,2]*

[1]Institute for Bioinnovation, Biomedical Sciences Research Centre "Alexander Fleming", Vari, Greece.
[2]Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Athens, Greece
[3]Endocrinology Unit, First Department of Propaedeutic and Internal Medicine, Medical School, National and Kapodistrian University of Athens, 11527 Athens, Greece.

Receptor activator of nuclear factor-B ligand (RANKL) constitutes a key regulator in osteoclast development and bone resorption, while its inhibition by the monoclonal antibody Denosumab has been approved for the treatment of postmenopausal osteoporosis. Our lab has established a genetic mouse model of osteoporosis by overexpression of human RANKL in transgenic mice (TgRANKL). In the current study, we identified differentially expressed (DE) genes with potential clinical value in osteoporosis by performing RNA-Seq for mRNAs, miRNAs, and lncRNAs in flushed femurs from TgRANKL and control wild-type (WT) mice. Regarding mRNAs, we identified in total 2,747 DE mRNAs (|log2FoldChange| > 1, adjusted p-value < 0.05) in TgRANKL femurs compared to WT. Enrichment analysis of the upregulated genes revealed correlation with protein degradation, proteolytic enzymes, transport, response to cytokines, cell adhesion, apoptosis, and bone remodelling, while downregulated genes were mainly related to metabolism, transport, cytoskeleton organization, muscle structure, and oxidative phosphorylation. More than 50 upregulated genes have been validated in TgRANKL femurs with qPCR, while treatment with Denosumab return their expression to normal levels. Furthermore, the expression of such genes was examined in osteoclasts and osteoblasts to identify the cellular source. Concerning miRNAs, we identified 63 DE miRNAs (|log2FoldChange| > 1) and validated selected miRNAs in femurs and mouse sera with qPCR. We also identified 235 DE lncRNAs (|log2FoldChange| > 2), and the expression of selected lncRNA genes was validated in femurs of TgRANKL mice, while their conservation with human transcripts was also evaluated. Among the DE transcripts we identified genes already known to play critical role in bone remodelling, while others have not correlated so far with osteoporosis. Thus, the DE genes revealed in this study may serve as the basis for the discovery of novel pathogenic mechanisms, and the identification of novel biomarkers in osteoporosis or potential drug-targets.

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 4th Call for HFRI PhD Fellowships (Fellowship Number: 10704).

ST81

Metabolite driven thermoprotection in heat hardened Mediterranean mussels

Ioannis Georgoulis[1,2*], Christian Bock[3], Gisela Lannig[3], Hans O. Pörtner[3], Inna M. Sokolova[4], Konstantinos Feidantsis2[,5], Ioannis A. Giantsis[2,6], Basile Michaelidis[1,2*]

[1]Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece
[2]Environmental Control and Research Laboratory, Region of Central Macedonia, GR-54625 Thessaloniki, Greece
[3]Alfred Wegener Institute, Helmholtz-Centre for Polar and Marine Research, Integrative Ecophysiology, Postfach 120161, D-27515 Bremerhaven, Germany
[4]Department of Marine Biology, Institute of Biological Sciences, University of Rostock, D-18055 Rostock, Germany
[5]Department of Fisheries & Aquaculture, University of Patras, GR-26504 Mesolonghi, Greece
[6]Department of Animal Science, Faculty of Agricultural Sciences, University of Western Macedonia, 53100 Florina, Greece.

Temperature affects organisms' metabolism and ecological performance. Owing to climate change, sea warming constitutes a severe source of environmental stress for marine organisms. Rapid warming can exceed resilience of marine organisms leading to fitness loss and mortality. However, organisms can improve their thermal tolerance when briefly exposed to sublethal thermal stress (heat hardening), thus generating heat tolerant phenotypes. We investigated the "stress memory" effect caused by heat hardening on M. galloprovincialis metabolite profile in order to identify the underlying biochemical mechanisms, which enhance mussels' thermal tolerance. Thermal stress at 24°C, 26°C and 28°C, resulted to an increase of all examined metabolites. However, H mussels exhibited statistically significant higher levels of these metabolites compared to the NH ones. Specifically, the three examined BCAAs (valine, leucine and isoleucine), glutamate and glutamine showed a sharp increase in their levels in the mantle tissue in thermally stressed H mussels compared to the NH ones. Likewise, levels of trimethylamine-N-oxide (TMAO), n-acetylcysteine (NAC), hypotaurine and sn-glycero-3-phosphocholine followed a similar pattern with H mussels exhibiting higher levels of these metabolites compared to the NH individuals. All the examined metabolites serve a key cell-protective role, since they are involved in energy metabolism, amino acid biosynthesis, growth, stress signaling and redox homeostasis. The initial increase in metabolite levels during the first five days may serve as a preemptive defense mechanism to safeguard the structure and functionality of proteins, prior to the sufficient Hsp expression that provide protection during the later phase. This highlights the potential of heat hardening as a highly promising approach in combating the impacts of global warming on cultivated and economically valuable bivalves, providing increased endurance to thermal stress. However, the energy costs associated with this response and its potential benefit on mussels' thermotolerance during prolonged exposure to elevated temperatures remain uncertain.

ST82

Characterization of Friend of GATA1 (FOG-1) co-factor functions in beta-globin gene regulation and erythropoiesis

Ioannis Marios Roussis[1,2,3], Grigoris Tsaknakis[1], Umar Niazi[4], Sagi Mansoor[4], John Strouboulis[3,*],

[1] Institute of Molecular Biology and Biotechnology, Foundation of Research & Technology Hellas, Heraklion, Crete, Greece.
[2] Department of Biology, University of Crete, Heraklion, Crete, Greece
[3] School of Cancer and Pharmaceutical Sciences, Faculty of Life Sciences and Medicine, King's College London, London, UK.
[4] Translational Bioinformatics, National Institute for Health Research Biomedical Centre, Guy's and St Thomas' NHS Foundation Trust and King's College London, London, UK.

Despite the significance of FOG-1 in erythropoiesis and megakaryopoiesis, as demonstrated by the Zfpm1 gene knockout, the molecular mechanisms underlying its functions have remained a long-lasting enigma. In our quest to shed light in this mystery, we conducted a comprehensive investigation of FOG-1's interactome and expression profile in both WT and FOG-1 KO erythroid cells. Our efforts in characterizing and validating FOG-1 protein complexes revealed that FOG-1 interacts with CTCF and members of the cohesion complex, known key players of chromatin looping. To further illuminate the role of FOG-1, we generated a FOG-1 KO cell line and utilized artificial zinc finger tethering to anchor FOG-1 to the inactive β-globin promoter. This experiment demonstrated that FOG-1 not only interacts with CTCF and Cohesins but also acts as an intermediate that facilitates their interaction with GATA1. Thus, providing a potential mechanism for the previously described FOG-1/GATA1 function in mediating DNA looping in target genes1,2. Expression profiling of the FOG-1 KO cells revealed another potential novel function of FOG-1 in cholesterol homeostasis. This finding illuminates a potential mechanism of how cholesterol levels are regulated in erythropoiesis, which remains largely uncharted despite early studies from 1980s3,4. Specifically, occupancy profiling in MEL cells, demonstrated that FOG-1 (with GATA1) occupy the promoters of cholesterol transporters. Consistent with this finding, our RNA-seq and western blot analyses suggest that FOG-1 may function as a repressor for Abca1 and Ldlr cholesterol transporters. Notably our quantification of intracellular cholesterol indicates that the de-represion of Abca1 and Ldlr transporters in FOG-1 KO cells leads to the accumulation of intracellular lipid droplets, which in turn has an overall effect on the cell's membrane fluidity. Collectively our findings, although preliminary for the function of FOG-1 in cholesterol transport, pave the way for further research aiming to a more comprehensive understanding of FOG-1 functions. (300 max)

References

1. *Vakoc, C. R. et al. (2005) 'Proximity among distant regulatory elements at the β-globin locus requires GATA-1 and FOG-1', Molecular Cell, 17(3), pp. 453–462. doi: 10.1016/j.molcel.2004.12.028*
2. *Jing, H. et al. (2008) 'Exchange of GATA Factors Mediates Transitions in Looped Chromatin Organization at a Developmentally Regulated Gene Locus', Molecular Cell, 29(2), pp. 232–242. doi: 10.1016/j.molcel.2007.11.020.*
3. *Rittmann, L. S. et al. (1982) 'Lipid composition of Friend leukemia cells following induction of erythroid differentiation by dimethyl sulfoxide', Journal of Cellular Physiology, 110(1), pp. 50–55. doi: 10.1002/jcp.1041100109.*
4. *Tsiftoglou et al. (1986) 'Plasma clot clonal assay for determination of the proportion of committed cells. Plasma clot culture', 889, pp. 251–261.*

ST83

Transcriptional dynamics and alternative splicing events in Systemic Lupus Erythematosus: A comprehensive investigation of intron retentions

Sofia Papanikolaou[1,2]*, George Bertsias[1,3], Christoforos Nikolaou[2]

[1] School of Medicine, University of Crete, Voutes, Heraklion, Greece
[2] Biomedical Sciences Research Center "Alexander Fleming", Institute of Bioinnovation, Athens, Greece
[3] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology - Hellas (FORTH), Heraklion, Greece

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disorder characterized by production of autoantibodies against nuclear and cytoplasmic antigens and multisystem inflammation. Previous studies revealed that genes implicated in disease pathogenesis are subject to alternative splicing (AS)[1,2]. In addition to increasing the complexity of the transcriptional output, alternative RNA splicing can lead to the reduction of mRNA translation or the production of non-functional or malfunctional proteins, thus representing a vital component of gene regulation process[3].

The aim of our study was to identify and characterize alternative splicing events in individuals with Systemic Lupus Erythematosus (SLE) compared to healthy counterparts.

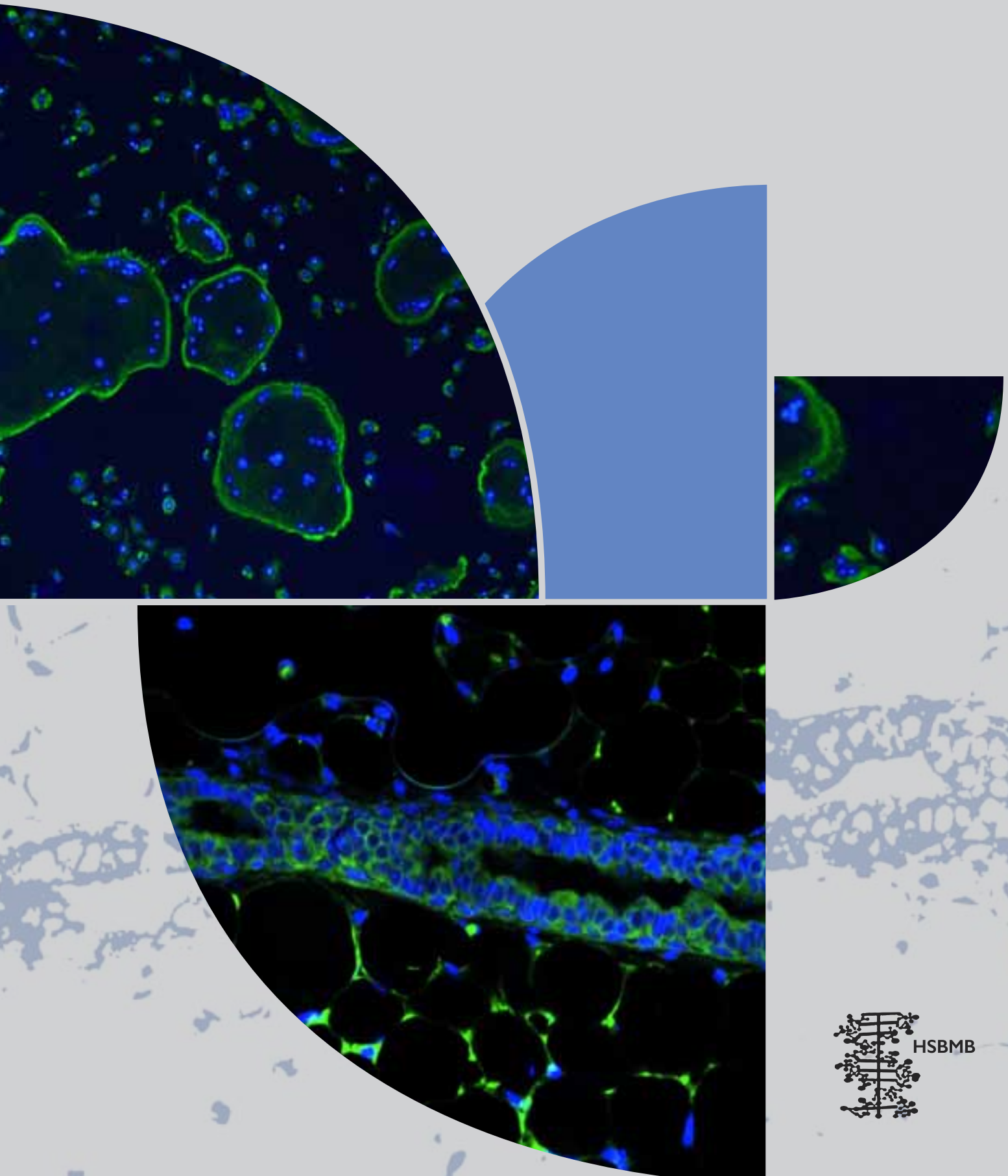
This analysis encompassed a range of specific cell types as well as whole blood samples from patients exhibiting variable levels of disease activity. Through the implementation of a computational pipeline on publicly available and our own RNA-sequencing data, we performed differential splicing analysis and uncovered significant alterations in transcription dynamics that affected a substantial number of genes.

Alternative splicing impacted a distinct set of genes as compared to those identified as differentially expressed. Differential splicing analysis between SLE versus healthy individuals revealed substantial cell type-specificity of splicing events. We observed a prevalence of intron retention events, with the majority leading to the introduction of premature stop codons, implying potential gene repression. Notably, intron retentions were detected in transcripts of healthy samples and this phenomenon was diminished in the context of SLE. We examined intrinsic properties of the retained introns, including their GC content and length. Functional assessment of genes influenced by alternative splicing pointed towards specific roles in metabolism and histone acetylation as areas of potential significance.

Overall, our findings emphasize the critical role of incorporating alternative splicing analyses in the molecular characterization of complex diseases like SLE.

1. *Dam, E.M.; Habib, T.; Chen, J.; Funk, A.; Glukhova, V.; Davis-Pickett, M.; Wei, S.; James, R.; Buckner, J.H.; Cerosaletti, K. The BANK1 SLE-risk variants are associated with alterations in peripheral B cell signaling and development in humans. Clin. Immunol. 2016.*
2. *Mamegano, K.; Kuroki, K.; Miyashita, R.; Kusaoi, M.; Kobayashi, S.; Matsuta, K.; Maenaka, K.; Colonna, M.; Ozaki, S.; Hashimoto, H.; et al. Association of LILRA2 (ILT1, LIR7) splice site polymorphism with systemic lupus erythematosus and microscopic polyangiitis. Genes Immun. 2008.*
3. *Djebali, S.; Davis, C.A.; Merkel, A.; Dobin, A.; Lassmann, T.; Mortazavi, A.; Tanzer, A.; Lagarde, J.; Lin, W.; Schlesinger, F.; et al. Landscape of transcription in human cells. Nature 2012*

POSTER SESSIONS



HSBMB

P1

A mechanism for actin patch formation to prevent chromatin bridge breakage in cytokinesis

Eleni Petsalaki[1]*, Sofia Balafouti [1], George Zachos[1]

[1]Department of Biology, University of Crete, Heraklion, Greece
**Email: grad600@edu.biology.uoc.gr*

Chromatin bridges are strands of incompletely segregated DNA connecting the anaphase poles or daughter nuclei and have been linked to carcinogenesis. If unresolved, chromatin bridges can break in cytokinesis leading to micronuclei formation and accumulation of DNA damage. To prevent this, human cells delay completion of cytokinesis (abscission) and form accumulations of polymerized actin (actin patches) at the base of the intercellular canal to stabilize chromatin bridges; however, the molecular mechanisms of actin patch formation are incompletely understood. In the present study, we identify small GTPases, which control the growth or contraction of filamentous actin fibers, that localize to actin patches and are required for stable chromatin bridges in cytokinesis. Inhibition of these actin regulators impairs actin patch formation and promotes chromatin bridge breakage, by confocal microscopy analysis of fixed cells and live-cell fluorescence micros-copy. Furthermore, chromatin breakage in cells deficient for the above proteins is not caused by premature abscission, but correlates with reduced actin patches compared with wild-type cells. This study identifies a novel signaling pathway that prevents chromatin bridge breakage by promoting actin patch formation in cytokinesis in human cells. Because chromatin breakage can lead to genomic instability that is associated with cancer formation or progression, understanding how cells stabilize chromatin bridges may help us understand mechanisms of tumorigenesis.

Acknowledgements
This work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers" (Project Number: 629).
George Zachos was supported by the "2nd Call for H.F.R.I. Research Projects to support Faculty Members and Researchers" (Project Number: 2486).

P2

The plasma membrane associated protein remorin6.6: in silico characterization and protein purification under native conditions

Veronica Giourieva[1], Nestoras Kargios[2], Murray Grant[2], Rigini Papi[3], George Komis[1]*

*email: gkomis.bio.auth.gr
[1]Department of Botany, School of Biology, Aristotle University of Thessaloniki, Greece
[2]School of Life Sciences, University of Warwick, Coventry, United Kingdom
[3]Department of Biochemistry, School of Chemistry, Aristotle University of Thessaloniki, Greece

In plants, cell division plane orientation is determined by the formation of a cortical microtubule annulus, the preprophase microtubule band (PPB). The PPB is physically associated with the plasma membrane via the recruitment of specific microtubule associated proteins (MAPs) devoid of lipid binding domains. It is proposed that such cell division zone localized MAPs, are themselves confined to the plasma membrane by means of scaffold proteins with dual affinity for both specific lipids of the plasma membrane and for MAPs. Most membrane bound protein scaffolds are peripheral and preferentially localize in sterol- and sphingolipid-rich membrane nanodomains. Remorins are plant specific plasma membrane associated proteins with prominent scaffold function and prospective role in cell division plane orientation. As an example, remorin6.6 was found to colocalize with myosin VIII at the plasma membrane implicated a possible role to plasma membrane and cytoskeleton(1). It has been observed that remorin6.6 is conditionally recruited to microtubules via its N-terminus. This project aims to structurally characterize the remorin6.6 and to identify protein interactors of this protein during cell division plane establishment. Through in silico modeling, remorin6.6 was found to consist of short α-helices and more than 50% was disorganized. The C-terminal domain was conserved and is specific to remorin family proteins while the N-terminal domain is proline rich and more variable. No additional specific domains were identified. The coding sequence of remorin6.6 from Arabidopsis thaliana leaves was cloned to expression vector resulting in a recombinant 6xhistidine tagged protein and purified to be used in pull down assay and subsequent LC-MS/MS analysis. The expression and solubility of recombinant protein was evaluated and optimized with specific Escherichia coli strains, different temperatures and IPTG concentrations.

References

(1) Einat Sadot et al. (2017) The 51st Annual Meeting of ISM, Weizmann Institute of Science, Rehovot

Acknowledgements

This work was supported by 4th call of Hellenic Foundation for Research and Innovation (H.F.R.I.) scholarships for PhD students and by the Greek-British short-term scholarship programme 2023

P3

Identification of the protein interactome of Escherichia coli Glutaredoxin 3

Charalampos N. Bompas[1], Eleni Poulou-Sidiropoulou[1], Martina Samiotaki[2], Alexios Vlamis-Gardikas[1]*

[1]Department of Chemistry, University of Patras, 26504, Rion, Patras, Greece
[2]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", 16672, Vari, Attica, Greece.

The glutaredoxin (Grx) and thioredoxin (Trx) systems participate in antioxidant defenses and maintain redox homeostasis in all living cells including viruses. The Trx system of Escherichia coli (E. coli) consists of Trx1 and 2, both reduced by thioredoxin reductase (TrxR). The respective Grx system is comprised of Grx1, 2, 3, and 4 with the first three reduced by glutathione (GSH), while Grx4 is reduced by TrxR. GSH is maintained at its reduced state by GSH reductase while NADPH+ is the electron donor for both systems. Grxs 1, 3, and 4 have similar molecular weights (~10 kDa), while Grx2 is an atypical Grx of 24,3 kDa. E. coli Grx3 (9,1 kDa, encoded by grxC) is relatively abundant amounting up to 0,4 % of the total soluble protein. Its active site contains the conserved motif CPYC that forms a disulfide in the oxidized form of the molecule. The vicinal cysteines of the active site catalyze thiol-disulfide exchange reactions between protein substrates and GSH. Levels of Grx3 are generally stable with changes of the external redox conditions known to affect them. In this study, affinity chromatography with bound monothiol Grx3 in the immobile phase was used to trap and identify possible interactors of Grx3 using as input cell extracts from different growth phases (exponential, stationary). Bound proteins were eluted under different conditions (high salt, acid, DTT) and identified through LC-MS/MS. To assess the biological function of Grx3, proteins detected in the eluates were further analyzed by bioinformatics (Perseus, Cytoscape). In another set of experiments, total proteomes of null mutants for grxC were compared to those of the wild type. The results from affinity chromatography and the null mutants suggest that Grx3 is implicated in basic metabolic procedures (respiratory chain, carbohydrate metabolism, amino acid metabolism), protein synthesis (ribosome assembly, initiation and termination of translation) and stress responses (oxidative stress, temperature stimuli). These novel perspectives for Grx3 correlate the molecule with many more functions than the hitherto known reduction of ribonucleotide reductase.

P4

Identification of putative protein interactors of Escherichia coli Glutaredoxin 2

Eleni Poulou-Sidiropoulou[1], Charalampos N. Bompas[1], Martina Samiotaki[2], Alexios Vlamis-Gardikas[1]*.

[1]Department of Chemistry, University of Patras, 26504, Rion, Patras, Greece
[2]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", 16672, Vari, Attica, Greece.

In all types of cells, the cytosol is maintained at a reduced state by glutaredoxin (Grx) and thioredoxin (Trx) systems. Grxs comprise a group of glutathione (GSH)-disulfide oxidoreductases that structurally belong to thioredoxin superfamily. The Grx system of Escherichia coli (E. coli) consists of $NADPH^+$ which reduces GSH reductase, that maintains GSH continuously reduced to donate electrons to Grxs1-3. A fourth Grx in E. coli (Grx4) is reduced not by GSH but by Trx reductase. While the smaller Grx1 and 3 (about 10 kDa) can reduce ribonucleotide reductase, the larger Grx2 (24 kDa) and Grx4 (15 kDa) cannot. Grx2 contributes more that 80 % of GSH mediated redox activity on cellular extracts and protects cells from oxidative damage resulting from the formation of carbonyls. It may constitute up to 1 % of total soluble protein in the stationary phase and its levels are known to be affected by external redox conditions. However, Grx2 function remains to be elucidated. The current study employed affinity chromatography of cell lysates through columns with immobilized monothiol/athiol Grx2 mutants, to identify potential interactors of Grx2. In addition, lysates from E. coli null mutants for grxB (the gene encoding Grx2) were compared to those of the wild type. All proteomic analyses were performed by LC-MS/MS followed by bioinformatics and gene ontology evaluations. The affinity chromatography results suggest that Grx2 is involved in protein synthesis, nucleotide and organonitrogen compound metabolism and stress responses. Comparative analysis of the proteomes of the wild type and null mutants for Grx2 implicated the protein in translation, response to stress, DNA damage, the β-barrel assembly machinery complex, organonitrogen compound metabolism, rRNA metabolic process and transcription, corroborating the functions proposed by the affinity chromatography experiments. Grx2 appears in essence as a multifunctional protein involved in many more biological pathways than its known antioxidant function.

P5

Multi-responsive sodium alginate-based hydrogels for injectable and 3D-printing bioapplications

Sofia – Falia Saravanou[1], Thomai Samouilidou[2], Constantinos Tsitsilianis[1], Stavros Taraviras[2], George Pasparakis[1]

[1]Department of Chemical Engineering, University of Patras, Greece
[2]Department of Physiology, School of Medicine, University of Patras, Greece

We present self-healing, pH/thermo-responsive injectable and 3D printable semi- interpenetrated networks based on sodium alginate formulations, which behave as soft gels at room temperature and as strong ones at physiological body temperature. We combine two different gelation mechanisms: 1. the alginate was conjugated with amino-functional boronic acid (NaALG-g-BA) where the gelation mechanism was developed by the boronic ester structure along the alginate-backbone and 2. the graft copolymer of alginate bearing thermosensitive side chains of Poly(N-isopropylacrylamide-co-N-tert-butylacrylamide) P(NIPAM-co-NtBAM), [NaALG-g- P(NIPAM-co-NtBAM)] where the gel is formed via hydrophobic associations upon heating. Surprisingly, our alginate-based networks were found to constitute excellent cell-spheroid formation matrices by simply mixing cells with gels. Notably, cell- aggregations with average diameter up to 65 μm are formed in less than 24h. The viscous gels retain the cells inside their volume favouring the cell-cell adhesion process. The reversible thermo-responsiveness and shear-thinning properties of the gels render them promising candidates for cell-spheroids growth through injection/3D printing strategies which could find potential applications in tissue regeneration.

P6

Novel interactions of two NuRD complex components, CHD4 and HDAC2, with the mitotic spindle apparatus

Evgenios Eftalitsidis[1], Lito Karkaletsou[1], Christos Efstathiou[1], Stylianos Didaskalou[1], Maria Koffa[1]*

[1]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece

The Nucleosome Remodeling and Deacetylase (NuRD) complex is an ATP-dependent chromatin remodeling complex, involved in many cellular processes, such as transcription, chromatin assembly, cell cycle progression and genomic stability. The NuRD complex comprises different protein subunits and combinatorial assembly of these subunits determines the function of specific NuRD sub-complexes. Chromodomain Helicase DNA binding protein 4 (CHD4) is a core component of NuRD and plays an important role in epigenetic transcriptional repression. CHD4 interacts with other NuRD components, including Histone Deacetylase 2 (HDAC2), an enzyme responsible for the removal of acetyl groups from lysine residues of the core histones, facilitating the formation of transcription repressor complexes.

The complexity and heterogeneity of NuRD complexes are highlighted by the fact that some of its core components maintain additional roles in the cell and participate in other complexes, such as those involved in mitotic spindle assembly. Sakai et al (2002) showed that NuRD components co-localize with the mitotic kinase Aurora-A at centrosomes during early mitosis. We have shown (Yokoyama et al, 2013) that CHD4 acts as a RanGTP-regulated Microtubule-Associated Protein (MAP) during mitosis, and is required for proper spindle assembly.

In this study, we further demonstrate that both CHD4 and HDAC2 localize on the mitotic spindle, towards the centrosomes. CHD4 directly interacts with HURP, a Ran-GTP regulated MAP that is crucial for proper spindle formation. Changes observed in mitotic spindle formation after depleting CHD4 by RNA interference may be attributed to perturbations in NuRD sub-complexes during interphase. To address this, we employed an alternative strategy to rapidly remove CHD4, specifically during mitosis. By applying the knocksideways technique (Robinson et al, 2013) in HeLa Kyoto cells, we efficiently sequestered CHD4 in mitochondria, at specific times during mitosis, thus enabling temporal resolution of its role during the spindle assembly process.

- *Sakai et al. MBD3 and HDAC1, Two Components of the NuRD Complex, Are Localized at Aurora-A-positive Centrosomes in M Phase. J Biological Chemistry, 2002.*
- *Yokoyama H, Nakos K, Santarella-Mellwig R, Rybina S, Krijgsveld J, Koffa MD, Mattaj IW. CHD4 is a RanGTP-dependent MAP that stabilizes microtubules and regulates bipolar spindle formation. Current Biology 2013*
- *Robinson MS, Hirst J. Rapid inactivation of proteins by knocksideways. Current Protocol Cell Biology 2013.*

P7

McIdas localizes at centrioles to control centriole numbers in cycling and multiciliated cells

Marina Arbi[1]*, Vasiliki Bakali[1†], Lydia Koufoudaki[1†], Margarita Skamnelou[1], Spyridoula Bournaka[1], Sihem Zitouni[2], Aikaterini Tsika[3], Georgios Spyroulias[3], Monica Bettencourt-Dias[2], Stavros Taraviras[4], Zoi Lygerou[1]*

[1]Department of General Biology, School of Medicine, University of Patras, Greece
[2]Instituto Gulbenkian de Ciência, Oeiras, Portugal
[3]Department of Pharmacy, University of Patras, Greece
[4]Department of Physiology, School of Medicine, University of Patras, Greece
[†] These authors contributed equally to this work

The chromosome and centrosome cycles in proliferating cells must be coordinated to ensure that genome and centriole duplication occurs only once per cell cycle. Aberrations in centriole numbers lead to genomic instability and cancer[1], however, such aberrations can be part of the normal life-cycle of specific cell types. Multiciliated cells (MCC) best exemplify the deviation from a normal centriole cycle. During MCC formation, centrioles are massively amplified, bypassing the rule for once-per-cell cycle centriole duplication, and are then docked to the apical membrane where they generate multiple motile cilia. The mechanisms controlling cell choice between duplicating their centrioles once or hundreds of times remain poorly characterized. Recent studies highlighted Geminin and the evolutionarily-related proteins McIdas and GemC1, as important regulators of this cell fate decision. They play key roles in the cell cycle[2-3] and centriole amplification in MCC[4-8]. Here, we characterized McIdas as a protein important for maintaining correct centriole numbers in cycling and multiciliated cells.

Bioinformatic analysis predicted two possible Nuclear Export Signal (NES) sequences9 of the McIdas protein and three mutant constructs were created. Immunofluorescence combined with mutant analysis was performed to assess McIdas mode of function. The above data identified the functional NES sequence, corresponding to the mutant showing a statistically significant decrease in the cytoplasmic signal of McIdas. Our data also suggest that McIdas is important for centriole number control, as its overexpression induces centriole overduplication and consistently, McIdas depletion reduces centriole amplification.

1. *Gönczy P, Nat Rev Cancer, (2015) 15(11):639-52.*
2. *Pefani DE et al, JBC, (2011) 286(26):23234-46.*
3. *Balestrini A et al, NCB, (2010) 12(5):484-91.*
4. *Stubbs JL et al, NCB, (2012) 14(2):140-7.*
5. *Kyrousi C et al, Development, (2015) 142(21):3661-74.*
6. *Zhou F et al, Curr Biol, (2015) 25(24):3267-73.*
7. *Arbi M et al, EMBOR, (2016) 17(3): 400-13.*
8. *Terré B et al, EMBOJ, (2016) 35(9):942-60.*
9. *Ossareh-Nazari B et al,Traffic, (2001) 2(10): 684–689*

This project has received funding from the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT), under grant agreement No 1302.

P8

Unconventional protein secretion: cytoplasmic galectin-1 hijacks the exocytic organelles of endothelial cells

Panagiotis Lentzaris[1,2,#], Evangeli Goula[1,2,#], Panagiotis Botsios[1,2,#], Vasiliki Lazani[1,2], Styliani Tsiagka[1,2], Alexandra Papafotika[1,2], Michalis Aivaliotis[3,4], Savvas Christoforidis[1,2,*]

[1] Biomedical Research Institute, Foundation for Research and Technology-Hellas, Ioannina, Greece
[2] Laboratory of Biological Chemistry, Department of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece
[3] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Greece
[4] Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.
[#] These authors contributed equally
**Corresponding author: Savvas Christoforidis, email: savvas_christoforidis@bri.forth.gr, schristo@uoi.gr*

Galectins constitute a family of carbohydrate-binding proteins1. They serve a variety of purposes, including cell to cell or matrix interactions, intracellular signaling, proliferation, differentiation, metastasis and cancer. Although they are cytosolic proteins, they are also found extracellularly. However, as they lack a typical secretory signal peptide, their secretion is considered unconventional, that does not involve the ER-to-Golgi secretory route2.

Here, unexpectedly, using proteomics analysis, we identified galectin-1 in the secretome profile of activated endothelial cells (HUVECs). Since in endothelial cells most secreted proteins are stored inside the Weibel-Palade bodies (WPBs) and are secreted from them upon stimulation3, we investigated whether galectin-1 is present in WPBs. Interestingly, using confocal and STED microscopy we found that galectin-1 is localized in a sub-population of cells, in a sub-group of WPBs. Given that galectin-1 is a cytoplasmic protein, the WPB-localized pool of this protein could be either present at the membrane facing the cytosolic side of WPBs, or in their lumen. To address the exact topology of galectin-1 in WPBs, we altered the pH of the vesicles, which increases their diameter, thereby allowing us to resolve between the lumen and the membrane of the WPB. Intriguingly, we found that galectin-1 is stored within the lumen of WPBs, suggesting that it constitutes a cargo molecule of these vesicles. Furthermore, we found that high cell confluence augmented the number of galectin-1 positive WPBs. As increased cell confluence leads to WPB homotypic fusion and polymerization of its cargo vWF, which could cause WPB membrane damage, it is possible that transient membrane leakage might be the cause of entry of galectins into WPBs.

The above data reveal galectin-1 as a novel WPB cargo molecule and provide a potential new method for unconventional secretion of cytoplasmic proteins, by hijacking the secretory pathway, using the exocytic organelles as vehicles.

References

1. *Barondes, S. H., Castronovo, V., Cooper, D. N., Cummings, R. D., Drickamer, K., Feizi, T., Gitt, M. A., Hirabayashi, J., Hughes, C., & Kasai, K. (1994). Galectins: a family of animal beta-galactoside-binding lectins. Cell, 76(4), 597–598.*
2. *Popa, S. J., Stewart, S. E., & Moreau, K. (2018). Unconventional secretion of annexins and galectins. Seminars in cell & developmental biology, 83, 42–50.*
3. *Rondaij, M. G., Bierings, R., Kragt, A., van Mourik, J. A., & Voorberg, J. (2006). Dynamics and plasticity of Weibel-Palade bodies in endothelial cells. Arteriosclerosis, thrombosis, and vascular biology, 26(5), 1002–1007.*

Acknowledgments

This work was supported in part by the Call EDBM34, Ministry of Finance, Operational Programme "Development of Human Resources, Education and Lifelong Learning", (NSRF 2014-2020); the project "BioImaging-GR: The Greek Research Infrastructure for the Imaging and Monitoring of Fundamental Biological Processes" (MIS 5002755), under the "Action for the Strategic Development on the Research and Technological Sector", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund); and the "Flagship actions in interdisciplinary scientific areas with a special interest in connecting to the productive industry" in the context of the National Recovery and Resilience Plan, Greece 2.0, Component 4.5 "Promote Research and Innovation".

P9

HURP protein promotes breast cancer cell proliferation and contributes to increased CIN

Christos Efstathiou[1], Lito Karkaletsou[1], Stylianos Didaskalou[1], Maria Koffa[1]*

[1]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece

HURP (Hepatoma Up Regulated Protein) is a Microtubule Associated Protein that regulates proper spindle formation. HURP is overexpressed in many types of cancers, such as hepatocellular carcinoma, lung adenocarcinoma, colorectal, ovarian and breast cancer, indicating that it could promote carcinogenesis through certain molecular mechanisms. During metaphase, HURP is localized predominantly on kinetochore microtubules with higher concentration in the vicinity of chromosomes. HURP bundles and stabilizes k-fibers in order to ensure faithful chromosome congression and segregation. Alterations during mitosis may lead to increased Chromosomal Instability (CIN). One of the main consequences of CIN is aneuploidy, where daughter cells end up having an abnormal number of chromosomes after the completion of cell division. Several alterations during mitosis may lead to aneuploidy such as, multipolar spindles, inefficient chromosome congression and bi-orientation, as well as, defective mitotic checkpoint. Here we examine whether HURP protein increases CIN in breast carcinogenesis, through causing several spindle defects.

Three well-characterized breast cell lines that reflect different stages of carcinogenesis were employed to address the possible significance of HURP expression: normal immortalized human epithelial cell line MCF10A; non-metastatic ER and PR positive tumor cell line T47D; and triple negative (-ER/PR/HER2) metastatic tumor cell line MDA-MB231. HURP total protein levels and spindle-bound levels were found elevated in the cancer cell lines T47D and MDA-MB231 compared to MCF10A. In addition, HURP expression is correlated with several mitotic abnormalities during metaphase. HURP silencing suppresses cell proliferation and induces cell death in synergy with Taxol or Mitomycin-C treatment. Interestingly, while no significant differences were observed in cell migration upon HURP silencing in MDA-MB 231 cells, the generated Taxol resistance cell line MDA-MB231-R exhibited decreased HURP levels and decreased migration, compared to the parental cell line.

A mechanistic understanding of the spindle assembly factor HURP and its effect on CIN could provide a better biomarker involved in prognosis and response to treatment in cancer and potentially lead to new drug targets.

P10

Tracking algorithm reveals epithelial cell mechano-chemical properties during gastrulation in Drosophila embryo

Efsevia Neonaki[1], George Pavlidakis[1], Vangelis Kostalas[2], Amsha Proag[3], Katerina Vakaloglou[1], Christos Zervas[1], Demosthenes Mitrossilis[1].

Affiliation
[1]BRFAA,
[2]Omilia Natural Language Solutions Ltd,
[3]Scalian Digital Systems.

Tissues are composed of cells that are connected to one another. In particular, cadherin-mediated adhesions couple the contractile acto-myosin cytoskeleton of cells together to form the mechanical architecture of tissues. Local changes of cell shape and their mechanical properties can drive significant tissue shape change and functionality. For instance, epithelial folding during animal development determines cell fate and tissue topology, leading to the generation of diverse organs. Gastrulation is the first major morphogenetic process during embryogenesis, which includes cell shape changes and movements, leading to the formation of three germ layers, mesoderm, endoderm and ectoderm in a snail & twist dependent manner. The role of biochemical information in the production of mechanical forces and the formation of new shapes is quite well understood. Conversely, it is less known how mechanical cues developed by the cells and by the morphogenetic movements influence gastrulation. To address the above, following that apical cell deformation generates a mechanical force that activates gastrulation in Drosophila (Mitrossilis et al. Nat Commun, 2017), we perform confocal live imaging microscopy and we develop an image analysis software to perform robust tracking of individual cells and quantitative analysis. Our image analysis software implements image preprocessing, segmentation of epithelial cells and spatiotemporal tracking of cells. Finally, by extracting the necessary cellular information we aim to determine how the spatio-temporal conditions regulate the myosin apical stabilization and the cadherin adhesion during gastrulation. Collectively, our cutting-edge tracking algorithm will unambiguously provide to the biomedical community a useful tool to map cell fate in development and cancer.

Acknowledgements
This work was funded by Hellenic Foundation Research & Innovation (#01281).

P11

GCN5 plays multiple regulatory roles in Arabidopsis thaliana root development

Christos Tersenidis[1], Emmanuel Panteris[1]*, Konstantinos Vlachonasios[1,2].

[1]Department of Botany, School of Biology, Faculty of Science, Aristotle University of Thessaloniki, Greece
[2]Natural Products Research Centre of Excellence (NatPro-AUTh), Center of Interdisciplinary Research and Innovation of Aristotle University of Thessaloniki (CIRI-AUTh), Thessaloniki, Greece

Mitotic activity in the apical meristem provides new cells and establishes cell patterning in developing Arabidopsis thaliana roots. Although root development is defective in the gcn5-1 mutant, the involvement of GCN5 histone acetyltransferase in meristematic cell division and root anatomy remains obscure. Accordingly, cell cycle progression and patterning were studied in gcn5-1 roots compared to wild-type Ws-2. Our data suggest that an overall lower mitotic index characterizes gcn5-1 root meristem, while individual mitotic phase frequency is not altered. It appears, therefore, that GCN5 may promote the initiation of mitosis but not participate in its progression. Cell patterning was also aberrant, particularly in the root cap, probably stemming from alterations in the quiescent center and initials organization. Ultrastructural observations revealed incomplete cell plate formation in specific cytokinetic cells, resulting in gaps in the nascent cell walls of post-cytokinetic cells. The results support that GCN5 plays multiple regulatory roles in Arabidopsis thaliana root development.

P12

Chondrocyte differentiation potential of dog adipose-derived mesenchymal stem cells in artificial matrix-based bioscaffolds

Dimitra Bainantzou[1], Kyriazopoulou Maria[1]#, Natsiou Aggeliki[1]#, Zoi Piperigkou[1,2], Rigini Papi[3], Theodora Choli-Papadopoulou[3], Nikos K. Karamanos[1,2], Achilleas D. Theocharis[1]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Foundation for Research and Technology-Hellas (FORTH)/Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece
[3]Laboratory of Biochemistry, School of Chemistry, Aristotle University of Thessaloniki, Greece
 # Equal contribution

Articular cartilage is a type of connective tissue found on the articular surfaces of the bones providing resistance to mechanical forces and frictionless movement and consists of chondrocytes embedded in an extracellular matrix (ECM). Due to the lack of blood vessels and nerves, cartilage has a limited ability to regenerate in degenerative diseases such as osteoarthritis (OA). In recent years, tissue engineering has created new hope in the treatment of osteoarthritis by combining mesenchymal stem cells (MSCs), artificial 3D scaffolds that can mimic the ECM and biological agents to regenerate damaged cartilage by promoting chondrogenesis. In the present study, we examined the differentiation of canine adipose-derived mesenchymal stem cells (ASCs) into chondrocytes in biomimetic scaffolds both in the presence and in the absence of chondrogenic culture medium. Specifically, the synthesized artificial bioscaffolds are an elastin-silk-mussel-like-polypeptide (ELP) combined either with transforming growth factor β1 (TGFβ1) peptide or with arginine-glycine-aspartic acid (RGD) motifs. The above scaffolds were also combined with collagen type II. The effect of these scaffolds on gene and protein expression of major ECM molecules, biomarkers and transcription factors implicated in chondrogenic differentiation and cartilage hypertrophy, and the activation of signaling pathways implicated in the chondrogenic differentiation was studied. ASCs were cultured on synthesized crosslinked bioscaffolds under normal or chondrogenic differentiation conditions for 24 days. Collectively, the ELP bioscaffold combined with RGD as well as its combination with collagen type II exhibited a chondrogenic potential, especially in the presence of chondrogenic culture medium, suggesting a promising role for cartilage regeneration in OA.

Reference

Piperigkou Z, Bainantzou D, Makri N, Papachristou E, Mantsou A, Choli-Papadopoulou T, Theocharis AD, Karamanos NK (2023) Enhancement of mesenchymal stem cells' chondrogenic potential by type II collagen-based bioscaffolds, Mol Biol Rep. 50(6):5125-5135. doi: 10.1007/s11033-023-08461-x.

P13

McIdas is fundamental for the generation of ependymal cells

Georgia Lokka[1], Maria Bakogianni[1], Konstantina Kaplani[1], Ioanna Papadionysiou[1], Maria-Eleni Lalioti[1], Zoi Lygerou[2] , Stavros Taraviras[1]

[1]Laboratory of Physiology, Medical School, University of Patras, Greece
[2]Laboratory of General Biology, Medical School, University of Patras, Greece

Ependymal cells constitute a very important cell population for the appropriate function of the brain. They line the walls of the ventricles and most of them bear multiple motile cilia on their apical surface that beat coordinately, contributing to cerebrospinal fluid flow. Defects in their differentiation, has been related to hydrocephalus, a severe pathological condition. A subpopulation of ependymal cells has also been identified, bearing two cilia and distinguishable basal bodies.They are called E2 ependymal cells and are found in higher numbers in the third ventricle , the aqueduct and the fourth ventricle.

Previous findings of our laboratory, have demonstrated McIdas and GemC1 as key regulators of multiciliogenesis in the mouse brain. Overexpression and knockdown experiments have shown that these two factors, are crucial for the commitment and the differentiation of multiciliated ependymal cells. Our data show that McIdas deletion in mice (McIdas KO/KO), causes growth retardation and enlargement of the brain ventricles, due to hydrocephalus development. In McIdas KO mice, the expression of p73 and Foxj1, two factors that are important for multiciliated ependymal cell differentiation, is retained, indicating that radial glial cells are committed towards the ependymal lineage. However, immunofluorescence experiments in coronal brain sections and SVZ whole mounts, revealed that upon McIdas deletion, multiciliogenesis is blocked at the early stages during ependymal cell differentiation as the progenitor cells are unable to generate multiple basal bodies and multiple cilia. Interestingly, the number of cells with characteristics of E2 ependymal cells increases in the SVZ, while preliminary data support that there is increase in the aqueduct as well.

To conclude, our data suggest that McIdas is not necessary for the commitment towards the ependymal lineage, but plays a key role in multicilliogenesis and may regulate the balance between ependymal cell populations.

P14

Deciphering the role of OFD1, a primary's cilium protein, in cortical development and malformations using mouse models

Athanasia Rapti[1,2], Panagiotis Politis[3], Stavros Taraviras[4], Silvia Cappello[5], Christina Kyrousi[1,2]

[1] 1st Department of Psychiatry, Medical School, National and Kapodistrian University of Athens, Greece.
[2] UMHRI University Mental Health, Neurosciences and Precision Medicine Research Institute "Costas Stefanis", Athens, Greece.
[3] Biomedical Research Foundation of the Academy of Athens, Greece.
[4] Department of Physiology, Medical School, University of Patras, Greece.
[5] Department of Developmental Neurobiology, Max Planck Institute of Psychiatry, Munich, Germany.

The cerebral cortex development is a process that involves the coordinated sequence of neural progenitor proliferation, neuronal differentiation and migration. Possible dysregulation of such processes results in malformations of cortical development (MCDs) such as periventricular heterotopias and polymicrogyria which are characterized by morphological and functional cerebral complications. MCDs can be caused by gene mutations, many of which with unknown functions. Recent evidence suggests the involvement of some MCDs' causative genes with the organization and function of the primary cilium (PC). Aiming to shed light on this direction we selected OFD1, a centriole and centriolar satellite protein coding gene, reported mutated in a patient with extensive polymicrogyria and heterotopia. By comparing single-cell RNA sequencing datasets, we observed that species-specific differences in its expression are suggestive of its plausible important role in human corticogenesis and malformations. Next, we sought to investigate OFD1's role in cortical development by manipulating its expression in vivo. Our data suggest that ectopic OFD1 manipulation leads to apical and basal progenitors' numbers and distribution changes in the developing cortex. To ascertain if PC plays role in the prementioned differences, we studied PC's length using appropriate cilia markers and we observed that PC's length seems disrupted upon OFD1 manipulation. Knowing that MCDs cannot be fully recapitulated in animal models, our current and future strategy is to unmask human-specific mechanisms of cortical development using human brain organoid models.

P15

The role of long non-coding RNAs in mammalian brain development

Dimitrios Gkikas, Elpinickie Ninou, Nikos Malissovas, Maximilianos Elkouris, Daphne Antoniou, Artemis Michail, Valeria Kaltezioti, Panagiotis Politis

Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, 4 Soranou Efesiou, 115 27, Athens, Greece

With the advent of new sequencing technologies, a growing list of formerly unknown regulatory RNA species has come into the spotlight. Among them, long non-coding RNAs (lncRNAs) have been found to control stem cell pluripotency, carcinogenesis, and the development and function of several tissues and organs. Although thousands of lncRNAs are expressed in the adult mammalian brain in a highly patterned and specific manner, they remain poorly characterized, and their roles in brain development have not yet been studied. To this end, we performed RNA-Seq analysis in the developing nervous system of the mouse embryo. Based on this analysis, we identified many lncRNAs highly expressed in neural cells. We focused our efforts on lncRNAs, which are transcribed from genomic loci in close proximity to protein coding-genes, encoding for transcription factors (TFs) with critical roles in brain development. We hypothesized that these lncRNAs may be implicated in the regulation of neighbouring TF genes. Thus, we characterized the changes in the expression profile of the most interesting of the identified lncRNA-TF pairs during the development of the mouse brain (telencephalon). In this study, we further investigated the functional role of three lncRNAs, TCONS_00034309, LockD and AK142161, in the differentiation of neural stem cells by in vitro and in vivo overexpression and knock-down studies. Our data suggest critical roles for these lncRNAs in neuronal differentiation and astrogliogenesis during brain development. Collectively, our study provides insights into the involvement of lncRNAs in brain organogenesis and shows how lncRNAs and protein-coding genes form regulatory networks with important functions in neural cells.

This work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Num- ber: 1782) and Fondation Santé Biomedical Research grants.

P16

Exploring GemC1's Impact on Murine Hippocampal Development

Anna Bimpli[1], Nikoletta Triantopoulou[1], Konstantina Kaplani[1], Maria-Eleni Lalioti[1], Georgia Lokka[1], Zoi Lygerou[2], Stavros Taraviras[1]

[1]Department of Physiology, Medical School, University of Patras, Greece
[2]Department of General Biology, Medical School, University of Patras, Greece

The murine hippocampus is a complex brain structure located within the inner part of the mammalian temporal lobe. It is comprised of two main regions: the Cornu Ammonis (CA) and the Dentate gyrus (DG). Within the Dentate gyrus, a narrow layer of cells called the subgranular zone (SGZ) functions as a site for neurogenesis. Proper hippocampal maturation is of utmost importance, as structural irregularities within the hippocampus have been linked to numerous neurogenetic diseases.

Our research is committed to exploring the function of GemC1, a member of the Geminin superfamily, in the development of the hippocampus. For this purpose, we have generated GemC1 knockout mouse models within our laboratory. GemC1 plays a crucial role in the process of multiciliogenesis in mice. More specifically, GemC1 regulates the transcriptional activation of p73, an essential factor for the maturation of radial glial cells into multiciliated cells in the lateral ventricles. Concurrently, experiments on mouse models have shown that p73 is essential for normal hippocampal development and its depletion leads to severe hippocampal dysgenesis. Our study, as of yet, both in embryonic and early postnatal stages suggests that GemC1 controls the expression of p73 during the development of the hippocampus. Preliminary data also show that GemC1 deficiency leads to changes in various cell populations of the hippocampus, including astrocytes and neurons, highlighting its importance on normal hippocampal development.

We hope that these findings will assist in revealing the molecular mechanisms behind hippocampal formation and support the development of optimal models for researching hippocampus-related diseases and disorders.

P17

Unravelling the role of Transgelin in Drosophila intestine

Irene Kagianni[1,2], Katerina M. Vakaloglou[1], Athena Keramidioti[1,3], Danai Fida[1], Ismini Kloukina[1], Christos G. Zervas[1], *

[1]Biomedical Research Foundation, Academy of Athens, Center of Basic Research, Athens, Greece
[2]University of Ioannina, Department of Biological Applications & Technology, Ioannina, Greece
[3]University of Thessaly, Department of Biochemistry & Biotechnology, Larissa, Greece
**:czervas@bioacademy.gr*

The intestine of Drosophila melanogaster is a single layer of epithelial cells where their apical surface emerges into the lumen and shapes finger-like membrane protrusions known as microvilli. These apical protrusions are densely packed and mechanically supported by an actin meshwork created right beneath the membrane surface in all enterocytes, constituting the brush border. The morphology and function of microvilli highly depends on the tight regulation of this actin meshwork, however the underlying mechanisms are largely unknown. Shedding a light to the functions of actin regulators in the gut epithelium will provide us with a better understanding on how this machinery works.

Transgelins form a well conserved family of actin-binding proteins involved in cytoskeletal remodeling, cell contractility and cell shape. We have recently characterized the spatiotemporal differential expression pattern of the three Transgelin proteins in Drosophila development (Vakaloglou et al., 2021). In this study, we have focused on the Transgelin Chd64 protein, which is highly expressed in the gut epithelium. To identify the functional role of Chd64 protein, we engineered CRISPR-indel null mutants for Chd64. We identified that Chd64 is required for the maintenance of the gut epithelia architecture. Our on-going work aims to reveal a mechanistic insight of how Chd64 function in the gut and thus provide a genetic model for intestinal brush border pathologies.

Reference

- *Vakaloglou, K.M., Mouratidou, M., Keramidioti, A., Zervas, C.G. (2021). Differential Expression of Drosophila Transgelins Throughout Development. Front. Cell Dev. Biol.9():648568.*

P18

Dynamics of antibody response to S antigen in individuals infected with and/or vaccinated against SARS-CoV-2

Dimitra Georgakopoulou, Maria Rodi, and Athanasia Mouzaki

Laboratory of Molecular Diagnosis of Infectious Agents, University of Patras, Patras, Greece

COVID-19 disease caused by the SARS-CoV-2 virus has spread rapidly throughout the world, creating a pandemic. An important element in the containment of this pandemic is the achievement of herd immunity, which is mainly achieved by vaccinating the population with durable effective vaccines. In Greece, the mRNA vaccines BNT162b2 (Pfizer) and mRNA-1273 (Moderna) and the viral vector vaccines AZD1222 (AstraZeneca) and Ad26.COV2.S. (Janssen-Johnson and Johnson) have been used to date. However, despite achieving some herd immunity, SARS-CoV-2 infections still occur after vaccination. In parallel with the emergence of new SARS-CoV-2 variants in the population, the need arose to investigate the efficacy of vaccination.

In the present study, 1499 serum samples from individuals infected with and/or vaccinated against SARS-CoV-2 were analyzed. Neutralizing anti-S IgG antibodies were detected in these samples and quantified by ELISA. In addition, to determine SARS-CoV-2 variants, nasopharyngeal samples were genotyped by sequencing and multiple RT-PCR. In this way, the sequence of variants in the population during the time that the serum samples were collected and analyzed becomes clear.

According to the results of the present study, individuals who were vaccinated with one or two doses of a vaccine have statistically significantly higher antibody titers than individuals who were not vaccinated but were infected with the virus. In addition, antibody titers were found to decrease with time after vaccination. A negative correlation was also found between the age of the individuals and the antibody titer. In addition, differences were found between the different vaccine types. However, after the third dose and subsequent two booster vaccinations with BNT162b2 vaccine, vaccine efficacy appears to increase maximally and reach a plateau that has remained stable for more than one year. Nevertheless, SARS-CoV-2 infections occur even in fully vaccinated individuals, albeit less frequently, suggesting that herd immunity has not yet been fully achieved and more effective vaccines are needed.

P19

Versican expression in lung fibroblasts controls their invasive properties and regulates pulmonary fibrosis

Paraskevi Kanellopoulou, Ilianna Barbayianni, Dionysios Fanidis, Vassilis Aidinis

Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center Alexander Fleming, Athens, Greece

Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease, with dismal prognosis and lack of effective treatment. The pathogenesis of IPF is characterized by the activation and accumulation of lung fibroblasts and excessive deposition of ECM components, leading in the distortion of lung architecture and the impairment of respiratory functions. ECM is a dynamic structure which regulating cell behaviour and various (patho)physiological processes. The lung ECM, that plays a multifaceted role in the homeostasis of the lung, is rich in proteoglycans that are characterized by an assortment of glycosaminoglycan side chains, that influence tissue mechanics, as well as cellular properties of both stromal and immune cells. Increased Versican (Vcan) expression, the most abundant proteoglycan in the lung ECM, has been detected in animal models of lung cancer [1], while pulmonary fibrosis is a major risk factor for cancer development. Therefore, in this report, we investigated a possible role for Vcan in pulmonary fibrosis. Increased VCAN expression was detected in silico in IPF patients, and validated in animal models, predominantly expressed from macrophages and lung fibroblasts. Accordingly, TGF-β1, the major pro-fibrotic factor, was found to induced VCAN expression in human and mouse primary lung fibroblasts and cell lines. Haploinsufficient Vcan+/- mice were found to develop exacerbated bleomycin (BLM)-induced pulmonary fibrosis, suggesting a protective role of Vcan in pulmonary fibrosis. Bone marrow transfer experiments and chimeric knock out mice indicated a distinct contribution of Vcan in stromal and immune cells. Disease exacerbation in Vcan+/- mice correlated with increased lung stiffness, as determined with atomic force microscopy, that led to the formation of podosomes in lung fibroblasts, recently shown to be an inherent property of fibrotic lung fibroblasts, stimulating ECM invasion [2].

Reference List

1. *Magkrioti, C., et al., The Autotaxin-Lysophosphatidic Acid Axis Promotes Lung Carcinogenesis. Cancer Res, 2018. 78(13): p. 3634-3644.*
2. *Barbayianni, I., et al., SRC and TKS5 mediated podosome formation in fibroblasts promotes extracellular matrix invasion and pulmonary fibrosis. Nat Commun, 2023. 14(1): p. 5882.*

P20

Investigating the role of SFRP2 in the pathogenesis of Idiopathic Pulmonary Fibrosis

Maria Shira[1], Dionysios Fanidis[1], Paraskevi Kanellopoulou[1], Vassilis Aidinis[1]

1.Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center Alexander Fleming, Athens, Greece.

Idiopathic pulmonary fibrosis (IPF) is a disease of the connective lung tissue. It is characterized by excessive extracellular matrix (ECM) deposition and tissue scarring leading to permanent tissue injury, organ malfunction, disruption of gas exchange, and death from respiratory failure. Tissue injury leads to proliferation, activation, and differentiation of fibroblasts via several signaling pathways, of which the Wnt signaling pathway is of utmost importance. Wnt activity can be modified by a variety of modifiers such as Secreted Frizzled-Related Protein 2 (SFRP2). SFRP2 is a protein that can bind to Wnt ligands and modulate Wnt pathway activity, ultimately controlling cell proliferation and differentiation. It has been linked to various diseases such as colorectal and breast cancer, melanoma, cardiac and skin fibrosis, psoriasis and osteoarthritis. In this study, machine learning inquiries revealed that SFRP2 was the most deregulated gene in IPF, whereas single-cell analysis from the Human Lung Cell Atlas (HLCA) revealed exclusive expression of SFRP2 in adventitial and peribronchial IPF fibroblasts. In vitro, TGF-β1, a major profibrotic factor, and lysophosphatidic acid (LPA), which is also implicated in pulmonary fibrosis, dramatically decreased the expression of Sfrp2 in mouse lung fibroblasts (LFs). Moreover, the expression of Sfrp2 was also downregulated in LFs isolated from mice after administration of bleomycin (BLM), a well-known mouse model resembling human IPF disease in vivo. In conclusion, the decrease of Sfrp2 expression under fibrotic conditions indicates its key role in fibrosis, most probably through processes such as fibroblast proliferation and differentiation into myofibroblasts, where the Wnt pathway is implicated. Ongoing experiments and generation of $Sfrp2^{-/-}$ KO mice will demonstrate the potential of this gene as a therapeutic target in IPF.

P21

Versican V1 upregulation in murine hepatic fibrosis

Stefanos Smyrniotis[1], Christiana Magkrioti[1], Vassilis Aidinis[1]

1Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center "Alexander Fleming", 16671, Athens, Greece

Chronic liver disease (CLD) constitutes a global health care burden, since it can lead to liver fibrosis, cirrhosis and cancer, causing approximately 2 million deaths annually. During the procedure of fibrosis, there is an intense modification in several pathophysiological mechanisms contributing to the main fibrosis trait, which is the excessive accumulation of extracellular matrix (ECM) components (e.g. fibronectin and collagen). Versican (VCAN) is a large chondroitin sulfate proteoglycan that is also an ECM component. It consists of different isoforms (V0, V1, V2, V3 & V4), depending on the presence or absence of two glycosaminoglycan (GAG) attachment regions. VCAN interacts with hyaluronan and other ECM components via specific domains in its core protein and plays a role in cellular processes, such as migration, adhesion, invasion, proliferation, apoptosis and tissue hydration. Previously in the lab we have found that VCAN plays a role in pulmonary fibrosis, therefore we sought to investigate its expression in liver fibrosis using the murine model of carbon tetrachloride (CCL4). First, we administered CCL4 in C57Bl6 mice for different time points (4, 8 & 12 weeks). Then, we analyzed the induction of liver fibrosis with the help of histochemistry (collagen measurements – Sirius-red staining) and RT-qPCR in order to determine the collagen type I, III and Versican V1 levels. Our results showed that VCAN transcription is upregulated upon liver fibrosis. Finally, we isolated primary murine hepatocytes and hepatic stellate cells (HSCs) and showed that, out of the two populations, VCAN mRNA levels are higher in HSCs, which are fibroblasts responsible for the initiation and perpetuation of liver fibrosis. These results demonstrate that VCAN could be used as a liver fibrosis biomarker that should be further investigated in the liver context.

P22

A new role for Fbw7 in pancreatic β-cell maintenance and regulation of glucose homeostasis

Adriana Papadimitropoulou[1], Chrysanthi Charalampous[1], Paraskevi Kogionou[1,2], Dimitrios Troumpoukis[1], Ioannis Serafimidis[1]*

[1]Center of Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece.
[2]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece
**email: iseraf@bioacademy.gr*

T1D (Type-1 diabetes) is a chronic condition characterized by insulin deficiency due to the destruction of the insulin-producing pancreatic β-cells, resulting in deregulation of glucose homeostasis and severe hyperglycemia. Clinical studies have shown that diabetic symptoms can be permanently alleviated by restoring a sufficient mass of functional β cells, and one way this can be achieved is through in-vivo stimulation of β-cell proliferation. Fbw7 is a ubiquitin ligase that targets key regulators of cell division and growth, including Cyclin E, c-Myc, c-Jun and Notch. To investigate the role of Fbw7 in β cell physiology, we inactivated Fbw7 in the entire pancreatic epithelium ($Fbw7^{c/c}$;$Pdx1^{Cre}$ mice). We observed that $Fbw7^{c/c}$;$Pdx1^{Cre}$ mice become increasingly hyperglycemic and progressively more intolerant to glucose compared to their wild-type littermates. We demonstrated that the number and size of islets in these mice progressively decreased, without however affecting the overall size and morphology of the organ. Immunofluorescent stainings for Ki67 indicated no major differences between $Fbw7^{c/c}$;$Pdx1^{Cre}$ and WT in all embryonic stages tested. However, in the early post-natal stages (P1-P15), both Ki67 staining and EdU incorporation measurements indicated that β-cell proliferation rates were severely compromised, thus explaining the subsequent loss of β-cell mass in later adult stages. To investigate whether the observed diabetic phenotype is autonomously attributed to the loss of Fbw7 in the endocrine compartment, we generated mice where Fbw7 is exclusively ablated in endocrine progenitor cells and their progeny, using Ngn3, the key regulator of endocrine cell specification, as a driver ($Fbw7^{c/c}$;$Ngn3^{Cre}$). Preliminary evidence indicate that these mice also become severely hyperglycemic postnatally, in a manner similar to, but even more pronounced than $Fbw7^{c/c}$;$Pdx1^{Cre}$ mice, as they die from diabetic complications at earlier adult stages. Collectively, our data provide intriguing evidence that Fbw7 may regulate β-cell proliferation postnatally, making it a potential target for diabetes therapies.

P23

Establishment of CYLD-deficient human induced pluripotent stem cells for the development and characterization of relevant mammary organoid systems

Georgios Katsipis[‡], Angeliki Daiou[‡], Konstantinos E. Chatzistergos, Georgios Mosialos*

School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[‡] Equally contributing authors
**Author to whom correspondence should be addressed*

CYLD is a deubiquitinating enzyme that regulates poly-ubiquitinoylation of crucial targets for the regulation of cell proliferation, survival and differentiation. CYLD is now recognized as a tumor suppressor protein, as its inactivation or downregulation has been implicated in the development of several types of cancer including breast cancer. Indeed, reduced Cyld expression in breast cancer cell lines has previously linked with enhanced cell proliferation, higher motility, and reduced response to chemotherapy. Furthermore, CYLD inactivation in immortalized mammary epithelial cells can induce epithelial-to mesenchymal transition-a process that is crucial for metastasis. The employment of induced pluripotent stem cells (iPSCs) as an in vitro modelling system comes with several advantages, such as bypassing the need for receiving human tissues and the generation of multiple types of tissues including mammary epithelia. In this study, the CRISP-Cas9 system has been utilized for targeted inactivation of Cyld in human iPSCs. Successful targeting was verified by relative quantitation of mRNA and protein levels. Following that, iPSCs were differentiated into mammary spheroids for 10 days, using a commercial differentiation medium. These spheroids are currently characterized for gene expression, survival, growth and morphological perturbations associated with CYLD inactivation. These experiments will permit the characterization of the role of CYLD in human mammary epithelia oncogenic transformation and serve as a platform for the identification of targeted therapeutic approaches.

This research has been supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: HFRI-FM17-1731).

P24

In vivo microglial Bin1 deletion following LPS stimulation regulates neuroinflammation in the mouse hippocampus and Adult Hippocampal Neurogenesis

Maria Anna Roussaki[1]*, Maria Margariti[1], Irini Thanou[1], Evangelia Xingi[2], Vasiliki Kyrargiri[3], Marcos Costa[4, 5], Dimitra Thomaidou[1]

[1]Hellenic Pasteur Institute, Neural Stem Cells and Neuro-imaging Group, Department of Neurobiology, Athens, Greece,
[2]Hellenic Pasteur Institute, Light Microscopy Unit, Athens, Greece,
[3]Hellenic Pasteur Institute, Laboratory of Molecular Genetics, Microbiology Department, Athens, Greece,
[4]Institut Pasteur de Lille, Univ. Lille, Inserm, CHU Lille, Lille, France,
[5]Federal University of Rio Grande do Norte, Brain Institute, Natal, Brazil

The hippocampal formation and its cortical inputs -crucial areas for memory formation- are affected early in the development of Late Onset Alzheimer's Disease (LOAD). Adult Hippocampal Neurogenesis (AHN), taking place in the hippocampal Dentate Gyrus (DG), declines drastically during early stages of LOAD, via unknown mechanisms, and correlates to the cognitive status of LOAD patients. At the same time, numerous Single Nucleotide Polymorphisms (SNPs) linked to LOAD by Genome-wide Association Studies (GWAS) concern genes which express isoforms in microglia– the innate immune cells that actively remodel AHN. Notably, SNPs near the Bridging Integrator 1 (Bin1) gene, a member of the BAR protein family, have been significantly associated with an elevated risk for LOAD development, surpassed only by Apolipoprotein E. Microglial Bin1 is related to the endolysosomal network, a system which is necessary for microglial modulation of AHN in the hippocampal Subgranular Zone (SGZ). To this end, we generated a double transgenic mouse model ($Cx3CR1^{Cre}$-ERT2//$Bin1^{fl/fl}$) which allows the conditional knockout of Bin1 in microglial cells. To investigate microglial Bin1 deletion in the murine hippocampus in homeostasis and inflammation, we treated both control and microglial Bin1 cKO mice with lipopolysaccharide (LPS), which triggers an inflammatory response. Immunochistochemical experiments indicated that microglial Bin1 deletion leads to elevated proliferative capacity of DG microglia upon LPS stimulation. Simultaneously, exposure to LPS-induced neuroinflammation led to a pronounced upregulation of pro-inflammatory genes, in hippocampi lacking microglial Bin1. Importantly, the conditional knockout of microglial Bin1 mediated a rise in the number of neuroblasts in the hippocampal SGZ, along with alterations in the numbers of Neural Stem Cells (NSCs) and Intermediate Progenitor Cells (IPCs). Taken together, microglial Bin1 is implicated in LPS-induced microglial proliferation in the mouse hippocampus, while its absence accounts for shifts in the populations of cells within the neuronal lineage, under homeostatic conditions.

P25

Suppression of chemically-induced mammary cancer by early-life oral administration of cholera toxin in mice is associated with aberrant regulation of Bmp and Notch signaling pathways

Dimitris G. Argyris[1], Hara Afaloniati[1], Maria Markaki[1], Theofilos Poutahidis[2], Katerina Angelopoulou[1]*

[1]Laboratory of Biochemistry and Toxicology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece,
*[2]Laboratory of Pathology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece, *e-mail: kangelop@vet.auth.gr*

Currently, significant attention has been drawn towards the potential efficacy of cholera toxin (CT) - an exotoxin produced by the small intestine pathogenic bacterium *Vibrio cholera* - in modulating cancer-promoting events. This interest stems from CT's well-documented immunomodulatory and anti-inflammatory properties. In a recent study of our group, we demonstrated that early-life oral administration of non-pathogenic doses of CT in mice suppressed chemically-induced carcinogenesis in tissues distantly located from the gut. The carcinogen used was the 7,12-dimethylbenzanthracene (DMBA), known to cause mainly mammary, but also lung, skin and non-glandular stomach cancers. In the mammary gland, CT pretreatment was shown to reduce tumor multiplicity, increase apoptosis in cancer prone tissues and alter the expression of several cancer-related molecules. Continuing our effort to unravel the molecular mechanisms underlying CT's anti-cancer outcome in the mammary gland, in the present study we delved into the gene expression patterns of key components of the Bmp and Notch signaling pathways, i.e. ligands, receptors, transcriptional regulators and target genes. Although both pathways have been widely implicated in the development of tumors, their roles in carcinogenesis remain a subject of debate. Our results revealed that CT anti-tumor effects significantly correlated with deregulation of crucial BMP pathway elements; downregulation of Bmp7 ligand and BmpR2 receptor as well as upregulation of inhibitory Smad6 were the most prominent alterations observed. Concerning Notch signaling pathway, significantly elevated gene expression levels in the CT-treated DMBA mice, as compared to their non-treated counterparts, were identified in key components, such as Jag2 and Dll4 ligands. Overall, these findings suggest that CT tumor protective effects in the mammary gland are associated with an aberrant regulation of both Bmp and Notch signaling pathways.

P26

Pancreatic Cancer-Associated Depression (PCAD) is linked to adult neurogenesis impairment

Dimitrios Troumpoukis[1], Adriana Papadimitropoulou[1], Effrosyni Koronaiou[2], Paraskevi Kogionou[1,3], Chrysanthi Charalampous[1], Alexia Polissidis[2], Nicolas Nicolaides[2,4], Yassemi Koutmani[2*], Ioannis Serafimidis[1*]

[1]Center of Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[2]Center of Experimental Surgery, Clinical and Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[3]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece
[4]First Department of Pediatrics, National and Kapodistrian University of Athens, Athens, Greece
[*]emails: ykoutmani@bioacademy.gr, iseraf@bioacademy.gr

Pancreatic Cancer (PC) is a very aggressive type of cancer, associated with a high incidence of major depression (PCAD), which manifests well before formal diagnosis. Although the connection between PC and depression is well-recognised, the biological factors underpinning this correlation remain unknown. In this study, we aim to elucidate how PC affects adult neurogenesis, shedding light on the mechanisms leading to depression in PC patients. We initially conducted behavioral tests on a PC mouse model, generated by orthotopically injecting human pancreatic Panc-1 cells in immunocompromised (NOD-SCID) mice. Our results demonstrated that these mice exhibit a depressive-like phenotype when compared to sham-operated controls. High-performance Liquid Chromatography (HPLC) analysis on brain lysates, for analytes previously associated with depression, demonstrated an unprecedented imbalance in the serotonin levels, both at the prefrontal cortex and the hippocampus of PC mice. To investigate the connection between PC and hippocampal neurogenesis, we employed a genetic mouse model ($Pdx1^{Cre}$-$AKras^{G12D}$) that mimics human PC development by constitutively expressing $Kras^{G12D}$ specifically in the pancreas. HPLC analysis of brain lysates from these mice confirmed the imbalance in the serotonin pathway. Moreover, immunofluorescent analysis on brain cryosections showed impaired hippocampal neurogenesis, as demonstrated by the reduced number of DCX^+ and $GFAP^+$/radial glia-like neural stem cells, compared to controls. To assess the involvement of systemic factors on adult hippocampal neural stem cell (NSCs) behavior during progression of PC, NSCs isolated from the dentate gyrus of wild-type mice were cultured in the presence of sera collected from $Pdx1^{Cre}$-$AKras^{G12D}$ or control mice. Exposure of NSCs to $Pdx1^{Cre}$-$AKras^{G12D}$ serum reduced their proliferative capacity and survival as indicated by BrdU incorporation and TUNEL assays respectively. Our findings suggest that PC affects the brain by altering the serotonin pathway and by reducing the neurogenic capacity of hippocampal NSCs, possibly via the secretion of systemic factors, leading to PCAD.

P27

Extracellular vesicles produced by the gut microbiome and their effect on mental disorders

Effrosyni Louka, Vassiliki Lila Koumandou*

Department of Biotechnology, Agricultural University of Athens, Greece

Extracellular vesicles (EVs), produced by prokaryotes, are recognized as having an important role in intra and interspecies communication. EVs are clinically important as their cargo can include toxins associated with bacterial virulence and toxicity; additionally, they have been proposed as efficient vaccine agents. Importantly, bacterial EVs can also affect the nervous system. Modulating the gut microbiome has emerged as a potential way to improve resilience to stress and overall mental health[1–3]. This project deals with the issue of the correlation between the gut microbiome and stressors that can lead to mental disorders, studying bacterial EV secretion and the mechanisms by which EVs are involved in the Gut-Brain Axis (GBA). The mechanistic details behind EV biogenesis, cargo selection and release are still poorly understood. A recent study from our lab identified cargo proteins which are common among EVs from various taxa by comparing published data on EV proteomes from 42 species[4]. The role of these prominent EV cargo proteins in EV biogenesis will be tested via knockouts and over-expression of the corresponding genes. The effect of stress signals (e.g. starvation, heat-shock, antibiotics) on the biogenesis and cargo content of EVs will also be tested. The ultimate goal of the thesis is both the in-depth deciphering of the biochemical pathways that characterize the communication of the nervous and digestive systems, as well as the generation of hypotheses for an alternative approach to the way mental illnesses are handled and, consequently, treated. Stress is hard to avoid in today's modern competitive lifestyle and chronic stress is fast becoming a global social challenge[5]. The contribution of the present study lies in clarifying the gaps that arise in the already existing research with the ultimate vision of contributing to the improvement of the well-being that is so threatened nowadays.

1. *Bibbò, S. et al. Gut microbiota in anxiety and depression: Pathogenesis and therapeutics. Front. Gastroenterol. 1, 1019578 (2022).*
2. *Yang, B., Wei, J., Ju, P. & Chen, J. Effects of regulating intestinal microbiota on anxiety symptoms: A systematic review. Gen. Psychiatry 32, e100056 (2019).*
3. *Noonan, S., Zaveri, M., Macaninch, E. & Martyn, K. Food & mood: a review of supplementary prebiotic and probiotic interventions in the treatment of anxiety and depression in adults. BMJ Nutr. Prev. Health 3, 351–362 (2020).*
4. *Stathatos, I. & Koumandou, V. L. Comparative Analysis of Prokaryotic Extracellular Vesicle Proteins and Their Targeting Signals. Microorganisms 11, 1977 (2023).*
5. *Morales, P., Mencher, A., Tronchoni, J. & Gonzalez, R. Extracellular vesicles in food biotechnology. Microb. Biotechnol. 14, 8–11 (2021).*

P28

The effect of osmotic stress on mean platelet volume and on aggregation of platelets

Despoina Pantazi*[1], Athina Stratou[2], Anastasios Petrou[3], Evangelia Dounousi[4], Alexandros D. Tselepis[1]

[1]Laboratory of Biochemistry/Atherothrombosis Research Centre, Department of Chemistry, University of Ioannina, Ioannina, Greece;
[2]Department of Cardiothoracic Surgery, University Hospital of Ioannina, Ioannina, Greece;
*[3]Anesthesiology Department, University Hospital of Ioannina, Ioannina, Greece, 4Department of Nephrology, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, 45110 Greece, *dpantazi@uoi.gr*

Introduction: Plasma osmolality is the concentration of osmotically active substances. Hyperosmotic or hypoosmotic conditions affect various cellular functions and are associated with several adverse effects. Thus, osmolality and mean platelet volume (MPV) may be significantly associated with perioperative mortality in cardiac surgery patients.

Aim: To investigate the effect of osmotic stress on the mean platelet volume (MPV), the platelet indices MPV, PDW, and P-LCR as well as on platelet aggregation induced by the agonists arachidonic acid (AA, 500 μM), ADP (10 μM) and TRAP-6 (10 μM).

Materials and Methods: We induced experimental variations of serum osmolarity in heparinized whole blood (WB) samples by adding hypertonic and hypotonic solutions. Aggregation of platelets in whole blood was tested with impedance aggregometry (IA) using ADP (10 μM), TRAP-6 (10 μM), and AA (500 μM) as agonists. Osmolality (in mOsm/Kg) and MPV (in fL) were measured on each occasion.

Results: Platelet indices MPV, PDW, and P-LCR did not show statistically significant changes due to osmotic stress. The hypoosmotic environment caused a decrease in ADP-induced accumulation by 50% (p=0.056), while the TRAP-6-induced decrease by 31% (p=0.017) compared to control samples. We did not observe changes in the aggregation of platelets induced by AA.

Conclusions: Hypotonicity reduced ADP- and TRAP-6-induced platelet aggregation in whole blood. The underlying mechanisms as well as the clinical relevance in our ongoing in vivo study are under investigation.

P29

Effect of simvastatin on Hs578T breast cancer cells: functional properties, and expression of stem and EMT markers of selected extracellular proteases

Constantine Papadimatos, Ioanna Christodoulou, Spyridon S. Skandalis, Nikos K. Karamanos and Demitrios H. Vynios

Biochemistry, Biochemical Analysis & Matrix Pathobiochemistry Research Group, Department of Chemistry, University of Patras, 26504 Patras, Greece

Οι εξωκυτταρικές πρωτεάσες εκκρίνονται τόσο από καρκινικά όσο και από στρωματικά κύτταρα και συμβάλλουν στην τροποποίηση του μικροπεριβάλλοντος του όγκου με πολλαπλούς μηχανισμούς. Στην οικογένεια αυτή υπάγεται μεγάλος αριθμός ενζύμων, με ενδιαφέρουσες ιδιότητες. Μια χαρακτηριστική υπο-οικογένεια αποτελούν οι σύνθετες μεταλλοπρωτεάσες που ονομάζονται ADAMTS και περιλαμβάνουν 19 μέλη. Δεύτερη υπο-οικογένεια είναι οι καθεψίνες και περιλαμβάνουν 15 μέλη. Στην παρούσα εργασία ελέγχθηκε η επίδραση της σιμβαστατίνης, ενός αντι-υπερλιπιδαιμικού φαρμάκου που όμως εμφανίζει πλειοτροπικές ιδιότητες και μεταξύ αυτών αντικαρκινική δράση, στα καρκινικά κύτταρα τριπλά αρνητικού καρκίνου του μαστού, Hs578T. διερευνήθηκαν οι μεταβολές στις λειτουργικές ιδιότητες των κυττάρων και οι αλλαγές στην έκφραση δεικτών βλαστικότητας και EMT, ώστε να χαρακτηριστεί η επίδραση της σιμβαστατίνης στον καρκινικό χαρακτήρα αυτών των κυττάρων. Παράλληλα, διερευνήθηκαν οι μεταβολές στην έκφραση των ADAMTS και των καθεψινών. Διαπιστώθηκε ότι δεν μεταβάλλεται η κυτταρική μορφολογία, αλλά οι δείκτες βλαστικότητας KLF4, SNAIL2, TWIST1 αυξάνονται σε πολύ χαμηλές συγκεντρώσεις σιμβαστατίνης, όπως και ο δείκτης EMT, VIM, ο οποίος σχεδόν τριπλασιάζεται. Από τις ADAMTS με κολλαγονολυτική δραστηριότητα η ADAMTS3 και η ADAMTS14 αυξάνονται παρουσιάζοντας ανάλογη εικόνα των δεικτών βλαστικότητας, σε αντίθεση με την ADAMTS2, η οποία μειώνεται. Από τις ADAMTS με δράση έναντι της COMP, μόνο η ADAMTS12 εμφανίζει μικρή αύξηση. Η συγκεκριμένη πρωτεάση βέβαια είναι η μοναδική που παρουσιάζει χαρακτηριστικά ισχυρού κλινικού βιοδείκτη για τον καρκίνο του μαστού. Από τις καθεψίνες, φαίνεται ότι η CTSL παρουσιάζει αύξηση, ενώ η CTSO χαρακτηριστική μείωση. Τα αποτελέσματα καταδεικνύουν ότι η σιμβαστατίνη, ναι μεν διαθέτει κυτταροτοξική δράση για τα κύτταρα καρκίνου του μαστού, όμως, ειδικά έναντι κυττάρων τριπλά αρνητικού καρκίνου του μαστού, δεν παρουσιάζει τα χαρακτηριστικά εκείνα που αντιπροσωπεύουν αντικαρκινική δράση.

P30

Study of the anticancer effects of fatty acid lithium salts from Thamnidium elegans and Mortierella alpina on prostate cancer cell lines

Georgios Kalampounias[1], Panagiotis Dritsas[1], Dimitris Karayiannis[2], George Aggelis[1], Chrysavgi Gardeli[3], Seraphim Papanikolaou[2], Panagiotis Katsoris*[1]

[1]Division of Genetics, Cell Biology and Development, Department of Biology, School of Natural Sciences, University of Patras, 26504 Patras, Greece
[2]Laboratory of Food Microbiology and Biotechnology, Department of Food Science and Human Nutrition, Agricultural University of Athens, 11855 Iera Odos Athens, Greece
[3]Laboratory of Food Chemistry and Analysis, Department of Food Science and Human Nutrition, Agricultural University of Athens, 11855 Iera Odos, Athens, Greece

Thamnidium elegans and Mortierella alpina are two oleaginous Zygomycetes that accumulate γ-linolenic acid and arachidonic acid, respectively, both of which are credited with possible anticancer properties. Growing the fungi on industrial-grade glycerol - a byproduct of the agricultural industry, cost-effective and available in vast quantities - resulted in significant lipid production and storage. After extracting the lipids and transforming them into fatty acid lithium salts, we created a water-soluble, absorbable form of the lipids and subsequently administered them to DU-145 and PC-3 cells. The two cell lines, both long-established prostate cancer models, indicated increased susceptibility to the lipid extracts, exhibiting reduced viability and proliferation rates, as well as impaired migratory capabilities. Our results indicate that the possible admission of microbial fats from these organisms into human nutrition could have possible health effects, as has already been shown regarding the consumption of eicosapentaenoic acid in the form of cod oil.

Acknowledgements
The current investigation was financially supported by the project entitled "Biotransformation of glycerol into high pharmaceutical-value poly-unsaturated fatty acids (PUFAs)" (Acronym: Glycerol2PUFAs, project code HFRI-FM17-1839) financed by the Hellenic Foundation for Research & Innovation (H.F.R.I.), Nea Smyrni – Greece (project action: "1st Call for H.F.R.I. Research Projects to Support Faculty Members & Researchers and Procure High-Value Research Equipment").

P31

MAPK signaling and autophagy activation as key points of Bortezomib resistance on the PC-3 cell line

Georgios Kalampounias[1], Kalliopi Zafeiropoulou[1,2], Spyridon Alexis[2], Theodosia Androutsopoulou[1], Argiris Symeonidis[2], Panagiotis Katsoris[1]*

[1]Division of Genetics, Cell Biology and Development, Department of Biology, School of Natural Sciences, University of Patras, 26504 Patras, Greece
[2]Division of Hematology, General University Hospital of Patras, University of Patras, Patras 26504, Greece

Bortezomib is a proteasome inhibitor used clinically to treat multiple myeloma and mantle cell lymphoma. Proteasome inhibitors base their efficacy on the cancer cells' dependence on the ubiquitin-proteasome system to maintain their higher metabolic state and low intracellular stress levels. Impairment of this equilibrium induces the apoptotic death of cancer cells; however, after prolonged exposure to the drug, resistance emerges. Preliminary data on a Bortezomib-resistant clone of the DU-145 cell line from our laboratory indicated elevated phosphorylation of MAPKs in the resistant cells as well as augmented autophagy. These findings, combined with the decreased oxidative stress levels observed in the resistant cells, were confirmed in this study to also appear in resistant clones derived from the PC-3 cell line. The universality of such alterations indicates a generic way of establishing Bortezomib resistance, thus elucidating novel therapeutic targets able to surpass the current treatment obstacles.

P32

In vivo study of the effect of microglial BIN1 deletion in homeostatic and neuroinflammatory conditions

Maria Margariti[1]*, Irini Thanou[1#], Elsa Papadimitriou[1#], Alexandre Pelletier[2], Maria Anna Roussaki[1], Evangelia Xingi[3], Maria Avloniti[4], Vasso Kyrargiri[4], Marcos Costa[5,6], Dimitra Thomaidou[1]

[1]Neural Stem Cells and Neuro-imaging Group, Department of Neurobiology, Hellenic Pasteur Institute
[2]Univ. Lille, Inserm, CNRS, CHU Lille, Institut Pasteur de Lille, U1283-UMR 8199 EGID, F-59000, Lille, France
[3]Hellenic Pasteur Institute, Light Microscopy Unit, Athens, Greece
[4]Hellenic Pasteur Institute, Laboratory of Molecular Genetics, Microbiology Department, Athens, Greece
[5]Univ. Lille, Inserm, CHU Lille, Institut Pasteur Lille, U1167 - RID-AGE - Facteurs de risque et déterminants moléculaires des maladies liées au vieillissement, F-59000, Lille, France.
[6]Brain Institute, Federal University of Rio Grande do Norte, Natal, Brazil
#equal contribution

Genome-Wide Association Studies have identified several Single Nucleotide Polymorphisms (SNPs) strongly associated to increased risk of developing LOAD, many of which are related to microglial activation. SNPs in the locus harboring Bridging Integrator 1 (Bin1) gene show the strongest association with AD, after Apolipoprotein E. BIN1 is an adaptor protein implicated in cell membrane modelling dynamics. Although, its role in neurons has been studied both in vitro and in vivo, the role of BIN1 in microglial activation state and its contribution in LOAD pathology remains to be clarified. To this end we developed a conditional double transgenic Cx3CR1 Cre-ERT2//Bin1 fl/fl mouse, in which BIN1 is knocked-out in microglial cells. Furthermore, we have challenged Bin1 cKO mice with LPS, to investigate the effect of microglia-specific BIN1 deletion under homeostatic and inflammatory conditions. We performed snRNA-Seq in somatosensory cortex in our model to reveal novel targets related to microglial Bin1. Our analysis indicates that a number of signaling pathways regulated by microglia are differently impacted by LPS treatment in Bin1 cKO and control animals. Bin1 deletion resulted in the enrichment of microglial subpopulations exhibiting enhanced proliferative capacity and IFN-type I - mediated inflammatory response after LPS treatment, findings that were confirmed by subsequent real time RT-PCR and immunohistochemical analysis. Moreover, Bin1 deletion in resting microglia was sufficient to elicit transcriptional changes in astrocytes related to the expression levels of other LOAD risk factors. In parallel, we are focusing on the hippocampal region to investigate the potential region-specific effects of microglial Bin1 deletion. Our preliminary observations regarding the inflammatory properties of microglia are in agreement with our results from the cortex. We also observed a trend of an increase in the number of adult-born hippocampal neuroblasts, after Bin1 deletion, highlighting the role of microglia in the regulation of hippocampal neurogenesis.

P33

Establishment of a bone metastasis model in TgRANKL mice and evaluation of therapeutics

Evi Gkikopoulou[1,2], Christos Chrysovalantis Syrigos[1,2], Ioanna Mantogiannakou[2], Chrysa-Eleni Petraki[1,2], Melina Dragolia[2], Martina Rauner[3], Vasileios Ntafis[2], Eleni Douni[1, 2*]

[1]Department of Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece
[2]Institute for Bioinnovation, B.S.R.C. "Alexander Fleming", Fleming 34, 16672, Vari, Greece
[3]Department of Medicine III, Faculty of Medicine, TU Dresden, Dresden, Germany.

Receptor activator of nuclear factor-κB ligand (RANKL) is considered to be the main mediator of osteoclastogenesis, and bone resorption, while lately it has been associated with hormone-driven breast carcinogenesis and bone metastasis. Drugs targeting either RANKL directly such as Denosumab, a monoclonal antibody that binds to human RANKL, or osteoclast activity, such as Zoledronic acid that induces the apoptosis of osteoclasts, have been approved for postmenopausal osteoporosis and are currently evaluated in clinical trials for bone metastasis prevention or therapy. In the present study, our aim was to establish a breast cancer derived bone metastasis model in osteoporotic TgRANKL mice overexpressing the human RANKL gene and investigate bone metastasis progression upon anti-resorptive drug administration. For establishing our model, we injected EO771 mouse breast cancer cells systemically through the caudal artery of the tail. Since this cell line is stably transduced with the firefly luciferase gene, we were able to monitor early bone metastasis by in vivo bioluminescence imaging. Our data revealed that TgRANKL mice displayed an early onset of bone metastasis and severe bone loss when compared to their WT littermates. Then, we examined whether Denosumab or Zoledronic acid administration could prevent bone metastasis as a prophylactic therapy. Indeed, both drugs managed to delay bone metastasis occurrence similarly to the WT level and, also, prevented osteolysis. In contrast, therapeutic Denosumab administration did not reduce bone metastasis in the TgRANKL mice, while it minimized metastasis induced bone loss and osteolysis. Collectively, our results demonstrated that prophylactic administration of anti-resorptive drugs effectively delays bone metastasis and its effects in TgRANKL mice, while therapeutic Denosumab administration could protect their bones of secondary skeletal related events.

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship Number: 05346).

P34

Investigating the role of RANKL in mammary and prostate gland pathophysiology

Christos Chrysovalantis Syrigos[1,2], Evi Gkikopoulou[1,2], Dimitrios Lymperopoulos[2,3], Anthi Kolokotroni[1,2], and Eleni Douni[1,2]*

[1]Department of Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece
[2]Institute for Bioinnovation, B.S.R.C. "Alexander Fleming", Fleming 34, 16672, Vari, Greece
[3]School of Medicine, European University Cyprus, Nicosia, Cyprus

Receptor activator of nuclear factor-κB ligand (RANKL) is the main regulator of bone loss through the induction of osteoclast differentiation. Although it has been associated with hormone-induced carcinogenesis in the mammary gland, the mechanisms of carcinogenesis and metastasis involving RANKL remain unclear. Bone metastasis is a quite common phenomenon in advanced stages of several types of cancer, including breast and prostate cancer which are leading causes of cancer-related death. In the present study, we examined the effect of RANKL protein on the mammary and prostate glands of transgenic TgRANKL mice that overexpress human RANKL (huRANKL). Our results have recently shown that TgRANKL mice overexpress huRANKL at the mammary gland and display increased mammary density with expansion of epithelial ducts and ductal branches. Our qPCR results also revealed a significant increase in the expression of breast cancer associated genes such as Akt1, Vegfa, Stat5a, Ccnd1, Adam9 and Marco in the mammary glands of TgRANKL mice compared to those of (wild type) WT, confirming previous RNA-Seq analysis results between mammary glands from TgRANKL and WT mice. As regards the prostate gland, histological analysis did not reveal gross structural differences in the four types of paired prostatic lobes (anterior, ventral, lateral, dorsal) between WT and TgRANKL mice. However, huRANKL is expressed locally at the prostate gland of TgRANKL mice, while the levels of endogenous RANKL gene and its cognate receptors RANK and LGR4 genes are similar to those of WT as shown by qPCR analysis. Furthermore, we examined the expression level of stem cell and proliferation markers to identify initial stages of tissue proliferation. The analysis of the mammary and prostate glands in TgRANKL mice provides insights about the correlation of RANKL with gland-specific pathophysiology and possibly with cancer development.

P35

Fibroblast heterogeneity and functions in the regenerating intestine

Paraskeva Christina[1], Stavropoulou Athanasia[1,2], Chalkidi Niki[1], Samiotaki Martina[2], Nikolaou Christoforos[2], Koliaraki Vasiliki[1]

[1]Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece
[2]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece

Tissue damage and inflammation in the intestine is followed by a series of events that result in the restoration of normal organ function. These involve both intrinsic epithelial responses and microenvironmental alterations that are tightly regulated[1]. Failure in any of these processes can lead to serious implications, including chronic inflammatory disorders and fibrosis[2]. Fibroblasts in the intestinal stroma play important homeostatic roles, including the regulation of epithelial proliferation and differentiation, immune responses, and extracellular matrix (ECM) synthesis and remodeling. However, their contribution in terms of cellular and molecular mechanisms during tissue regeneration remains poorly understood[3]. In this study, we utilized the acute Dextran Sodium Sulfate (DSS)-induced colitis mouse model to examine the heterogeneity of fibroblasts during intestinal damage and repair and the role of individual subsets in the orchestration of a regeneration-permissive niche. DSS administration in mice results in colonic damage and inflammation, while its removal allows tissue repair[4]. To gain mechanistic insights into these functions, we analyzed the different stages of the disease using high-throughput approaches, including single cell RNA (scRNA) sequencing of the intestinal stroma and proteomic analysis of the decellularized tissue. Integration of published and in-house single cell transcriptomic data[5] and downstream bioinformatic analysis showed similar stromal heterogeneity across disease progression. However, deregulated gene expression analysis revealed significant differences in fibroblast subset activation during intestinal damage and repair, which was further corroborated for ECM-related molecules at the protein level[6]. In more detail, fibroblast-secreted inflammatory mediators, which were enriched in the initial stage of inflammation were downregulated and were followed by overexpression of specific ECM regulators at the regeneration stage[7]. In sum, our results provide insight into the activation and properties of fibroblasts during the damage-repair stages of colitis and indicate that they could actively support the restoration of intestinal integrity.

1. *Hageman, J. H., Heinz, M. C., Kretzschmar, K., van der Vaart, J., Clevers, H., & Snippert, H. J. G. (2020). Intestinal Regeneration: Regulation by the Microenvironment. Dev Cell, 54(4), 435-446. https://doi.org/10.1016/j.devcel.2020.07.009*
2. *Koliaraki, V., Prados, A., Armaka, M., & Kollias, G. (2020). The mesenchymal context in inflammation, immunity and cancer. Nat Immunol, 21(9), 974-982. https://doi.org/10.1038/s41590-020-0741-2*
3. *Chalkidi, N., Paraskeva, C., & Koliaraki, V. (2022). Fibroblasts in intestinal homeostasis, damage, and repair. Front Immunol, 13, 924866. https://doi.org/10.3389/fimmu.2022.924866*
4. *Wirtz, S., Popp, V., Kindermann, M., Gerlach, K., Weigmann, B., Fichtner-Feigl, S., & Neurath, M. F. (2017). Chemically induced mouse models of acute and chronic intestinal inflammation. Nat Protoc, 12(7), 1295-1309. https://doi.org/10.1038/nprot.2017.044*
5. *Ho, Y. T., Shimbo, T., Wijaya, E., Kitayama, T., Takaki, S., Ikegami, K., Tamai, K. (2021). Longitudinal Single-Cell Transcriptomics Reveals a Role for Serpina3n-Mediated Resolution of Inflammation in a Mouse Colitis Model. Cell Mol Gastroenterol Hepatol, 12(2), 547-566. https://doi.org/10.1016/j.jcmgh.2021.04.004*
6. *Giobbe, G. G., Crowley, C., Luni, C., Campinoti, S., Khedr, M., Kretzschmar, K., . . . De Coppi, P. (2019). Extracellular matrix hydrogel derived from decellularized tissues enables endodermal organoid culture. Nat Commun, 10(1), 5658. https://doi.org/10.1038/s41467-019-13605-4*
7. *Naba, A., Clauser, K. R., Hoersch, S., Liu, H., Carr, S. A., & Hynes, R. O. (2012). The matrisome: in silico definition and in vivo characterization by proteomics of normal and tumor extracellular matrices. Mol Cell Proteomics, 11(4), M111.014647. https://doi.org/10.1074/mcp.M111.014647*

P36

Pharmacogenetic analysis of MIR21 rs1292037 and ITGAM rs4597342 polymorphisms with response to anti-TNFa treatment in Greek psoriasis patients

Adam Akritidis[1], Aikaterini Patsatsi[2], Elisavet Lazaridou[2], Efterpi Zafiriou[3], Angeliki Roussaki-Schulze[3], Sophia Georgiou[4], Katerina Grafanaki[4], Yiannis Vasilopoulos[1]*

[1]Laboratory of Genetics, Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, 26504 Patras, Greece,
[2]2nd Dermatology Department, Medical School, Papageorgiou Hospital, Aristotle University, Greece,
[3]Department of Dermatology, University General Hospital Larissa, University of Thessaly, Greece,
*[4]Dermatology Department, Medical School, University of Patras, Greece, *e-mail: iovasilop@upatras.gr*

The contribution of tumor necrosis factor (TNF) in the pathogenesis of psoriasis has established the anti-TNFa monoclonal agents as ideal therapeutic approaches in the clinical routine. Despite their efficacy, inadequate response is observed in patients1, partially mediated by genetic factors. A prime example of the underlying molecular mechanisms includes the interaction of microRNA-21 with the 3' untranslated region of the ITGAM gene's mRNA in the presence of the rs4597342 T allele2. Here, we investigated the pharmacogenetic association between the MIR21 rs1292037 and ITGAM rs4597342 variants with the response to anti-TNFa treatment in Greek patients with psoriasis. One-hundred patients received anti-TNFa therapy for 6 months, where treatment response was assessed with the Psoriasis Area Severity Index (PASI). After DNA isolation, genotyping was performed via the PCR-RFLP method, using TspRI enzyme for the MIR21 rs1292037 and HindIII for the ITGAM rs4597342 variants. Statistical significance was set at P 0.05. Patients' mean age was 45 years, while mean age of disease onset was 38 years. Sixty-eight patients were considered as responders and the remaining 32 were non-responders. Neither of the polymorphisms showed statistically significant association with treatment response. However, the allelic (P=0.078) and recessive (P=0.0501) models of inheritance of the ITGAM rs4597342 showed a nominal association with etanercept response. Despite the absence of association between the variants under study and response to treatment, expansion of our cohort is appropriate to validate the above results. Furthermore, the putative associations between the ITGAM rs4597342 polymorphism and response to etanercept imply further stratification of patients according to the prescribed therapeutic approach to unveil possible pharmacogenetic biomarkers.

1 *Ovejero-Benito, M.C., Muñoz-Aceituno, E., Reolid, A. et al. Pharmacogenetics and Pharmacogenomics in Moderate-to-Severe Psoriasis. Am J Clin Dermatol 19, 209–222 (2018). https://doi.org/10.1007/s40257-017-0322-9.*
2 *Hruska P, Kuruczova D, Vasku V, Bienertova-Vasku J (2019) MiR-21 binding site SNP within ITGAM associated with psoriasis susceptibility in women. PLoS ONE 14(6): e0218323. https://doi.org/10.1371/journal. pone.0218323.*

P37

Preclinical imaging of therapeutic alpha-emitters

Sofia Lagoumtzi[1*], Maria Georgiou [1*], Eleftherios Fysikopoulos[1], Izabela Tworowska[2], Leo Flores[2], Xuewei Qu[2], Maritina Rouchota[1], Efthimis Lamprou[1], George Loudos[1]

[1]BIOEMTECH, Lefkippos Attica Technology Park - NCSR Demokritos, Athens, Greece, EL
[2]Radiomedix Inc, Houston, Texas, US

Introduction Alpha and beta emitters exhibit significant potential as therapeutic agents in clinical settings. [1], [2]. Novel radionuclide-based compounds like Pb-212 and Ac-225 are undergoing preclinical testing for future radiopharmaceutical development. Quantitative imaging of such compounds is possible via imaging of the gamma rays produced by complex decay schemes. Understanding the alpha and beta emitting radiopharmaceuticals biokinetics, in preclinical studies, is of major importance to evaluate its safety and efficacy [3], [4]. Here, we present in vivo and phantom imaging studies of Pb-212 and Ac-225.

Materials and Methods In vivo animal studies were performed on a dedicated bench top, mouse-sized, planar scintigraphy system (γ-eye™, BIOEMTECH, Greece). The system's ability to quantify activity variations was assessed using phantom experiments. It uses position-sensitive photomultiplier tubes, a CsI(Na) pixelated scintillator, and a high-sensitivity tungsten collimator with hexagonal holes for multiple gamma-emitting isotopes.

To evaluate system's sensitivity to activity variations, three cylindrical phantoms filled with Pb-212 and Ac-225 were imaged. A mouse phantom (fillable mouse phantom, BIOEMTECH, Greece) with different isotope activities was used to further assess the system's accuracy.

In vivo Pb-212 static scans with 10 min duration were performed at different time points, providing information on the distribution on the same animal. The total administered activity was <1 Mbq. During imaging, mice were anesthetized using isoflurane, under constant temperature (37° C).

Results Phantom studies results showed the capability of γ-eye™ to obtain accurate quantitative information of alpha emitting radiopharmaceuticals distribution in preclinical studies.

In vivo studies proved that successful tumor targeting with a Pb-212 compound is feasible in low activity and limited-time scans.

Conclusions Non-invasive imaging of alpha emitters, by detecting their gammas and positrons, can lead to a derived imaging method. Specialized scintigraphy tools enable long-term tracking, potentially speeding up preclinical research.

[1] M. Lassmann et al. Ann.ICRP, 47 (2018):187-195
[2] ES Delpassand Targeted et al. J Nucl Med (2022)
[3] Y. Seo, Nucl Med Mol Imaging, 53 (2019):182-188
[4] Poty S. et al. J Nucl Med, 59 (2018)

P38

Quantitative detection of Non Helicobacter pylori Helicobacter (NHPH) species in human gastric biopsies

Beatriz Martinez-Gonzalez[1], Ioanna Nanou[1,2], Nikolaos Moustakas[1], Stylliani Xenaki[1], Ioannis Karayiannis[1], Panagoula Kollia[2] and Dionyssios N. Sgouras[1]*

[1]Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens, Greece
[2]Department of Genetics and Biotechnology, Faculty of Biology, School of Physical Sciences, University of Athens, Athens, Greece
**correspondence: sgouras@pasteur.gr*

Helicobacter pylori (Hp) infection, mainly acquired during infancy, is involved in gastrointestinal pathology and is presumed to be transmitted between humans. In contrast, gastric Non Helicobacter pylori Helicobacter (NHPH) species, naturally colonize the stomach of animals and have been suggested to have a pathophysiological involvement in human gastric disease. Routine microbiological detection of NHPHs by culture is fastidious and difficult to be established, whereas, real-time quantitative polymerase chain reaction (qPCR) can offer a quick, reliable, and easy detection alternative. The present study objective was to develop and validate a SYBR-Green-based qPCR strategy for the rapid and accurate determination of the most prevalent gastric NHPH species transmitted to humans namely, H. heilmannii, H. bizzozeronii, H. salomonis, H. ailurogastricus, H. felis and H. suis. Specific primer combinations for detection of the ureA, ureB, ureAB and IpsA genes were utilized and the preparation of respective internal control standards was materialized by Topoisomerase-based (TOPO) cloning of the specific amplicons. qPCRs were optimized and validated for their accuracy and precision and were employed to determine the prevalence and significance of zoonotic gastric NHPHs in gastric biopsies derived from symptomatic Greek adults (N=105) and children (N=150). The species-specific analysis exhibited a high degree of diagnostic specificity and analytical sensitivity, as no cross-reactivity was detected with DNA from other Helicobacter spp. and the bacterial load was determined at copy number level. Molecular analysis revealed H. pylori presence in 16,5% of gastric samples (42/255),

33,3% in adults (35/105) and 4,7% in children (7/150), however, no NHPH species were detected in any of the gastric biopsies analyzed. These early results provide no evidence of transmission of NHPH infection in symptomatic Greek adults and children referred for upper gastroduodenal endoscopy. Further research should be performed in a larger sample size from people with occupational exposure to animals.

P39

RNA editing alterations in Amyotrophic Lateral Sclerosis (ALS)

Eirini Kanata[1], Korina Karagianni[2], Konstantinos Kyriakidis[1], Spyros Pettas[1,2], Konstantinos Xanthopoulos[1], Dimitra Dafou[2], Theodoros Sklaviadis[1]*

[1]School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece,
[2]School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece

RNA editing introduces RNA to DNA differences (RDDs). Deamination of Adenosine (A) to Inosine (I) or Cytosine (C) to Uracil (U), mediated by ADARs (Adenosine deaminases acting on RNA) and APOBECs (Apolipoprotein B mRNA Editing Catalytic Polypeptide-like) respectively, is the prevalent editing type in mammals. RNA editing may affect RNA:RNA or RNA:protein interactions, alter transcript processing (e.g. splicing, miRNA maturation), stability, translational efficiency, cellular localization and function (e.g. miRNA retargeting) or result in amino acid alterations (protein recoding). Studying RNA editing to better understand disease pathogenetic mechanisms has gained much attention. RNA editing alterations in neurodegenerative disorders, including Amyotrophic Lateral Sclerosis (ALS), the most common form of the motor neuron diseases, have been reported. ALS presents as familial (fALS, 5-10%) or sporadic (sALS, 90-95%). Aberrant RNA editing profiles have been reported in fALS, however global editing (editome) in sALS is missing. We aimed to determine RNA editomes in sALS and provide experimental validation of editing alterations in disease related transcripts. We utilized publicly available RNA-seq data and a rigorous bioinformatics pipeline combined with stringent filtering steps and subsequent quality control analyses, to identify high confidence RNA editing events. Comparative analysis of control and sALS editomes revealed reduced overall RNA editing frequency in sALS. Moreover, we noticed enrichment of editing events in 3'UTRs at the expense of intronic editing. Pathway analysis of differentially edited transcripts (mean editing frequency with a p-value 0.05 between sALS and controls) highlighted enrichment in the glutamatergic synapse pathway, suggesting that aberrant RNA editing is involved in synaptic dysfunction, an early event in ALS pathogenesis. Editing alterations in the CACNA1C transcript, encoding the alpha-1 subunit of a voltage dependent calcium channel, was experimentally verified through targeted re-sequencing in human autopsy material. In vitro studies on the functional effects of these alterations are in progress.

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers" (Project Number:01146). The authors would like to acknowledge the MRC UK Brain Bank Network Tissue, University of Edinburgh, Old College, South Bridge, Edinburgh, for providing human autopsy material from ALS and control cases used in this study

P40

Investigating the contribution of stromal cells in a novel mouse model of Psoriatic Arthritis

Vagelis Rinotas[1], Dimitris Konstantopoulos[1], Kalliopi Iliaki[1], Marietta Armaka[1]*

[1]BSRC "Al.Fleming", Vari, Greece
**Corresponding author. armaka@fleming.gr*

Psoriatic arthritis (PsA) is a challenging rheumatic disease affecting 0.25% of the population. PsA combines main manifestations from the peripheral and axial joints and the skin with multiple co-morbidities. Genetic associations and immunophenotyping of affected tissues led to the effective employment of anti-TNF and anti-IL17 therapies for most clinical features. However, not all patients respond to current treatments indicating additional pathogenic mechanisms. Owing to our poor understating of PsA etiology and the emerging evidence on the significant stromal contributions (e.g. Synovial Fibroblasts (SFs)), in other arthritides, we focus on appreciating the role of stromal cells in the PsA synovium. We employed a newly generated mouse carrying mutations in A20 gene ($A20^{Znf7KI}$), which develops spontaneous IL-17/TNF-dependent PsA-like pathology, characterized by peripheral arthritis, dactylitis, and mild bowel and liver disease, similar to human disease[1-3].

The diseased joints of $A20^{Znf7KI}$ mice exhibit proportional expansion of both sublining SFs and lining SFs in synovium compared to disease-free joints. Myeloid cells dominate, while effector and memory T and B cells are also increased in synovium compared to naïve counterparts, consistent with the splenic analyses. By generating reciprocal bone-marrow chimeras of WT and $A20^{Znf7KI}$ mice, we show that $A20^{Znf7KI}$ haemopoietic cells have the capacity to transfer the enteric and liver disease, but not the arthritic pathology in lethally-irradiated WT recipients. These results suggest that arthritogenic responses of radio-resistant/stromal cells are necessary for the development of arthritis in $A20^{Znf7KI}$ mice. Ex vivo analysis revealed that the $A20^{Znf7KI}$ SFs robustly activate NFkB and MAPKs in response to TNF rather than IL17, compared to WT SFs. Our ongoing scRNA-seq analysis of sorted $A20^{Znf7KI}$ and WT SFs shed light into the affected pathways in diseased SF subsets. In conclusion, our functional genetic and genomic studies in modelled $A20^{Znf7KI}$ disease provide novel insights on the stromal-mediated pathogenic pathways operating in PsA.

References

1. *Polykratis, A. et al. A20 prevents inflammasome-dependent arthritis by inhibiting macrophage necroptosis through its ZnF7 ubiquitin-binding domain. Nat Cell Biol (2019) 21, 731–742. https://doi.org/10.1038/s41556-019-0324-3*
2. *Martens A, et al. Two distinct ubiquitin-binding motifs in A20 mediate its anti-inflammatory and cell-protective activities. Nat Immunol. (2020) 21(4):381–7. doi:10.1038/s41590-020-0621-9*
3. *Razani B, et al. Non-catalytic ubiquitin binding by A20 prevents psoriatic arthritis–like disease and inflammation. Nat Immunol. (2020) 21(4):422–33. doi:10.1038/s41590-020-0634-4*

Acknowledgements

This study was funded by a FOREUM grant to MA (StroPHe, #061)

P41

Development of an antigen-specific therapy for myasthenia gravis

Eleni Ntoukaki, Vasiliki Baltatzidou, Konstantinos Lazaridis*

Department of Immunology, Hellenic Pasteur Institute, Athens, Greece.

Myasthenia gravis (MG) is an antibody-mediated autoimmune disorder targeting proteins at the neuromuscular junction. In the majority of MG patients, the autoimmune response is targeted mainly against the muscle acetylcholine receptor (AChR). Currently, MG is usually treated with long-term administration of non-specific immunomodulatory agents, which may have several side effects. Therefore, the development of novel therapeutics is imperative. To this end, antigen-specific therapies aiming towards tolerance reestablishment are a compelling strategy. We aim towards the development of an approach based on the administration of AChR domains. We have previously reported the expression of a mutated form of the extracellular domain of the human AChR alpha-1 subunit (hα1-ECD) in yeast, with increased solubility and good antigenicity. In vivo studies in rats with experimental autoimmune MG (EAMG) have shown the therapeutic potency of hα1-ECD administrated intravenously after disease induction, resulting in a significant and long-lasting reduction of EAMG symptoms. However, intravenously administered hα1-ECD was shown to have a short serum half-life. Therefore, improving the pharmacokinetic characteristics of hα1-ECD, could further increase its therapeutic potency. Thus, we firstly compared the treatment efficiency of hα1-ECD by different administration routes. In addition, we explored different strategies to improve the protein pharmacokinetics. Studies aiming towards the elucidation of the mechanisms of action are currently also underway. Overall, tolerance reestablishment against antigenic targets via the intravenous route is a promising therapeutic strategy for MG.

P42

The nuclear receptor NR5A2 as a potential regulator of HIF-1α

Panagiota Milioti, Dimitrios Gkikas, Eliana Markidi, Valeria Kaltezioti, Panagiotis K. Politis

Biomedical Research Foundation of the Academy of Athens, Soranou Efesiou 4, 11527, Athens, Greece

Glioblastoma multiforme (GBM) is one of the most fatal primary brain tumors with extremely low patient survival rates. A key feature of glioblastoma is the altered tumor metabolism. In order to support rapid proliferation, cancer cells favor glycolysis even in the presence of oxygen (Warburg effect) to provide the necessary macromolecules for the synthesis of nucleotides, fatty acids and amino acids, rather than fueling the citric acid cycle and oxidative phosphorylation. Hypoxia-inducible factor-1α (HIF-1α) is a main regulator of this metabolic adaptation and its high expression indicates poor prognosis in glioblastoma. Therefore, understanding the GBM adaptive HIF-1α signaling pathway might be critical for improving therapeutic strategies against malignant tumors. In this study, we show that NR5A2 (Nuclear Receptor Subfamily 5 group A Member 2) is sufficient and necessary to repress HIF-1α protein expression in GBM cells independently of prolyl hydroxylase (PHD) inhibition-mediated stabilization. Consistently, NR5A2 inhibits the glycolytic pathway as well as associated genes, which are HIF-1α direct targets. Moreover, NR5A2 exhibits strong antiproliferative, antigliogenic, and anti-tumorigenic activity in these cells. Most importantly, DLPC, a well-established agonist of NR5A2, can recapitulate these effects by repressing HIF-1α expression and tumor-associated properties in GBM cells. These data suggest that NR5A2 action in HIF-1α holds a protective role against GBM.

This work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Num- ber: 1782) and Fondation Santé Biomedical Research grants.

P43

Differential response of unfolded protein response pathway in multiple myeloma cells

Dimitra Bainantzou[1], Markos Sousis[1#], Nikolitsa Kritsioni[1#], Giannis Rigopoulos[1], Dionysia Psarreou[1], Vasiliki Labropoulou[2], Achilleas D. Theocharis[1*]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Department of Internal Medicine, Division of Hematology, University of Patras Medical School, Patras, Greece
[#] Equal contribution
**email: atheoch@upatras.gr*

Multiple myeloma (MM) is a hematological malignancy derived from plasma cells, a type of white blood cell vital for the immune response and antibody production. Although various therapeutic agents already exist, a complete understanding of the pathology and biochemical mechanisms of MM will be a crucial asset in developing more effective forms of treatment. Of particular interest is the relationship between unfolded protein response (UPR) pathway and induction of endoplasmic reticulum (ER) stress in MM cells. ER stress is associated with the accumulation of misfolded proteins in the ER, resulting in a disruption of its balance and triggering the UPR, which aims at adaptation and cell survival. Aberrant activation of UPR induces cell apoptosis. Our study aims to examine the UPR pathway in H929 and U266 MM cells in the presence of tunicamycin, a substance capable of inducing ER stress. More specifically, the study of the gene and protein expression of the target genes belonging to the three main UPR pathways mediated by IREa1, PERK and ATF6 revealed that H929 cells are particularly resistant to tunicamycin and were able to adapt to tunicamycin induced ER-stress. In contrast, the U266 cells are not able to regulate the expression of UPR genes in the presence of tunicamycin. Our data suggest a differential response of MM cells concerning UPR activity and regulation upon ER stress induction.

P44

Study of neutrophil responses in Helicobacter pylori infection

Aristotelis Petris[1, 2], Elena Petrou[1], Stavros Naoum[2], Yiannis Karayiannis[1], Beatriz Martinez-Gonzalez[1], Dionyssios Sgouras[1], Konstantinos Kambas[2]

[1]Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens, Greece
[2]Laboratory of Molecular Genetics, Hellenic Pasteur Institute, Athens, Greece

Helicobacter pylori (Hp) is a pathogen colonizing the human gastric mucosa through lifetime, increasing the risk of gastric cancer development. Although neutrophil infiltration in the lamina propria is the hallmark of Hp infection, it fails to clear the infecting bacteria. Considering the capacity of neutrophils to exert a big array of antimicrobial responses, we investigated the in vitro interactions of freshly isolated human peripheral blood neutrophils with Hp strains, in terms of phagocytosis and neutrophil extracellular traps (NETs) release, visualized by confocal microscopy, while ROS generation was detected by flow cytometry. We documented the capacity of neutrophils to phagocytose all Hp strains, irrespective of CagA phosphorylation status and produce ROS, albeit at significantly lower levels to those observed when phagocytosing E. coli. Interestingly, Hp induced NETs which appeared to be degraded and demonstrated only faint presence of NET-related proteins compared to controls. To validate these findings, a live imaging protocol was carried out on Hp-infected neutrophils by using SYTOX-GREEN and Hoechst 33342 stains and observed in confocal microscope. Images were captured every 2 to 3 minutes and demonstrated progressive reduction of extracellular staining in Hp-induced NETs, compared to NETs released by sterile stimulus, suggesting a degradation process due to Hp presence. These findings suggest possible immune-evasion strategies of Hp to host neutrophilic response.

P45

Evaluation of the therapeutic potential of AK057887 lncRNA inhibition in CD133+/CD44+ stem-like pancreatic cancer cells

Elisavet Deligianni[1], Giasemi Eptaminitaki[2], Vasiliki Stravokefalou [1], Apostolos Zaravinos[3,4], Panagiota Stamou[1], Christos Chochos[1], Stavroula Baritaki*[2], Dimitris Stellas*[1]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, 11635 Athens, Greece
[2]Laboratory of Experimental Oncology, Division of Surgery, School of Medicine, University of Crete, 71003 Heraklion, Greece
[3]Department of Life Sciences, School of Sciences, European University Cyprus, 2404 Nicosia, Cyprus
[4]Basic and Translational Cancer Research Center (BTCRC), Genomics and Systems Biology Laboratory, Cancer Genetics, 1516 Nicosia, Cyprus

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies. Its aggressive phenotype is often related to cancer stem cells (CSCs). It has been shown that several long non-coding RNAs (lncRNAs) in CSCs play a pivotal role in promoting tumor growth or resistance to chemotherapy. In the present study, we investigated the role of AK057887 lncRNA in the aggressiveness of $CD133^+/CD44^+$ stem-like PDAC cells and evaluated its inhibition in vitro and in vivo. First, we sorted a cell subpopulation expressing both surface antigens CD133 and CD44, indicative of CSCs, from the human PDAC cell lines PANC1 and MIAPACA2. We next evaluated its chemoresistance against chemotherapy, using cell viability and immunofluorescence assays. We further analyzed its transcriptomic profile focusing on the expression of lncRNAs. Finally, we explored a potential therapeutic intervention by inhibiting a unique and highly expressed lncRNA in this population, named AK057887, and verified these results in PDAC xenografts. The sorted population of $CD133^+/CD44^+$ stem-like PDAC cells could establish the initial tumor heterogeneity in vitro and in vivo. Our proliferation assays revealed that the $CD133^+/CD44^+$ subpopulation was resistant to chemotherapy. Transcriptomic analysis of the $CD133^+/CD44^+$ cells revealed differential expression of many lncRNAs. We chose the lncRNA AK057887 because it exhibited high expression in all PDAC cell lines. Finally, in vitro, and in vivo inhibition of the AK057887, resensitized the tumors to chemotherapy revealing a novel therapeutic intervention. CD133+/CD44+ stem-like PDAC cells can generate initial tumor heterogeneity both in vitro and in vivo, and they exhibit chemoresistance. Inhibition of the AK057887 lncRNA resensitizes PDAC tumors to chemotherapy, offering a novel therapeutic approach.

Acknowledgements
The authors gratefully acknowledge Dr. Gkikas and Dr. Politis from Biomedical Research Foundation of the Academy of Athens for their help in designing the anti Ak057887 oligos.

P46

A "pulse-chase" method for the isolation and characterisation of the quiescent cells of the adult pancreas

Chrysanthi Charalampous[1], Kisara Gjolena[1,2], Paraskevi Kogionou[1,3], Adriana Papadimitropoulou[1], Argiris Efstratiadis[4], Ioannis Serafimidis[1]*

[1]Center of Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[2]Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, Greece.
[3]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece
[4]Center of Experimental Surgery, Clinical and Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
**email: iseraf@bioacademy.gr*

Adult stem cells contribute to organ repair after injury, however their role in homeostatic tissue regeneration is not always apparent, especially in tissues with inherently low rates of cell turnover. The pancreas is a particularly striking example of this controversy, with only limited evidence for the existence of cells possessing unequivocal stem cell properties. We designed an in vivo "pulse-chase" method to isolate the rare population of quiescent cells of the adult pancreas and investigate how this correlates with our recently identified Aldh1b1^{+} putative stem cell population. To achieve this, we generated a Pdx1^{tTA};Tet^{H2BGFP};Aldh1b1^{LacZ} triple transgenic line: Pdx1 is a transcription factor expressed in the entire pancreatic epithelium during embryonic development, and in the Pdx1^{tTA} mice, the coding region for the tTA$_{off}$ transcriptional activator has replaced the coding region of the endogenous Pdx1 gene. The Tet^{H2BGFP} mice conditionally express a fusion protein of histone-2B with GFP, under the control of a Tet-responsive element (TRE). In Pdx1^{tTA};Tet^{H2BGFP} mice therefore, the tTA$_{off}$ transactivator, expressed exclusively in Pdx1^{+} cells, activates expression of H2BGFP via TRE, and thus labels the nuclei of Pdx1+ cells with green fluorescence. In the presence of doxycycline however, H2BGFP expression is turned off, so the number of GFP+ cells gradually decrease as the ongoing proliferation dilutes the labelling without replenishment from newly expressed H2BGFP. Cells with high proliferation rates loose GFP labeling quickly, whereas quiescent or very slowly proliferating cells retain the label, and thus become trackable. We explored different schemes of dox-treatment, applied both during embryonic development and postnatally, in order to precisely identify the label-retaining quiescent cells of the adult pancreas. We demonstrated that these cells largely co-express Aldh1b1 (LacZ detection) and, importantly, we FACS-isolated these cells by virtue of their GFP expression and performed RNA-sequencing in order to identify possible new transcripts that they uniquely express.

P47

Exploring the heterogeneity of adult pancreatic stem cells

Paraskevi Kogionou[1,2], Despoina Gkavali[1], Adriana Papadimitropoulou[1], Chrysanthi Charalampous[1], Ioannis Serafimidis[1*]

[1]Center of Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece.
[2]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece
**email: iseraf@bioacademy.gr*

The presence of stem cells in the adult pancreas, and their potential involvement in homeostatic tissue regeneration, remain a hotly debated issue. We have recently shown that mouse ductal cells expressing the mitochondrial enzyme Adlh1b1 fulfill many of the requirements for their characterization as adult pancreatic stem cells. Nevertheless, it is becoming increasingly evident that Aldh1b1+ cells are not a homogeneous population, as they only partially co-express other markers allegedly specific for adult pancreatic stem cells (Hes1, Sox9 etc). We seek to address this heterogeneity issue and question whether Aldh1b1+ cells are further divided in subpopulations of more restricted or unipotent progenitors, identifiable by specific markers that they uniquely express.

The Wnt signaling receptor Lgr5 is an established marker of adult stem cells in several gastrointestinal tract organs such as the small intestine, colon and stomach, however, in the pancreas, Lgr5 is only expressed after pancreatic duct ligation, an injury model that activates regeneration. We generated an $Lgr5^{CreERT2}/ROSA26^{LSLtdTomato}/ Aldh1b1^{lacZ}$ triple transgenic line, to label and lineage-trace $Lgr5^{+}$ cells. We demonstrated that Lgr5 is not expressed in cerulein-induced pancreatitis, an alternative regeneration model associated with strong expansion of $Aldh1b1^{+}$ cells, indicating that Lgr5 and Aldh1b1 mark distinct populations of pancreatic progenitor/stem cells.

In order to explore the heterogeneity within the Aldh1b1 population, we initially performed a series of pancreas whole-mount immunofluorescent stainings with potential duct-specific cell surface markers. Our analysis revealed a clear distinction between large and small ducts at the protein expression level, and confirmed that the Aldh1b1 population is confined to the small ducts. Subsequently, we designed a multi-step FACS scheme to isolate $Aldh1b1^{+}$ cells, successively purified from the whole-duct ($CD133^{+}$) and small-duct ($Cldn8^{+}$) populations. We are currently performing single-cell RNA seq analysis on this refined Aldh1b1 population, in order to identify novel markers that address its heterogeneity.

P48

Disrupted hippocampal neurogenesis following brain chemical lesion induced by the chemotherapeutic agent Ara-C

M. Fourmouzi[1], I. Thanou[1], E. Makarouni[1], M. Margariti[1], D.Thomaidou[1]

[1]Hellenic Institute Pasteur, Neurobiology Department, Athens, Greece

Despite the therapeutic role of chemotherapeutic agents, a number of secondary adverse effects occur after their administration. These effects are detected in the physiology and cognitive function of the brain contributing to the syndrome referred to as “ChemoBrain”. Previous studies have highlighted the reduction in proliferation of neural stem cells (NSCs) of brain’s adult neurogenic niches with possible involvement in memory loss following antimitotics administration. Thus, the aim of our study was to investigate the dynamic changes of adult hippocampal neurogenesis in response to antimitotic agents. To this end, we performed a protocol of repeated intraventricular stereotactic injections of the cytotoxic agent cytosine arabinoside (Ara-C) and studied its effect on hippocampal neurogenesis in 3 distinct time-points of 4, 15 days and 6 weeks later. Our immunofluorescence analysis indicates that Ara-C led to a significant reduction in the populations of NSCs/radial glia (GFAP+/SOX2+), intermediate progenitors (TBR2+) and neuroblasts /immature neurons (DCX+) during the first 15 days. However, after 6 weeks the population of DCX+ neuroblasts significantly increased and exceeded the equivalent population in control animals. Despite the recovery of the population, many DCX+ cells were unexpectedly misplaced deeper within the granule cell layer (GCL) of the hippocampus towards the molecular layer. This observation along with the reduced dendritic complexity of DCX+ population indicates defects during the neuronal maturation process. Our data collectively suggest that the administration of Ara-C leads to dynamic changes in the populations of the neural lineage of the dentate gyrus of the hippocampal niche. Our future studies aim to elucidate the causes of their delayed maturation and potential defects on their functional integration into the system.

P49

The regulatory role of the interaction between platelets and postnatal brain Neural Stem and Progenitor Cells of the Subependymal Zone

Maria Anesti[1*], Cédric Ghevaert[2], Ilias Kazanis[1]

[1] Laboratory of Developmental Biology, Division of Genetics Cell and Developmental Biology, Department of Biology, University of Patras, Patras, Greece
[2]Wellcome Trust – MRC Cambridge Stem Cell Institute & Department of Haematology, University of Cambridge, Cambridge, UK
**e-mail: anestimaria@gmail.com*

Postnatal brain Neural Stem and Progenitor cells (NSPCs) reside in specialized microenvironments, called stem cell niches, one of which is the Subependymal Zone (SEZ) of the lateral walls of the lateral ventricles. Previous in vivo work has revealed that focal demyelination in the adjacent corpus callosum (CC) induces specific platelet aggregation within the activated vasculature and that exposure of NSPCs to platelet lysate enhances their survival [1]. Here, we investigate direct cell-to-cell interactions, performing cocultures between platelets and NSPCs, as typical monolayer cultures, or using a polymorphic assay in which we created a range of microenvironments, classified either as "niche-like" 3D, or as "parenchyma-like" 2D areas [2]. Our analysis revealed that platelets affect NSPC behavior, by increasing their undifferentiated/progenitor state in the absence of growth factors and by enhancing the distribution of neuroblasts and proliferating cells in the proximity of niche-like structures, but without any changes in apoptotic markers. However, when we exposed NSPCs in a medium that has been enriched with platelet factors, the progenitor state was not affected, reinforcing the role of cell-to-cell interactions between the two populations. We also performed experiments of focal demyelinating lesion on the CC in animals with reduced platelet numbers, using two different approaches: transient chemical platelet depletion and thrombocytopenic Nbeal2 knockout mice. Histological analysis showed increased oligodendrocyte density in both the SEZ and CC, and a reduction in the percentage of proliferating oligodendrocyte progenitor cells (OPCs) in the SEZ of chemical platelet-depleted mice, without changes in neurogenesis. Finally, we successfully grafted platelets directly into the CC in order to investigate their direct interaction with progenitors in homeostasis. In conclusion, we demonstrate a functional role of cell-to-cell interaction between platelets and NSPCs in vitro, and an altered behavior of OPCs in the niche and the adjacent CC of thrombocytopenic mice after demyelination.

Acknowledgements
The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT) to I.K, as well as PhD scholarships from both the Bodossaki Foundation and the State Scholarships Foundation (IKY) to M.A

References

1. *Kazanis, I., Feichtner, M., et al.., Rivera, F. J. (2015). Lesion-induced accumulation of platelets promotes survival of adult neural stem/progenitor cells. Experimental neurology, 269, 75-89.*
2. *Anesti M., et al., Kazanis I. (2022). Divergence between Neuronal and Oligodendroglial Cell Fate, in Postnatal Brain Neural Stem Cells, Leads to Divergent Properties in Polymorphic In Vitro Assays. Cells, 11, 1743.*

P50

Disrupted Neurogenesis and Ectopic Neuroblast Migration in Both Neurogenic Niches Following AraC administration

I. Thanou[1], , M. Fourmouzi[1*] , E.Makarouni[1*#]. P. N. Koutsoudaki[2], M. Margariti[1], F. Luzzati[3], S. Havaki[2], V. G. Gorgoulis[2], D. Thomaidou[1]

[1]Hellenic Institute Pasteur, Neurobiology, Athens, Greece corresponding author: thomaidou@pasteur.gr
[2]National and Kapodistrian University of Athens, School of Medicine, Laboratory of Histology-Embryology, Athens, Greece
[3]Neuroscience Institute Cavalieri Ottolenghi (NICO),University of Turin, Italy
**equal contribution #current address: Biozentrum - University of Basel*

Recent research has identified side effects of chemotherapy on brain function. These include reduced neural stem cell growth, white matter degeneration, and brain inflammation, collectively known as 'Chemobrain.' In our study we focus on the impact of intraventricular infusion of chemotherapeutic mito-toxic agent arabinoside-C (Ara-C) on adjacent neurogenic and non-neurogenic brain areas. Characterization of the spatio-temporal distribution of neuronal and glial cells at multiple time points following the Ara-C administration (4, 15 days and 6 weeks) revealed that it leads to persisting ependymal cell layer disruption and impaired neurogenesis in both neurogenic niches (Subventricular Zone and Subgranular Zone). Moreover, it triggers doublecortin+ (DCX+) neuroblasts' ectopic presence clustered within white matter tracts of striatal parenchyma, as well as in deep areas of the granule layer of dentate gyrus (DG). Furthermore, noted defects in dendritic arborization suggest a perturbation in the maturation process, which may lead to the observed accumulation of neuroblasts in both regions. Using transmission electron microscopy (TEM) to evaluate brain micro-structure we observed myelination defects and axon loss which is accompanied by impairment of the oligodendrocyte lineage. Our data also support a strong neuroinflammatory response with extensive astro- and microgliosis and presence of CD3+ T cells in the SVZ and choroid plexus, suggesting a disruption of the blood–CSF barrier in this site. Our studies are ongoing to follow the lineage trajectories, origin and molecular profile of ectopic neuroblasts.

References

- *Gibson EM, Nagaraja S, Ocampo A, et al. Methotrexate Chemotherapy Induces Persistent Tri-glial Dysregulation that Underlies Chemotherapy-Related Cognitive Impairment. Cell. 2019;176(1-2):43-55.e13. doi:10.1016/j.cell.2018.10.049*
- *Beera KG, Li YQ, Dazai J, et al. Altered brain morphology after focal radiation reveals impact of off-target effects: implications for white matter development and neurogenesis. Neuro Oncol. 2018;20(6):788-798. doi:10.1093/neuonc/nox211*

P51

Description and quantification of apoptosis during in vitro mouse brain Neural Stem Cell differentiation

Konstantina Mastori[1], Maria Anesti[1], Aggeliki Dimopoulou[1], Ilias Kazanis[1*]

[1]Laboratory of Developmental Biology, Department of Biology, University of Patras, 26504 Patras, Greece

One of the best-described neurogenic regions of the adult mammalian brain is the Subependymal Zone (SEZ), which is located at the lateral walls of the lateral ventricles. Neural Stem Cells (NSCs) found therein are the descendants of the embryonic NSCs called Radial Glia cells and remain mainly in an inactive (quiescent) state. Because of their ability to preserve their proliferation and differentiation potential, NSCs can be isolated and cultured in vitro. The absence of growth factors from the culture medium, can lead to NSCs' differentiation towards Neural Progenitor Cells, which can give rise to astrocytes, oligodendrocytes and neurons[1]. However, the removal of pro-mitotic factors can have a negative effect on stem cells, in general, either via apoptosis or necrosis[2].

In this study we followed for three days adult mouse NSCs, cultured in differentiation conditions in order to describe and quantify apoptotic cell death. Apoptotic nuclei and apoptotic bodies were identified based on DAPI staining, while at the same time the expression of SOX2 and NESTIN (to mark progenitor cells), GFAP (to mark astrocytes), DCX (to mark neuroblasts) and OLIG2 (to mark oligodendrocytes) was assessed immunocytochemically. The results indicated a significant increase of apoptosis after 24 and 48h in culture, with a parallel significant switch in the presence of apoptotic bodies at the expense of whole apoptotic nuclei. In addition, we observed a surprising fluctuation in the cell profile of the cultures; a finding that has to be thoroughly investigated.

1. *Kriegstein A, Alvarez-Buylla A. (2009) The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci. 32:149-84. doi: 10.1146/annurev.neuro.051508.135600.*
2. *Ceccatelli S, Tamm C, Sleeper E, Orrenius S. (2004) Neural stem cells and cell death. Toxicol Lett. 149(1-3):59-66. doi: 10.1016/j.toxlet.2003.12.060.*

This work was funded by the Hellenic Foundation for Research and Innovation (HFRI-FM17-3395) to IK.

P52

Neural Stem Cells and Platelets: allies or rivals? an investigation of cell viability, apoptosis, mitosis and neurogenesis in co-cultures

F. Katsaitis[1,2*], Maria Anesti[1], Aggeliki Dimopoulou[1], I. Kazanis[1*]

[1]Laboratory of Developmental Biology, Department of Biology, University of Patras, 26500, Patras, Greece
[2]Current Affiliation: Laboratory of Cell Signaling and Molecular Pharmacology, Institute of Biosciences and Applications, NCSR "Demokritos", 15343, Athens, Greece
Corresponding E-mails: filippos.katsaitis@upnet.gr or ikazanis@upatras.gr

Neural Stem Cells (NSCs) are multipotent (undifferentiated) cells, capable of generating the three major cell types of the Nervous System. Pools of postnatal brain NSCs are clustered in specialized microenvironments called stem cell niches. One such niche is located at the Subependymal Zone (SEZ, or Ventricular - Subventricular Zone, V-SVZ) at the lateral walls of the lateral ventricles. Platelets (PLTs) are small, oval-shaped fragments that circulate through the Vasculatory System; they store many bioactive molecules, which are released during platelet activation, mainly to create blood clots. The SEZ is characterized by very specialized vasculature that participates in the regulation of NSC activity (Kazanis et al., 2010). We have previously shown the specific aggregation of PLTs within the vasculature of the niche in response to a focal demyelinating lesion in the adjacent corpus callosum and the pro-survival effects that PLT-derived factors exert on NSCs in vitro (Kazanis et al., 2015). In this project we investigated the effects of the direct co-culture of NSCs with different densities of PLTs, by investigating basic cellular functions of NSCs, such as viability, apoptosis, mitosis, maintenance of stemness and induction of neurogenesis. We found that the highest PLTs density led to decreased viability of NSCs, while the percentage of apoptotic cells was not affected by the presence of PLTs. This may indicate that PLTs affect NSCs when maintained in direct contact, depending on their density. High densities of PLTs are toxic to NSCs, indicating the emergence of necrotic cells death. Additionally, the highest density of PLTs led to increased levels of mitosis, which may be enhanced by the secretion of platelet-derived factors that have to be identified. Finally, the percentage of Doublecortin+ cells (DCX+), as well the maturation of neuroblasts (assessed by the co-expression of DCX and Sox2 transcription factor) was not affected by the PLTs.

The work was funded by a grant from the Hellenic Foundation for Research and Innovation (HFRI-FM17-3395) to IK.

1. *Kazanis, I. (2009). The subependymal zone neurogenic niche: A beating heart in the center of the Brain: A Journal of Neurology, 132(Pt 11), 2909–2921. https://doi.org/10.1093/brain/awp237*
2. *Kazanis, I. et al. (2015). Lesion-induced accumulation of platelets promotes survival of adult neural stem / progenitor cells. Experimental Neurology, 269, 75–89. https://doi.org/10.1016/j.expneurol.2015.03.018*

P53

Activin A Signalling in Human Pluripotent Stem Cells

Elena Rakovoliou[1*], Angelos Papadopoulos[2], Athena Kyrkou[1], Nikoleta Kostopoulou[1], Sofia Bellou[1,3], Aikaterini Apostolidi[1], Maria Margariti[4], Maria Markou[1], Eleni Bagli[1], Panagiota Chira[4], Eleni Tschari[5], Philippe Chavrier[5], John Heath[2], Theodore Fotsis[1,4], and Carol Murphy[1]**

[1]Biomedical Research Institute, Foundation of Research and Technology-Hellas, University Campus, 45110 Ioannina, Greece
[2]School of Biosciences, College of Life and Environmental Sciences, University of Birmingham, UK.
[3]Confocal Laser Scanning Microscopy Unit, Network of Research Supporting Laboratories, University of Ioannina, 45110, Greece.
[4]Laboratory of Biological Chemistry, Medical School, University of Ioannina, 45110, Greece
[5]Centre de Recherche, Institut Curie, CNRS, UMR 144, 26 rue d'Ulm, 75248 Paris Cedex 05, France
**Presenting author: Rakovoliou Elena, email: elenarakovoliou@gmail.com **Corresponding author, email: carol_murphy@bri.forth.gr*

Pluripotency in human stem cells is known to be regulated primarily by components of the TGFβ superfamily. Activin A and TGFβ ligands act through heteromeric complexes of type I and type II transmembrane serine/threonine kinase receptors by activating SMAD2 and SMAD3 proteins which oligomerise with SMAD4 in the cytoplasm. The SMAD2/3/4 complex translocates to the nucleus and regulates transcription using a large network of interactions with transcription factors, co-activators and co-repressors. Unpublished results from our lab have established that the small GTPase ADP-Ribosylation Factor 6 (ARF6) is directly implicated in TGF-β and Activin A signalling. As ARF6 cycles through its active (GTP-bound) and inactive (GDP-bound) form, it regulates cell surface ligand internalisation, post internalisation, trafficking along the endocytic pathway, endosomal recycling and actin remodeling. To dissect the mechanism of action of ARF6 in Activin A/TGFβ signalling we identified an ARF6-GAP which interacts with the type 1 activin receptor (ACVR1B , ALK4). We verified the interaction by co-immunoprecipation, FRET analysis and colocalization experiments by confocal microscopy. We present our results and discuss the importance of these findings.

P54

Direct cellular reprogramming of mESCs and astrocytes towards the ependymal lineage

Maria Kanellopoulou[1*], Georgios Kritikos[1], Konstantina Kaplani[1], Stella Vassalou[1], Maria-Eleni Lalioti[1], Georgia Lokka[1], Evangelia Parlapani[1], Anna Chantzara[1], Despoina Korrou-Karava[1], Zoi Lygerou[2], Stavros Taraviras[1]

[1]Department of Physiology, Medical School, University of Patras, Greece
[2]Department of General Biology, Medical School, University of Patras, Greece

During mammalian brain development, neural progenitors undergo a transformation, shifting from an undetermined state to a more determined one. This transition is orchestrated by lineage-specific molecular pathways. Direct reprogramming, on the other hand, enables the cell fate conversion from one lineage into another without transitioning through an intermediary pluripotent state. We have previously provided evidence that two proteins of the Geminin superfamily, GemC1 and McIdas, govern the ependymal cell fate molecular program. Ependymal cells are ciliated-epithelial cells that develop along the surface of the ventricles of the brain, and they play a critical role in the circulation of the cerebrospinal fluid and in the homeostasis of the brain. Our study aims to determine the reprogramming potential of GemC1 and McIdas towards the ependymal lineage.

Experiments on embryonic stem cells (ESCs) showed that, through the expression of GemC1 or McIdas, mouse ESCs can be programmed into the ependymal lineage. In addition, preliminary results show that combined expression of GemC1 and inhibition of the polycomb repressive complex show the potential enhancement of reprogramming efficiency towards ependyma. Ectopic expression of these proteins in cortical astrocytes indicate the successful direct reprogramming towards ependyma. The reprogrammed cells displayed unique morphological and functional features of mature ependymal cells.

Overall, we believe that further understanding of the role of GemC1 and McIdas in the molecular pathway that promotes ependymal cell reprogramming could provide new evidence for the creation of new therapeutic approaches for the directed production of ependymal cells against neurodegenerative diseases.

P55

Artificial matrix-based bioscaffolds promote the chondrogenic differentiation potential of mesenchymal stem cells

Maria Barmpoutsi[1#], Olga Florou[1#], Spyros Kremmydas[1], Zoi Piperigkou[1,2*], Rigini Papi[3] Theodora Choli-Papadopoulou[3], Achilleas D. Theocharis[1], Nikos K. Karamanos[1,2]

[1]Biochemistry, Biochemical Analysis and Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece
[2]Foundation for Research and Technology-Hellas (FORTH)/Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece
[3]Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece
 # Equal contribution

Tissue engineering, using innovative techniques like the development of artificial biocompatible scaffolds, is a growing field in regenerative medicine, with broad potential applications in various diseases, including degenerative joint disease. Osteoarthritis (OA), the most common type of arthritis, is characterized by the progressive destruction of cartilage, leading to structural and functional changes within the joint with only a few effective treatments available. During OA progression, hyaline cartilage cannot repair the joint due to lack of nerves and blood vessels. Artificial biomimetic scaffolds, synthesized from natural polymeric molecules and recombinant matrix proteins, replicate the necessary biological and mechanical properties of the extracellular matrix (ECM). Biomimetic scaffolds can be used as 3D structures, where mesenchymal stem cells (MSCs) can be seeded, expanded, and then implanted in vivo, facilitating the release of bioactive molecules to induce MSC differentiation into chondrocytes, promoting cartilage regeneration. Our study aims to investigate the ability of biomimetic artificial matrix-based scaffolds to induce chondrogenic differentiation in human umbilical cord mesenchymal stem cells (human Wharton's jelly MSCs). The impact of chondrogenic differentiation culture medium is also investigated. The biotechnologically synthesized bioscaffolds are based on elastin-silk-mussel-like polypeptide (ELP), modified with either the transforming growth factor β1 (TGFβ1) peptide or arginine-glycine-aspartic acid (RGD) motifs. The above scaffolds were also combined with type II collagen. The effect of these scaffolds on gene and protein expression of major ECM biomarkers, transcription factors implicated in chondrogenic differentiation and specific biomarkers for cartilage hypertrophy, and the phosphorylation of proteins involved in signaling pathways important for chondrogenic differentiation is studied. Wharton's jelly MSCs are cultured on the synthesized crosslinked bioscaffolds under normal or chondrogenic differentiation conditions for 24 days. Our results pinpointed that both ELP-TGFβ1 and ELP-RGD bioscaffolds can induce MSC differentiation into chondrocytes under certain conditions, suggesting their promising role as novel therapeutic approaches for OA.

Reference

- *Piperigkou Z, Bainantzou D, Makri N, Papachristou E, Mantsou A, Choli-Papadopoulou T, Theocharis AD, Karamanos NK (2023) Enhancement of mesenchymal stem cells' chondrogenic potential by type II collagen-based bioscaffolds, Mol Biol Rep. 50(6):5125-5135. doi: 10.1007/s11033-023-08461-x.*

P56

Generation of vascularized human pluripotent stem cells derived retinal organoids as a platform to study retinogenesis and disease modeling

Katerina Apostolidi [1,2], Maria Markou [1], Sofia Bellou [1,4], Theodore Fotsis [1,3], Carol Murphy[1*], Eleni Bagli [1*]

[1]Foundation of Research and Technology-Hellas, Biomedical Research Institute, University Campus, 45110 Ioannina, Greece
[2]Department of Biological Applications and Technology, University of Ioannina, 45110 Ioannina, Greece
[3]Laboratory of Biological Chemistry, Medical School, University of Ioannina, 45110 Ioannina, Greece
[4]Confocal Laser Scanning Microscopy Unit, Network of Research Supporting Laboratories, University of Ioannina, Ioannina, 45110, Greece

Retinal diseases such as diabetic retinopathy and age-related macular degeneration are the major causes of blindness nowadays. It has been recently shown that the dysfunction in the relationship between the neuroretina and the vascular system (neurovascular unit-NVU) plays a crucial role in the pathophysiology of these diseases. In vitro retinal model development has gained momentum due to the inadequacy of animal models in replicating the structure and function of the human retina. Human embryonic and induced pluripotent stem cell (iPSC)-derived retinal organoids (ROs) have demonstrated diverse applications, such as investigating human retinogenesis, modeling diseases, drug discovery, and potential cell therapy. Multiple protocols have been established to generate retinal organoids, aligning with fundamental principles of forebrain and eye development, in which the consistent laminar organization and the presence of all neural cell types within the retinal structures is significant. However, ROs that have been generated and differentiated from pluripotent stem cells (PSCs) lack vascularization and thus their maturation is impaired. Therefore, the advancement of reliable experimental model systems in order to study the NVU is an urgent need and the generation of human retinal organoids (ROs) is the ideal approach to do this, given the limitations of the use of experimental animal models. Our work is focused on the generation of ROs from human PSCs consisting of both neuronal and vascular cells (endothelial and mural cells- ECs, MCs). We have already generated and extensively characterized ECs and MCs derived from hPSCs/hiPSCs. Furthermore, hPSCs/hiPSCs derived ROs have been generated and characterized using a sequential step strategy, mimicking the spatio-temporal development of the retina in vivo. Our plan is to vascularize these ROs in order to develop the retinal NVU (rNVU) in the best anatomical layout. Furthermore, our in vitro rNVU will serve as a model to elucidate the pathophysiology of Retinitis Pigmentosa (RP) (an inherited disease causing blindness) using patient-derived iPSCs with a PRPF31 mutation, known to be responsible for RP development.

P57

Effect of graphene and graphene oxide on human pluripotent and vascular cells

Maria Markou, Kostas Spyrou, Athanasia Zoi Pappa, Sofia Bellou, Eleni Bagli, Dimitrios Gournis, Theodore Fotsis, Carol Murphy*

[1]Biomedical Research Institute (BRI) -FORTH, Ioannina, Greece.
[2]Department of Material Sciences and Engineering, University of Ioannina, Greece.
[3]Laboratory of Biological Chemistry, Medical Department, School of Health Sciences. University of Ioannina, Greece.
Corresponding author: Carol Murphy, email: carol_murphy@bri.forth.gr

The goal of tissue engineering is to assemble functional constructs that restore, maintain, or improve damaged tissues or whole organs. A major requirement for viability and function of the implantable construct is the availability of blood vessels to support its in vivo growth. Regenerating tissue over 100–200μm exceeds the capacity of nutrient supply and waste removal by diffusion, and requires a vascular network. It takes several weeks for a scaffold to become fully vascularised in vivo, and without a rapid and high level of vascularisation of the transplanted grafts, the majority of cells fail to survive the early post-transplantation phase. Blood vessels consist of two main cell types, endothelial (ECs) and mural cells (MCs), such as pericytes and vascular smooth muscle cells (vSMCs), and both cell types must be incorporated to achieve a stable vasculature.

Biomaterial scaffolds are increasingly used due to their ability to provide a desirable micro-environment, guiding and enhancing the proliferation and differentiation of cells into specific tissue types. One such biomaterial, graphene, offers numerous benefits for tissue engineering including electrical conductivity, flexibility, and the ability to absorb protein and other low molecular weight molecules, altering stem cells differentiation and proliferation. In the present work, we investigated the employment of graphene nanomaterials as scaffolds capable of being vascularised.

Towards this purpose, we differentiated human pluripotent stem cells (hPSCs) to ECs and vSMCs and tested the effect of graphene on (i)the expression of pluripotent stem cell markers, (ii)the mitotic potential of ECs and vSMCs, (iii)the expression of MC phenotype markers and (iv)finally the formation and sprouting of vascular organoids.

Additionally, we have addressed the internalisation of graphene and found no damage to intracellular organelles. In conclusion, we suggest that 30μm diameter graphene at 1μg/ml is the optimal for use employing hPSCs, ECs, vSMCs and vascular organoids.

P58

Novel roles of ILK in Drosophila follicle epithelium

Athena Keramidioti[1,2], Irene Kagianni[1,3], Demosthenes Mitrossilis[1], Maria Boulougari[1,4], Elisavet Lotsi[1], Yiannis Gallos[5], Katerina Magiorou[4], Katerina M. Vakaloglou[1], Christos G. Zervas[1,*]

[1]Biomedical Research Foundation, Academy of Athens, Center of Basic Research, Athens, Greece
[2]University of Thessaly, Department of Biochemistry & Biotechnology, Larissa, Greece
[3]University of Ioannina, Department of Biological Applications & Technology, Ioannina, Greece
[4]Agricultural University, Department of Biotechnology, Athens, Greece
[5]National Technical University of Athens, School of Applied Mathematical & Physical Sciences, Athens, Greece

In the developing animal, cells change shape, reorganize their adhesion properties and assemble in distinct layers to form three dimensional tissues and organs. A major engine of tissue morphogenesis is the mechanical force, generated by molecular motors such as the non-muscle myosin II pulling on filamentous actin (F-actin). Different actin networks are formed in cells, facilitated by cell-ECM adhesion and reorganize upon tension, namely cortical actin, stress fibers, filopodia and lamellipodia. The different intracellular actin networks necessitate fine regulation in space and time, as misplaced or untimely transitions impair organ formation and lead to pathologies including tumor invasion. This highlights further the need to elucidate the molecular mechanisms of how the cell-ECM adhesion regulates actin networks in vivo and understand how forces fine-tune different actin networks in the entire tissue.

Here, we have analyzed how Integrin-Linked Kinase- a highly conserved integrin adhesome member- orchestrate the spatiotemporal organization of the actin-myosin network and maintain the epithelial sheet architecture during the morphogenesis of Drosophila egg chamber. The latter is an elegant model system that consists of the oocyte encircled by a monolayer of somatic follicle epithelial cells, which undergo specific cell shape changes and collective movements that sculpt the tissue. We have combined the powerful genetic tools of Drosophila and employed high-resolution live-imaging of egg chambers. Our data uncovered novel roles of ILK in epithelia: a) ILK tunes the pace of the acto-myosin oscillations in the basal side of the epithelium and b) ILK stabilizes integrin adhesions and thus preventing uncontrolled collective cell movements that disrupt the epithelial architecture.

P59

Crosstalk between Neural Stem Cells and Platelets: Insight from co-cultures under differentiation conditions

Maria Nousia[1*], Maria Anesti[1], Aggeliki Dimopoulou[1], Ilias Kazanis[1*]

[1]Laboratory of Developmental Biology, Department of Biology, University of Patras, Patras, Greece

In the postnatal brain of mammals, there are neurogenic niches where undifferentiated, self-renewing cells, called Neural Stem Cells (NSCs), are located. The lateral walls of the lateral ventricles host one of these niches, the Supebendymal Zone (SEZ). The SEZ microenvironment is supported by specialized vascularization, which delivers nutrients and signaling molecules across the blood-brain barrier. Platelets (PLTs), which are small, disk-shaped cell fragments, besides forming the haemostatic plug, are also involved in tissue regeneration through the release of stored bioactive molecules. It has been demonstrated that PLTs aggregate in the SEZ after a focal demyelinating lesion in the supraventricular corpus callosum of mice (Kazanis et al., 2015). In this study, we isolated NCSs and PLTs from mice and cocultured them for three days under NSC differentiation conditions investigating three different PLT densities. We assessed cell viability and the occurrence of apoptotic cell death, using DAPI staining. In addition, we determined the differentiation potential of NSCs through the immunocytochemical labeling of neuroblasts (that express doublecortin (DCX)) , astrocytes (that express GFAP) and cells of the oligodendrocyte lineage (that express OLIG2). We also examined the NSC progenitor identity using the expression of SOX2 transcription factor. Our results add more information into the ongoing investigation of the presence of a functional interaction between PLTs and NSCs and the effects of different platelet densities.

The work was funded by a grant from the Hellenic Foundation for Research and Innovation (HFRI-FM17-3395) to IK.

1. *Kazanis, I. et al. (2015). Lesion-induced accumulation of platelets promotes survival of adult neural stem / progenitor cells. Experimental Neurology, 269, 75–89. https://doi.org/10.1016/j.expneurol.2015.03.018*

P60

A cell competition CRISPR screen to identify genes regulating fitness in primary esophageal keratinocytes

Argyro Kalogeropoulou[1*], Ujjwal Banerjee[1], Albert Herms[1], Phil Jones[1]

[1]Wellcome Sanger Institute, Hinxton, Cambridge, UK

Somatic mutations are accumulated in human esophageal epithelium (EE) progressively with age. By middle age, EE is a patchwork of mutant clones under strong positive selection, meaning that mutations confer a competitive advantage over wild-type cells[1]. Mutated genes that increase fitness of normal cells have been identified through sequencing studies of aging human tissues or though long-term experiments in appropriate mouse models. Here, we apply a CRISPR/Cas9 gene KO screen in 3D epithelioid cultures of mouse esophagus to identify the genetic networks that modulate cellular fitness. We have recently shown that the esophageal epithelioids reach a homeostatic state in which proliferation and differentiation of keratinocyte progenitors are balanced, permitting the analysis of cell competition for long periods[3]. Based on gene expression profiling, we designed a custom library of 65.000 sgRNAs targeting every gene expressed (>0.01 FPKM) in epithelioids. The lentiviral gRNA library was transduced into the cells in low MOI (~ 0.5) so each cell would be infected with a single gRNA. Following transduction, mutant and wild-type progenitor cells compete for the limited space in the basal layer of the culture, to the physiological condition in esophagus. Cells were collected 3 weeks post transduction and genomic DNA sequenced to identify gRNA abundance. The results validated genes known to be positively selected in sequencing studies of ageing epithelium in vivo and identified new regulators of cell fitness. The genes cluster into pathways that regulate progenitor cell fate. Overall, this system of cell competition CRISPR screening provides a new platform to explore the genetic network of competitive fitness and offers new grounds of research towards cancer prevention strategies.

1. *Martincorena I, et al. Somatic mutant clones colonize the human esophagus with age. Science. 2018 Nov 23;362(6417):911-917. doi: 10.1126/science.aau3879.*
2. *Colom B, et al. Spatial competition shapes the dynamic mutational landscape of normal esophageal epithelium. Nat Genet. 2020 Jun;52(6):604-614. doi: 10.1038/s41588-020-0624-3.*
3. *Herms A, et al. Epithelioids: Self-sustaining 3D epithelial cultures to study long-term processes. BioRXiv 2023. doi: https://doi.org/10.1101/2023.01.03.522589*

P61

Structural investigation of DTX3L domains

Nikolaos K. Fourkiotis#[1], Aikaterini C. Tsika#[1], Konstantina P. Kravvariti[1], Sofia-Antigoni Tsatsouli[1], Maria Birkou[1], Georgios A. Spyroulias[1*]

[1]Department of Pharmacy, University of Patras, 26504 Patras, Greece

Human Deltex (DTX) proteins belong to the family of RING-type E3 ligases that recently gained increased attention because they regulate a lot of signalling pathways and are implicated, among others, in cancer and viral infections. The DTX family is comprised of five members, which are characterized by a C-terminal region containing a RING domain and a Deltex C-terminus (DTC) domain. The latter is a feature that groups DTX proteins into two evolutionary clades, with DTX1, 2, and 4 belonging to the first and DTX3 and Deltex3-like (DTX3L) to the second. The RING domain that precedes it sequentially is responsible for mediating the interaction with E2 enzymes and the subsequent transfer of ubiquitin to the target proteins. Regarding their N-termini, DTX1, 2, and 4 contain WWE domains, that facilitate the interaction with PARylated substrates. Instead, at the respective region of DTX3L are found two domains called D1 and D2, responsible for oligomerization of the protein, while the proline-rich central region of DTXs is replaced by a distinctive D3 domain, which is the interaction region with its intracellular partner PARP9. Taken all the above into consideration, DTX3L is the most divergent member of the DTX family. There is only one available experimental structure submitted in PDB concerning its DTC domain.

Herein, we present the preliminary structural and dynamical characterization of human DTX3L

polypeptides through high-resolution NMR spectroscopy at the atomic level. The results of our study are important for the further biochemical characterization of this molecule since they provide the basis to map and define the crucial domain regions and residues for interaction with PARP9. Note that these data can contribute to the design of molecules that can alter the specific complex formation.

Acknowledgements
We acknowledge support the project "INSPIRED" (MIS 5002550), under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020), and by the European Union's HE programs (2021-2027), "REMOTE-NMR" (GA 101058595) and "ESPERANCE" ERA Chair (GA 101087215).

References

[1] Vela-Rodríguez C, Lehtiö L. Activities and binding partners of E3 ubiquitin ligase DTX3L and its roles in cancer. Biochem Soc Trans. 2022 Dec 16;50(6):1683-1692. doi: 10.1042/BST20220501. PMID: 36421918.
[2] Zhu K, Suskiewicz MJ, Hloušek-Kasun A, Meudal H, Miko A, Aucagne V, Ahel D, Ahel I. DELTEX E3 ligases ubiquitylate ADP-ribosyl modification on protein substrates. Sci Adv. 2022 Oct 7;8(40):eadd4253. doi: 10.1126/sciadv.add4253. Epub 2022 Oct 5. PMID: 36197986.
[3] Scalia P, Williams SJ, Suma A, Carnevale V. The DTX Protein Family: An Emerging Set of E3 Ubiquitin Ligases in Cancer. Cells. 2023 Jun 21;12(13):1680. doi: 10.3390/cells12131680. PMID: 37443713; PMCID: PMC10340142

P62

Structural biology-based targeting of viral and human macrodomains

Aikaterini C. Tsika[#1], Nikolaos K. Fourkiotis[#1], Angelo Gallo[1], Christos Sideras- Bisdekis[1], Sofia-Antigoni Tsatsouli[1], Danai Moschidi[1], Athanasios Papakyriakou[2], Georgios A. Spyroulias[1*]

[1]Department of Pharmacy, University of Patras, 26504 Patras, Greece
[2]Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", 15341 Agia Paraskevi, Athens, Greece
**Correspondence to: G.A.Spyroulias@upatras.gr*
[#]Contributed equally

Macrodomains (MDs) constitute a structural family of modules with a distinctive a/b/a sandwich fold, found in all kingdoms of life and viruses as standalone entities or parts of large proteins. Despite their architectural similarities, they display functional variations, and their role as "readers" and/or "erasers" is indicative of their significance to the ADP-ribosylation process. In viruses, MDs are parts of large non-structural proteins. Recently, their biological function, linked to their de-MARylation capacity and thus to the inhibition of PARP-mediated antiviral activity, has rendered them potential drug targets. MacroPARPs refers to PARP9, PARP14, and PARP15 due to the tandem MDs that are embedded in their sequence, which are responsible for macroPARPs subcellular localization and their interaction with other cellular components.[1,2]

Herein, we present a comparative study of viral and human macroPARPs MDs with the objective of characterizing the structure, the dynamic properties, and the biochemical characteristics of previously unexplored members. Also, we aim to unravel subtle differences that can lead to the discovery and development of selective binders through targeted drug design, overcoming the sequence identity challenge between the MD family members. Using in silico screening, NMR-driven approaches, biophysical techniques, and biochemical assays, we discovered GS-441524 as a de-MARylation inhibitor that binds selectively to the SARS- CoV-2 MD in comparison to the other viral MDs and elucidated sequence elements that modify the binding affinity.[3] Furthermore, our research expanded to the quest for small molecules as MDs' ligands, spanning a wide range of organic scaffolds, with the aim of identifying new compounds as MDs' inhibitors.

Acknowledgements
We acknowledge support the project "INSPIRED" (MIS 5002550), under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020), and by the European Union's HE programs (2021-2027), "REMOTE-NMR" (GA 101058595) and "ESPERANCE" ERA Chair (GA 101087215).

References

1. *Rack JGM, Zorzini V, Zhu Z, Schuller M, Ahel D, Ahel I. Viral macrodomains: a structural and evolutionary assessment of the pharmacological potential. Open Biol. 2020 Nov;10(11):200237. doi: 10.1098/rsob.200237. Epub 2020 Nov 18. PMID: 33202171; PMCID: PMC7729036.*
2. *Rack JG, Perina D, Ahel I. Macrodomains: Structure, Function, Evolution, and Catalytic Activities. Annu Rev Biochem. 2016 Jun 2;85:431-54. doi: 10.1146/annurev-biochem-060815-014935. Epub 2016 Jan 29. PMID: 26844395.*
3. *Tsika AC, Gallo A, Fourkiotis NK, et al. Binding Adaptation of GS-441524 Diversifies Macro Domains and Downregulates SARS-CoV-2 de-MARylation Capacity. J Mol Biol. 2022;434(16):167720. doi:10.1016/j.jmb.2022.167720.*

P63

Structure guided discovery of new generation antimalarial drugs

Gerorge A. Stravodimos, Anastasia S. Tsagkarakou, Symeon M. Koulas, Demetres D. Leonidas, Anastasia L. Kantsadi*

Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis Campus, 41500, Larissa, Greece

Plasmodium falciparum, the human pathogen responsible for the most dangerous malaria infection, survives and develops in mature erythrocytes through the export of proteins needed for remodeling of the host cell. This process is essential for the development of the parasite and is associated with the pathology of the infection. Among the exported proteins are heat shock proteins, functioning as molecular chaperones that are proposed to be highly adapted to the malaria parasite lifecycle [1,2]. Many studies have shown that the exported PfHsp70-x chaperone and its co-chaperones Hsp40s PFE0055c and PFA0660w are involved in facilitating protein folding, stabilization, degradation, and translocation across membranes. Changes in the protein profile of parasites are one of the major challenges to the development of robust antimalarial drugs and hence targeting chaperones involved in protein folding and function could represent a new avenue for antimalarial drug development [3,4]. Here we present our progress towards the structure-guided development of a new generation of antimalarial drugs to protect the infection of human erythrocytes. More specifically, we report our efforts combining in silico and structural biology methods with the aim to develop novel compounds by specifically targeting different subsites within the PfHsp70-x catalytic domain. Moreover, we report the first crystal structure of the Hsp40 co-chaperone PFE0055c. These results will help us to gain insight into the complexity of the Hsp70- Hsp40 interactions and utilize the potential of designing compounds with enhanced and selective inhibitory potency against PfHsp70–PfHsp40 over the human orthologues. Such an understanding may allow us to not only develop and validate new leads for therapeutic development to sustain current disease control strategies but also to use them as tools to dissect the network of chaperone proteins in the infected erythrocyte.

Acknowledgements

The research project is supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "3rd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers" (Project Number: 7453). This work has been also supported by iNEXT-Discovery (PID 24711), funded by the Horizon 2020 program of the European Commission.

References

[1] Day, J., et al., 2019, FASEB J. 33, 14611-14624
[2] Daniyan M. O., et al., 2016, PLoS One 11, e014851
[3] Külzer S., et al., 2012, Cell. Microbiol. 14, 1784-1795
[4] Harm H. Kampinga and Elizabeth A. Craig, 2010, Nat Rev Mol Cell Biol. Aug; 11(8): 579-592

P64

Structure based inhibitor design studies against human liver Glycogen Phosphorylase, a pharmaceutical target for diabetes type 2

Symeon M. Koulas [1], Rachel Mathomes[2], Ioannis Tsialtas[1], George A. Stravodimos[1], Panarat Arunrattiyakorn[3], Anna-Maria G. Psarra[1], Joseph M. Hayes[2], Demetres D. Leonidas[1]*

[1]Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis Campus, 41500, Larissa, Greece
[2]School of Pharmacy & Biomedical Sciences, University of Central Lancashire, Preston, PR1 2HE, United Kingdom
[3]Department of Chemistry, Srinakharinwirot University, Bangok, Thailand
**Correspondence to: ddleonidas@bio.uth.gr*

Diabetes mellitus is a chronic metabolic disorder characterized by high levels of fasting and post prandial glucose in the bloodstream because of insulin resistance and relative insulin deficiency[1]. Patients suffering with diabetes will eventually develop multiple complications such as nephropathy, neuropathy, retinopathy, diabetic foot ulcers, ketoacidosis, and even high risk of cardiovascular diseases like hypertension etc.[2] Inhibition of glycogenolysis has been proposed as a therapeutic strategy for the treatment of type 2 diabetes[3]. Glycogen phosphorylase (GP; E.C. 2.4.1.1) is the most well studied enzyme in glycogen metabolism[4]. It catalyzes the first step in the intracellular degradation of glycogen to yield α-D-glucose 1-phosphate5. Because of its central role in glucose homeostasis, GP has been exploited for the discovery of potent and specific inhibitors[4, 6-8] which may be used as anti- hyperglycemic agents[9]. Apart from the catalytic site, X-ray crystallography has revealed a number of different GP binding sites (allosteric, new allosteric, inhibitor, quercetin, and glycogen storage)[3]. GP is an allosteric protein and transition from T to R state is achieved by modulators such as AMP, ATP, IMP or glucose-6-phosphate, which induce a disordering of the 280s loop (residues 282-287) that blocks the entrance to the active site[10].

In this study a series of polyphenol compounds have been tested against human liver GPa (hlGPa), the pharmaceutical target, and the crystal structures of rmGPb in complex with the most efficient compounds have been determined through X-ray crystallography to elucidate the structural basis of their inhibitory potency. Baicalein was revealed as a good inhibitor of hbGPa (Ki = 32.5 μM), a low micromolar inhibitor of rmGPb and hlGPa (Kis < 10 μM) and was effective in reducing glycogenolysis (IC50 was 119.6 μM) in a hepatocarcinoma HepG2 cellular model. X-ray crystallography determination of the rmGPb – baicalein complex revealed binding at the inhibitor site and the interactions responsible for the observed potency[11]. This study further contributes to the development of novel and potent inhibitors through structure- based inhibitor design to combat type 2 diabetes.

Acknowledgements
This research is co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme "Human Resources Development, Education and Lifelong Learning" in the context of the Act "Enhancing Human Resources Research Potential by under- taking a Doctoral Research" Sub-action 2: IKY Scholarship Programme for PhD candidates in the Greek Universities implemented by the State Scholarships Foundation (IKY). This work has been also supported by iNEXT-Discovery, grant number 871037, funded by the Horizon 2020 program of the European Commission.

References

1. *DeFronzo, R. A.; Ferrannini, E.; Groop, L.; Henry, R. R.; Herman, W. H.; Holst, J. J.; Hu, F. B.; Kahn, C. R.; Raz, I.; Shulman, G. I.; Simonson, D. C.; Testa, M. A.; Weiss, R., Type 2 diabetes mellitus. Nat Rev Dis Primers 2015, 1, 15019.*
2. *Rocha, S.; Aniceto, N.; Guedes, R. C.; Albuquerque, H. M. T.; Silva, V. L. M.; Silva, A. M. S.; Corvo, M. L.; Fernandes, E.; Freitas, M., An In Silico and an In Vitro Inhibition Analysis of Glycogen Phosphorylase by Flavonoids, Styrylchromones, and Pyrazoles. Nutrients 2022, 14 (2).*
3. *Leonidas, D. D.; Hayes, J. M.; Kato, A.; Skamnaki, V. T.; Chatzileontiadou, D. S.; Kantsadi, A. L.; Kyriakis, E.; Chetter, B. A.; Stravodimos, G. A., Phytogenic Polyphenols as Glycogen Phosphorylase Inhibitors: The Potential of Triterpenes and Flavonoids for Glycaemic Control in Type 2 Diabetes. Curr Med Chem 2017, 24 (4), 384-403.*
4. *Oikonomakos, N. G., Glycogen phosphorylase as a molecular target for type 2 diabetes therapy. Curr Protein Pept Sci 2002, 3 (6), 561-86.*
5. *Newgard, C. B.; Hwang, P. K.; Fletterick, R. J., The family of glycogen phosphorylases: structure and function. Crit. Rev. Biochem. Mol. Biol. 1989, 24 (1), 69-99.*
6. *Somsak, L.; Czifrak, K.; Toth, M.; Bokor, E.; Chrysina, E. D.; Alexacou, K. M.; Hayes, J. M.; Tiraidis, C.; Lazoura, E.; Leonidas, D. D.; Zographos, S. E.; Oikonomakos, N. G., New inhibitors of glycogen phosphorylase as potential antidiabetic agents. Curr Med Chem 2008, 15 (28), 2933-83.*
7. *Drakou, C. E.; Gardeli, C.; Tsialtas, I.; Alexopoulos, S.; Mallouchos, A.; Koulas, S. M.; Tsagkarakou, A. S.; Asimakopoulos, D.; Leonidas, D. D.; Psarra, A. G.; Skamnaki, V. T., Affinity crystallography reveals binding of pomegranate juice anthocyanins at the inhibitor site of glycogen phosphorylase: the contribution of a sugar moiety to potency and its implications to the binding mode. J. Agric. Food. Chem. 2020, 68 (37), 10191-10199.*
8. *Fischer, T.; Koulas, S. M.; Tsagkarakou, A. S.; Kyriakis, E.; Stravodimos, G. A.; Skamnaki, V. T.; Liggri, P. G. V.; Zographos, S. E.; Riedl, R.; Leonidas, D. D., High Consistency of Structure- Based Design and X-Ray Crystallography: Design, Synthesis, Kinetic Evaluation and Crystallographic Binding Mode Determination of Biphenyl-N-acyl-beta-d-Glucopyranosylamines as Glycogen Phosphorylase Inhibitors. Molecules 2019, 24 (7).*
9. *Rines, A. K.; Sharabi, K.; Tavares, C. D.; Puigserver, P., Targeting hepatic glucose metabolism in the treatment of type 2 diabetes. Nat. Rev. Drug Discov. 2016, 15 (11), 786-804.*
10. *Barford, D.; Johnson, L. N., The allosteric transition of glycogen phosphorylase. Nature 1989, 340 (6235), 609-16.*
11. *Mathomes, R. T.; Koulas, S. M.; Tsialtas, I.; Stravodimos, G.; Welsby, P. J.; Psarra, A. G.; Stasik, I.; Leonidas, D. D.; Hayes, J. M., Multidisciplinary docking, kinetics and X-ray crystallography studies of baicalein acting as a glycogen phosphorylase inhibitor and determination of its' potential against glioblastoma in cellular models. Chemico-biological interactions 2023, 382, 110568.*

P65

Large Scale Molecular Dynamics Simulations Shed Light on Steps of the Catalytic Cycle of TYK2 Kinase: Implications in Diseases

Nastazia Lesgidou, Metaxia Vlassi*

Protein Structure & Molecular Modeling laboratory, Institute of Biosciences & Applications, National Center for Scientific Research "DEMOKRITOS", Athens, 15310, Greece
**Corresponding author: (MV) meta@bio.demokritos.gr*

Eukaryotic protein kinases are highly dynamic enzymes with elaborate and specific interactions that control key processes in human cells. As they pass through their catalytic cycle, their regulation mechanism relies on their conformational plasticity and concerted motions that create a dynamic allosteric network of communications within their catalytic domains (KD).

Tyrosine Kinase 2 (TYK2) is a non-receptor tyrosine kinase that belongs to the Janus Kinase family (JAK) and is involved in various signaling pathways with major role in the pathogenesis of several diseases, including autoimmune diseases and many types of cancer. In order to elucidate the atomic details of TYK2 regulation mechanism it is vital to explore the dynamics and the structural changes along different stages of its catalytic cycle. This can be achieved through the application of Molecular dynamics (MD) simulations. MD simulations have evolved into a powerful tool that allows the prediction of large conformational changes of proteins, over time, when it is not possible experimentally.

In previous work (Lesgidou et al. 2018), we employed MD simulations to explore the dynamics of the APO form of the TYK2 KD compared to that of a variant (P1104A) that affects kinase activity and is associated with cancer albeit protective against autoimmune diseases. In the work presented here, we used large-scale (microsecond-scale; μs), all-atom MD simulations and investigated the conformational dynamics of the TYK2-KD along three different stages of its catalytic cycle (APO, ATP.1Mg, ATP.1Mg/substrate-peptide). Analysis of the corresponding MD-trajectories identified flexible and highly dynamic areas that are linked to kinase activity and pointed to amino-acids with key roles in its regulation mechanism. Validating our findings, several of the identified amino-acids are associated with cancer-related mutations.

References

- *Lesgidou N, Eliopoulos E, Goulielmos G. and Vlassi M. (2018) Insights on the alteration of functionality of a tyrosine kinase 2 variant: a molecular dynamics study. Bioinformatics, 34(17), i781–i786*

Acknowledgments

We acknowledge support of this work by the projects: "INSPIRED-The National Research Infrastructures on Integrated Structural Biology, Drug Screening Efforts and Drug target functional characterization" (MIS 5002550) implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", and "SANITURA-Target Identification and Development of Novel Approaches for Health and Environmental Applications" (MIS 5002514) implemented under the Action for the Strategic Development on the Research and Technological Sectors, both funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).The computational time required for this work was granted from the Greek Research & Technology Network (GRNET) in the National HPC facility -ARIS- under project IDs: KIN_IMMUNMD_II to _IV.

P66

Expression, isolation and crystallization of the 3'-5' exonuclease of the nucleoprotein of the LCMV virus

Angelos Kontarinis[1*], Maria Spiliopoulou[1], Nicolas Papageorgiou[2,3], Maria Alevizou[1], Bruno Canard[2,3,4], François Ferron[2,3,4], Irene Margiolaki[1]

[1]Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, GR-26500, Patras, Greece.
[2]Aix-Marseille Université, AFMB UMR 7257, 13288 Marseille.
[3]Architecture et Fonction des Macromolécules Biologiques, CNRS - UMR 7257, Polytech Case 925, 13009 Marseille, France.
[4]European Virus Bioinformatics Center, Leutragraben 1, 07743 Jena, Germany.

Lymphocytic Choriomeningitis virus (LCMV) is a member of the Arenaviridae family, which includes some of the world's deadliest viruses and encode a 3'– 5' exonuclease in their genome. LCMV causes the disease of aseptic lymphocytic choriomeningitis and is in a small percentage fatal. In this project we are studying LCMV exonuclease (ExoN). This ExoN is contributing to the silencing of innate immunity and to a yet unclear process is seemingly playing a role in replication [1]. Wild type (WT) and mutant (MT) forms of this ExoN have successfully been expressed, while a mutation associated with the deletion of a 12-residue flexible region ('basic loop') above the active site of the protein is also examined. The precise role of this region is unclear, but mutation of its basic residues decreases the exonuclease activity [2]. So, the structural and functional exploration of these proteins could constitute as an ideal target for antiviral medications and vaccines for LCMV.

In this study we present crystallization screens of the WT 3'-5' exon protein via the vapor diffusion method through the sitting drop technique around bibliographic conditions. This work is in continuation with previous studies of our research team, based on the LCMV wild type ExoN, where single crystal diffraction data allowed the identification of the monoclinic crystal symmetry (space group P21) and the refinement of the protein structure led to a model of 2.8 Å resolution (PDB ID: 8cnl) [3]. In addition, we present the obtained results using commercial crystallization screening kits.

Our aim is to produce high quality protein crystals, for Synchrotron X-ray Single Crystal and Powder Diffraction measurements in order to investigate the protein's polymorphism upon variation of its physicochemical environment while high resolution structural models will constitute a key step in elucidating the intrigued function of the protein both in wild type and mutant, may lead to new strategies for drug development.

[1] Papageorgiou, N., Spiliopoulou, M., Nguyen, T.-H. V., Vaitsopoulou, A., Laban, E. Y., Alvarez, K., Margiolaki, I., Canard, B., & Ferron, F. (2020). Brothers in Arms: Structure, Assembly and Function of Arenaviridae Nucleoprotein. Viruses, 12(7), 772. https://doi.org/10.3390/v12070772.
[2] Hastie, K. M., Kimberlin, C. R., Zandonatti, M. A., MacRae, I. J., & Saphire, E. O. (2011). Structure of the Lassa virus nucleoprotein reveals a dsRNA-specific 3 to 5 exonuclease activity essential for immune suppression. Proceedings of the National Academy of Sciences, 108(6), 2396–2401. https://doi.org/10.1073/pnas.1016404108.
[3] Spiliopoulou, M. (2023), Structural Study of Biological Macromolecules for the creation of Pharmaceutical Targets [PhD Thesis, University of Patras].

P67

Alphaionic, a python script for implementing metal additions on Alphafold protein structure predictions

Spyridon Marios Giatro[1], Michail Papadourakis[2], Vasilis Panagiotopoulos[1,2], Minos-Timotheos Matsoukas[1]

[1]Department of Biomedical Engineering, University of West Attica, Egaleo, Greece
[2]Cloudpharm Private Company, Greece

Alphafold is a deep learning software that provides 3D structural models of proteins given their amino acid sequence with an unprecedented accuracy. To enrich Alphafold's models, Alphafill adds potential ligands, cofactors and (metal) ions to Alphafold models based on sequence identity with experimentally resolved structures from Protein Data Bank. The confidence for each addition is evaluated by certain scores regarding the alignment, the sequence identity and the Van de Waals overlaps. However, those structures cannot be subjected to molecular simulations such as docking or dynamics. To overcome this limitation, we created a python-based pipeline, that removes all the heteroatoms added except Fe, Mn, Co, Ni, Mg, Zn, Ca, K, Cu, Na, and the cofactor Heme, and retains the ones with high confidence scores. Proximal additions are removed if they don't originate from the same pdb. Following this automatic process, metals or the ferric atom of heme are subjected to geometric and steric overlap evaluation adn are further refined. Finally, the structural model is automatically prepared, and energy minimized, using Gromacs, a molecular dynamics software, to avoid unnatural repulsions and steric clashes. The automated pipeline was originally performed and evaluated on a selected representative (~1000) dataset of protein models containing metal ios.

1 *Jumper, John, et al. "Highly accurate protein structure prediction with AlphaFold." Nature 596.7873 (2021): 583-589.*
2 *Hekkelman, Maarten L., et al. "AlphaFill: enriching AlphaFold models with ligands and cofactors." Nature Methods 20.2 (2023): 205-213.*

P68

The molecular recognition of carbohydrates by the human Starch Binding Domain-Containing Protein 1 (STBD1).

Serafeim Alexopoulos[1], Aikaterini I. Argyriou[2], Panagiotis Mastorakis[1], Georgios A. Spyroulias[2], Vasiliki Skamnaki[1*].

[1]Department of Biochemistry and Biotechnology, University of Thessaly, Larisa, Greece.
[2]Department of Pharmacy, University of Patras, Patras, Greece.
**corresponding author email address: vskamnaki@uth.gr*

Starch binding domain-containing protein 1 (STBD1) is a highly conserved carbohydrate-binding protein which is predominantly expressed in muscle and liver, and it is implicated in the metabolism and cellular trafficking.[1,2] Previous studies have shown that dysregulation of STBD1 causes multiple diseases, including cardiovascular disease, metabolic disease, and even cancer.[3] The human STBD1 has 358 amino acids and a MW of 39 KDa. In silico analysis has recognized an N-terminal hydrophobic sequence that allows STBD1 to bind the ER and a C-terminal CBM20 carbohydrate binding domain. It has been shown that the C-terminal domain binds glycogen related carbohydrates (e.g., amylose, amylopectin, and polyglucosans), allowing STBD1 to act as a carbohydrate cargo protein as well as glycogen related proteins, such as glycogen synthase (GS), glycogen debranching enzyme (DBE), and laforin. In addition, a mutation of a conserved tryptophan residue (W293) in CBM20 domain eliminated the ability of STBD1 to bind the carbohydrate amylose and caused the protein to degrade rapidly, pointing to the domain's importance in maintaining protein stability4. To date, our knowledge on the structural basis of carbohydrate recognition by STBD1 remains elusive. Towards this, our group has produced CBM20 protein of high yield and purity (6,5 mg/ml, purity>95%) suitable for biophysical and structural studies. Circular dichroism analysis reveals secondary structure motifs, mostly antiparallel β-sheets but no helical conformations. The binding of glucose, sucrose, β-cyclodextrin and glycogen is assessed using a combination of circular dichroism, fluorescence spectroscopy and isothermal calorimetry. The determination of the 3D structure of the CBM20-b-cyclodextrin complex is further pursued and crystallization trials are in progress.

1. *Jiang, S. et al. Starch binding domain-containing protein 1/genethonin 1 is a novel participant in glycogen metabolism. J Biol Chem 285, 34960-34971, doi:10.1074/jbc.M110.150839 (2010).*
2. *Bouju, S. et al. Molecular cloning and functional expression of a novel human gene encoding two 41-43 kDa skeletal muscle internal membrane proteins. Biochem J 335 (Pt 3), 549-556, doi:10.1042/bj3350549 (1998).*
3. *Tang, Q,Liu M, Zhao H, Chen L. Glycogen-binding protein STBD1: Molecule and role in pathophysiology J Cell Physiol. 2023 Sep;238(9):2010-2025. doi: 10.1002/jcp.31078. Epub 2023 Jul 12.*
4. *Zhu, Y., Zhang, M., Kelly, A. R., & Cheng, A. (2014). The carbohydrate binding domain of overexpressed STBD1 is important for its stability and protein protein interactions. Bioscience Reports, 34(4), e00117.*
5. *Lytridou, A. A. et al. Stbd1 promotes glycogen clustering during endoplasmic reticulum stress and supports survival of mouse myoblasts. J Cell Sci, doi:10.1242/jcs.244855 (2020).*

P69

High Resolution Structure and In Situ Structural Investigation of the Pharmaceutical Peptide Octreotide Upon Controlled Relative Humidity and Temperature Variation

Christina Papaefthymiou[1*], Maria Athanasiadou[1], Angelos Kontarinis[1], Maria Spiliopoulou[1], Dimitris Koutoulas[1], Marios Konstantopoulos[1], Stamatina Kafetzi[1], Natalia Dadivanyan[2], Detlef Beckers[2], Thomas Degen[2], Kleomenis Barlos[3], Kostas K. Barlos[3], Fabia Gozzo[4], Catherine Dejoie[5], Irene Margiolaki[1*]

[1]Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, GR-26500, Patras, Greece
[2]Malvern Panalytical BV, Lelyweg 1, 7602 EA Almelo, The Netherlands
[3]CBL-Patras, Patras Industrial Area, Block 1, Patras, Greece
[4]Excelsus Structural Solutions (Swiss) AG, Park Innovaare, Villigen, 5234, Switzerland.
[5]ESRF, 71 Avenue des Martyrs, CS40220, 38043 Grenoble Cedex 9, France

Octreotide is a somatostatin analog with eight amino acids and a widely used pharmaceutical activity. The successful analysis of octreotide's polycrystalline precipitates, lead to its debut structural deposition in the PDB (ID: 6vc1) that was revealed via X-ray Powder Diffraction (XRPD) 1,2. This study focuses on the recent high-resolution data obtained in the upgraded ID22 in the ESRF and the response exhibited by octreotide microcrystals when exposed to varying humidity levels, with specific attention directed towards the stability of the polycrystalline sample. To gather in-situ diffraction data, a series of measurements were conducted using the Anton Paar MHC-trans humidity chamber, which is integrated within a laboratory X'Pert Pro diffractometer manufactured by Malvern Panalytical. The primary function of the humidity chamber is to meticulously regulate and uphold accurate humidity and temperature conditions for samples during X-ray Powder Diffraction (XRPD) measurements 3,4. Following a comprehensive series of experimental measurements encompassing different levels of relative humidity and temperature, the analysis of collected data sets using the Pawley method, uncovered the impressive stability of the polycrystalline octreotide samples.

This examination disclosed the absence of any phase transitions in a range of 35 to 95% rH as well as 21 to 45 °C. However, a distinct structural transformation, evidenced by alterations in lattice parameters and unit cell volume, materializes when the sample undergoes controlled variations in temperature at different relative humidity levels. These findings underscore the imperative significance of accounting for environmental influences, such as humidity and temperature, within the framework of structure-based drug design and storage conditions of pharmaceuticals. In doing so, this research substantially contributes to the advancement of more efficacious and stable pharmaceutical products.

References

[1] Spiliopoulou, M. et al. New perspectives in macromolecular powder diffraction using single-photon- counting strip detectors: high-resolution structure of the pharmaceutical peptide octreotide. Cover article of Acta Crystallogr A Found Adv 77, 186–195 (2021) & Press Release at Scientific Highlights of the Paul Scherrer Institute (PSI-Switzerland).
[2] Fili, S. et al. Revisiting the structure of a synthetic somatostatin analogue for peptide drug design. Acta Crystallogr B Struct Sci Cryst Eng Mater 75, 611–620 (2019).
[3] Trampari, S. et al. In situ detection of a novel lysozyme monoclinic crystal form upon controlled relative humidity variation. J Appl Crystallogr 51, 1671–1683 (2018).
[4] Logotheti, S. et al. Unit-cell response of tetragonal hen egg white lysozyme upon controlled relative humidity variation. J Appl Crystallogr 52, 816–827 (2019)

P70

The 'linchpin' Arg983 of E3 Ubiquitin Ligase Arkadia and its role in the ubiquitination machinery

Georgia N. Delegkou[1], Nefeli Fragkaki[1], Maria Birkou[1], Tamara Toro[1], Konstantinos D. Marousis[1], Vasso Episkopou[2], Georgios A. Spyroulias[1*]

[1]Department of Pharmacy, University of Patras, 26504 Patras, Greece
[2]Department of Brain Sciences, Imperial College, London W12 0NN, UK
**e-mail: G.A.Spyroulias@upatras.gr*

Ubiquitin-mediated proteasomal degradation is a fundamental and tightly coordinated process that controls the cellular concentration of proteins. The process is catalyzed by the concerted action of activating (E1), conjugating (E2) and ligating (E3) enzymes. E3 ubiquitin ligases are responsible for substrate recognition, and their deregulation is associated with various diseases, most notably, cancer. The E3 ligase Arkadia (RNF111) targets for proteasomal degradation negative regulators of the TGF-β SMAD2/3 signaling pathway i.e., the inhibitory SMAD7[1], transcriptional co-repressor SKI and its close homologue SNON (SKIL)[2] and poly-SUMOylated proteins e.g., Promyelocytic Leukaemia protein (PML)[3]. Despite the pivotal role of Arkadia in many pathways, little is known about the features that modulate its enzymatic function. We have previously shown that, unlike most other E3 ligases, RING domain of Arkadia is not fully active and requires external elements to activate an E2~Ub complex[4]. This study attempts to provide insights into the role of Arg983, a key residue of Arkadia that is conserved amongst other RING domains. In this context, we demonstrate that although the introduction of R983A and R983K mutations to Arkadia did not affect the interaction with its physiological E2 partner UbcH5B, it disabled both ubiquitylation and oxyester hydrolysis assays. We also found that both Arkadia mutants delayed the initiation of ubiquitin chain assembly by the MMS2/UbcH13 E2 complex that acts synergistically with Arkadia in DNA damage response. Overall, we suggest that Arg983 controls the enzymatic activity of Arkadia serving as a so-called 'linchpin'5 that contributes to the shift in E2~Ub conformational equilibria toward the most favored 'closed conformation'.

Acknowledgments
This research is financed by the Hellenic Foundation for Research and Innovation (HFRI) under the 4th Call for HFRI PhD Fellowships (Fellowship Number: 11147).

References

1. *Daizo Koinuma et al. Arkadia amplifies TGF-b superfamily signalling through degradation of Smad7. EMBO J 22, 6458–6470 (2003).*
2. *Nagano, Y. et al. Arkadia induces degradation of SnoN and c-Ski to enhance transforming growth factor-β signaling. Journal of Biological Chemistry 282, 20492–20501 (2007).*
3. *Erker, Y. et al. Arkadia, a Novel SUMO-Targeted Ubiquitin Ligase Involved in PML Degradation. Mol Cell Biol 33, 2163–2177 (2013).*
4. *Birkou, M. et al. Unveiling the Essential Role of Arkadia's Non-RING Elements in the Ubiquitination Process. Int J Mol Sci 23, 10585 (2022).*
5. *Pruneda, J. N. et al. Structure of an E3:E2 Ub Complex Reveals an Allosteric Mechanism Shared among RING/U-box Ligases. Mol Cell 47, 933–942 (2012).*

P71

Insights into the mechanism and E2 specificity of E3 ubiquitin ligase Arkadia 2C

Christina Panagiotopoulou[1], Maria D. Politi[1], Maria Birkou[1], Konstantinos D. Marousis[1], Vasso Episkopou[2], Georgios A. Spyroulias[1]

[1]Department of Pharmacy, University of Patras, Patras, Greece
[2]Imperial College, London, United Kingdom

Cellular ubiquitination homeostasis is accomplished by the synchronized interplay of E1, E2 and E3 enzymes. Ub is activated by an Ub-activating (E1) enzyme and then is transferred to the active cysteine residue of an Ub-conjugating enzyme (E2). At the final step, Ub is transferred to a substrate via an Ub-ligating (E3) enzyme. E3 ligases can produce Ub links of various lengths and structures[1]. Mechanistically, E3 ligases are categorized in HECT, RING and RING-in-between-RING ligases (RBR). RING E3s, the largest class, tranfer Ub directly from E2~Ub to the substrate without the formation of a covalent E3-ub intermediate[2].

E3 ubiquitin ligase Ark2C is necessary for effective motor axon extension in the dorsal forelimb by enhancing the transcriptional responses of the Smad 1/5/8 effectors, which are activated downstream of the Bone Morphogenetic Pathway (BMP)[3]. Ark2C contains a characteristic RING-H2 domain in its C-terminus (canonical RING) that binds two zinc ions. RBRs contain RING1 (non-canonical RING) domains that are structurally similar yet mechanistically distinct from canonical RING domains. The fundamental difference is a short extension of 2-4 amino acids at the Zn (II) binding loop. Both types of E3 bind E2~Ub via their RINGs but canonical RING E3s promote closed E2~Ub conformations required for direct Ub transfer from the E2 to substrate, while RBR RING1s promote open E2~Ub to favor Ub transfer to the E3 active site. These distinct strategies used by structurally similar domains allow E3s to modulate E2 reactivity to specific biological outcomes[4].

Our study attempts to shed light on Ark2C specific features, that modulate its activity towards ubiquitination compared to its homologues, the non-canonical RING domains. Here we show via NMR titration experiments that when a standard Zn-binding loop is replaced by a “non-canonical” one, (like in HHARI, RBR ligase) then the RING may preserve its conformational characteristics, but may abolish its functional properties.

References

1. *Xu G, Jaffrey SR. Proteomic identification of protein ubiquitination events. Biotechnol Genet Eng Rev. 2013;29(1):73-109. doi: 10.1080/02648725.2013.801232. PMID: 24568254; PMCID: PMC3937853.*
2. *Yang, Quan et al. “E3 ubiquitin ligases: styles, structures and functions.” Molecular biomedicine vol. 2,1 23. 30 Jul. 2021, doi:10.1186/s43556-021-00043-2*
3. *Kelly CE, Thymiakou E, Dixon JE, Tanaka S, Godwin J, Episkopou V (2013) Rnf165/Ark2C Enhances BMP-Smad Signaling to Mediate Motor Axon Extension. PLoS Biol 11(4): e1001538.*
4. *Dove, Katja K et al. “Structural Studies of HHARI/UbcH7 Ub Reveal Unique E2 Ub Conformational Restriction by RBR RING1.” Structure (London, England : 1993) vol. 25,6 (2017): 890-900.e5. doi:10.1016/j.str.2017.04.013*

Acknowledgments

We acknowledge support of this work by the project “European Union’s HE program (2021-2027), “ESPERANCE” ERA Chair (GA 101087215).

P72

Structural determination of Phe964 Arkadia mutants via NMR Spectroscopy

Apostolos N. Koutsodimas1, Georgia N. Delegkou1, Maria Birkou1, Vasso Episkopou2, Georgios A. Spyroulias1

[1]Dept of Pharmacy, U of Patras, GR-26504, Greece,
[2]Department of Brain Sciences, Imperial College, London W12 0NN, UK
e-mail: G.A.Spyroulias@upatras.gr

Ubiquitination is a post-translational modification responsible for a variety of cellular functions including DNA repair, apoptosis, and protein degradation. Initially, ubiquitin is activated by the E1 enzyme in an ATP-depended manner. Afterwards, the activated ubiquitin is transferred to the catalytic cysteine of the E2 conjugating enzyme. In the final step, the E3 ligase facilitates the transmission of ubiquitin to the substrate. The target protein may undergo several types of ubiquitination each of which results in diverse outcomes[1].

Arkadia is an E3 ubiquitin ligase that positively regulates the transforming growth factor-β (TGF-β) pathway by promoting degradation of Smad7, c-Ski and SnoN. Arkadia possesses a RING-H2 domain at its C-terminus, which exhibits a canonical RING ββα topology consistent to other RING domains. The 3-turn a-helix and the two zinc-binding loops of Arkadia RING domain play a crucial role in the interaction with the E2 enzymes[2]. We have previously demonstrated that mutations in conserved residues of the RING domain result in structural alterations of the domain and therefore its interaction with the E2 enzyme[3]. In this study, we investigate the pivotal role of Phe964 of Arkadia, a sequentially and structurally conserved residue in many RING domains, via NMR spectroscopy conformational analysis. This residue is located at the hydrophobic core of the protein and is considered as a "linker" between the second and third metal binding motif. Phe964Arg mutant led the protein to an unfolded state. Phe964Ala mutant imposed chemical shift changes in the 1H-15N HSQC signal dispersion compared with the wild type one. The comparison of the NMR solution structures of the wt and the Phe964Ala RING mutant suggest that the replacement of Phe964 in the hydrophobic core by a smaller nonpolar residue, significantly change the RING structure. In order to define the interaction interface between the Phe964Ala mutant and the E2 UbcH5b NMR titration experiments were carried out.

Acknowledgments
This research is supported by the project "INSPIRED-The National Research Infrastructures on Integrated Structural Biology, Drug Screening Efforts and Drug target functional characterization" (MIS 5002550) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

References

1. *Pickart CM.et al. (2004). Ubiquitin: structures, functions, mechanisms. Biochimica et Biophysica Acta 1695, 55-72*
2. *Chasapis CT.et al. (2012). NMR-based insights into the conformational and interaction properties of Arkadia RINGH2 E3 Ub ligase. Proteins 80(5), 1484-9*
3. *Birkou M.et al. (2017). A residue specific insight into the Arkadia E3 ubiquitin ligase activity and conformational plasticity. Journal of Molecular Biology 429, 2373-2386.*

P73

Physicochemical and spectroscopical study of Nostoc sp. bacterial homolog of β1 H-NOX domain of human soluble Guanylyl Cyclase (sGC) in complex with sGC activators

Stefanos I. Gravalos*, Aikaterini I. Argyriou, Styliani A. Chasapi, Georgios A. Spyroulias

Department of Pharmacy, University of Patras, Patras, Greece
e-mail: G.A.Spyroulias@upatras.gr

Heme-Nitric oxide/Oxygen binding (H-NOX) domains are a family of gas-sensing hemoproteins that bind diatomic gases. In mammals, H-NOX domains are part of soluble Guanylyl Cyclase (sGC) and function as a sensor of NO, resulting in a series of conformational and charge changes which lead to the signal transduction and the enzymatic activation of sGC. sGC is a heterodimer enzyme composed of two subunits, alpha and beta. H-NOX domain, which is located at the N-terminus of the regulatory β1 subunit of sGC and bears a heme b molecule, seems to be crucial in catalysis of conversion of GTP (guanosine 5 -triphosphate) to cGMP (cyclic guanosine 3,5 -monophosphate) to this effect downstream changes in cellular homeostasis. The NO/sGC/cGMP signaling pathway is dysfunctional in many diseases, such as cardiovascular diseases. Due to sGC's essential role in NO/sGC/cGMP signaling pathway, significant research is being done to discover and develop therapeutic compounds that will restore and enhance enzyme's activity under pathological conditions. Nonetheless, the intricate structural mechanisms governing the recognition and positive modulation of sGC by these compounds remain shrouded in limited comprehension. In this direction, we examine the interaction and conformational changes of sGC activators with the bacterial stand-alone H-NOX protein from Nostoc sp. (Ns H-NOX) via Nuclear magnetic resonance (NMR). This bacterial domain closely resembles the H-NOX domain of sGC, both in terms of sequence and ligand binding, making it an excellent tool to investigate how the redox state of heme b determines the binding of activators. Our study attempts to identify which amino acids are involved in this binding, to elucidate conformational alterations and explain how these changes in key-regions/residues contribute to sGC's activation.

References

- *Argyriou, A.I. et al. (2021) 'Replacement of heme by soluble guanylate cyclase (sGC) activators abolishes heme-nitric oxide/oxygen (H-NOX) domain structural plasticity', Current Research in Structural Biology, 3, pp. 324–336. Available at: https://doi.org/10.1016/j.crstbi.2021.11.003.*
- *Chasapi, S.A., Argyriou, A.I. and Spyroulias, G.A. (2022) 'Backbone and side chain NMR assignment of the heme-nitric oxide/oxygen binding (H-NOX) domain from Nostoc punctiforme', Biomolecular NMR Assignments [Preprint]. Available at: https://doi.org/10.1007/s12104-022-10107-1.*
- *Makrynitsa, G.I. et al. (2021) 'Backbone and side chain NMR assignments of the H-NOX domain from Nostoc sp. in complex with BAY58-2667 (cinaciguat)', Biomolecular NMR Assignments, 15(1), pp. 53–57. Available at: https://doi.org/10.1007/s12104-020-09982-3.*

Acknowledgements

R-NMR project, funded from the European Union's Horizon Europe research and innovation programme (2021-2027), under grant agreement 101058595.

P74

Expression, isolation, and characterization of recombinant full binding sites of the human neuronal nicotinic acetylcholine receptor.

Anna Kordela[1,2], Marios Zouridakis[1*], Aikaterini I. Argyriou[3], Georgios A. Spyroulias[3], Elias Eliopoulos[2], Petros Giastas[1,2*]

[1]Structural Neurobiology Research Group, Department of Neurobiology, Hellenic Pasteur Institute, Athens, Greece
[2]Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Greece
[3]Department of Pharmacy, University of Patras, Greece
**Correspondence to: petrosgiastas@aua.gr; mzouridakis@pasteur.gr*

The nicotinic acetylcholine receptors (nAChRs) are ligand-gated cation channels, members of the Cys-loop superfamily. They are membrane glycoproteins that configure homo- or hetero-pentameric assemblies comprised of homologous or even identical subunits. In humans, the group of neuronal nAChRs consists of eight α (α2-7, α9-10) and three β (β2-4) subunits, bearing a N-terminal extracellular domain (ECD), four transmembrane domains, an intracellular loop and an extracellular C-terminal domain. nAChRs bind the naturally occurring neurotransmitter acetylcholine and other nicotinic agonists or antagonists. The binding site is located at the interface of two adjacent ECDs, contributing its principal (+) or complementary (-) sides. The (+) side determines the binding affinity of the bound ligand, while the far less conserved (-) side dictates the ligand selectivity of each nAChR subtype. Neuronal nAChRs are abundant in the central and peripheral nervous system, playing key roles in synaptic neurotransmission and serving as important pharmacological targets due to their involvement in several neurological disorders. In the present study, we expressed, isolated and characterized recombinant human nAChR subunit ECDs to understand better the pharmacological properties of fully assembled binding sites. For this cause, we expressed an α9-α9 ECD concatemer, the α9 ECD fused with a Leucine Zipper (α9LeuZip), and an α2-β2 ECD concatemer. The expression was performed in the eukaryotic system Pichia pastoris in the presence or absence of nicotine in the culture media. The formation of properly assembled dimers, in all cases, was confirmed by size exclusion chromatography and electrophoretic analysis. We also confirmed the formation of fully formed α9(+)/α9(-) binding sites in the α9-α9 concatemer and α9LeuZip constructs, using isothermal calorimetry with the antagonist methyllycaconitine as a binder. Our results show that this approach of concatenated or dimerization-prone nAChR subunit constructs leads to suitable material for crystallographic studies for the structural elucidation of fully assembled binding sites.

P75

Biochemical characterization of the odorant degrading carboxylesterase 7 from Bactrocera oleae

Christina E. Drakou[1*], Iraklis Koutmanis[1], Antonia Spanomitrou[1], Kostas D. Mathiopoulos[1], Demetres D. Leonidas[1]

[1]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, 41500, Greece

The olive tree, Olea europaea, is one of the most characteristic trees of the Mediterranean region. In addition to its nutritional importance, olive trees are of great social and economic importance to the people cultivating them. Olive orchards are threatened by various pests and especially by the olive fruit fly Bactrocera (Dacus) oleae (Rossi), since it is a homodynamic monophagous insect which reproduces and develops during the year in favorable climates [1].

Female flies lay their eggs in olive fruits just before they become ripe. Emerging larvae feed upon the pulp of the fruit causing serious damage to fruits (premature drop and weight loss) resulting in serious quantitative and qualitative damage to table products and olive oil production [2].

Traditional suppression methods to control infestation of olive orchards, such as sprays with organophosphate and pyrethroid based insecticides, had serious side effects to the environment and to human health, and led to the development of insecticidal resistance to some populations. Metabolic resistance to pyrethroids and organophosphates has been associated to carboxyl-esterase (CXE) mediated hydrolytic degradation [3].

Odorant CXEs represent the first step in odorant and pheromone degradation, and the detoxification of the insect olfactory system [4]. Biochemical characterization of insect odorant CXEs is therefore essential, since inhibiting this step may prove crucial in finding new ways to control insect pests.

Toward this end, we studied the expression profile of B. oleae's odorant carboxylesterase 7 (BolEST7) in all the developmental stages of wild and laboratory grown insects. We also developed a protocol that allows the heterologous production of significant amounts of purified BolEST7 by liquid chromatography. Kinetic experiments are in progress to reveal substrate specificity.

Acknowledgements

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers" (Project Number: 01254)

References

[1] Tzanakakis M.E. et.al. Applied Entomology Library (2006)
[2] Malheiro R. et. al. Trends Food Sci. Technol. (2015) 44, 226-242
[3] Wheelock C.E. et. al. Journal of Pesticide Science (2005) 30(2): p. 75-83
[4] Chertemps T. et. al. Front. Physiol. (2015) 6, 315

P76

Biochemical and Structural Studies on Mitogen Activated Protein Kinase Phosphatase 7

Maria-Evgenia Politi[1,2], Athanasios Papakyriakou[3], Marios Zouridakis[2], Petros Giastas[1,2*]

[1]Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Greece
[2]Structural Neurobiology Research Group, Department of Neurobiology, Hellenic Pasteur Institute, Athens, Greece
[3]Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Athens, Greece
**Correspondence to: petrosgiastas@aua.gr*

Mitogen activated protein kinases phosphatases (MKPs) are a subfamily of dual specificity phosphatases that target and deactivate Mitogen Activated Protein Kinases (MAPKs). The MKP subfamily consists of 10 members [1]. Their target, MAPKs, are components of signal transduction pathways mediating the cellular response to several extracellular stimuli, e.g., oxidative stress and radiation, and, hence, they play an important role in pathways regulating gene expression and cellular proliferation, differentiation or apoptosis [2]. Overexpression of MKPs has been associated with many cancer types and cellular resistance to chemotherapy; therefore, their inhibition is of high importance. Since recently, such inhibition was considered infeasible because of the lack of selective inhibitors [3]. Novel studies on MKP5, a member of the MKP subfamily, have raised interest in targeting a potential allosteric site for selective inhibition of MKPs located in the catalytic domain of the enzyme [4]. Our particular study, utterly, aims at identifying a homologous allosteric site for inhibition of MKP7, another member of MKPs, whose catalytic domain shows high similarity to that of MKP5. To accomplish this, obtaining high quality of MKP7 crystals is crucial. In the presented study, we overexpressed in BL21DE3 E. coli cells the catalytic domain of MKP7, which, as predicted by homology modelling, contains the potential allosteric site. The protein was purified with metal affinity chromatography based on its C-terminal 6-His tag, followed by gel filtration analysis. The purified MKP7 was then subjected to crystallization trials using commercial crystallization screening kits, leading to formation of MKP7 crystals, which however proved fragile with weak diffraction. So, optimization of the crystallization conditions is being conducted for increasing the size of the crystals and their diffraction power. Our future studies include evaluating various synthetic inhibitors for binding to the potential allosteric site.

[1] Masuda, K.; Shima, H.; Watanabe, M.; Kikuchi, K. MKP-7, a Novel Mitogen-Activated Protein Kinase Phosphatase, Functions as a Shuttle Protein. J. Biol. Chem. 2001, 276, 39002–39011, doi:10.1074/jbc.M104600200.
[2] Liu, X.; Zhang, C.-S.; Lu, C.; Lin, S.-C.; Wu, J.-W.; Wang, Z.-X. A Conserved Motif in JNK/P38-Specific MAPK Phosphatases as a Determinant for JNK1 Recognition and Inactivation. Nat. Commun. 2016, 7, 10879, doi:10.1038/ncomms10879.
[3] Caunt, C.J.; Keyse, S.M. Dual specificity MAP Kinase Phosphatases (MKPs): Shaping the Outcome of MAP Kinase Signalling. FEBS J. 2013, 280, 489–504, doi:10.1111/j.1742-4658.2012.08716.x.
[4] Gannam, Z.T.K.; Min, K.; Shillingford, S.R.; Zhang, L.; Herrington, J.; Abriola, L.; Gareiss, P.C.; Pantouris, G.; Tzouvelekis, A.; Kaminski, N.; et al. An Allosteric Site on MKP5 Reveals a Strategy for Small-Molecule Inhibition. Sci. Signal. 2020, 13, eaba3043, doi:10.1126/scisignal.aba3043.

P77

Insights into the structure and function of Hepatitis E virus Open Reading Frame 1 (ORF1)

Maria D. Politi1, Aikaterini Angelopoulou 1, Angelo Gallo2, Georgios Bouras1, Maria Birkou1, Elvira Koutsouki1, Bruno Canard3, Bruno Coutard3, and Georgios A. Spyroulias1*

[1]Department of Pharmacy, University of Patras, GR-26504 Patras, Greece
[2]Department of Chemistry, University of Torino IT-10126 Torino, Italy.3
[3]Unité des Virus Émergents (UVE : Aix-Marseille Univ-IRD 190-Inserm 1207), Marseille, France.

Hepatitis E virus (HEV) has been identified as the most common cause of acute viral hepatitis worldwide. HEV infection is estimated to cause 70,000 deaths from acute liver failure. There are seven genotypes (HEV1-HEV7), yet only the first four genotypes are responsible for human infection. According to the statistical incidence data, 20 million cases of HEV infection occur annually, while approximately 3.3 million of them result in symptomatic infections. Transmission occurs mainly through the fecal-oral route, but also through contaminated blood transfusions, transplants, and from mother to embryo1. HEV belongs to the Hepeviridae family and its genome is a 7.2Kb ss(+) RNA. It consists of a 5' untranslated region (5'-UTR), 3 open reading frames (ORF's) and a 3' untranslated region(3'-UTR) which ends in a polyadenine tail2. Each ORF has a crucial role for successful viral transmission and human infection.

Our study attempts to elucidate the physicochemical and structural properties of two components of the ORF-1, known as replication complex, which consists of 7 functional domains, which are necessary for RNA replication. These functional domains are the methyltransferase (vMT) and the macro domain (vMD). HEV vMT catalyzes the transfer of the methyl group from S-adenosyl methionine to GTP, resulting in m7GTP, a procedure known as mRNA capping. mRNA capping is essential for virus life cycle and the multiplication process. Additionally, HEV vMD serves a central role in a variety of cellular activities, including de-MARylation and de-PARylation3. The current investigation encompassed a diverse array of experimental factors to optimize the expression, folding and stability of recombinant polypeptides of varying amino acid sequence lengths of vMD and vMeT. In order to acquire a deeper understanding of the biophysical characteristics of the vMD, we performed interaction studies using heteronuclear NMR Spectroscopy, Isothermal Titration Calorimetry (ITC), and biological assays employing western blot analysis.

References

1. *Debing, Yannick, et al. "Update on hepatitis E virology: Implications for clinical practice." Journal of hepatology 65.1 (2016): 200-212.*
2. *Wißing, Michael H., et al. "Virus–Host Cell Interplay during Hepatitis E Virus Infection." Trends in Microbiology (2020).*
3. *S. Anang et al., "Identification of critical residues in Hepatitis E virus macro domain involved in its interaction with viral methyltransferase and ORF3 proteins," Sci Rep, vol. 6, no. 1, p. 25133, Jul. 2016*

Acknowledgments

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant (Fellowship Number: 663). We also acknowledge support of this work by the project "European Union's HE program (2021-2027), "ESPERANCE" ERA Chair (GA 101087215).

P78

Lon protease the guardian of mitostasis

Fengoula Avgeri[1*], Dikran Tsitsekian[1], Gerasimos Daras[1], Dimitris Templalexis[1], Martina Samiotaki[2], Polydefkis Hatzopoulos[1], Stamatis Rigas[1]

[1]Department of Biotechnology, Agricultural University of Athens, Athens, Greece
[2]Sciences Research Center "Alexander Fleming", Athens, Greece
** e-mail: favgeri@aua.gr*

As "power plants" of the cell, mitochondria are organelles of energy production through aerobic respiration, which consequently results to ROS accumulation, causing oxidative damage to cellular biomolecules. Protein Quality Control (PQC) is a vital defense mechanism against oxidative stress in eukaryotic cells, aiming to remove damaged polypeptides and maintain genome integrity or gene expression. While Lon protease is a PQC component, our knowledge about the mechanism of Lon substrate recognition and processing still remains elusive. In Arabidopsis, Lon1 loss-of-function mutants bear dysfunctional mitochondria with abnormal morphology and reduced respiratory capacity. These defects result in a growth retardation phenotype of lon1 mutants. Here we report an in vivo trapping approach to identify protein substrates of Arabidopsis Lon1 protease in mitochondria. A proteolytically inactive Lon1 variant was generated by substitution of a Serine-Lysine catalytic dyad with Alanine residues at the C-terminal domain to trap the interacting protein substrates in the proteolytic chamber. The trapped substrates were identified by Mass Spectrometry-based proteomics, after pulling down the whole complex by FLAG-epitope tagging. Interestingly, a set of PentatricoPeptide-Repeat (PPR) proteins, known to be involved in RNA processing and maturation, were identified as Lon1 substrates. These PPR proteins were validated as bona fide Lon1 substrates by applying an in cell protein degradation assay. This assay monitors the rate of protein degradation, based on heterologous co-expression of the substrate with the active or the inactive Lon1 proteolytic variant in E. coli lon- cells. Complementary to these findings, a transcriptomic analysis (RNA-seq) revealed impaired splicing of group II introns of lon1 mitochondrial genes. These intron retention events constitute a convergence point tightly coupling Lon1-PPR physical interaction with mitochondrial RNA processing and expression. In conclusion, our results shed light on Lon1-substrate specificity and reveal Lon1 control in mitochondrial RNA splicing, supporting a critical role in maintenance of mitochondrial homeostasis.

P79

Differential response of A. thaliana VISUAL cell cultures of vpnb1 mutants and overexpression lines during induced xylogenesis

Stavroula Statiri, Evaggelos Charalampidis, Varvara Podia, Eleni Giannoutsou, Ioannis-Dimosthenis Adamakis, Kosmas Haralampidis*

National and Kapodistrian University of Athens, Biology Department, Section of Botany, 15772 Athens, Greece

The Arabidopsis thaliana VPNB1 gene locus encodes for a protein with “armadillo” repeat domains, which is exclusively expressed in the plant vascular system. Previous research has shown that vpnb1 mutant lines exhibit a delayed growth phenotype and an abnormal xylem pattern in stems and roots, while the overexpression lines (35S::VPNB1) display an inverse phenotype of increased deposition of vascular tissue elements. This study focused on the comparative analysis of the xylem elements ontogenesis between vpnb1 mutants, 35S::VPNB1 lines, and wild type plants (Col-0) by using the VISUAL cell culture system. Cotyledons, before and after induction, were chemically fixed, resin embedded, and transverse and tangential sections were stained with toluidine blue and calcofluor white. Moreover, cotyledons incubated in chloral hydrate were observed. The results showed that the vpnb1 lines exhibited a delayed growth/differentiation of xylem elements, smaller cells, and in general an abnormal-incomplete vascular pattern compared to that of wild type plants. The above data verified the effectiveness of the VISUAL system in the study of the genetically modified AtVPNB1 plants, confirmed the involvement of the corresponding gene in xylogenesis, and provide a better insight into the involvement of the VPNB1 protein during cell differentiation and plant vascular system ontogenesis.

P80

melinoe mutant uncovers the role of AtFMN/FHy enzyme as a biochemical hub for flavin synthesis in plants

Gerasimos Daras[1*], Dikran Tsitsekian[1], Dimitris Templalexis[1], Montana Rayburn[2], Fengoula Avgeri[1], Sanja Roje[2], Polydefkis Hatzopoulos[1], Stamatis Rigas[1]

[1]Department of Biotechnology, Agricultural University of Athens, Athens, Greece
[2]Institute of Biological Chemistry, Washington State University, Pullman, USA
**Correspondence: gdaras@aua.gr*

Riboflavin, or vitamin B2, is the biosynthetic precursor of flavocoenzymes, which are essential for the cell. As the central component of FMN and FAD cofactors, riboflavin controls the function of flavoproteins, being indispensable for growth and cellular metabolism. These cofactors sustain photosynthesis, mitochondrial electron transport, fatty acid oxidation, photoreception and secondary metabolism. While animals depend on exogenous resources, riboflavin is mostly biosynthesized in plants and microorganisms. In the model species Arabidopsis thaliana, the key enzyme AtFMN/FHy has both riboflavin kinase and FMN hydrolase activities. Phylogenetic analysis suggests that AtFMN/FHy proteins occur in algae and land plants. AtFMN/FHy 3D structure displays two distinct domains representing the different enzymatic properties. Here, we report the characterization of the FMN/FHy mutant, which displays abnormal plant growth and leaf chlorosis. Due to the evident phenotype, the mutant was called melinoe (mel) after the Greek godness “Μηλιόν”, who had a pale skin illuminated in the moonlight. A nucleotide transition at an intron-exon junction of the FMN/FHy gene of mel homozygous plants results in alternative 3’ splicing, skipping twelve amino acids within the FMN phosphatase domain. Leaf variegation was apparent during the entire life cycle of mel homozygous plant that showed a specific gradient pattern of chlorosis beginning from the center of the leaves and extending to the entire leaf lamina. The growth of mel homozygous plants ceased upon transition from the reproductive to the vegetative stage. Biochemical approaches revealed that mel mutant seedlings accumulated riboflavin to exceptionally increased levels. Further, the knockout mutant of fmn/fhy is lethal, underlining the pivotal role of the enzyme in flavin metabolism. Translational fusion to YFP, showed subcellular localization of FMN/FHy in the cytosol and FMN/FHy was constantly expressed in various organs upon distinct developmental stages. Overall, the results shed light on the regulatory role of FMN/FHy in intracellular flavins biosynthesis.

This project was funded by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “2nd Call for H.F.R.I. Research Projects to support Faculty Members and Researchers” (grant no: 02457).

P81

Improving biodesulfurization via targeted re-insertion of the flavin reductase DszD in the genome of Rhodococcus qingshengii IGTS8

Fotios Klenias[1], Olga Martzoukou[1] , Dimitris G. Hatzinikolaou[1]

[1]Enzyme and Microbial Biotechnology Unit, Department of Biology, National and Kapodistrian University of Athens, Athens, Greece

Biodesulfurization poses as an ideal replacement to the high cost hydrodesulfurization of the recalcitrant heterocyclic sulfur compounds, such as dibenzothiophene (DBT) and its derivatives. Bacteria of the genus Rhodococcus may be the most promising biocatalysts for biodesulfurization, as they are able to withstand the harsh conditions of a biphasic process on an industrial scale, however, the increasingly stringent limits on the sulfur content of fuel intensify the need for improved biocatalysts, without sacrificing the calorific value of the fuel. Rhodococcus qingshengii IGTS8 is a model biocatalyst, responsible for the removal of sulfur through the 4S metabolic pathway, which includes a plasmid-borne dszABC operon, encoding enzymes that catalyze the formation of 2-hydroxybiphenyl (2-HBP) from DBT with simultaneous removal of sulfur as SO_3^{2-}, as well as the chromosomal gene for flavin-dependent reductase, dszD. However, naturally occurring biocatalysts do not exhibit the required biodesulfurization activity to be useful for industrial applications and for this reason, genetic modifications to the genome of strain IGTS8 are currently being explored. Here, the effect of increased reductive power was assessed by constructing a genetically modified R. qingshengii IGTS8 strain, which carries an additional copy of the dszD gene under the control of the strong P_{kap1} rhodococcal promoter, inserted in the neutral genetic locus carA2. We conduct a comparative study of the growth and biodesulfurization capabilities of P_{kap1}-dszD, wild-type and carA2Δ strains, grown on different types and concentrations of carbon and sulfur sources. The Dsz-mediated desulfurization from DBT was monitored at three growth phases, through HPLC analysis of end-product levels. The results highlight ethanol and dimethyl sulfoxide as the preferred carbon and sulfur sources, respectively, for the P_{kap1}-dszD strain. Importantly, a significant enhancement of the maximum calculated biomass and biodesulfurization activity of the Pkap1-dszD strain is also revealed in the presence of DBT as the sole sulfur source, paving the way for further studies that could lead to a more viable commercial biodesulfurization process.

P82

Histone acetyltransferase GCN5 and transcriptional coactivator ADA2b regulate plant hormone metabolism and homeostasis in Arabidopsis thaliana inflorescence

Christina Balouri[1,2], Stylianos Poulios[1], Konstantinos Vlachonasios[1,2,3]

[1]Department of Botany, School of Biology, Faculty of Science, Aristotle University of Thessaloniki, 54121, Thessaloniki, Greece
[2]M.Sc. in Biological Applications, "Biotechnology – Molecular and Microbiological Analysis of Products and Food", School of Biology, Faculty of Science, Aristotle University of Thessaloniki, 54121, Thessaloniki, Greece
[3]Natural Products Research Centre of Excellence, Center of Interdisciplinary Research and Innovation, Aristotle University of Thessaloniki, 57001, Thessaloniki, Greece

Histone acetyltransferases (HATs) can modify the N-terminal tails of the core histone proteins via acetylation. The General Control Non-derepressible 5 (GCN5) protein is a HAT that acetylates Lys residues in the N-terminal tail of histone H3 in Arabidopsis. GCN5 has been associated with cell division and differentiation, meristem function, root, stem, foliar and floral development, and environmental responses. GCN5 interacts with the Alteration / Deficiency in Activation 2b (ADA2b) functioning in two transcription adaptor complexes, ADA and Spt-Ada-Gcn5-Acetyltransferase (SAGA) complex. ADA2b protein enhances the ability of GCN5 to acetylate histones. Flowers of ada2b-1 mutant and early flowers of gcn5-1 mutant display stamen shorter than carpels and thus reduced fertility relative to the wild type plants, while gcn5-1 mutant also shows indeterminate flower meristem. This research aims to study the genetic interaction of ADA2b and GCN5 proteins on gene expression of genes involved in plant hormone metabolism and homeostasis of Arabidopsis thaliana inflorescence. Through RNA-sequencing in ada2b-1 and gcn5-1 inflorescence, the differentially expressed genes (DEGs) were identified from 27054 genes. About 42%, 5146 upregulated and 6329 downregulated genes, and 24%, 2862 upregulated and 3747 downregulated genes, of identified genes showed differential expression in ada2b-1 and gcn5-1 mutant, respectively, relative to wild-type plants. From the identified DEGs in ada2b-1 and gcn5-1 mutant, 168 genes are involved in plant hormone metabolism and homeostasis. Most genes in plant hormone biosynthesis and homeostasis are downregulated in both mutants, whereas a different expression pattern is shown between catabolism genes. These results suggest that ADA2b and GCN5 are positive regulators of genes in plant hormone biosynthesis and homeostasis, and gene-specific transcription regulators in plant hormone catabolism, regarding Arabidopsis thaliana inflorescence.

This work is supported by the Hellenic Foundation for Research and Innovation (HFRI) Grant Number 73333. PI Konstantinos Vlachonasios, Greece

P83

A transcriptomic analysis to decipher olive antioxidants biosynthesis during olive fruit ripening of the "Koroneiki" cultivar

Dikran Tsitsekian[1], Gerasimos Daras[1], Dimitrios Templalexis[1], Fengoula Avgeri[1], Anthi Panara[2], Nikolaos S Thomaidis[2], Polydefkis Hatzopoulos[1], Stamatis Rigas[1*]

[1]Laboratory of Molecular Biology, Department of Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece.
[2]Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, 15771 Athens, Greece.
**e-mail: srigas@aua.gr*

Olive fruit and their derivatives are essential components of the Mediterranean diet and their protective role has been attributed to distinctive fatty acid composition and elevated levels of bioactive secondary metabolites, like phenolics, terpenes and sterols. "Koroneiki" cultivar is among the most popular Greek oil-producing varieties. Olive fruits accumulate oil at a percentage of up to 30% and are rich in secondary metabolites. Oleuropein and vitamin E are among the most important phenolic compounds with antioxidant properties. Oleuropein has a high antioxidant capacity and scavenging activity against ROS, while vitamin E has been associated with the promotion of health and the prevention or amelioration of severe chronic diseases, such as cancer, cardiovascular and neurodegenerative disorders. Due to these properties, production of olive oil with high content of such metabolites draws high attention. The elucidation of the genes involved in the antioxidants biosynthetic pathways is of high importance. In this study, we applied a holistic and targeted approach to determine the expression profile of genes involved in the biosynthetic pathways of these important antioxidant compounds during olive fruits ripening of the "Koroneiki" cultivar. Olive fruits were sampled at four different stages, 17, 21, 26 and 30 weeks after flowering (waf), to perform RNA-seq analysis. Validation of this dataset with a set of known genes from olive or model species within these pathways, showed a gradual increase of gene expression during fruit ripening. Hence, the dataset provides a toolbox to identify novel unknown genes involved in these biosynthetic pathways, complementary to the metabolomic profile, upon during fruit ripening. Overall, an integrated analysis of transcriptomic and metabolomic data will enable us to initiate approaches to engineer the phenolic antioxidant content and enhance olive oil organoleptic properties or value for human nutrition and health.

This project was funded by the program Agricultural European Innovation Partnership – EIP AGRI, "M16.1 support for the establishment and operation of operational groups of the EIP for agricultural productivity and sustainability and M16.2 support for pilot projects, and for the development of new products, practices, processes and technologies" Grant no. M16SYN2-00125

P84

Biological activities of extracts isolated from symbiotic Lotus japonicus plants

Foteini D. Kalousi[1], Michail Tsakos2, Christina N. Nikolaou[3], Aikaterini Perati[1], Eleftheria Bazouli[1], Marios I. Valmas[1], Daniela Tsikou[1,*], Anna-Maria G. Psarra[1,*]

[1]University of Thessaly, Larissa, Greece
[2]National and Kapodistrian University of Athens, Athens, Greece
[3]Agricultural University of Athens, Athens, Greece
[*]Correspondence: dtsikou@uth.gr; ampsarra@uth.gr

Plant secondary metabolism produces a variety of compounds that are studied for useful biological activities. In the present study, we examined the presence of putative bioactive molecules in ethyl-acetate extracts of shoot and root tissues of the model-legume plant Lotus japonicus. This plant engages into symbiotic relationships with beneficial soil bacteria and fungi, which causes changes in both the root and shoot metabolome. We examined the putative production of bioactive molecules in L. japonicus plants after single or double inoculations with arbuscular mycorrhizal fungi and nitrogen-fixing bacteria (rhizobia), as well as non-inoculated plants. Plant extracts were tested for cytotoxic, apoptotic and anti-inflammatory effects on human HEK293 cell cultures. Moreover, the plant extracts were tested for antifungal activity against selected plant pathogens. Our results suggest that symbiotic L. japonicus plants are enriched with metabolites that have interesting biological activities and could be further explored for putative future use both in the pharmaceutical and agricultural sector.

P85

Sulcatol affects gravitropism in Arabidopsis thaliana root by interfering with auxin response

Dimitris Pappas[1,2*], Stylianos Poulios[1], Konstantinos Vlachonasios[1], Spyros Gkelis[1], Ioannis-Dimosthenis S. Adamakis[3], Emmanuel Panteris[1]

[1]Department of Botany, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greec
[2]Plant Systems Biology, School of Life Sciences, Technical University of Munich, Freising, Germany
[3]Section of Botany, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece
**correspondence: dimitrios.pappas@tum.de*

Sulcatol (6-methyl-5-hepten-2-ol), also known as coriander heptenol, is an organic compound produced by various organisms, including prokaryotes (such as cyanobacteria), insects and plants. Although sulcatol may become accessible to crops irrigated by freshwater rich in cyanobacteria, currently, there is a lack of published data regarding its effects on plants. Our previous data showed that sulcatol could induce aberrant gravitropism in Arabidopsis thaliana roots. In this study, we further investigated the effects of sulcatol treatment on the gravitropic response of Arabidopsis thaliana roots, focusing on auxin distribution and activity. Four-day-old seedlings expressing the auxin response reporter DR5-GFP were first treated with different sulcatol concentrations for 24 h and then gravistimulated for various time periods to determine DR5-GFP lateralization during the gravitropic response by confocal microscopy. Additionally, seedlings expressing GFP-tagged PIN-FORMED (PIN) efflux carriers (PIN1, PIN2, PIN3) were examined to check polar auxin transport after treatment. The expression of genes involved in auxin biosynthesis in roots (YUCCA5, YUCCA8, YUCCA9, TAA1) was also tested with qRT-PCR. In sulcatol-treated root tips, the level of DR5-GFP fluorescence intensity was significantly higher compared to the control. Interestingly, gravistimulated sulcatol-treated roots exhibited abnormally extended DR5-GFP lateralization, which could be observed even 24 h after gravistimulation. Unaltered gene expression levels and PIN-dependent auxin transport, along with DR5-GFP enhanced signal and lateralization defects, suggest a possible defect in the auxin signaling pathway due to sulcatol treatment.

P86

A Novel Ultrastable Carbonic Anhydrase for Efficient CO2 Sequestration discovered through Large-Scale Metagenomic Analysis

Konstantinos Rigkos[1,2], Georgios Filis[1,3], Pavlos Saridis[1,4], Dimitra Zarafeta[1*], Georgios Skretas[1,5*]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]Department of Biological Applications and Technologies, University of Ioannina, Ioannina, Greece
[3]Department of Informatics and Telecommunications, National and Kapodistrian University of Athens, Athens, Greece
[4]Faculty of Biology, National and Kapodistrian University of Athens, Greece
[5]Institute for Bio-innovation, Biomedical Sciences Research Centre "Alexander, Fleming", Vari, Greece

Surging anthropogenic CO_2 emissions have exacerbated the greenhouse effect, making global warming a critical concern. The European Commission's 2019 pledge to achieve net-zero emissions by 2050, underscores the need for implementing innovative and sustainable CO_2 Capture and Storage Technologies to address this global crisis. Currently, capture technologies rely heavily on amines but their high toxicity and energy-demanding regeneration process raise environmental concerns. Consequently, researchers worldwide are actively seeking eco-friendly alternatives. One promising green technology for CO_2 sequestration involves carbonic anhydrases (CAs), enzymes that accelerate CO2 dissolution, playing vital roles in biological processes in all kingdoms of life. Yet, despite their abundance in nature, conventional enzymes cannot be utilized in carbon capture pipelines as they fail to endure the harsh conditions present in industrial settings (high temperatures, high alkalinity etc.). To address this problem, the identification of ultra-stable CAs is necessitated. In this study we describe the discovery of a novel CA, termed CA-KR1, which we have discovered through large-scale bioinformatic analysis. An in-house developed computational pipeline was developed to screen extensive metagenomic datasets retrieved from the NIH Sequence Read Archive (SRA) and the Joint Genome Institute (JGI) open-access databases. CA-KR1 was identified and produced recombinantly in E. coli. The novel CA is ultra-thermostable, and exceptionally alkali-stable, exhibiting a 24-hour half-life at 80 °C, while remaining soluble and catalytically active after prolonged exposure to the standard CO2 capture medium (20% K_2CO_3, pH 11.5). CA-KR1 is one of the most thermostable and alkali-stable CAs known to date, making it an exceptional candidate biocatalyst for industrial CO_2 sequestration.

Acknowledgements
This research has been co financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (T2EΔK-02899/ MIS5134532). Furthermore, we acknowledge support for the Twin4Promis project, which is funded by the "Widening Participation & Spreading Excellence" component of Horizon Europe Programme (call Twinning "HORIZON-WIDERA-2021-ACCESS-03-1"), under Grant Agreement No. 101079363.

Bibliography
[1] Ozensoy Guler O, Capasso C, Supuran CT. J Enzyme Inhib Med Chem. 2016, 31(5):689-94.
[2] Angeli A, Supuran CT. J Enzyme Inhib Med Chem. 2023, 38(1):2166503.
[3] Hanifa M, Agarwal R, Sharma U, Thapliyal PC, Singh LP. J CO2 Util. 2023, 67:102292.
[4] Olabi AG, Wilberforce T, Sayed ET, Shehata N, Alami AH, Maghrabie HM, et al. Energies. 2022, 15(22):8639.
[5] Dutcher B, Fan M, Russell AG. ACS Appl Mater Interfaces. 2015, 7(4):2137-48.
[6] Anderson C, Harkin T, Ho M, Mumford K, Qader A, Stevens G, et al. Energy Procedia. 2013, 37:225 32.
[7] Barzagli F, Giorgi C, Mani F, Peruzzini M. J CO2 Util. 2017, 22:346-54.
[8] Talekar S, Jo BH, Dordick JS, Kim J. Curr Opin Biotechnol. 2022, 74:230-40.

P87

Study of the role of Hsp90 proteins in the organization and development of the vascular system in A. thaliana

Vassiliki Basdeki[1], Stavros Chatzirvasanis[2], Varvara Podia[1], Ioannis-Dimosthenis Adamakis[1], Eleni Giannoutsou[1], Despoina Samakovli[1], Dimitra Milioni[2], Kosmas Haralampidis[1]*

[1]National and Kapodistrian University of Athens, Biology Department, Section of Botany, 15772 Athens, Greece.
[2]Agricultural University of Athens, School of Applied Biology and Biotechnology, Department of Biotechnology, Iera Odos 75, Athens 11855.

HSP90 is considered a highly specialized molecular chaperone that functions in plant development and cellular homeostasis. HSP90s play an essential role in many processes ranging from hypocotyl elongation, stomatal differentiation, root growth, flowering and seed germination to hormonal signaling and the circadian clock.

In the present study, wild-type Arabidopsis thaliana plants and HSP90 RNA interference (RNAi) lines under the control of the Rac2/ROP7 tissue-specific promoter were analyzed. The Rac2/ROP7 gene is expressed in the primary xylem of roots, hypocotyl, cotyledons, shoots and leaves and has been associated with secondary cell wall formation. These silenced RNAi lines show a pleiotropic phenotype. More specifically, the mutants show delayed growth, absence of apex dominance, large variation in the height of the central shoot, in the number of lateral shoots, an increased number of rosette leaves as well as variations in the number of siliques and abnormalities in the siliques structure and development. The RNAi lines analyzed show altered organization of vascular bundles and a reduction in the number of total phloem, procambium and xylem cells. The above data indicate the involvement of HSP90 in vascular tissue organization thus opening a new field of research to elucidate the molecular mechanisms of HSP90 function in vasculature patterning.

P88

HSP90 is a key regulator of the GA signaling pathway

Konstantinos Panagiotopoulos[1], Despina Samakovli[1,2], Panagiota Konstantinia Plitsi[1], Aggeliki Rambou[1,3], Polydefkis Hatzopoulos[1], Dimitra Milioni[1]

[1]Agricultural University of Athens, School of Applied Biology and Biotechnology, Department of Biotechnology, Iera Odos 75, Athens 11855.
[2]National and Kapodistrian University of Athens, Biology Department, Section of Botany, 15772 Athens, Greece.
[3]Laboratory of Virology, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, 14561 Athens, Greece.

Multiple signaling systems allowing for the integration of environmental and hormonal cues, coordinate plant development and physiology. Gibberellins (GA) are a group of essential phytohormones that control complex plant growth and developmental processes such as germination, hypocotyl and root elongation, leaf expansion, pollen maturation, flower induction and adaptation to environmental stresses. In land plants, GA signaling is mediated via GIBBERELLIN-INSENSITIVE DWARF1 (GID1) cytoplasmic receptor. GA-bound GID1 receptor interacts with DELLA proteins which are negative regulators of development, promoting their degradation. The HSP90 molecular chaperone is essential under normal and stress conditions for both eukaryotic and prokaryotic organisms. It was recently reported that the HSP90 actively participates in the GA-mediated signaling pathway controlling the hypocotyl elongation of etiolated seedlings via the interaction with DELLA proteins.

In the present study we show that HSP90 physically interacts with key components of the GA signaling pathway and we investigate the impact of the HSP90 pharmacological or genetic depletion on GID1 receptor levels.

P89

Investigation of the immune-mediated anticancer effect of Origanum vulgare ssp. hirtum essential oil in a syngeneic colon cancer model

Georgioa Aindelis[1], Katerina Spyridopoulou[1], Aggeliki Tiptiri-Kourpeti[1], Stavros Fragias[2], Katerina Chlichlia[1*]

[1]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece
[2]PAXMAN Ltd., Patras Industrial Zone, Ag. Stefanos, Patras, Greece

In recent times, plant-derived secondary metabolites have been identified as potential therapeutic agents, with a wide array of biological properties. Oregano is a widespread herb in the Mediterranean area, integrated in the local diet for thousands of years and has been shown to exert anti-inflammatory, antimicrobial and antitumor activity, as we have previously shown. The aim of this project was to further investigate the mechanisms involved in the in vivo anticancer properties of the essential oil extracted from the plant Origanum vulgare ssp. hirtum. Consumption of the essential oil had a profound growth inhibitory effect in a BALB/c mouse tumor model and an 80% reduction in tumor volume was observed. The infiltration of effector CD8+ and CD4+ T cells and natural killer cells in the tumor was evaluated, and their activity was assessed by examining the expression of cytokines mediating antitumor immune responses, like IFN-γ, IL-12 and IL-1β, as well as markers of reduced proliferative capacity and apoptotic death of cancer cells, such as ki67, caspase 3 and PARP-1. Systemic antitumor immune responses in blood circulation were evaluated by detection of the respective cytokines. In order to confirm the effect of the essential oil in more real conditions, a lower concentration of an essential oil emulsion in tomato sauce was prepared and administered in mice. As was expected, the effect was less prominent, however, an almost 45% reduction in tumor volume was still detected.

Part of this research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment" (Project Number: HFRI-FM17C3-2007).
Part of this research project was co-financed by the European Union (European Regional Development Fund–ERDF) and Greek national funds through the Operational Program 'BILATERAL COOPERATION GREECE–CHINA' (Project No. 12CHN_409).

P90

Lactococcus lactis ssp. lactis bacteriocins exert cytotoxic effects on colon cancer cells

Christina Thoda[1], Eleni Gounari[2], Dimitris Kontoyiannis[1], George Koliakos[2,3], Maria Touraki[1]

[1]Department of Genetics, Development and Molecular Biology, School of Biology, Faculty of Sciences, Aristotle University of Thessaloniki, Thessaloniki, 54124
[2]Biohellenika, Biotechnology Company, Thessaloniki, 57001
[3]Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, 54124

Colorectal cancer (CRC) represents the most prevalent type of gastrointestinal malignancy worldwide. Due to the increased toxicity of current treatment strategies and their ineffectiveness to completely eradicate malignant cells, probiotic derived bioactive compounds have been proposed as novel anticancer agents. Among these compounds bacteriocins, the cationic ribosomally synthesized antimicrobial peptides have attained scientific interest for their potential antiproliferative properties [1]. Most studies focus on the commercially available nisin produced by Lactococcus lactis subsp. lactis [2, 3]. However, the synergistic cytotoxic activity of other purified bacteriocins of L. lactis on CRC cells has not been investigated yet. In the present study, the effect of the purified bacteriocins from L. lactis ATCC 11454 on the viability of CRC cells (Caco-2 and RKO) and normal Wharton's Jelly mesenchymal stem cells (WJ-MSCs) was investigated via MTT and Crystal violet assay. Briefly, the cell-free culture supernatant (CFS) collected from a 24h culture was subjected to a dual purification protocol for bacteriocin isolation, including ammonium sulfate precipitation and organic solvent extraction. The resulted two different fractions, namely sample 1 containing mainly nisin A and sample 2 containing nisin Z, lacticins, and lantibiotic T2C9F0, were further subjected to desalting, ion exchange chromatography, solid phase extraction and lyophilization. Each sample was administered separately to cells following reconstitution in DMEM. Both samples resulted in significant reduction in CRC cell viability in a dose- and time-dependent manner, while cytotoxicity against normal cells was low. Additionally, increased apoptosis levels were revealed in the case of Caco-2 and RKO cells after 24h and 48h treatment corresponding to the IC50 values of each sample, while leaving normal cells intact, indicating the specificity of bacteriocins towards CRC cells. In conclusion, bacteriocins may serve as selective cytotoxic agents that can be exploited in the future as anticancer therapy.

References

[1] Thoda, C., & Touraki, M. (2023). Probiotic-Derived Bioactive Compounds in Colorectal Cancer Treatment. Microorganisms, 11(8), 1898. https://doi.org/10.3390/microorganisms11081898
[2] Ahmadi, S., Ghollasi, M., & Hosseini, H. M. (2017). The apoptotic impact of nisin as a potent bacteriocin on the colon cancer cells. Microbial pathogenesis,111, 193–197. https://doi.org/10.1016/j.micpath.2017.08.037
[3] Norouzi, Z., Salimi, A., Halabian, R., & Fahimi, H. (2018). Nisin, a potent bacteriocin and anti-bacterial peptide, attenuates expression of metastatic genes in colorectal cancer cell lines. Microbial pathogenesis,123, 183–189. https://doi.org/10.1016/j.micpath.2018.07.006

P91

Unveiling Microbial Diversity in Biobeds: Impact on Fungicide Dissipation and Groundwater Protection

Eleni Sakka[1], Paraskevas Parlakidis[2], Maria Tokamani[1], Anastasia Bouchorikou[1], Athanasios Toros[1], George Adamidis[1], Zisis Vryzas [2*], Raphael Sandaltzopoulos[1*]

[1]Department of Molecular Biology and Genetics, Faculty of Health Sciences, Democritus University of Thrace, University Campus, Bldg. #10 "Fotis Kafatos", 68100 Alexandroupolis, Greece
[2]Laboratory of Agricultural Pharmacology and Ecotoxicology, Faculty of Agricultural Development, Democritus University of Thrace, 193 Pantazidou, 68200 Orestias, Greece

Biobeds systems are employed for the treatment of pesticide-containing wastewaters. High throughput DNA sequencing of bacterial 16S rRNA gene can provide high resolution identification of microbial communities, allow a great insight into community dynamics and diversity during the pesticide removal, and aid in future biobed design and optimization. In this study, an amended biomixture (active core of biobeds) under co-bioaugmentation with the plant growth-promoting rhizobacteria Pseudomonas putida, the artificial contamination with a model fungicide mixture (fluopyram, myclobutanil and triticonazole) and the planting of Trifolium repens L. was investigated for monitoring alterations of both bacterial and archaeal communities under different treatment conditions, during a 60-day experiment. For the 16S rRNA gene amplicon analysis of the original biomixture (B), the biomixture contaminated with fungicides (B+F), the bioaugmented biomixture (B+F+P), and the planted biomixture with clover (B+F+T+P), the V4 16S rRNA gene region was amplified. The amplified products were sequenced with the aid of Ion Torrent S5 system. According to α-diveristy results, the biomixtures planted with Trifolium repens L. showed the highest microbial diversity. Regarding β-diversity analysis, the presence of Trifolium repens L. enhanced the abundance of nitrogen-fixing bacteria and archaea, whereas fungicide mixture and bioaugmentation had no significant influence on microbial communities. Between samples including and excluding Trifolium repens, the phyla Actinobacteriota and Proteobacteriota showed the most significant differences. The aforementioned findings underscore the crucial role of T. repens L. in shaping and preserving the microbial diversity in biomixtures, which could potentially redirect interest towards research aimed at maximizing biobeds effectiveness.

P92

Development of specialized Escherichia coli strains for high-level recombinant membrane protein production

Eleni Vasilopoulou[1,2,3], Dimitra Gialama[1,2], Myrsini Michou[1,3], Georgios Skretas[1,2*]

[1]Institute for Bio-innovation, Biomedical Sciences Research Center "Alexander Fleming", 16672, Vari, Greece
[2]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, 11635, Greece
[3]Department of Biochemistry and Biotechnology, University of Thessaly, Viopolis, Larisa, 41500, Greece
[*]e-mail: skretas@fleming.gr

Membrane proteins (MPs) are basic components of cell membranes with highly important functions and constitute more than half of all known targets for drug development. As a result, there is a huge need for access to large amounts of MPs to achieve detailed characterization of their structure and function, and therefore expedite the discovery of new pharmaceuticals that target such proteins. The required MP quantities are typically produced recombinantly in heterologous hosts such as E. coli. However, in the case of heterologous expression, recombinant MP production in bacteria is accompanied with severe toxicity for the host and low overall yields. To address this, we have performed a genome-wide screen to identify suppressors of MP- induced toxicity and have identified rraA, the gene encoding for RraA, an inhibitor of the mRNA-degrading activity of E. coli RNase E, as a potent such suppressor. Based on this, we have generated a new modified rraA-overexpressing E. coli strain, termed SuptoxR, which is particularly effective in suppressing toxicity caused by the MP- overexpression process, and in dramatically enhancing the cellular and volumetric accumulation of recombinant MPs of both prokaryotic and eukaryotic origin. Then, after an evaluation of homologous RraA proteins from other bacteria and plant chloroplasts, we identified improved RraA variants which we have utilized to develop second- generation SuptoxR strains, termed SuptoxR2.1 and SuptoxR2.2, which can achieve even more enhanced levels of MP production. Finally, we investigated whether a variety of strains expressing certain mutations of RNase E, exhibiting reduced ribonucleolytic activity, can promote MP production in a manner resembling the conditions of rraA overexpression and we found a single E. coli strain, termed SuptoxRNE22, which can achieve enhanced levels of recombinant MP production. Thus, E. coli strains SuptoxR2.1, SuptoxR2.2 and SuptoxRNE22 are highly promising hosts for achieving high-level recombinant MP production.

This work was financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme "Human Resources Development, Education and Lifelong Learning" in the context of the Act "Enhancing Human Resources Research Potential by undertaking a Doctoral Research" Sub-action 2: IKY Scholarship Programme for PhD candidates in the Greek Universities and by the Horizon Europe Programme under the "Widening Participation & Spreading Excellence" component (call Twinning "HORIZON-WIDERA-2021-ACCESS-03-1"); Project "Twin4Promis"; Grant Agreement No. 101079363

P93

Deciphering the molecular basis of drought tolerance in olive trees.

Georgia Pantidi[1], Margarita Thomopoulou[1], Aikaterini Dimopoulou[1], Ioannis Giannoukos[1], Nickolas Xideros-Malefakis[1], Ioanna-Angeliki Stathaki[1], Konstantinos Koudounas[1,2], Polydefkis Hatzopoulos[1*]

[1]Laboratory of Molecular Biology, Department of Biotechnology, Agricultural University of Athens, 11855 Athens, Greece
[2]Laboratory of Agricultural Chemistry, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
**e-mail: phat@aua.gr*

The Mediterranean basin has been identified as one of the most affected regions of climate change globally. The impacts will exert additional pressure on already strained ecosystems and therefore is expected to seriously affect valuable crops in the coming decades. One emblematic crop of the Mediterranean Basin is the olive tree (Olea europaea L., Oleaceae) with high nutritional value for humans and of great economic significance since more than the 90% of the global olive oil production is sourced from this region. Olive tree has been domesticated thousands of years ago and as a result of selection, a plethora of cultivars with different agronomic characteristics is currently available. In order to shed light in the molecular basis of differential responses among olive cultivars under drought stress, we used olives (cv. Kalamon) grafted on different rootstocks and water deficiency was introduced by irrigating with high concentration of polyethylene glycol 6000 (PEG 6000). Samples from both leaves and roots were collected at different timepoints and RNAs were extracted in order to perform a comparative transcriptomic analysis. Both phenotypic and morphometric differences were recorded and our results suggest that select rootstocks may enhance the drought tolerance of olive trees.

P94

Overexpression of the Arabidopsis thaliana ULCS1 gene results in pleiotropic phenotypes during vegetative and reproductive development

Alexandra Vallianou, Stylianos Spyropoulos, Mantalena Rovoli, Despoina Beris, Georgios Kapolas Varvara Podia, Ioannis-Dimosthenis Adamakis, Eleni Giannoutsou, Kosmas Haralampidis

National and Kapodistrian University of Athens, Biology Department, Section of Botany, 15772 Athens, Greece

WD40-repeat-containing proteins (WDRs) are highly abundant in all eukaryotes. Several have been implicated as subunits of multi-protein CRL E3 ligase complexes that regulate ubiquitination-mediated protein degradation and thus various cellular and developmental processes. Impairment of the WDR protein ULCS1 from Arabidopsis causes pleiotropic phenotypes during plant development, including reduced lignification, anther indehiscence, and sterility. The present study shows that 35S::ULCS1 upregulation results in a slow-growing phenotype compared to WT plants, and in contrast to the ulcs1 silencing mutants, which reach their vegetative to reproductive transition point later than WT plants. The overexpression plants display also fewer numbers of branches, secondary shoots, and siliques. Furthermore, they exhibit significantly enlarged flower spines and flower organs. Although siliques are also enlarged, they contain a normal number of ovules per silique. Toluidin staining and immunolabeling of various cell wall components revealed cell wall modifications and increased lignification of the vascular tissue of the siliques. The percentage of fertilized ovules in the overexpression lines is also lower than that of Col-0 plants, while the seeds produced display high heterogeneity in shape and size. Furthermore, a lower germination rate of the 35S::ULCS1 seeds compared to Col-0 is observed. The above data indicate that ectopic and constitutive elevated levels of ULCS1 result in pleiotropic alteration during flower, siliques, and seed development, underlining the significant role of ULCS1 both in vegetative and reproductive development.

P95

"Sustainable Hydroponic Waste Management Through Microalgae Bioremediation: A Study on Nutrient Removal and Biomass Valorization"

Danae Ifanti[1*], Georgios-Chrisovalantis Prattis[1*], Maria-Eleftheria Zografaki[1], Sofia Marka[1], Alexandros Ntzouvaras[1], Ioannis Karavidas[2], Theodora Ntanasi[2], Gabriel Vasilakis[3], Georgia Ntatsi[2], Emmanouil Flemetakis[1]

[1]Agricultural University of Athens, Laboratory of Molecular Biology, Department of Biotechnology, Iera Odos 75, 11855, Athens, Greece
[2]Agricultural University of Athens, Laboratoty of Vegetable Crops, Department of Crop Science, Iera Odos 75, 11855, Athens, Greece
[3]Agricultural University of Athens, Laboratory of Food Microbiology and Biotechnology, Department of Food Science and Human Nutrition, Iera Odos 75, 11855, Athens, Greece
**email: stud318113@aua.gr*

Given the current environmental and energy crises, it is imperative need to establish a more sustainable approach to hydroponic waste management. Microalgae can effectively utilize for wastewater purification and serve as a biological treatment for an effective nitrite and phosphate removal offering a sustainable solution for bioremediation. In the present study, effluent water from tomato hydroponic cultures was used to cultivate two isolated microalgae strains. More specifically, two isolated microalgae classified as Chlorella sp. and class Trebouxiophyceae indigenous in hydroponic effluents were cultivated in Walnes common medium and hydroponic wastewater in industrial photobioreactor under environmental conditions. During the culture period pH, conductivity, biomass production and photosynthetic activity were monitoring daily to evaluate the growth rate of the microalgae in hydroponic effluent. In addition, nitrite and phosphate content were measured photometrically daily in the culture medium to evaluate their potential for nutrient removal. The produced biomass was harvested and lyophilized for further biochemical analysis of primary and secondary metabolites. The protein content was determined by Kjeldahl method, while total lipids and polysaccharides were evaluated spectrophotometrically. Methanolic extracts were also prepared to evaluate the antioxidant capacity via FRAP Assay, total phenolics with the Folin-Ciocalteu method, flavonoids with aluminum chloride assay and pigments content determined photometrically. Our findings emphasized the effective removal of nutrients as well as the high yield biomass of the isolated strains using hydroponic effluents. Extracts produced by species cultured in wastewater provide new insights into their beneficial role as sustainable feedstock of value-added biomass for many applications.

- *Ajeng, A. A., Rosli, N. S. M., Abdullah, R., Yaacob, J. S., Qi, N. C., & Loke, S. P. (2022). Resource recovery from hydroponic wastewaters using microalgae-based biorefineries: A circular bioeconomy perspective. Journal of Biotechnology, 360, 11–22. doi.org/10.1016/j.jbiotec.2022.10.011*

P96

Microalgae-Based Wastewater Purification and Resource Valorization: A Study on Chlamydomonas sp. and Scenedesmus sp. in Tomato Hydroponic Effluents

Georgios-Chrisovalantis Prattis[1*], Danae Ifanti[1], Sofia Marka[1], Maria-Eleftheria Zografaki[1], Alexandros Ntzouvaras[1], Ioannis Karavidas[2], Theodora Ntanasi[2], Gabriel Vasilakis[3], Georgia Ntatsi[2], Emmanouil Flemetakis[1]

[1]Agricultural University of Athens, Laboratory of Molecular Biology, Department of Biotechnology Iera Odos 75, 11855, Athens, Greece
[2]Agricultural University of Athens, Laboratoty of Vegetable Crops, Department of Crop Science, Iera Odos 75, 11855, Athens, Greece
[3]Agricultural University of Athens, Laboratory of Food Microbiology and Biotechnology, Department of Food Science and Human Nutrition, Iera Odos 75, 11855, Athens, Greece
**stud318085@aua.gr*

Microalgae exhibit immense promise of wastewater purification, effectively reducing contaminants like eutrophic compounds[1]. Simultaneously, their biomass can be utilized in diverse fields of applications and technologies, including the food industry. This study focused on two microalgae strains isolated from hydroponic effluents, Chlamydomonas sp. and Scenedesmus sp., which were tested for their ability to bioremediate tomato hydroponic wastewater and their biomass was further characterized for their nutritional profile. Microalgae strains were grown in both hydroponic wastewater and commonly used culture medium in 50L industrial photobioreactors under environmental conditions. Throughout the culture period, we conducted daily measurements of pH, conductivity, photosynthetic activity and biomass production in microalgae cultures. To assess the microalgae potential for wastewater treatment, phosphate and nitrate removal was determined photometrically, while Zn, Cu, Mn, Ca, Mg and Zn removal were measured by atomic spectroscopy. Moreover, primary and secondary metabolites were determined in the freeze-dried microalgae biomass. Specifically, Kjeldahl method was used to determine their protein content whereas Dubois and phospho-vanillin assays were used to evaluate sugar and lipid contents. Additionally, total antioxidant activity, total phenolic and flavonoid content were estimated according to FRAP, Folin-Ciocalteau and Aluminium chloride assays, respectively. Our results underscored the effective removal of nutrients as well as the high yield biomass of the Chlamydomonas and Scenedesmus strains when using tomato wastewater.

1 *Geremia, E., Ripa, M., Catone, C. M., & Ulgiati, S. (2021). A review about microalgae wastewater treatment for bioremediation and biomass production—a new challenge for europe. In Environments - MDPI (Vol. 8, Issue 12).*

P97

Molecular and biochemical properties of 7-deoxyloganetic acid glucosyltransferase (7-DLGT) homologues from olive tree

Margarita Thomopoulou[1], Georgia Pantidi[1], Ioanna-Angeliki Stathaki[1], Ioannis Mpaxevanakis[1], Kali Baldou[1], Despina Samakovli[1], Konstantinos Koudounas[1,2], Polydefkis Hatzopoulos[1*]

[1] Laboratory of Molecular Biology, Department of Biotechnology, Agricultural University of Athens, 11855 Athens, Greece
[2] Laboratory of Agricultural Chemistry, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
**e-mail: phat@aua.gr*

Oleosides, are terpene-derived secoiridoids biosynthesized exclusively by members of the Oleaceae family. Over the last decade, extensive research has been conducted to characterize the enzymatic steps involved in the biosynthetic pathway of oleuropein, the predominant oleoside in olives (Olea europaea) that serves as a mighty chemical arsenal against herbivores but also shapes the organoleptic properties of olive oil. Glycosylation of secondary metabolites is an important enzymatic step observed in numerous biosynthetic pathways that increases water solubility, reduces chemical reactivity and improves chemical stability therefore modifying the physicochemical properties of compounds as well as their bioactivities. Although oleuropein is a glucoside, the upstream glycosylation step in the biosynthetic pathway has not been characterized, as yet. Glycosylation of 7-deoxyloganetic acid, an enzymatic hub identified and characterized in other plants biosynthesizing secoiridoids, has been suggested to be the key step in oleuropein biosynthesis. Here, we describe the molecular and biochemical properties of four 7-DLGT-like candidates from olive. Phylogenetic analysis grouped these four enzymes together with 7-DLGT from Catharanthus roseus and an iridoid-specific glucosyltransferase from Gardenia jasminoides. Subcellular localization studies of YFP-fused constructs in Nicotiana benthamiana revealed that these enzymes exhibit a nucleocytosolic localization and this result is in agreement with the characterized 7-DLGT from C. roseus. The expression profile of the four candidates was evaluated by RT-qPCR and revealed a differential expression pattern among olives' tissues. Additionally, in order to assess the involvement of the candidates in the biosynthesis of oleosides, we performed Virus-Induced Gene Silencing (VIGS) in olive plantlets and this analysis revealed that silencing of the transcripts encoding two of the four 7-DLGT-like enzymes resulted in reduced amount of oleuropein in planta suggesting a direct role of these candidates in biosynthesis of olive oleosides.

P98

Uncovering the role of HSP90 in Gene Transcriptional Regulation

Panagiota Konstantinia Plitsi[1], Rafail Gkritzas[1], Vasilios Apostolou[1], Loukia Roka[1], Despina Samakovli[1], Polydefkis Hatzopoulos[1], Dimitra Milioni[1*]

[1]Biotechnology Department, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

Heat shock protein 90 (HSP90) is an evolutionary conserved molecular chaperone. Its central role as a capacitor in molecular networks is well established in both animals and plants. The output of many signal transduction pathways converts at the transcriptional control of key regulatory or effector genes. Recently it has been established that HSP90 localizes at the nucleus of the cell, besides the cytoplasm, and in animals the molecular chaperone interacts with epigenetic factors and the RNA polymerase II complex (RNAPII), playing a key role in the transcriptional control of a vast number of genes. In plants, its roles and potential interacting partners exerting transcriptional control is significantly less studied. In the present study, using Yeast two Hybrid (Y2H) and Bimolecular Fluorescence Complementation (BiFC) we show that HSP90 interacts with CDC37, TERMINAL FLOWER2 (TFL2), SPLAYED (SYD) and RELATIVE OF EARLY FLOWERING6 (REF6). CDC37 is involved in the complex that stabilizes the RNAPII elongation complex and PAF1C promotes efficient transcription of genes by RNAPII linking transcriptional elongation with post-translational histone modifications. TFL2 is a member of the Polycomb Repressive Complex 1 (PRC1) and mediates its interaction with PRC2 while SYD is a plant SWI/SNF chromatin remodeler with ATPase activity and REF6 displays demethylase activity. SYD and REF6 promote gene expression by counteracting repressive methylation marks by the two PRC complexes. The biological significance of these interactions is the focus of our future studies.

P99

Novel quinolinones as potential inhibitors of the transcription factor NF- B

Rafaela G. Tsiakalidou[1], Panagiotis Ntavaroukas[1], Nikolaos Kollatos[1], Eleni Stampouloglou[1], Fani Kalala[2], Dimitrios Komiotis[1], Stamatia Papoutsopoulou[1*].

[1]Department of Biochemistry & Biotechnology, University of Thessaly, Larisa, Greece
[2]Department of Medicine, University of Thessaly, Larisa, Greece

The inflammatory bowel disease, IBD, encompassing Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory conditions with high prevalence worldwide. At the cellular level, the NF-B transcription factor pathway is one of the main dysregulated signaling pathways in the immune cells and in the intestinal epithelium (1). Targeting and blocking hyperactivation of the NF- B is a major pharmaceutical research area (2). Three different novel quinolinones* were tested on a NF- B luciferase reporter HeLa stable cell line for the initial screening. One of them, the 3K, showed the most inhibitory effect, and was chosen for further studies. In vitro stimulation of cells with tumour necrosis factor (TNF) induced cell death, as revealed by trypan blue staining. The 3K inhibitor did not impact on cell survival on its own, but in the presence of TNF the observed cell death was much higher, implying a synergistic effect. This could potentially be due to the inhibition of the canonical NF- B pathway that has a protective effect in TNF-treated epithelial cells (3). Activation of the NF-B/p65 protein is characterised by phosphorylation on serine 529, under the specific experimental conditions. Flow cytometry analysis of TNF-induced HeLa cells for phospho-NF- B/p65 showed a non-statistically significant reduction in the presence of the 3K inhibitor, but rather a trend that must be further investigated. Current studies involve DNA-binding and transcriptional activation assays to check whether the 3K inhibitor affects these functions of the NF- B transcription factor.

*The organic synthesis and storage of quinolinones was performed in the laboratory of BioOrganic chemistry, Department of Biochemistry and Biotechnology.

References

1. *Zaidi D, Wine E. (2018) Regulation of Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (NF-kappabeta) in Inflammatory Bowel Diseases. Front Pediatr.6:317.*
2. *Ramadass V, Vaiyapuri T, and Tergaonkar V. (2020) Small Molecule NF-kappaB Pathway Inhibitors in Clinic. Int J Mol Sci 21: (14):5164. doi: 10.3390/ijms21145164.*
3. *Kondylis V, Kumari S, Vlantis K, Pasparakis M. The interplay of IKK, NF-κB and RIPK1 signaling in the regulation of cell death, tissue homeostasis and inflammation. Immunol Rev. 2017 May;277(1):113-127. doi: 10.1111/imr.12550.*

Funding

The current research project was supported by the University of Thessaly Financial & Secretarial Administration of research grants (DEKA 5600.03.08.11).

P100

The effects of Bortezomib resistance on main cellular processes of the DU-145 prostate cancer cell line

Kalliopi Zafeiropoulou[1,2], Lydia Menounou[1*], Georgios Kalampounias[1], Spyridon Alexis[2], Anargyros Symeonidis[2], Panagiotis Katsoris[1]

[1]Division of Genetics, Cell Biology and Development, Department of Biology, University of Patras, 26504 Patras, Greece
[2]Hematology Division, Faculty of Medicine, University of Patras, 26504 Patras, Greece

The proteasome inhibitor Bortezomib (Velcade®, PS-341) is the first of its kind to be implemented clinically and is approved by the FDA for the treatment of multiple myeloma. Its mechanism of action involves the reversible and high specificity binding to the 26S proteasome β5 subunit, inhibiting its function in protein degradation. However, it has been shown that patients develop resistance during the time-course of treatment with bortezomib, compromising its effectiveness and, rendering the study of the underlying mechanisms crucial. In the current study, the human prostate cancer cell line DU-145 was utilized as a cancer model, Bortezomib-resistant cell clones were created and a cell cycle, proliferation, and Bortezomib-induced stress study was conducted. Our results demonstrate that Bortezomib resistance leads DU-145 cells to an alteration of cell cycle regulation mechanisms, activation of proliferation signaling, and reduced levels of stress-regulating proteins.

P101

Reassessment of PTEN inhibitors: A comparative study of bisperoxovanadium complexes and novel compounds.

Kyriaki Premeti[1*], Antonios E. Nadalis[1], Vasiliki Syropoulou[1], Danai Karagkiozeli[1], George Aggelis[1], MIhalis G. Papanikolaou[2], Themistoklis Kampanos[2], Charalampos Labrakakis[3,4], Katerina Antoniou[1,4], George Leondaritis[1,4]

[1]Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Ioannina, 45110 Ioannina, Greece
[2]Section of Inorganic and Analytical Chemistry, Department of Chemistry, University of Ioannina, Ioannina 45110, Greece
[3]Department of Biological Applications and Technology, School of Health Sciences, University of Ioannina, 45110 Ioannina, Greece
[4]Institute of Biosciences, University Research Center of Ioannina, 45110 Ioannina, Greece

The exploration of phosphatases as targets for pharmacological intervention has been comparatively understudied in contrast to kinases. Our research is focused on PTEN, a prominent lipid phosphatase of significant human physiology relevance, which regulates the PI3K/Akt/mTOR signaling pathway and is implicated in various human diseases and pathologies. Compounds reported to act as PTEN inhibitors have demonstrated encouraging results in enhancing functional recovery after nerve, lung, or cardiac injuries in both cell-based and animal experiments. However, the specificity and potency of PTEN modulators remain unelucidated, while attempts to target PTEN's catalytic activity or its interactions with membranes and other proteins, using small molecules have encountered notable challenges. In the present study we have undertaken a comprehensive re-evaluation of first-generation PTEN inhibitors, specifically bisperoxo-vanadium (V) complex (bpVs) compounds by focusing on their inhibitory efficacy and selectivity both in vitro and in vivo. For this reason, we synthesized multiple bpV compounds and assessed their impact on, bacterially expressed, PTEN's phosphatase activity in vitro and we evaluated the PI3K/Akt pathway activity in PTEN wild-type and knock-out cell lines (A549, PC3, DU-145). bpVs exhibited varying levels of PTEN inhibition at the micromolar range that depends on the reducing conditions. Moreover, bpVs result in downstream activation of Akt and mTORC1 in vitro and in vivo, but also enhance a PTEN independent activation of Erk1/2. Additionally, bpV(phen) caused a suppressive effect on motility and exploratory behavior of Wistar rats after acute and subchronic administrations. While 1st generation PTEN inhibitors, particularly bpVs, are widely used, a consensus on their pharmacological profile was elusive. Our ongoing research focuses on understanding even more PTEN modulators especially peptide molecules that may affect its activity, conformation, or interaction with other proteins. Ultimately, we aim to underscore the significance of pharmacological PTEN targeting and establish a framework for the design of novel therapeutics.

P102

Ca2+ signaling dysregulation in human iPSC-derived astrocytes and neurons from Parkinson's disease patients

Anastasios Kollias[1], Christina Paschou[1], Konstantina Charmpi[1], Olympia Apokotou[2], Panagiotis Chandris[1], Martina Samiotaki[3], George Panagiotou[3], Erasmia Taoufik[1], Rebecca Matsas[1], Florentia Papastefanaki[*1, 2]

[1]Laboratory of Cellular and Molecular Neurobiology-Stem Cells, Hellenic Pasteur Institute, Athens, Greece
[2]Human Embryonic and Induced Pluripotent Stem Cell Unit, Hellenic Pasteur Institute, Athens, Greece
[3]Institute of Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece

Accumulating evidence demonstrates that astrocytic Ca2+ signals play critical roles in important brain functions, partially mediated through neuromodulatory systems, while their dysregulation may contribute in neurodegenerative conditions(1). Parkinson's disease (PD) models suggest that disturbances in neuronal and astrocytic Ca^{2+} signaling may lead to disruptive neuronal networks and provide insights into PD pathology(2,3). Here, we differentiated human induced pluripotent stem cells toward midbrain neurons (iNeurons)(4) and astrocytes (iAstrocytes) to investigate whether the autosomal dominant mutation p.A53T in alpha synuclein (αSyn), which is causal to PD, may affect the intrinsic Ca^{2+} activity of astrocytes or that of co-cultured neurons. Within this scope, after Fluo4-AM labeling, we acquired time-lapse images of live iAstrocytes in monocultures or of iNeurons co-cultured with iAstrocytes. iAstrocytes displayed spontaneous and ATP-induced activation through Ca^{2+}, validating their successful differentiation and maturation. Interestingly, the p.A53T-αSyn iAstrocytes displayed spontaneous Ca^{2+} activity with decreased amplitude and peak latency and increased frequency, as compared with healthy iAstrocytes, without considerable differences in their response to ATP. Proteomic analysis of healthy and p.A53T-αSyn iAstrocytes highlighted several differentially expressed proteins with critical roles in important Ca^{2+}-mediated intracellular pathways. Furthermore, in a mixed neuron-astrocyte setup, the p.A53T-αSyn iNeurons presented with increased peak amplitude and firing frequency, as compared with healthy iNeurons, regardless of the co-cultured iAstrocytes, in line with our previous work(5). Of note, in the presence of p.A53T-αSyn astrocytes, both healthy and p.A53T-αSyn iNeurons displayed augmented firing frequency. Altogether, our data support that iAstrocytes bearing the pA53T-αSyn mutation present malfunctions in Ca^{2+} homeostasis that alter their spontaneous Ca2+ activity. Moreover, they contribute to abnormal neuronal Ca^{2+} firing, in relevance with PD pathology. Next, along with our proteomic data, we will investigate the ER, mitochondria, and plasma membrane of the p.A53T-αSyn iAstrocytes to elucidate the observed Ca^{2+} disturbances and explore neuron-astrocyte cross-talk to uncover novel therapeutic targets.

Funding
Funded by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment" (Project 1019-DiseasePhenoTarget; PI: R. Matsas)

References

1. *Mustaly-Kalimi, S., et al.. "Calcium Signaling Deficits in Glia and Autophagic Pathways Contributing to Neurodegenerative Disease". Antioxidants & redox signaling (2018), 29(12), 1158–1175. https://doi.org/10.1089/ars.2017.7266*
2. *Aflaki E, et al. "A characterization of Gaucher iPS-derived astrocytes: Potential implications for Parkinson's disease." Neurobiol Dis. (2020). doi: 10.1016/j.nbd.2019.104647*
3. *Ramos-Gonzalez P, et al. "Astrocytic atrophy as a pathological feature of Parkinson's disease with LRRK2 mutation." NPJ Parkinsons Dis. (2021) doi: 10.1038/s41531-021-00175-w*
4. *Kouroupi G, et al. "Defective synaptic connectivity and axonal neuropathology in a human iPSC-based model of familial Parkinson's disease". PNAS (2017). doi: 10.1073/pnas.1617259114*
5. *Zygogianni O, et al. "In vivo Phenotyping of Familial Parkinson's Disease with Human Induced Pluripotent Stem Cells: A Proof-of-Concept Study". Neurochemical Research (2019) doi: 10.1007/s11064-019-02781-w*

P103

Fibroblasts' central role in the cell-to-cell communication network of intestinal cancer

Athanasia Stavropoulou[1, 2], Niki Chalkidi[2], Christoforos Nikolaou[1], Vasiliki Koliaraki[2]

[1]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Athens, Vari, Greece
[2]Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center "Alexander Fleming", Athens, Vari, Greece

Fibroblasts exert multiple functions during intestinal homeostasis, damage-repair, inflammation, and tumorigenesis[1]. Cancer-associated fibroblasts (CAFs) are found both inside and adjacent to tumours. They affect the progression of cancer by regulating immunological responses and extracellular matrix (ECM) remodelling, acquiring either tumour-supporting or tumour-suppressive properties[2,3,4] and are, therefore, considered as promising targets for therapy. However, the extent of their heterogeneity and the functions of individual CAF subsets in colorectal cancer is still unclear. To elucidate the roles of CAFs in intestinal cancer, we have computationally integrated in-house single cell RNA sequencing data of stromal, epithelial and immune cells isolated from two distinct murine tumor models: AOM/DSS and $Apc^{min/+}$. Based on marker gene expression, we found CAFs to be classified into three major subsets, similar to the normal tissue. Nevertheless, they exhibited a transcriptionally activated state, characterised by enrichment of ECM- and wound healing-related pathways. A unique pre-CAF state was also detected in $Apc^{min/+}$ tumors. Cell-to-cell communication inference analysis revealed multiple potential interactions between different celltypes in the tumours and a central role for fibroblasts in the inferred interaction networks, acting as the major signal-senders to other fibroblasts, macrophages, and cancer cells. Enriched communication pathways in the two murine models indicate both model-specific and common activation patterns that can help us decipher the mechanisms that drive early intestinal carcinogenesis. These data constitute a first step towards the deeper understanding of intratumoral cell communication and CAF subset function in intestinal cancer in the effort to identify novel strategies towards CAF therapeutic reprogramming.

Bibliography

1. *Chalkidi, N., Paraskeva, C. & Koliaraki, V. Fibroblasts in intestinal homeostasis, damage, and repair. Front. Immunol. 13, 924866 (2022).*
2. *Koliaraki, V., Prados, A., Armaka, M. & Kollias, G. The mesenchymal context in inflammation, immunity and cancer. Nat. Immunol. 21, 974–982 (2020).*
3. *Chen, Y., McAndrews, K. M. & Kalluri, R. Clinical and therapeutic relevance of cancer-associated fibroblasts. Nat. Rev. Clin. Oncol. 18, 792–804 (2021).*
4. *Elyada, E. et al. Cross-Species Single-Cell Analysis of Pancreatic Ductal Adenocarcinoma Reveals Antigen-Presenting Cancer-Associated Fibroblasts. Cancer Discov. 9, 1102–1123 (2019).*

P104

Notch3 expression characterizes intestinal tumor pericytes

Niki Chalkidi[1,*], Athanasia Stavropoulou[1,2,*], Maria Sakkou[2], Christoforos Nikolaou[2], Vasiliki Koliaraki[1]

*[1]Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece, 2Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece,*equal contribution*

The Notch signaling pathway plays an important role in development, homeostasis, and disease, such as cancer, where its therapeutic potential is under consideration. However, the role of individual receptors, their cell-specific functions, and their implications in cancer therapy are not yet fully understood. Notch3 is of particular interest, as it is overexpressed in colorectal cancer (CRC) and associated with worst prognosis. To determine the cell specificity of Notch3 expression in CRC, we performed single-cell RNA sequencing in tumor stromal cells from two mouse models of intestinal carcinogenesis (AOM/DSS, $Apc^{min/+}$). These data showed that Notch3 and downstream targets were expressed specifically in tumor pericytes, which was further verified by immunohistochemistry and flow cytometry analysis. In addition, Notch3+ pericytes were significantly enriched in murine intestinal tumors and could be further divided in four subclusters, indicating diverse origins and functions. Similar analysis of published human single cell data verified restricted Notch3 expression in tumor pericytes from CRC patients and comparable heterogeneity in the pericyte population. Preliminary in vitro experiments further showed that Notch3 activation in mouse fibroblasts resulted in changes in fibroblast proliferation and migration. We are currently using lineage tracing and cell-specific genetic Notch3 activation in animal models to decipher the origin of tumor pericytes in colorectal cancer, the mechanisms that drive pericyte-specific Notch3 activation, and its role in carcinogenesis. These experiments can provide a better understanding of the role of Notch3 in CRC and the potential of its inhibition in the clinic.

P105

Biochemical and NMR analysis revealed the regulatory role of the mitochondrial estrogen receptor beta in mitochondrial transcription and metabolism in SH-SY5Y and N2A neuroblastoma cells

Georgantopoulos Achilleas[1a], Tsialtas Ioannis[1a], Chasapi A. Styliani[2], Kalousi D. Foteini[1], Spyroulias A. Georgios[2], Psarra G. Anna-Maria[*1]

[1]Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis, 41500 Larissa, Greece.
[2]Department of Pharmacy, University of Patras, 26504 Patras, Greece
[a]equal contribution

Estrogens are steroid hormones that regulate cell growth, differentiation, metabolism, and physiology of the reproductive system, via their cognate receptors, the estrogen receptors (1). Estrogens are also known for their neuroprotective actions. These actions are proposed to be associated, among others, with the mitochondrial localization of ERβ, affecting mitochondrial transcription, oxidative phosphorylation, enzyme biosynthesis, reactive oxygen species production and apoptosis (2, 3). In this study, the direct involvement of the mitochondrial ERβ in the regulation of mitochondrial transcription and mitochondrial metabolism of neuroblastoma SH-SY5Y and N2A cells was documented by real-time PCR, ATP measurements and NMR-based mitochondrial metabolomic analysis. Our results revealed mitochondrial transcription activation, and oxidative phosphorylation enzymes (OXPHOS) biosynthesis, in the presence or absence of estrogen, even in the presence of α-amanitin, a specific inhibitor of the DNA-dependent RNA polymerase II in ERβ positive SH-SY5Y cells and in N2A cells stably overexpressing a mitochondrial targeted ERβ (N2AmtGFPERβ), revealing direct involvement of the mitochondrial ERβ in mitochondrial transcription. This action is followed by increased pyruvate-triggered activation of the mitochondrial metabolism, as revealed by NMR-based mitochondria metabolomics analysis, in isolated mitochondria from N2AmtGFPERβ compared to the N2AmtGFP ones. Specifically, NMR analysis showed increased hormone-dependent pyruvate metabolism, GTP production, amino acids synthesis and decreased ADP and NAD levels in isolated mitochondria from N2AmtGFPERβ compared to the N2AmtGFP ones. These results are in accordance with the increased mitochondrial transcription and ATP production in mtERβ overexpressing cells. Our results substantiate the direct involvement of mtERβ in mitochondrial transcription, which is accompanied by increased mitochondrial metabolism leading to increased amino acids synthesis and consumption, increased OXPHOS biosynthesis, Krebs cycle activation and ATP production.

1. *Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, et al. Estrogen receptors:: How do they signal and what are their targets. Physiol Rev. 2007;87(3):905-31.*
2. *Tsialtas I, Georgantopoulos A, Karipidou ME, Kalousi FD, Karra AG, Leonidas DD, et al. Anti-Apoptotic and Antioxidant Activities of the Mitochondrial Estrogen Receptor Beta in N2A Neuroblastoma Cells. Int J Mol Sci. 2021;22(14).*
3. *Psarra AMG, Sekeris CE. Glucocorticoid receptors and other nuclear transcription factors in mitochondria and possible functions. Bba-Bioenergetics. 2009;1787(5):431-6.*

P106

The effects of Aticaprant, a κ-opioid receptor antagonist, in stress-induced deficits in mood and cognition

Alexandra Symeonof, Anastasia Vamvaka-Iakovou, Anastasia Megalokonomou, Christos Karoussiotis, Ioannis Sotiropoulos, Zafiroula Georgoussi

[1]Laboratory of Cellular Signaling & Molecular Pharmacology, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", 15310 Athens, Greece

Kappa opioid receptors (κ-ORs) are extensively investigated for their emerging role in anxiety and depression, as κ-OR blockade impedes the effects of stress in animal studies (1). Aticaprant, a selective κ-OR antagonist is currently in phase III clinical trials and is used for treatment of depression (2). In the present study, mice were subjected to unpredictable chronic stress, followed by administration of Aticaprant and behavioral assessments were performed, including elevated plus maze, open field, novel object recognition, Y-maze and forced swim test. Our results demonstrate that Aticaprant produced an anxiolytic and antidepressant effect, reversed stress-induced impairments in long-term memory, but was ineffective on short-term memory deficits. Recent studies from our laboratory have demonstrated that κ-OR induces autophagy (3), a homeostatic mechanism that degrades dysfunctional proteins to modulate the morphology of neuronal cells and alter synaptic plasticity (4). We have shown that activation of κ-OR mediates the autophagic machinery via a Gαi/o-ERK1,2-CREB pathway resulting in the decrease of hippocampal synaptic proteins, under acute stress conditions (3). In this respect, herein, we demonstrate that the levels of the hippocampal synaptic proteins spinophilin, PSD95 and SNAP25 in stressed animals were restored in Aticaprant-treated animals. Moreover, Aticaprant altered the levels of the autophagic markers in chronic stressed animals compared to naïve ones, with a concomitant alteration of the ERK1/2 signaling pathway. Our data provide evidence for the mechanism via which Aticaprant exerts its therapeutic effects as a putative novel drug to alleviate stress-related disorders.

1. *Browne CA, et al. Targeting opioid dysregulation in depression for the development of novel therapeutics. Pharmacol Ther. 2019 Sep;201(9):51-76. doi: 10.1016/j.pharmthera.2019.04.009.*
2. *Jacobson ML, et al. The kappa opioid receptor antagonist aticaprant reverses behavioral effects from unpredictable chronic mild stress in male mice. Psychopharmacology 2020 Dec;237(12):3715-3728. doi: 10.1007/s00213-020-05649-y. Epub 2020 Sep 7.*
3. *Karoussiotis C, et al. The κ-opioid receptor-induced autophagy is implicated in stress-driven synaptic alterations. Front Mol Neurosci. 2022 Nov;16: 15:1039135. doi: 10.3389/fnmol.2022.1039135.*
4. *Nikoletopoulou V, et al. Autophagy in the physiology and pathology of the central nervous system. Cell Death Differ. 2015 Mar;22(3):398-407. doi: 10.1038/cdd.2014.204. Epub 2014 Dec 19.*

P107

Cell signaling pathways during the early development of reared greater amberjack (Seriola dumerili, Risso 1810)

Nikolas Panteli[1], Konstantinos Feidantsis[2], Christina Chatsatourian[1], Maria Demertzioglou[1], Konstantinos Kormas[3], Elena Mente[4], Efthimia Antonopoulou1

[1]School of Biology, Aristotle University of Thessaloniki, Greece
[2]School of Agricultural Sciences, University of Patras, Mesolonghi, Greece
[3]School of Agricultural Sciences, University of Thessaly, Volos, Greece
[4]School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

Somatic growth and ontogenic changes are coordinated in response to endogenous and environmental cues, which are mediated via several signal transduction pathways to target tissues for activation of proliferation and differentiation processes. Although aquaculture ensures the simulation of fish natural environment, rearing conditions may interfere with the developmental cues, potentially affecting growth rate and development. Reproductive dysfunctions of captive-reared greater amberjack (Seriola dumerili), which constitute the primary bottleneck to its commercialization, are speculated to emerge due to captivity-induced stress or nutritional deficiencies in fishfeed. Thus, the main aim of the present study was to explore the involvement of several components and downstream targets of signaling mechanisms during the development of reared greater amberjack. For this purpose, activation of insulin-like growth factor type 1 receptor (IGF-1R), protein kinase B (Akt) and mitogen-activated protein kinases (MAPKs), and induction of heat shock proteins (Hsps) were evaluated through SDS-Page/immunoblot analysis during five developmental stages: 1 day prior to hatching fertilized eggs (D-1), hatching day (D0), 3 days post-hatching larvae (D3), 33 (D33) and 46 (D46) days post-hatching juveniles. According to the results, IGF-1R/Akt pathway was significantly activated during D-1 and D33 stages, which may recruited for the transduction of hypertrophic growth signals. On the contrary, MAPKs phosphorylation was prominent during the post-hatching stages, potentially involved in the transduction of cell proliferation and differentiation signals to facilitate the organogenesis in postembryonic period. Compared to earlier stages, Hsps exhibited higher induction at D3 and onwards, while Hsp90 seems to play pivotal role in post-hatching ontogeny of greater amberjack. Collectively, the present findings demonstrate the changes in pivotal for the ontogeny signaling pathways during the development of reared S. dumerili.

Acknowledgements
The project is funded by the Operational Program "Competitiveness, Entrepreneurship and Innovation 2014-2020 (EPAnEK)" (project code: 6-00068, MIS: 5045873, Acronym: MAGIATIKO), which is co-funded by Greece and the European Union.

P108

Serglycin-WISP-1 interplay affects tumorigenic signaling and aggressiveness in glioblastoma

Eleftherios N. Athanasopoulos[1], Dimitra Manou[2], Panos Fountas[3], Zoi Karagiorgou[1], Giannis Giotis[1], Dimitra Bainantzou[1], Alexios Aletras[1], Achilleas D. Theocharis[1]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Brain Tumor Biology, Division of Translational Cancer Research, Lund University, Sweden
3Biochemistry and Molecular Biology, Lund University – Malmo, Sweden

WNT-inducible signaling pathway protein-1 (WISP-1, also known as CCN4) belongs to the CCN gene family and encodes a secreted matricellular protein with diverse functions and interactions within the extracellular matrix. Recent findings indicate that WISP-1 contributes to the aggressive phenotype of various malignant tumors, including glioblastoma, the most aggressive brain tumor. The tethering of WISP-1 to the extracellular domain of heterodimeric transmembrane integrins has been shown to activate a plethora of signaling pathways, leading to enhanced cell proliferation, migration and invasion. Serglycin (SRGN) is an intracellular proteoglycan that can also be found in the extracellular matrix and plays an important role in the promotion of malignancies including glioblastoma. We established the overexpression of SRGN in glioblastoma cells and we have developed SRGN-suppressed LN-18 glioblastoma cells, which exhibit a diminished aggressive potential compared to control cells. In the present study we evaluated the association of WISP-1 and SRGN in glioblastoma progression. We found reduced levels of WISP-1 and integrin heterodimers in SRGN-suppressed LN-18 cells. Although cell proliferation and migration were induced in control SRGN-expressing LN-18 glioblastoma cells treated with exogenous WISP-1, SRGN-suppressed cells were not responsive to exogenous addition of WISP-1 most likely due to the low levels of integrins. Exogenous addition of WISP-1 was able to activate integrin/FAK/MAPK signaling axis and Wnt/β-catenin pathway only in control SRGN-expressing LN-18 glioblastoma cells and not in SRGN-suppressed LN-18 cells. Our data indicate an interplay between WISP-1 and SRGN in glioblastoma cells that affect their tumorigenic potential.

P109

Implication of the VEGFR2 pathway in the activation of endothelial cells by the β-adrenergic receptor agonist isoproterenol

Vasiliki Kanellopoulou*, Eleni Mourkogianni, Evangelia Papadimitriou

*Laboratory of Molecular Pharmacology, Department of Pharmacy, School of Health Sciences, University of Patras, Greece; *e-mail: kanellopoulouvasia@gmail.com*

Adrenergic receptors (ARs) have a wide distribution and affect numerous functions. The catecholamines norepinephrine and epinephrine are the endogenous ligands of ARs and have been shown to induce the proliferation and migration of endothelial cells, thus promoting angiogenesis. Based on these observations, propranolol and other β-adrenergic receptor antagonists are being investigated as anti-angiogenic and anticancer therapeutic options. Although there are some efforts to identify the mechanism(s) through which ARs affect angiogenesis, the existing data are incomplete. Based on a structural study that supports a potential direct interaction between β_2-adrenergic receptors (β_2AR) and VEGFR2 , the present work studies the potential crosstalk of βARs with VEGFR2 signaling in endothelial cells and its impact in endothelial cell functions. We used endothelial cells isolated from the human umbilical vein (HUVEC) and mouse lungs (lung microvascular endothelial cells, LMVEC). The effect of βARs agonists and antagonists on cell proliferation and migration was studied using direct measurement of cells and the transwell assay, respectively. Activation of downstream signaling pathways was studied by Western blot. The non-selective βARs agonist isoproterenol was found to significantly induce both proliferation and migration of HUVEC. The nonselective βARs antagonist propranolol, but not the selective β_1ARs antagonists nebivolol and atenolol, decreased both unstimulated and isoproterenol induced endothelial cell proliferation and migration, suggesting that these effects were not mediated by the β1ARs. Propranolol did not affect $VEGFA_{165}$-induced endothelial cell proliferation and ERK1/2 activation. On the other hand, the selective VEGFR2 tyrosine kinase inhibitor SU1498 decreased the stimulatory effect of isoproterenol on endothelial cell proliferation. Isoproterenol enhanced tyrosine phosphorylation of VEGFR2, an effect that was unaffected by propranolol and abolished by SU1498. Interestingly, isoproterenol-induced ERK1/2 activation was abolished by propranolol but unaffected by SU1498, while Akt activation was decreased but not abolished by both inhibitors. Collectively, these data support the notion that the β_2AR cross talks with VEGFR2 and that βAR antagonists, such as propranolol, may have a beneficial effect when added in the therapeutic scheme to control angiogenesis that is due to adrenergic stimulation.

P110

The effect of EGFR and JAK/STAT signaling pathways on glypican expression in breast cancer cells

Paraskevi Ioannou[1], Kyriaki Tzaferi[1], Christos Koutsakis[1], Zoi Piperigkou[1, 2], Nikos K. Karamanos[1,2]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Foundation for Research and Technology-Hellas (FORTH) / Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece

Breast cancer is the most prevailing form of cancer in women, with the extracellular matrix (ECM) and its dynamic remodeling contributing to disease progression. Among the major components of the ECM are proteoglycans (PGs). Glypicans (GPCs) are heparan sulfate PGs anchored to the cell surface via GPI anchor and function as growth factors as well as cytokine co-receptors. An important regulator in the context of breast cancer progression is the JAK/STAT signaling pathway, particularly JAK2/STAT3. This pathway oversees the expression of genes associated with cancer cell characteristics, while growth factor receptors like the epidermal growth factor receptor (EGFR) are also pivotal in this process. The aim of this study is to evaluate the effect of the EGFR and JAK/STAT signaling pathways on GPCs in breast cancer cells with different estrogen (ER) status. To this end, ERα-positive MCF-7, and ERβ-positive MDA-MB-231 cell lines were treated with EGFR and JAK/STAT inhibitors, namely AG-1478 and AG-490 respectively. The functional properties of the two different cell lines, as well as the expression of GPCs 1-6 were studied. According to the results, the inhibitors mitigate cell proliferation and migration, while increasing the adhesion on collagen type I. Furthermore, the inhibitors seem to have a cell-line-dependent effect on GPCs expression, as in MCF-7 cells their expression is mostly downregulated with exceptions towards GPC-4 and GPC-5. Conversely, in MDA-MB-231 cells, glypican expression exhibits an upregulation pattern. Additionally, using bioinformatic tools, particularly STRING analysis, the small leucine-rich PG decorin emerges as a putative link between all GPCs and EGFR. Subsequently, a deeper understanding on the effect of EGFR and JAK/STAT signaling may shed light into the role of GPCs and decorin in breast cancer progression and could potentially contribute to novel therapeutic solutions.

P111

Mechanistic insights into the anticancer effect of sulfated hyaluronan in aggressive cancer cells

Christos Koutsakis[1], Sylvia Mangani[1], Nikolaos Ef. Koletsis[1], Zoi Piperigkou[1,2], Martyna Maszota-Zieleniak[3], Sergey A. Samsonov[3], Nikos K. Karamanos[1,2]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Foundation for Research and Technology-Hellas (FORTH) / Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece
[3]Faculty of Chemistry, University of Gdansk, Gdansk, Poland

Hyaluronan (HA), a central component of the extracellular matrix (ECM), is a linear non-sulfated glycosaminoglycan that plays key roles in various physiological processes, as well as in malignancies. Our research group has previously shown that chemically modified sulfated hyaluronan (sHA) exhibits a promising anticancer effect on breast cancer cells. The aim of the current study is to investigate the mechanism of action of sHA and thus evaluate its effect in highly metastatic breast cancer cells. To this end, MDA-MB-231 cells were treated with either HA or sHA fragments of 50 kDa. Gene expression and protein levels of HA receptors were analysed by real-time PCR, Western blot, and immunofluorescence in both 2D cultures and 3D spheroids. Furthermore, the functional properties of the cells were studied following the selective blocking of CD44, the major HA receptor. Additionally, to test their degradation potential, the HA and sHA fragments were incubated with exogenous hyaluronidases. Finally, in silico studies were performed to examine the molecular docking of HA and sHA to CD44 and HYAL1. According to the results, CD44 expression is not affected by either treatment at the gene or protein level, while RHAMM appears downregulated after treatment with sHA. Blocking of the CD44 receptor significantly ameliorated the observed effects for both HA and sHA, suggesting that sHA's action is mainly CD44-mediated. The degradation assay revealed that sHA cannot be cleaved by hyaluronidases at the same rate as HA, while data from the simulation studies suggested that the binding of HA to CD44 is more stable than sHA, and sHA binding to HYAL1 is rather improbable due to electrostatic repulsion. Conclusively the obtained data demonstrate that the sulfation of 50 kDa HA alters the functional breast cancer cell properties via its effects in ECM remodeling enzymes, gene expression and receptor binding properties.

P112

Effect of direct agonists of soluble Guanylyl Cyclase (sGC) on Platelet-Derived Growth Factor (PDGF) responses in vascular smooth muscle cells

Charalampos Paixos[1], Maria Stratoudaki[1], Anna-Sophia Parianou[1], Nikiforos Chandrinos[1], Konstantinos Salagiannis[1], Konstantinos Laimos-Geranios[2], Aggeliki Christopoulou[2], Konstantinos Toufas[2], Dionisis-Panagiotis Kintos[2], Manolis Fousteris[2], Stavros Topouzis[1]

[1]Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, GR-26504, Greece
[2]Laboratory of Medicinal Chemistry, Department of Pharmacy, University of Patras, GR-26500, Greece

The mitogens PDGF-BB and -DD are known to elicit phenotypic switching and mobilization in vascular smooth muscle cells (VSMCs), resulting in vascular dysfunction. The axis of NO/sGC/cGMP has been described to suppress VSMC proliferation and/or migration and therefore, molecules that can directly stimulate sGC could be promising therapeutics in atherosclerosis, pulmonary hypertension or (re)stenosis.

The Aim of the present study was to evaluate the ability of novel sGC direct agonists (sGC "stimulators") to modulate the effects of PDGF-BB (acting on both PDGFR-αβ and -ββ) and PDGF-DD (acting only on PDGFR-ββ) in VSMCs.

We first characterized the potential sGC stimulator DPK 399, rationally designed and synthesized by us. Subsequently, we compared the effects of the NO donor SNP, the known sGC stimulator BAY 41-2272 and DPK 399, on PDGF-induced proliferation (MTT assay), migration (scratch wound healing assay) and PDGFR downstream signaling (phosphorylation of p44/42 MAPK – Erk1/2 at Thr202/Tyr204 and Src at Tyr416 by western blotting) using as a model the rat aortic VSMCs line A7r5.

DPK 399 activated only the reduced-heme form of sGC and strongly synergized with NO, and therefore is a bona fide sGC stimulator. All three sGC agonists significantly reduced A7r5 proliferation triggered by PDGF-DD, but not by PDGF-BB, showing PDGFR-selectivity, despite comparable PDGF-BB and -DD responses. In addition, BAY 41-2272 strongly diminished A7r5 migration initiated by PDGF-DD in the scratch wound healing assay. Lastly, the direct sGC agonists BAY 41-2272 and DPK 399 suppressed the Erk and Src phosphorylation downstream of PDGFR-ββ, in response to PDGF-DD.

In conclusion, direct sGC agonists can suppress signaling by the potent VSMCs mitogen PDGF-DD, supporting their therapeutic use in vascular fibroproliferative disorders such as (re)stenosis and atherosclerosis.

We acknowledge support of this work by the project ***"INSPIRED-The National Research Infrastructures on Integrated Structural Biology, Drug Screening Efforts and Drug Target Functional Characterization"*** *(MIS 5002550) which is implemented under the Action "Reinforcement of Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and European Union (European Regional Development Fund).*

P113

NR2F2 is essential for endothelial angiogenic responses to Fibroblast Growth Factor-2 (FGF-2) in vitro

Anna-Sofia Parianou[1*], Maria Stratoudaki[1], Charalampos Paixos[1], Paraskevi-Ioanna Kyrgyridi[1], Nikiforos Chandrinos[1], Vasiliki Vazoura[1], Christina Dafni[1], Dionisis-Panagiotis Kintos[2], Emmanouil Fousteris[2], Stavros Topouzis[1]

[1]Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, GR-26504, Greece
[2]Laboratory of Medicinal Chemistry, Department of Pharmacy, University of Patras, GR-26500, Greece

NR2F2 (aka Chicken Ovalbumin Upstream Promoter Transcription Factor II, COUP-TF II) is a member of the steroid/thyroid nuclear hormone receptor superfamily, with a well-characterized role in organogenesis. NR2F2 is highly expressed in venous endothelium and determines its identity. Its role in angiogenesis has been previously studied using mainly VEGF as a prototypic stimulus. However, additional such stimuli are known and characterized, evidenced by the patchy effectiveness of anti-VEGF agents in cancer neo-angiogenesis, which is frequently bypassed by responses to alternative pro-angiogenic factors. Among the best-known is the polypeptide FGF-2 (bFGF).

The aim of this study was to investigate whether inhibition of NR2F2 is able to modulate the pro-angiogenic responses of human umbilical vein endothelial cells (HUVECs) to bFGF in vitro.

To reduce NR2F2 activity, we used the selective NR2F2 micromolecular inhibitor CIA2. We examined its effects on HUVEC proliferation by MTT assay, on migration by scratch wound healing and Transwell® assays and on pseudo-vessel network formation in Matrigel®. In addition, the activation by phosphorylation at Thr202/Tyr204 of ERK1/2 was assessed by western blotting, after either NR2F2 knock-down by a specific siRNA or by its pharmacological inhibition using CIA2.

The effect of bFGF in all of the above HUVEC functional responses was abrogated by CIA2. In agreement with the above, bFGF-induced phosphorylation of ERK1/2 was reduced by both NR2F2 knock-down and by CIA2 treatment.

We conclude that the anti-angiogenic effect of NR2F2 seems to extend to an additional bona fide pro-angiogenic factor (bFGF) and therefore inhibition of its activity by a small molecule should be considered as an anti-angiogenic therapeutic avenue that could overcome VEGF-blockade resistance.

P114

Initial in vitro characterization of KL-67, a novel, rationally designed direct agonist of soluble Guanylyl Cyclase (sGC)

Maria Stratoudaki[1*], Charalampos Paixos[1], Anna Sophia Parianou[1], Konstantinos Laimos-Geranios[2], Aggeliki Christopoulou[2], Konstantinos Toufas[2], Dionisios-Panagiotis Kintos[2], Manolis Fousteris[2], Stavros Topouzis[1]

[1]Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, GR-26504, Patras, Greece
[2]Laboratory of Medicinal Chemistry, Department of Pharmacy, University of Patras, GR-26500, Patras, Greece

The nitric oxide (NO)-sGC-cGMP axis plays a pivotal role in vascular homeostasis and development of cardiovascular disease and has been a promising therapeutic target. In recent years, a novel class of drugs, called direct sGC agonists have been developed. sGC "stimulators" require a reduced (Fe^{+2}) heme group associated with sGC and can act either independently of, or synergize with, NO. Their advantage over existing axon-modifying drugs is their ability to be effective in pathological conditions when there is a deficit of NO bioavailability.

The aim of the present work was the in vitro characterization of a novel sGC direct agonist, rationally designed to act as a "stimulator".

A candidate sGC stimulator bearing a multi-substituted pyrazolo[3,4-c]pyridin-7(6H)-one skeleton was synthesized and tested in cultured rat aortic smooth muscle cells (SMCs, line A7r5), for its ability to a) activate sGC by itself or to synergize with NO, by determination of cGMP levels with an ELISA assay kit, b) produce a characteristic cGMP-dependent VASP phosphorylation at Ser239, by western blotting, and c) modulate characteristic SMC responses to the well-known vascular smooth muscle mitogen, PDGF-BB, by assessing cell proliferation via MTT assay and cell migration, by scratch-wound and Transwell®-migration assays.

KL-67 exhibited weak sGC agonist properties on its own; however, it displayed strong synergism with the NO donor Sodium Nitroprusside and required a reduced heme, properties characteristic of sGC stimulators. The ability to elevate cGMP levels was further translated by increased phosphorylation of VASP at Ser239. Importantly, KL-67 significantly reduced both the migratory and proliferative effects of PDGF-BB in smooth muscle cells, potentially indicating a positive role in vascular homeostasis. Therefore, the novel, rationally-designed KL-67 is a bona fide sGC "stimulator", displaying vasculoprotective properties in vitro. Optimized derivatives of KL-67 are expected to allow the development of "lead" molecules with improved therapeutic potential.

*We acknowledge support of this work by the project "**INSPIRED-The National Research Infrastructures on Integrated Structural Biology, Drug Screening Efforts and Drug Target Functional Characterization"** (MIS 5002550) which is implemented under the Action "Reinforcement of Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and European Union (European Regional Development Fund)*

P115

Detection of neurodegeneration markers in the aftermath of SARS-CoV-2 infection

Despoina Olga Papaggeli[1§], Sofia Mazi[1§], Dimitrios S. Mysiris[2], Theodoros Mavridis[3], Georgia Papadopoulou[1], Maria Morakou[1,4], Georgia Xiromerisiou[5], Georgios D. Vavougios[6,7], Urania Georgopoulou[1], Eirini Karamichali[1*], Pelagia Foka[1*]

[1]Molecular Virology Laboratory, Hellenic Pasteur Institute, Athens, Greece
[2]Faculty of Medicine, University of Thessaly, Larissa, Greece
[3]1st Neurology Department, Eginition Hospital, Medical School, National & Kapodistrian University of Athens, Greece
[4]Molecular Microbiology & Immunology Laboratory, Department of Biomedical Sciences, University of West Attica, Athens, Greece.
[5]Department of Neurology, University Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Greece
[6]Laboratory of Pulmonary Testing and Rehabilitation, Department of Respiratory Medicine, Faculty of Medicine, University of Thessaly, Larissa, Greece
[7]Department of Neurology, Faculty of Medicine, University of Cyprus, Lefkosia, Cyprus
[§]These authors contributed equally to the work.

Although SARS-CoV-2 is considered to be a respiratory virus, neurological, dementia-like symptoms have emerged in infected patients. Cognitive decline has been associated with Long COVID, a syndrome with broad and prolonged clinical manifestations, afflicting many recovered individuals. So far, a mechanistic link between neurodegenerative diseases, such as Alzheimer's Disease (AD), and SARS-CoV-2 has not been clearly demonstrated. Exosomes are nanosized particles, serving as masters of intercellular communication. They are involved in physiological homeostatic processes by transferring cargos of biomolecules and signalling compounds between cells. Late research has shown that they hold diverse roles in viral infections, inflammatory and neurodegenerative diseases. We aimed to investigate the presence of neurodegenerative markers, indicative of a SARS-CoV-2-triggered initiation of neurodegenerative mechanisms, in the serum exosomal cargo of SARS-CoV-2 patients who experienced Long COVID-related symptoms after recovery. For this, we implemented a well-designed workflow that involved collection of a thoroughly characterised cohort of Long COVID sufferers with and without measurable cognitive decline, together with controls from healthy volunteers and AD patients. Representative sera samples were used to isolate total and neurospecific exosomal fractions that were characterised for size and concentration by nanoparticle tracking analysis. Then, they were tested for the presence of specific surface and internal exosomal markers. At the same time, we used differentiated SH-SY5Y neuron-like cells in the presence and absence of heavy metal ions to induce neuroinflammation that would allow the cells to express AD-related peptides. SH-SY5Y cell extracts were utilised as positive controls for peptide molecular weight verification alongside clinical samples in immunoblotting experiments. Our methodology allowed us to detect indications of neuropathic exosomal messages in the tested samples. Our results suggested that SARS-CoV-2 could promote the expression and circulation of certain neurodegenerative markers related to AD pathogenesis, an effect that may be preserved and possibly amplified after the end of viral infection.

P116

Receptor Protein Tyrosine Phosphatase zeta 1 (RPTPZ1) regulates primary osteoblast proliferation and differentiation in vitro

Athanasios Xanthopoulos[1*], Margarita Lamprou[1], Eleni Mourkogianni[1], Evangelia Papadimitriou[1]

*[1]Laboratory of Molecular Pharmacology, Department of Pharmacy, School of Health Sciences, University of Patras, Greece; *e-mail: athanasiosxan@gmail.com*

Receptor protein tyrosine phosphatase zeta 1 (PTPRZ1) belongs to the type V subfamily of receptor-type protein tyrosine phosphatases (RPTPs). Its primary expression occurs in the brain, although it is also found in endothelial cells, cancer cells, and fully differentiated osteoblasts. A DNA microarray analysis of primary osteoblasts revealed that among 10,000 genes, the Ptprz1 gene exhibited the most significant induction during differentiation, underscoring its role as a marker for terminally differentiated osteoblasts. In the present work, we used mice that are knockout for Ptprz1 ($Ptprz1^{-/-}$) and their corresponding wild-type mice ($Ptprz1^{+/+}$) to isolate primary calvaria osteoblasts and identify potential differences related to their proliferation and differentiation rate. $Ptprz1^{-/-}$ osteoblasts demonstrated an enhanced proliferation rate and elongated mitochondria when compared to $Ptprz1^{+/+}$ osteoblasts, in line with the increased activity of Akt and ERK1/2 kinases. Among known PTPRZ1 ligands such as pleiotrophin, vascular endothelial growth factor, and fibroblast growth factor-2 (FGF-2), only FGF-2 enhanced $Ptprz1^{+/+}$ osteoblast proliferation, and this effect was independent of the PTPRZ1 receptor since it was also observed in $Ptprz1^{-/-}$ osteoblasts. In osteogenic differentiation conditions, $Ptprz1^{+/+}$ osteoblasts exhibited a higher differentiation potential into mature osteoblasts compared to $Ptprz1^{-/-}$ osteoblasts, as indicated by higher alkaline phosphatase activity and the expression of osteoblast maturation and differentiation markers, such as collagen type I and Runx2. Our findings collectively suggest that PTPRZ1 acts as an endogenous brake of primary osteoblast proliferation and as an enhancer of osteoblast differentiation and ways to exploit this therapeutically will be discussed.

Acknowledgements
This research was partially co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning 2014-2020» in the context of the project "PLEIOBONE" (MIS 5047169). The Ptprz1-/- mice were a generous gift from Drs. H. Himburg and J.P. Chute, Division of Hematology/Oncology, Broad Stem Cell Research Center, UCLA, USA.

P117

Cell type-specific and senescence stimulus-dependent epigenetic signatures support heterogeneity in cellular senescence

Eleni Mavrogonatou[1], Katarzyna Malgorzata Kwiatkowska[2], Adamantia Papadopoulou[1], Paolo Garagnani[2,3], Chiara Pirazzini[4], Claudio Franceschi[2,5], Dimitris Kletsas[1*]

[1]Laboratory of Cell Proliferation and Ageing, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", 15341 Athens, Greece
[2]Department of Medical and Surgical Sciences (DIMEC), University of Bologna, 40126 Bologna, Italy
[3]IRCCS Azienda Ospedaliero-Universitaria di Bologna, 40138 Bologna, Italy
[4]IRCCS Istituto delle Scienze Neurologiche di Bologna, 40139 Bologna, Italy
[5]Laboratory of Systems Medicine of Healthy Aging, Institute of Biology and Biomedicine and Institute of Information Technology, Mathematics and Mechanics, Department of Applied Mathematics, N. I. Lobachevsky State University, 603022 Nizhny Novgorod, Russia
**e-mail: dkletsas@bio.demokritos.gr*
#Authors contributed equally to this work

Senescent cells play an important role in tissue homeostasis and the development of age-related pathologies. On the other hand, chromatin structure is essential for the regulation of cell function and epigenetic alterations (i.e., DNA methylation, histone modification and chromatin remodeling) contribute to the induction and maintenance of senescence. Aim of the present study was to provide a comprehensive characterization of whole genome DNA methylation patterns in replicative and ionizing irradiation- or doxorubicin-induced premature senescence, exhaustively exploring epigenetic modifications in three different human cell types: in somatic diploid skin fibroblasts and in bone marrow- and adipose-derived mesenchymal stem cells. With CpG-wise differential analysis, three epigenetic signatures were identified: (a) cell type- and treatment-specific signature; (b) cell type-specific senescence-related signature; and (c) cell type-transversal replicative senescence-related signature. Cluster analysis revealed that only replicative senescent cells created a distinct group reflecting notable alterations in the DNA methylation patterns accompanying this cellular state. Replicative senescence-associated epigenetic changes seemed to be of such an extent that they surpassed interpersonal dissimilarities. Enrichment in pathways linked to the nervous system and involved in the neurological functions was shown after pathway analysis of genes involved in the cell type-transversal replicative senescence-related signature. Although DNA methylation clock analysis provided no statistically significant evidence on epigenetic age acceleration related to senescence, a persistent trend of increased biological age in replicative senescent cultures of all three cell types was observed. Overall, this work supports the heterogeneity of senescent cells depending on the tissue of origin and the senescence inducer, implying diverse biological roles in tissue homeostasis and the development of age-associated diseases. Further characterization of these epigenetic signatures that could be linked to different degrees of sensitivity towards senotherapeutic compounds may prove extremely useful for designing novel therapeutic strategies.

Reference

Kwiatkowska K.M.#, Mavrogonatou E.# et al., Cells. 2023 Mar 17;12(6):927. doi: 10.3390/cells12060927.

P118

Molecular and functional characterization of a subset of human skin fibroblasts that resist UVB-mediated premature senescence

Asimina Fotopoulou, Maria Angelopoulou, Harris Pratsinis, Eleni Mavrogonatou, Dimitris Kletsas*

Laboratory of Cell Proliferation and Ageing, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", 15341, Aghia Paraskevi, Attiki, Greece
**dkletsas@bio.demokritos.gr*

Normal cells, when exposed to exogenous genotoxic stresses can become prematurely senescent. These cells express a specific senescence-associated secretory phenotype (SASP), which affects tissue homeostasis, including promotion of tumor growth. Ultraviolet (UV) radiation accelerates the ageing of the skin, a phenomenon called "photoageing". Previous publications have reported that repeated non-cytotoxic doses of UVB radiation provoke, in a short period, premature senescence (SIPS) to human skin fibroblasts (HSF). We first verified that 3 days after exposure to UVB radiation (10 doses of 35 mJ/cm^2) HSF express signs of SIPS; however, in long- term cultures we observed a mixed population composed by senescent and proliferating cells and this is in contrast to ionizing radiation (IR)-treated cells which form a population of exclusively senescent cells. These, proliferating, so-called "resistant", cells remain normal as they have a limited life-span and they respond similarly to young and IR-mediated premature senescent HSFs to several stresses, such as oxidative, mitochondrial or heat stress. However, these cells seem to be more resistant to an additional exposure to UVB radiation, in comparison to young and prematurely senescent HSFs. RNAseq analysis showed that resistant cells constitute an intermediate population between young and IR-induced senescent cells. Among the genes with altered expression there are several stress-related genes, including ERCC6, which is upregulated only in resistant HSFs, and plays crucial role in UVB-resistance, as its siRNA-mediated knocking down dramatically increases the cytotoxic effect of UVB. Other genes that have been tested are several classical SASP genes, such as those coding for inflammatory cytokines and matrix metalloproteases, among others. Interestingly, the latter are known from our previous work to enhance the growth of cancer cells in vitro and in vivo. In agreement, resistant cells enhance (at the borderline of statistical significance) the growth of A431 squamous cancer cells in vitro and in vivo.

P119

"Anti-Aging": Exploiting the Greek microbial diversity for the discovery and development of novel antiaging molecules.

Eirini Gkogkou[1], Despoina D. Gianniou[1], Konstantinos Gaitanis[2], Aikaterini Theodosopoulou[1], Nikolaos Tsafantakis[2], Stavroula Kaili[3], Paris Laskaris[3], Maria S. Manola[1], Xanthippi P. Louka[1], Evangelia Tsiokanos[2], Nikolas Fokialakis[2], Dimitrios G. Hatzinikolaou[3], Amalia D. Karagouni[3], Ioannis P. Trougakos[1*]

[1]Section of Cell Biology and Biophysics, Department of Biology, National and Kapodistrian University of Athens, Greece
[2]Section of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, National and Kapodistrian University of Athens, Greece
[3]Section of Botany, Department of Biology, National and Kapodistrian University of Athens, Greece
E-mail address: itrougakos@biol.uoa.gr

Greek ecosystems are a fertile yet under-explored ground for the study of Actinobacteria, known producers of bioactive compounds. By exploring this under-investigated biodiversity and chemodiversity of Greek actinobacterial strains (mostly of Streptomyces sp.), the "Anti-Aging" project aims to uncover potentially novel natural compounds with anti-aging activity, that can be formulated as cosmeceutical products. In total, 1000 isolates belonging to the Athens University Bacterial & Archaea Culture Collection were studied. A customized in-house library of 2000 extracts was generated (EtOAc and MeOH/H_2O), all of which were investigated for potential anti-aging properties. This biological evaluation was performed though elastase (anti-aging activity) and tyrosinase (whitening activity) inhibition in vitro assays. A small yet significant number of extracts demonstrated >50% elastase inhibitory activity, whereas a considerably larger number exhibited >50% tyrosinase inhibitory action. Interestingly, in some cases the bioactivity exceeded 80% in in vitro assays. The biological properties of ~100 non-cytotoxic extracts were also tested on human diploid fibroblasts (BJ) for their anti-aging activity, and on melanocytes (B16F10) for their whitening activity. Fractions (~120) of the most active, non-toxic extracts tested for elastase and tyrosinase inhibition activities in cell-free and cell-based in vitro assays. The most promising highly active fractions were subjected to compound isolation and bioactivity assessment. Isolated compounds tested for their ability to activate cyto-protective mechanisms in cell-based assays, while their anti-aging effects also tested in in vivo Drosophila melanogaster models, for their ability to induce antioxidant mechanisms through activation of the Nrf2/cncC transcriptional factor. Our promising results show a hidden yet powerful potential of harnessing Greek microbial wealth in the context of anti-aging.

Acknowledgments
This research has been co financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call "RESEARCH – CREATE – INNOVATE" (project code: T2ΕΔΚ-01410).

P120

Conserved transcriptomic signatures and protein markers in cellular senescence models

Sissy Skea[1,2], Christos Fotis[1,2], Nikos Tsolakos[2], Vicky Pliaka[2], Kleio-Maria Verrou[3], Leonidas G. Alexopoulos[1,2]

[1]Protavio Ltd,
[2]National Technical University of Athens,
[3]Center of New Biotechnologies & Precision Medicine

Cellular senescence is described as an irreversible cell cycle arrest induced in response to various stresses. Senescent cells are characterised by heterogeneous signalling alterations, complex secretory phenotype, known as senescence-associated secretory phenotype (SASP), and diverse transcriptomic profile. With the aim to investigate senescence heterogeneity and identify conserved transctiptomic alterations and senescence markers, we performed RNA-seq and multiplex proteomic analysis in proteasome inhibition-induced and stress-induced premature senescence models of HFL1 and BJ human fibroblasts. Our data revealed diverse transcriptomic signatures, but also, 231 common differentially expressed genes related to cell division and ECM remodelling, and enriched pathways that remained conserved among the different models with senescence onset. Moreover, we identified a subset of conserved protein senescence markers and validated them in replicative senescent models. These proteins are involved in cell cycle arrest and promote a pro-inflammatory environment in premature and replicative senescence models. We suggest that the simultaneous analysis of p21, p-c-JUN, BCL-xL and survivin in cellular lysates, and IL-8, GM-CSF, GDF-15 and GROa in culture supernatants can provide a powerful tool for the identification and monitoring of senescent cells and can support the assessment of the efficacy of potential senotherapeutic approaches.

P121

Age-dependent nuclear lipid droplet accumulation is a cellular hallmark of ageing

Eirini Mytilinaiou[1], Christina Ploumi[1], George Filippidis[2], Nektarios Tavernarakis[3], Konstantinos Palikaras[1]

[1]Department of Physiology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece
[2]Institute of Electronic Structure and Laser (IESL), Foundation for Research and Technology (FORTH), Heraklion, Crete, Greece
[3]Institute of Molecular Biology and Biotechnology (IMBB), Foundation for Research and Technology Hellas (FORTH), Heraklion, Crete, Greece

Lipid droplets have been shown to localize in most nuclear compartments, where they impinge on genome architecture and integrity. However, the significance of progressive nuclear lipid accumulation and its impact on nuclear morphology and organismal homeostasis remain obscure. Here, we implement non-linear imaging modalities to monitor and quantify age-dependent nuclear lipid deposition. We find that lipid droplets increasingly accumulate in the nuclear envelope, during ageing. Longevity-promoting interventions, such as low insulin signaling and caloric restriction, abolish the rate of nuclear lipid accrual and decrease the size of lipid droplets. Suppression of lipotoxic lipid accumulation in intestinal nuclei is dependent on the transcription factor HLH-30/TFEB and the triglyceride lipase ATGL-1. HLH-30 regulates the expression of ATGL-1 to reduce nuclear lipid droplet abundance in response to lifespan-extending conditions. Notably, ATGL-1 localizes to the nuclear envelope and moderates lipid content in long-lived mutant nematodes during ageing. Our findings indicate that the reduced ATGL-1 activity leads to excessive nuclear lipid accumulation, perturbing nuclear homeostasis and undermining organismal physiology, during ageing.

P122

Endogenous DNA damage and Oxidative Stress in Peripheral Blood Mononuclear Cells as predictors of Nivolumab efficacy in Head and Neck Squamous Cell Carcinoma

Christina Papanikolaou[1], Panagiota Economopoulou[2], Aris Spathis[3], Dimitra Mavroeidi[1], Amanda Psyrri[2], Vassilis L. Souliotis[1*]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, 11635 Athens, Greece
[2]Second Department of Internal Medicine, Medical Oncology Section, National and Kapodistrian University of Athens, Attikon University Hospital, Athens, Greece
[3]Second Department of Pathology, Attikon University Hospital, Athens, Greece

Accumulation of evidence highlights the cross-talk between the DNA damage response (DDR) network and the immune system. We sought to determine whether DDR-related signals, measured in peripheral blood mononuclear cells (PBMCs) from Head and Neck Squamous Cell Carcinoma (HNSCC) patients, correlate with the therapeutic benefit from immune checkpoint inhibitors.

DDR-related signals, including endogenous DNA damage [single-strand breaks (SSBs), double-strand breaks (DSBs)], DNA repair mechanisms [nucleotide excision repair (NER), DSB repair], oxidative stress and apoptosis rates were evaluated in PBMCs from 26 healthy controls (HC) and50 recurrent/metastatic HNSCC patients who participated in a phase II nivolumab trial (NCT03652142). PBMCs were obtained at baseline, after 4 weeks of nivolumab treatment, and at progression.

PBMCs from patients at baseline showed significantly higher levels of oxidative stress and endogenous DNA damage, increased DSB repair capacities and lower NER, as well as reduced apoptosis rates compared with HC (all $P<0.01$).Significantly lower endogenous DNA damage at baseline was associated with longer progression-free survival (PFS) and overall survival (OS), as well as a higher likelihood for response and clinical benefit from nivolumab therapy (all $P<0.02$). More importantly, lower NER and DSBs repair capacities of patients' PBMCs at baseline were associated with better PFS and OS, higher likelihood for response and clinical benefit (all $P<0.004$). Moreover, PBMCs that exhibited lower levels of oxidative stress at baseline correlated with improved clinical benefit ($P=0.011$).

To conclude, DDR-related signals and oxidative stress, measured in PBMCs from HNSCC patients, correlate with response to anti-PD-1 immunotherapy. These results provide future benefits for the incorporation of potential non-invasive biomarkers into immunotherapy in current practice and the combination of DNA-repair-based therapies with immune checkpoint blockade.

P123

DNA damage repair mechanisms in lung cancer

Dimitra Mavroeidi[1], Christina Papanikolaou[1], Konstantinos N. Syrigos[2], Vassilis L. Souliotis[1*]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]Oncology Unit, 3rd Department of Internal Medicine, School of Medicine, School of Health Sciences, National and Kapodistrian University of Athens, Greece

DNA Damage Response (DDR) comprises signaling pathways acting for detection and repair of DNA damage. Deregulated DDR is associated with the onset and progression of cancer, thus implicated in therapy outcome. Herein, we searched for DDR defects in lung cancer cell lines.

Lung cancer cell lines (A549, H1299) and normal cell lines (WS1, 1BR3hT) were studied in parallel. Endogenous DNA damage using alkaline comet assay, DNA repair mechanisms [Nucleotide Excision Repair (NER) following UV-C exposure and Interstrand Crosslinks Repair (ICL/R) following cisplatin treatment], oxidative stress and abasic (AP) sites were examined. Phosphorylated H2AX [γH2AX; marker of DNA double-strand breaks (DSBs)] was confirmed by Western blot.

Protein evaluation revealed γH2AX at untreated cancer cells but not in normal cells, indicating endogenous DSBs in lung cancer. Both cisplatin and UV-C irradiation induced γH2AX at 24h for all cell lines.

NER capacity, evaluated following UV-C exposure, showed DNA damage early after irradiation and continuous increase for 6h.

After cisplatin treatment, all cells showed maximal ICL levels within 6h, which decreased thereafter. No significant difference in NER and ICL/R capacities were found between normal and cancer cells.

Increased oxidative stress and AP-sites were observed in cancer cells at baseline relative to normal cells. After cisplatin treatment, oxidative stress was upregulated and restored after 3h for cancer cells, and 6h for normal cells. After UV-C irradiation, oxidative stress induction was noticed with no comeback. AP-sites in cancer cells were barely affected after cisplatin treatment, while normal cells demonstrated increased levels restored after 24h. UV-C irradiation resulted in augmented AP-sites at all cell lines that did not recuperate.

We conclude that lung cancer cells are characterized by increased endogenous DNA damage and higher oxidative stress compared with normal cells. These results could be exploited as therapeutic targets, biomarkers and for the design of novel genotoxic therapies.

P124

RNF113A as a potential drug target for brain tumors

Eliana Markidi, Dimitrios Gkikas, Panagiotis Politis

Center for Basic Research, Biomedical Research Foundation of the Academy of Athens (BRFAA), Soranou Efesiou 4, 11527, Athens, Greece

Glioblastoma multiforme (GBM) is a highly aggressive type of brain cancer with a poor prognosis and limited treatment options. The standard of care for GBM tumors has remained unchanged for decades, including surgery, followed by radiation and chemotherapy with alkylating agents. GBM tumors soon become resistant to chemotherapy, making it almost impossible to achieve long-term remission[1–4]. To this end, we propose here that RNF113A is a drug target that can sensitize GBM cells to alkylating agents. In particular, recent studies suggest that RNF113A is sensing alkylating DNA damage and initiating the DNA repair pathway [5,6]. Therefore, we hypothesize that RNF113A inhibition could sensitize GBM cells to alkylating agents and lead to more effective chemotherapy regimens for this disease. In accordance with our hypothesis, we show that knockdown of RNF113A induces apoptosis in human glioblastoma cell lines. Moreover, treatment of GBM cells with alkylating agents, such as temozolomide, and siRNA against RNF113A significantly enhances DNA damage. Although RNF113A exerts DNA repair action via its E3 ligase activity, this factor is currently undruggable. Fortunately, it can be indirectly targeted by inhibiting SMYD3 methyltransferase, which methylates RNF113A and activates its ability to sense alkylating DNA damage. Meta-analyzing data from the TCGA database, reveals a significant positive correlation between RNF113A and SMYD3 expression in GBM tumors, supporting their cooperative roles. Gene Set Enrichment Analysis of the co-expressed genes further indicates involvement in pathways like DNA repair and apoptosis. Most importantly, we show that treatment of human GBM cells with SMYD3 inhibitors strongly promotes the ability of alkylating agents to induce DNA damage and apoptosis. Our results demonstrate a beneficial role for SMYD3 and RNF113A inhibition in the context of GBM cancer, suggesting that pharmacological suppression of RNF113A may be a promising therapeutic approach to enhance the efficacy of alkylating agents in GBM treatment.

1. *Ostrom, Q. T. et al. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2011–2015. Neuro-Oncology 20, iv1–iv86 (2018).*
2. *Vartanian, A. et al. GBM's multifaceted landscape: highlighting regional and microenvironmental heterogeneity. Neuro-Oncology 16, 1167–1175 (2014).*
3. *Visser, O. et al. Survival of adults with primary malignant brain tumours in Europe; Results of the EUROCARE-5 study. European Journal of Cancer 51, 2231–2241 (2015).*
4. *Stupp, R. et al. Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. N Engl J Med 352, 987–996 (2005).*
5. *Tsao, N. et al. Aberrant RNA methylation triggers recruitment of an alkylation repair complex. Molecular Cell 81, 4228-4242.e8 (2021).*
6. *Lukinovi , V. et al. SMYD3 Impedes Small Cell Lung Cancer Sensitivity to Alkylation Damage through RNF113A Methylation–Phosphorylation Cross-talk. Cancer Discovery 12, 2158–2179 (2022).*

P125

Identification of factors that decrease the occurrence of oncogenic therapy-induced MLL fusions

Anna Athanasouli[1], Diana Kotini[1], Henrike Gothe[2], Vera Minneker[2], Jan Heidelberger[2], Petra Beli[2], Vassilis Roukos[1,2]

[1]Department of General Biology, Medical School, University of Patras, Patras, Greece
[2]Institute of Molecular Biology (IMB), Mainz, Germany

The topoisomerase II poison, etoposide, is widely used as chemotherapeutic; it is associated, however, with the development of therapy-induced leukemias caused by recurrent translocations of the MLL gene. We have recently shown that spatial chromosome folding and transcription, promote DNA fragility at translocation hot spots localized at chromatin loops anchors, within MLL gene and potential translocation partners, promoting the formation of tumorigenic fusions.1 In order to be able to reduce the occurrence of these tumorigenic fusions, we sought to perform an siRNA screening to identify factors whose downregulation decreases the formation of MLL fusions, but not etoposide cytotoxicity. Our screen identified the AAA ATPase p97 and the bromodomain containing protein, BRD4, as potential targets for inhibition to decrease the frequency of MLL breakage and translocations, upon etoposide treatment. We have characterized here the role of those proteins and relevant inhibitors, on their ability to influence MLL and translocation partners DNA fragility, and etoposide cytotoxicity, and acquired insights onto how this is performed mechanistically. Our observations shed light on cellular mechanisms that can be utilized to reduce the formation of oncogenic therapy-related MLL translocations.

References

1. *Gothe, H. J. et al. Spatial Chromosome Folding and Active Transcription Drive DNA Fragility and Formation of Oncogenic MLL Translocations. Mol Cell 75, 267-283.e12 (2019).*

P126

Geminin deletion induces DNA damage and inflammation activation in colon carcinogenesis

Maria Mougkogianni[1*], Thomas Kaltsounis[1], Michalis Petropoulos[1], Argyro Kalogeropoulou[1], Zoi Lygerou[2], Stavros Taraviras[1]

[1]Department of Physiology, School of Medicine, Patras University, Patras, Greece
[2]Department of General Biology, School of Medicine, Patras University, Patras, Greece

The maintenance of genome integrity is of fundamental importance since cases of its dysfunction have been linked to genomic instability. Thus, cells have developed strict mechanisms for the accurate and efficient genome duplication process during the cell cycle. Defects detected in DNA replication, including origin licensing aberrations, lead to under- or over-replicated DNA and are shown to cause replication stress. This phenomenon is considered as a hallmark of cancer and a driver of tumorigenesis, given its presence in most precancerous and cancerous cell types.

Geminin, one of the major regulators of the cell cycle, ensures genome replication only once per cell cycle by inhibiting the binding of the replication licensing factor Cdt1 onto replication origins and thus preventing the pre-replicative complex formation during the same cell cycle. According to previous in vivo studies of our research group, deletion of Geminin in experimental mice exacerbates colon and lung tumorigenesis through increased DNA damage and replication origins overlicensing[1]. Based on an exciting new concept, recent findings have provided mechanistic insights into how replication stress may induce inflammatory response in cancer through a cytosolic DNA sensing pathway, the cGAS-STING[2]. Our research goal focuses on studying the link between replication stress and inflammation activation and its role in cancer progression. Hence, we modified a human colon cancer cell line, utilizing the auxin-inducible degron system to conditionally deplete Geminin via rapid protein degradation[3]. Strikingly, after deleting Geminin for longer timepoints we identified an upregulation of type I interferon-stimulated genes and increased expression of cGAS-STING pathway markers.

Among the long-term goals of the proposed research work is firstly to further investigate the mechanism that activates inflammatory response in cases of genomic instability, as well as to elucidate how inflammation determines cancer progression and finally to reveal novel targets for anticancer therapy.

References

1. *Champeris Tsaniras, S. et al. Geminin ablation in vivo enhances tumorigenesis through increased genomic instability. J. Pathol. 246, 134–140 (2018).*
2. *Ragu, S., Matos-Rodrigues, G. & Lopez, B. S. Replication stress, DNA damage, inflammatory cytokines and innate immune response. Genes (Basel). 11, (2020).*
3. *Natsume, T., Kiyomitsu, T., Saga, Y. & Kanemaki, M. T. Rapid Protein Depletion in Human Cells by Auxin-Inducible Degron Tagging with Short Homology Donors. Cell Rep. 15, 210–218 (2016).*

P127

Identification of novel synthetic lethal interactions in cancer cells with DNA replication licensing aberrations

Elena Karydi[1], Nibal Badra-Fajardo[3], Ourania Preza[1], Stavros Taraviras[2], Zoi Lygerou[1*]

[1]Department of General Biology, School of Medicine, University of Patras, Greece.
[2]Department of Physiology, School of Medicine, University of Patras, Greece.
[3]Department of Physiology, School of Medicine, University of Seville, Spain.

Maintaining genome integrity requires strict regulation over DNA replication both in time and space. DNA licensing is a conserved mechanism that ensures the production of a full copy of the genetic material, restricting single-origin firing to once-per-cell-cycle[1]. CDT1 is an essential licensing factor for recruiting the MCM helicases onto the chromatin. To prevent illegitimate origin firing, licensing is restricted through tight regulation of CDT1 activity. One of the multiple mechanisms controlling CDT1 involves Geminin, a cell-cycle regulated protein that operates, binding to and inhibiting CDT1[2]. Previous studies propose that Geminin could function as a backup mechanism to limit CDT1 activity in highly proliferative cells, where CDT1 is found upregulated[3,4]. The potential dependency of highly proliferative cells on Geminin could be a significant genomic trait for the identification of novel therapeutic approaches in cancer treatment. Accordingly, previous results from our group showed that Geminin is critical for cancer cell survival, as its depletion leads to re-replication and DNA damage. In this context, we investigated the use of two chemical compounds, ATR, and PARP inhibitors, in combination with the depletion of Geminin to selectively sensitize cancer cells. U2OS cells, deprived of Geminin and PARP activity, demonstrated an increase in double strand breaks, shown by enhanced nuclear γH2AX intensity and 53BP1 foci formation. The viability of these cells was significantly reduced. Similarly, Geminin depletion combined with ATR inhibition led to the formation of micronuclei, and notably reduced cell viability. Interestingly, γH2AX nuclear intensities and 53BP1 foci formation were reduced, confirming cell cycle progression through the G2/M boundary upon ATR inhibition. Our findings suggest that these compounds could be potential candidates, acting synergistically with the depletion of Geminin, to selectively kill cancer cells. The utilization of compounds that phenocopy the depletion of Geminin would be a significant step towards novel anti-cancer therapies.

1 *Petropoulos, M., Champeris Tsaniras, S., Taraviras, S. & Lygerou, Z. Replication Licensing Aberrations, Replication Stress, and Genomic Instability. Trends Biochem Sci 44, 752-764, doi:10.1016/j.tibs.2019.03.011 (2019).*
2 *Pozo, P. N. & Cook, J. G. Regulation and Function of Cdt1; A Key Factor in Cell Proliferation and Genome Stability. Genes (Basel) 8, doi:10.3390/genes8010002 (2016)*
3 *Zhu, W. & Depamphilis, M. L. Selective killing of cancer cells by suppression of geminin activity. Cancer Res 69, 4870-4877, doi:10.1158/0008-5472.CAN-08-4559 (2009).*
4 *Karantzelis, N. et al. Small Molecule Inhibitor Targeting CDT1/Geminin Protein Complex Promotes DNA Damage and Cell Death in Cancer Cells. Front Pharmacol 13, 860682, doi:10.3389/fphar.2022.860682 (2022).*

P128

Cdt1 overexpression promotes colon cancer progression by generating DNA damage and licensing aberrations

Thomai Samouilidou[1] , Maria Mougkogianni[1], Michalis Petropoulos[2], Zoi Lygerou[2], Stavros Taraviras[1]

[1]Department of Physiology, School of Medicine, Patras University, Patras, Greece,
[2]Department of General Biology, School of Medicine, Patras University, Patras, Greece

The DNA replication machinery is under strict regulation throughout the cell cycle, since its reported defects favour replication stress. Replication stress (RS) is a complex phenomenon with serious implications for genomic stability and cell survival. RS appears commonly as under- or over-replicated DNA and fork collisions which contribute to the generation of DNA damage and consequently accelerate tumour progression.

Aberrations during replication licensing have reportedly been linked to compromised genomic integrity.
Cdt1 is a major licensing factor which binds to replication origins and leads together with other licensing factors to the formation of the pre-replication complex. Previous in vivo work of our lab has revealed that the overexpression of Cdt1 in colon of experimental mice has tumorigenic effects, alters the physiology of the murine colon tissue and reinforces the expression of DNA damage markers. In order to investigate the effects of Cdt1 overexpression in the regulation of replication licensing machinery, we measured the recruitment of the Mcm2-7 complex onto chromatin. We have shown that Cdt1 overexpression results in loading greater amounts of Mcm2-7, suggesting origin overlicensing. Furthermore, additional experiments of our lab have indicated that the overexpression of Cdt1 is strongly linked to the enrichment of cancer cells with stem cell properties in spheroid cultures. To further elucidate the effect of the ectopic expression of Cdt1 on cancer stem cell enrichment, as well as its contribution to DNA damage, we are currently constructing a stable human colon cancer cell line. In this context, the degradation of CDT1 is defective, and its transcription is dependent on a Tet-On system, allowing the conditional expression of the factor throughout the cell cycle. Along these lines, we aspire to underline the causative effects of the conditional and aberrant expression of CDT1 on cancer stemness and genomic integrity.

Reference

1. *Petropoulos, M., Champeris Tsaniras, S., Nikou, S., Maxouri, S., Dionellis, V. S., Kalogeropoulou, A., Karamichali, A., Ioannidis, K., Danalatos, I. R., Obst, M., Naumann, R., Delinasios, G. J., Gorgoulis, V. G., Roukos, V., Anastassiadis, K., Halazonetis, T. D., Bravou, V., Lygerou, Z., & Taraviras, S. (2023). Cdt1 overexpression drives colorectal carcinogenesis through origin overlicensing and DNA damage. Journal of Pathology, 259(1), 10–20.*
2. *Nishitani, H., Sugimoto, N., Roukos, V., Nakanishi, Y., Saijo, M., Obuse, C., Tsurimoto, T., Nakayama, K. I., Nakayama, K., Fujita, M., Lygerou, Z., & Nishimoto, T. (2006). Two E3 ubiquitin ligases, SCF-Skp2 and DDB1-Cul4, target human Cdt1 for proteolysis. EMBO Journal, 25(5), 1126–1136.*

P129

TOP2 topoisomerases remodel chromosome organization at boundaries of active, positively supercoiled and nuclear lamina-associated regions of the human genome

Anastasia Panagi[1*], Amalia Stavridou[1*], Gabe Longo[2], Sergi Sayols[2], Ting Xie[3], Argyris Papantonis[3], Diana Kotini[1], Vassilis Roukos[1,2]

[1]Department of General Biology, Medical School, University of Patras, Patras, Greece
[2]Institute of Molecular Biology (IMB), Mainz, Germany
[3]Institute of Pathology, University Medical Center Göttingen, Robert-Koch-Strasse 40, 37075 Göttingen
**equal contribution*

Changes in DNA topology must be coordinated with the 3D chromosome organization to maintain the stability of the genome. Fundamental cellular processes, such as replication and transcription alter DNA topology by the accumulation of torsional stress in the form of positive and negative DNA supercoiling, catenates and entanglements. Accumulation of torsional stress can be detrimental for the cell by stalling these essential processes and promoting genomic instability or cellular death. However, the cells have evolved a class of enzymes called topoisomerases to relieve torsional stress by the transient formation of DNA breaks. Previous work from the lab has shown that chromosome organization, DNA topology and genomic instability are interlinked through actions of type II topoisomerases (TOP2s) at chromatin loop anchors (Gothe et al., Mol Cell, 2019), however whether TOP2s shape chromosome organization per se, remains unclear. Here we performed HiC and MicroC experiments to profile chromatin interaction frequencies upon TOP2 depletion genome-wide. We found that TOP2s prevent the interaction of genome regions at boundaries of active genome regions with regions interacting with the nuclear lamina (Lamina-associated domains, LADs), leading to reorganization of chromatin-lamina interactions and gene expression changes. To profile positive supercoiling for the first time in human cells, we established GapRUN, a methodology to probe the occupancy of the bacterial protein GapR that binds positive supercoiling genome-wide. We found that positive supercoiling accumulates at highly transcribed, long genes and in enriched at boundaries of LADs with active genomic regions. Future steps include the profiling of positive supercoiling upon topoisomerase depletion, which requires further improvements of the GapRUN approach. Our work will lead to a better understanding of how chromosome organization is coordinated with genome topology to prevent genomic instability.

- *Gothe HJ, Bouwman BAM, Gusmao EG, Piccinno R, Petrosino G, Sayols S, Drechsel O, Minneker V, Josipovic N, Mizi A, Nielsen CF, Wagner EM, Takeda S, Sasanuma H, Hudson DF, Kindler T, Baranello L, Papantonis A, Crosetto N, Roukos V. Spatial Chromosome Folding and Active Transcription Drive DNA Fragility and Formation of Oncogenic MLL Translocations. Mol Cell. 2019 Jul 25;75(2):267-283.e12. doi: 10.1016/j.molcel.2019.05.015. Epub 2019 Jun 12. PMID: 31202576.*

P130

Molecular Determinants of CRISPR/Cas9 Scissile Profile for Precise and Predictable Genome Editing

Demetriana Hadjichristou[1], Gabriel M. C. Longo[2], Sergi Sayols[2], Andriana G. Kotini[1], Vassilis Roukos[1,2]

[1]Department of General Biology, Medical School, University of Patras, Patras, Greece
[2]Institute of Molecular Biology (IMB), Mainz, Germany

Genome engineering holds immense promise to treat or cure genetic disorders and embraces great potential in biomedicine. The CRISPR/Cas9 system has potential to modify DNA for successful gene therapy, offering life-saving solutions. Cas9 has a flexible scission profile, which might influence repair outcomes, however, what dictates the type of Cas9 incisions is largely unknown. To understand how Cas9 induces predictable repair genotypes it is important first, to characterize the scissile profile of Cas9 and second, shed light on how factors of the different repair pathways are able to process the different DNA end structures. To study the scissile profile of Cas9, we developed BreakTag, a versatile, highly parallel and scissile-aware methodology for the profiling of Cas9-induced DNA double strand breaks (DSBs) at nucleotide resolution across the genome. We used BreakTag to survey nearly 3,000 sgRNAs targeting human genes, generating a robust dataset containing >150,000 uniquely cleaved loci between on and off-targets with identified scissile profile. Our results indicate that Cas9 scissile profile is not random, but instead, it is highly dependent on the nucleotide sequence of the protospacer and the presence of gRNA-DNA mismatches. Comparing matched datasets of Cas9 incisions and repair outcomes, we established that Cas9-mediated staggered breaks are linked with precise, templated and predictable single-nucleotide insertions, indicating that controlling these cut profiles might allow prediction of repair genotypes with desirable indels. To identify the responsible factors mediating single-nucleotide insertions we developed a dual fluorescent cell-based system in which predicted repair of single nucleotide insertion reconstitutes a functional GFP gene. Our goal is to use this system to identify the relevant repair factors mediating these predictable insertions. Our work illuminates the fundamental characteristics of the Cas9 nuclease and lays the foundation for harnessing the flexible cut profiles of Cas9 and engineered variants for precise and personalized, template-free, genome editing.

P131

Effect of Escherichia coli co-infection on Helicobacter pylori-induced deregulation of DNA Damage Response in gastric epithelial cells

Maria-Georgia Klaoudatou[1,2], Emma Bergsten[3], Ioannis Karayiannis[1], Spyros Tastsoglou[4,5], Eleftherios Kontizas[1], Georgios Georgoulias[1], Beatriz Martinez-Gonzalez[1], Antonios Giakountis[2], Eliette Touati[3], Dionyssios Sgouras[1*]

[1]Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens 11521, Greece
[2]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa 41334, Greece
[3]Unit of Helicobacter Pathogenesis, Department of Microbiology, Institut Pasteur, Paris 75015, France;
[4]DIANA-Lab, Hellenic Pasteur Institute, Athens 11521, Greece
[5]Department of Computer Science and Biomedical Informatics, University of Thessaly, Lamia 35131, Greece
**correspondence email: sgouras@pasteur.gr*

Helicobacter pylori (Hp) and the characterization of the human gastric microbiome have challenged the idea of a sterile gastric environment. The interplay between bacterial and microbiota-derived factors, with host and environmental determinants can potentiate carcinogenesis, in effect by increasing inflammation-derived DNA damage and mutation, through increased oxidative stress and by deregulation of the host DNA damage repair(DDR) mechanisms. In this study we attempted to chart the effect of another bacterial member of the gastric microbiota, namely Escherichia coli (Ec), on the ability of Hp infection to deregulate a number of host genes involved in DDR. Human SV40-transformed gastric epithelial cells (GES-1), were Hp-infected for 24 hours and Ec-co-infected for the last 3hours of the incubation. RNA-Seq (QuantSeq-3'RNA) was performed on poly(A)-enriched transcripts extracted from each experimental condition, namely Hp; Ec; Hp/Ec co-infection; uninfected cells, in triplicates. Differential Expression Analysis included genes presenting |log2(FoldChange)| > 0.5 and a False Discovery Rate< 0.05.Our results suggested a significant deregulation of 74 genes during co-infection, 125 genes during Hp infection, and 50 genes during Ec infection alone. Focusing on DDR mechanisms, pathway-level differential abundance analysis (Fry) showed a significant upregulation of Nucleotide Excision Repair during infection with Hp or Ec alone. At a gene-level, Hp and to a lesser extent Ec infection, led to upregulation ofFEN1, BRCC3, ERRC3, RFC2, BIVM-ERCC5 and ERCC5, and downregulation of MPG and NTHL1.However co-infection did not significantly deregulate any of the above genes.DDR components of interest, as well as phosphorylated histone H2AX (γH2AX)a characteristic marker of double strand break formation, were further examined on a protein level, under the same infection protocol. Results indicate that Hp-induced replication stress on gastric epithelial cells may be attenuated or counteracted during concomitant infection with Ec, a suggestion that warrants further investigation.

This study was supported in part by the collaborative project PTR 332-20"CoPyMe" financed by Institut Pasteur entitled: "E. coli and H. pylori as models for the role of DNA methylation in the relation between bacteria and cancer".

P132

Correlation of complement pathway proteins with fibrosis and inflammation in early-stage type 1 diabetic kidney disease

Aggeliki Tserga[1], Jean Sebastien Saulnier-Blache[2,3], Konstantinos Palamaris[4], Despoina Pouloudi[4], Harikleia Gakiopoulou[4], Jerome Zoidakis[1,5], Joost-Peter Schanstra[2,3], Antonia Vlahou[1], Manousos Makridakis[1*]

[1]Department of Biotechnology, Biomedical Research Foundation, Academy of Athens, Soranou Efessiou 4, 11527 Athens, Greece.
[2]Institut National de la Santé et de la Recherche Médicale (INSERM), U1297, Institute of Cardiovascular and Metabolic Disease, 31432 Toulouse, France.
[3]Université Toulouse III Paul-Sabatier, 31062 Toulouse, France.
[4]First Department of Pathology, School of Medicine, National and Kapodistrian University of Athens, 11527 Athens, Greece.
[5]Department of Biology, National and Kapodistrian University of Athens, 15701 Zografou, Greece
**Corresponding author: Manousos Makridakis, PhD*
Email: mmakrid@bioacademy.gr

Introduction: Diabetic kidney disease (DKD) is characterized by histological changes including fibrosis and inflammation. Studies support that DKD development is mediated by the innate immune system and more specifically by the complement system. However, the mechanism of complement involvement in DKD progression is unknown. This study targets its better understanding and connection with inflammation and fibrosis in early DKD.

Methods: Proteomics data from kidney glomeruli of Ins2Akita mice (T1D model; with early and moderately advanced DKD) were extracted from previous publication. Correlation analysis of complement with inflammation and fibrosis- related protein expression was performed. Cross-omics validation of the results was performed using transcriptomics datasets from available repositories (Nephroseq) as well as by immunofluorescence of kidney sections from 43 DKD patients.

Results: Among the differentially expressed proteins of the published proteomic dataset, complement cascade components (C3, C4B, IGHM) were significantly increased in both early and later stages of DKD. Moreover, fibrosis- and inflammation-related proteins were mainly detected upregulated in early DKD. The expression levels of the detected complement, fibrosis and inflammation-related proteins were mostly positively correlated in early DKD. Investigation of 7 existent human and mouse transcriptomics datasets further supported this positive correlation of complement- with, fibrosis- and inflammation- related proteins in DKD. Immunofluorescence analysis of human kidney further confirmed the differential expression of complement related (C3, C1q and IGM) proteins in DKD and their correlation with fibrosis and inflammation mainly in advanced DKD due to limited number of early DKD samples. Conclusions: Our study shows for the first-time potential activation of complement cascade associated with inflammation-mediated kidney fibrosis in Ins2Akita T1D model. These results could provide new perspectives for the treatment of early DKD, as well as support the use of Ins2Akita in pre-clinical studies.

Keywords

Ins2Akita, Diabetes, Diabetic kidney disease, Proteomics, kidney, LC-MS/MS, Biomarker, complement, fibrosis, glomeruli

P133

Investigation of metabolic responses following treatment with a novel synthetic purine analog with antitumor activity in mouse model of breast cancer

Panagiotis Malamos[1*], Manolis Matzapetakis[1], Maria Georgiou[2], Nikolaos Lougiakis[2], Vassilis L. Souliotis[1], Nicole Pouli[2], Panagiotis Marakos[2], Dimitris Stellas[1], Maria Zervou[1]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]Division of Pharmaceutical Chemistry, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Athens, Greece

Targeting hyperactive cancer cell nucleotide metabolism has been established as a promising strategy towards the development of novel antineoplastic drugs [1]. A class of newly synthesized 1,4,6-tris-substituted pyrazolo[3,4-b]pyridines has shown potent in vitro cytotoxic activity against human breast (EO771) and murine pancreatic (KPC) tumor cell lines. The most potent analogue 9b featuring (4-methylpiperazin-1-yl)ethoxy substitution was found to strongly inhibit tumor growth and cell proliferation and induce apoptosis in murine breast cancer models without exhibiting any systemic toxicity nor interfering with the immune system of the animals [2]. In this study, we applied Nuclear Magnetic Resonance (NMR) tissue metabolomics in samples from breast tumors, kidneys and liver from mice, in order to identify metabolic responses following injections of the analogue 9b. Discriminant analysis revealed significant metabolic alterations in tissues from the breast tumors and pathway analysis identified purine biosynthesis and metabolism and interconnected pathways as significantly affected in response to treatment. On the other hand, no significant alterations were observed in kidneys metabolic signature between injected animals and the control group, suggesting the absence of treatment nephrotoxicity. As for the liver tissue, significant alteration was observed mainly in sugars metabolism, which deserves further investigation. The obtained results show that the analogue could be a promising candidate in the therapeutics for breast cancer. Further studies are in progress in an effort to uncover the mechanism of its antitumor action.

Acknowledgements
We would like to acknowledge support of this work relevant to the NMR infrastructure by the project "INSPIRED" (MIS5002550), under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme Competitiveness, Entrepreneurship and Innovation (NSRF 2014-2020).

References
[1] Mullen, N.J. et al. "Nucleotide metabolism: a pan-cancer metabolic dependency". Nat Rev Cancer (2023) 23, 275–294.
[2] Georgiou, M. et al. "Discovery of New 1,4,6-Trisubstituted-1H-pyrazolo[3,4-b]pyridines with Anti-Tumor Efficacy in Mouse Model of Breast Cancer". Pharmaceutics (2023) 15, 787.

P134

Chitosan-based nanoparticles with enclosed Salicylic acid induce defense mechanisms in A. thaliana against the phytopathogenic fungus Podosphaera xanthii

Theoni Margaritopoulou[1*], Martina Samiotaki[2], Jerome Zoidakis[3,4], Despina Tsiriva[5], Emilia Markellou[1]

[1]Mycology Laboratory, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, Kifissia, 14561, Greece
[2]Protein Chemistry Facility, Biomedical Sciences Research Center "Alexander Fleming", Vari, 166 72, Greece
[3]Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, Zografou, 15771, Greece
[4]Proteomics laboratory, Biomedical Research Foundation, Academy of Athens, Athens 11527, Greece
[5]Research and Development Department, Phytorgan SA, Nea Kifissia, Greece

The last decades, increased food demand, due to rapid population growth, and harsh environmental challenges, as a result of climate change, is expected to make crops more disease and stress vulnerable. Additionally, pesticide use reduction in Green Deal frame requires the development of new generation plant protection products safe for humans and environmentally sustainable. In this challenging era, the role of nanotechnology in crop protection needs to be elucidated. Here, chitosan nanoparticles (Cs-NPs) loaded with Salicylic acid (SA) were produced and tested for inducing host resistance on A. thaliana Col-0 plants against the phytopathogenic fungus Podosphaera xanthii. The defense stimulating effect was examined by ROS production through NBT and DAB staining. It was shown that in absence of the disease and after artificial inoculation with P. xanthii conidia, SA/Cs-NPs incorporated in the growth medium at the concentration of 5ppm, induced PR1 resistance marker gene and reduced disease severity on leaves of PR1prom::GUS transgenic plants. Proteomic analysis was performed with a Q-Exactive HF-X Orbitrap Mass analyzer and 6,163 unique proteins were detected. Clear protein differentiation was evident only between controls and SA/Cs-NPs-treated plants. In this comparison, 1489 differentially expressed proteins (DEPs) were detected, while in the same treatments but after pathogen inoculation, the number of DEPs was 559. Gene Ontology (GO) Analysis of differentially expressed proteins revealed enrichment in Systemic Acquired Resistance, Plant Pathogen Interactions and Pathogenesis GO Terms. PR1, PR2, PR4, PR5 and EDS1 proteins were significantly deregulated with similar expression patterns in both comparisons. Interestingly, EDS1 is a component of R-mediated disease resistance, has lipase activity and is involved in the SA signaling defense pathway, showing the interplay between membrane lipid and defense signaling. Moreover, EDS1 is associated with HSP90 suggesting a novel role of the molecular chaperone in defense signaling.

P135

Courgette defence induction by a botanical extract against Podosphaera xantii is triggered by glycerophospholipid production and is affected by host's genetic background

Margaritopoulou T.[1*], Baira E.[2], Anagnostopoulos C.[3], Vichou K.-E.[1], Markellou E.[1]

[1]Laboratory of Mycology, Scientific Directorate of Phytopathology, Benaki Phytopathological Insititute, Kiffisia, 14561, Greece
[2]Laboratory of Toxicological Control of Pesticides, Scientific Directorate of Pesticides' Control & Phytopharmacy, Benaki Phytopathological Insititute, Kiffisia, 14561, Greece
[3]Laboratory of Pesticide Residues, Scientific Directorate of Pesticides' Control & Phytopharmacy, Benaki Phytopathological Insititute, Kiffisia, 14561, Greece

Plant defense inducers (PDIs), a new generation of plant protection products, are molecules that activate host's defense mimicking pathogen attack. Their effectiveness against fungal pathogens is regulated by host susceptibility to a pathogen and host genetic resistance background. Here two different courgette genotypes: one sensitive (S) and one with Intermediate Resistance (IR) to Podosphaera xanthii (Px), a biotrophic fungal pathogen, were foliar treated with Regalia®, a botanical PDI. It was found that Regalia® application reduced leaf infection by Px and defense was activated by enhanced glycerophospholipid signaling and production especially in the S genotype, while the IR genotype was practically unresponsive due to inherent resistance (Pm-0, the Major Powdery Mildew Resistance Locus). Transcriptome profiling and differential expression analysis showed that in Regalia®-treated versus control comparison in S genotype cluster, 187 out of the 237 DEGs were more than 2-fold change (FC) upregulated, and many of them were strongly related to defense mechanisms. Moreover, many of these DEGs had also increased expression in the IR genotype. Gene Ontology (GO) analysis revealed enrichment in terms mainly oriented to plasma membrane such as Lipid Processes, Glycosyltransferase and Hydrolase Activities, Cell Wall and Carbohydrate-related Processes. Metabolomics approach annotated Lyso-Phospatidic acid (LPA) class with 16-FC in Regalia®- treated S samples and 7.5-FC in Regalia® and Px -treated S samples, and significant upregulation of Phosphatidylcholine (PC) metabolite in Regalia®- treated S samples. LPA and PC accumulation correlated with increased PHOSPHOLIPASE A (PLA) and PHOSPHOLIPASE D (PLD) protein expression. Pathogen inoculation also increased PLA and PLD expressions in S genotype, although to a lesser extent. Interestingly, high expression of both proteins was detected in IR control samples. Genetic and epigenetic analysis revealed enriched HeK4me3 and HeK27me3 histone modifications on ATS1 gene sequence of Pm-0 in the IR genotype. ATS1 is a G3P acyltransferase catalyzing LPA production.

P136

Does decellularization improve the analysis of the extracellular matrix?

Hanne Devos[1], Teresa Frattini[2], Manousos Makridakis[1], Jean-Sébastien Saulnier-Blache[2], Antonia Vlahou[1*], Joost Schanstra[2]

[1]Biomedical Research Foundation, Academy of Athens, Greece
[2]Institut National de la Santé et de la Recherche Médicale, U1048, Institute of Cardiovascular and Metabolic Disease, Toulouse, France; Université Toulouse III Paul-Sabatier, Toulouse, France.

The extracellular matrix (ECM) is a three-dimensional scaffold into which the cells reside, proliferate and communicate. ECM consists of fibrillar molecules such as collagens and fibronectin providing structural support but also non-fibrillar components including various matricellular proteins mediating interactions of cell surface receptors with structural ECM molecules.

In many chronic diseases the ECM loses its scaffold role and becomes overabundant leading to fibrosis. Reducing fibrosis is a major target in many chronic diseases but this necessitates a detailed understanding of the ECM. Mass-spectrometry (MS)-based methods offer a valuable means to explore this ECM composition. However, ECM proteins represent only about 1% of the whole tissue. Furthermore, they tend to aggregate with molecules creating macromolecules highly insoluble in aqueous solutions. These aspects make ECM analysis difficult.

Here we investigated the added value of applying a decellularization (DC) strategy in proteome analysis with the goal to enrich for ECM proteins.

With this aim mouse kidney samples were subjected to decellularization and decellularized (DC) samples were compared to non-decellularized samples (nonDC). Histological imaging clearly demonstrated effective removal of the cellular proteins in DC samples. DC and nonDC samples were then subjected to high resolution GeLC-MS/MS to analyse their protein composition. DC samples were clearly enriched for ECM proteins. However, DC also modified, among the ECM proteins, their relative abundance. 110 ECM proteins that were not detected in the nonDC samples were detected after decellularization. Details will be presented at the meeting.

In conclusion, decellularization increases the relative ECM content, unveils ECM proteins not detected without DC, but modifies the relative abundance of the ECM proteins in comparison to total proteome analysis which should be considered in comparative studies.

P137

Chronos webserver: An integrated platform for Clinical Genomics

Louis Papageorgiou[1,2], Dimitrios Vlachakis[1], Elias Eliopoulos[1,*]

[1]Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Athens 11855, Greece;
[2]Department of Biomedical Sciences, School of Health and Care Sciences, University of West Attica, Agioy Spyridonos, 12243, Egaleo, Greece;

Clinical genomics have brought about significant changes in the way hereditary traits are examined and explained in several diseases, as it yields information on genetic variants of the human genome. Nowadays, we are in the era of abundance of information in biological data, looking for new advanced techniques in order to analyze big data. This phenomenon is even more complex in clinical genomics, since we are still trying to decode the human genome by analyzing and correlating information from several fields in the "-omics" sciences. The key to this effort is a multilayer analysis of the collected genomic information regarding genetic polymorphisms using advanced AI and data mining techniques. In this study, the "Chronos-webserver for clinical genomics" is presented, as the result of an effort to understand the genetic and epigenetic basis of several diseases through their genomic grammar. The Chronos-webserver is an integrated platform of distinct applications, designed to assist medical doctors and researchers in the process of diagnosing and investigating diseases at the genomic level. By developing, evaluating and validating a series of online applications using data mining and semantic techniques the web-based applications contained in the Chronos-webserver identify the most probable genomic variants causing a number of specific target diseases, by utilizing a patient's provided genomic data. Each application is specialized to a particular disease, and each disease genomic information has been preprocessed through a state of the art bioinformatic pipeline, towards creating their distinct genomic grammar. Such are, the Demetra application for Endometriosis, Epione for Systemic Lupus Erythematosus, Panacea for Multiple Sclerosis and Aceso in investigating Crohn's disease. As further diseases are incorporated currently in the Chronos-webserver, we have correlated common genetic aspects between disease genomic grammars and analysed correlation trends in separate global populations. The Chronos-webserver for clinical genomics is available at http://geneticslab.aua.gr/.

Keywords

Bioinformatics; Genomics; Clinical Genomics; Genetics; Personalized medicine;

P138

A proteomics approach to investigate the underlying molecular mechanisms of the therapeutic properties of Chios Mastic Gum in zebrafish

Anastasiadou P., Margaritopoulou T., Agalou A., Kasiotis K.M., Machera K.

Benaki Phytopathological Institute, Kifisia Attiki, Greece

Chios mastic gum (CMG), the resin of the tree Pistacia lentiscus var. Chia, has been used for centuries as a natural remedy in the Mediterranean basin. The anti-inflammatory and antioxidant properties of CMG's components are getting growing attention from scientists and consumers the last decades. Based on evidence, it can be used for the prevention and healing of common health disorders, and even act as an inhibitor against cancer cells. Even though there is much evidence on CMG's protective and therapeutic role in human health, information about the underlying molecular mechanisms is extremely limited. Zebrafish, due to the plethora of its advantages, has emerged as a high throughput and low-cost model organism, widely used in research in recent years. To investigate the molecular pathways that are influenced by CMG administration, zebrafish embryos were exposed to non-toxic CMG concentrations from 3 to 96 hours post fertilization. Using a Q-Exactive Orbitrap Mass analyzer, proteomic analysis was performed and 410 differentially expressed proteins were detected in zebrafish embryos after exposure to 3 mg/L CMG. Among these, proteins related to antioxidant activity, like SOD1, SOD2 and GSTO2, to cytoskeleton organization, like GMFB and CNN3A and to lipid transport and hydrolysis, like CAV1 and PLA2G12B, were detected, and significantly upregulated. Immunodetection (Western blot analysis) of the heat shock protein 90 (HSP90) molecular chaperone, a constant research target for therapies against cancer and inflammatory-associated diseases, displayed noteworthy downregulation after CMG treatment. Moreover, STRING analysis showed a direct link of HSP90 with proteins of the antioxidant, cytoskeletal and lipid pathways. We believe that these findings contribute to the understanding of the molecular pathways that are influenced by CMG in zebrafish and indicate that CMG could be a potential naturally occurring HSP90 inhibitor. Further research is necessary.

P139

Dietary restriction and genetics drive expression levels of plasma proteins with key roles for human health: the FastBio study

Alexandros Simistiras[1*], Antigone Dimas[1,2*]

[1]Institute for Bioinnovation, Biomedical Sciences Research Center 'Alexander Fleming', Fleming 34, 16672, Vari, Greece
[2]Institute of Translational Genomics, Helmholtz Zentrum München – German Research Center for Environmental Health, 85764 Neuherberg, Germany
**Correspondence to: simistiras@fleming.gr dimas@fleming.gr*

Dietary restriction (DR) without malnutrition is one of the most prominent interventions that elongates lifespan and health span in various species (1). Studies in model organisms have highlighted genes, such as mTOR and FGF21, that are linked to biological mechanisms underlying ageing and age-related diseases upon DR (2). However, these genes and their effect have not been extensively investigated in human populations in a DR framework. The FastBio project (www.fastbio.gr) has profiled 200 Greek individuals that are voluntarily practicing a form of DR based on Eastern Orthodox Christian Church diet and 211 individuals who are continuously omnivorous as a control sample. DR-individuals are abstaining from animal products about 180~200 days annually, for at least ten years. Several biological layers have been quantified, genotypes, metabolites, proteins, for both groups at two timepoints, T1: a period of omnivory and T2: a period after 3 to 4 weeks of abstinence from meat, fish and their products for the DR group. Here we focus on the analysis of the protein expression values of 1218 Assays, captured through Olink's 1536 Explore panel. Our latest results indicate a prominent change in protein expression levels in the DR group compared to controls, with five times more differentially expressed proteins (DEPs=264) across timepoints. To investigate the genetic contribution to such changes we mapped cis-pQTLs (local protein Quantitative Trait Loci) at each group-timepoint. We report ~430 cis-pGenes (proteins with at least one pQTL) at each group-timepoint with 67% of them being shared. Upon examining the unique cis-pGenes in the DR-group at T2, we identified FGF21 being tagged by a DR-driven QTL rs4645881. FGF21 is nutrient sensing hormone that promotes metabolic health. Our study supports the existence of mediation effects of genetics to DR- response in human individuals. Harvesting these effects, we aim to provide insights in the "food as medicine" approach.

References

1. *C. Hine et al., Cell. 160, 132–144 (2015).*
2. *C. L. Green, D. W. Lamming, L. Fontana, Nat Rev Mol Cell Biol. 23, 56–73 (2022).*

P140

Precision Nutrition: Breakthrough of Multi-omics Precision Medicine

Olympia-Eirini Boulioglou[1], Giannis Vatsellas[2], Dimitris Thanos[2], Konstantinos N. Syrigos[3], Athanasios K. Anagnostopoulos[1]

[1]Department of Biotechnology, Center of Systems Biology, Biomedical Research Foundation of Academy of Athens, 11527, Athens, Greece
[2]Greek Genome Center, Biomedical Research Foundation of Academy of Athens, 11527 Athens, Greece
[3]3rd Department of Internal Medicine, Sotiria Hospital, School of Medicine, National and Kapodistrian University of Athens, 11527 Athens, Greece

Introduction: Precision nutrition is a pioneering field that offers multidimensional nutrition recommendations tailored to an individuals' genetic and endocrine background. Use of multi-omics platforms encompassing genomics, transcriptomics, proteomics and metabolomics allows analysis of an individual's genetic fingerprint, protein expression and metabolite presence leading to creation of customized dietary quota. The scope of the study is the modelization of a pipeline that leads from collection of biological material from an individual to production of a tailored nutrition plan. Materials and Methods: The approach entails distinct steps towards generation of vast datasets. The first node of the pipeline involves the precise analysis of biological samples (e.g. blood, urine, saliva, feces), by exhaustive multi-omics analysis integrating technologies such as DNA-seq, RNA-seq and Mass Spectrometry. The second and focal pivot of the pipeline is the implementation of bioinformatics tools towards integrative adaptation of previously collected data. Specifically, data pre-processing, quality control and integration of multi-omics data are combined towards the construction of a model. The final model, through utilization of machine learning algorithms, is used to predict individual dietary responses, risk factors as well as create personal nutrition plans. Conclusion: Precision nutrition, a sub-domain of personalized medicine, offers a new point of view to disease prevention and regulation. Multi-omics-derived data to are used create a predictive model for nutrition planning, the final goal being optimizing management of cancer and chronic or metabolic diseases, or offering a personalized plan to improve the individual's health status.

P140-A

Chemoselective enrichment strategy to identify endogenous protein and site-specific S-nitrosylation

Maria Symeonidou[1], Christos Gkougkousis[1], Paschalis-Thomas Doulias[1, 2 *]

[1]Laboratory of Biochemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece.
[2]Institute of Biosciences, University Research Center of Ioannina, Ioannina, Greece.
[*]Corresponding Author

The discovery of nitric oxide (NO) as a signaling molecule in the cardiovascular system was awarded the Nobel Prize in Physiology or Medicine in 1998. Signaling functions of NO are achieved through sGC-cGMP-dependent phosphorylation as well as through the selective and reversible modification of protein cysteine residues namely S-nitrosylation.

Herein, the selectivity and specificity of a chemoselective enrichment strategy for endogenous S-nitrosylated proteins and peptides are outlined. The strategy was developed a few years ago [(1-4)] and it has been recently adopted by our group. Thus, testing its performance and developing the best performing protocol was necessary before its implementation to address biological questions. In principle, the enrichment relies on the covalent binding of S-nitrosylated proteins or peptides to phenylmercury at pH=6.0. Our data documented that this reaction is selective and efficient for S-nitrosocysteine since no reactivity was documented for disulfides, sulfinic or sulfonic acids as well as S-alkylated cysteine residues. The specificity of enrichment as well as the detection limit of the method was assessed using biological and chemical negative and positive controls. Methodological advancements were placed into a biological context by detecting endogenous S-nitrosylation in heart homogenates from wild type, $eNOS^{-/-}$ and $nNOs^{-/-}$ mice. A pronounced reduction of global and protein specific S-nitrosylation was revealed in the heart of $eNOS^{-/-}$ mice indicating the dependency of cardiac S-nitrosylation from of eNOS-derived NO.

Overall, selective, sensitive and reproducible enrichment of S-nitrosylated proteins and peptides is achieved by the use of phenylmercury. The inclusion of appropriate negative controls secures the precise identification of endogenous S-nitrosylated sites and proteins in biological samples.

References

1, *Doulias PT, Greene JL, Greco TM, Margarita Tenopoulou, Seeholzer SH, Dunbrack RL, Ischiropoulos H (2010) Structural profiling of endogenous S-nitrosocysteine residues reveals unique features that accommodate diverse mechanisms for protein S-nitrosylation. Proc Natl Acad Sci U S A. 107: 16958-16963.*
2, *Doulias PT, Tenopoulou M, et al. (2013). Nitric oxide regulates mitochondrial fatty acid metabolism through reversible protein S-nitrosylation. Science Sig. 6: rs1*
3, *Doulias PT, Tenopoulou M, Raju K, Spruce LA, Seeholzer SH, Ischiropoulos H (2013). Site specific identification of endogenous S-nitrosocysteine proteomes. J Proteomics 92: 195-203*
4, *Doulias PT, Tenopoulou M, Zakopoulos I, Ischiropoulos H. (2021). Organic mercury solid phase chemoselective capture for proteomic identification of S-nitrosated proteins and peptides. Nitric Oxide 117: 1-6*

Acknowledgements

This work was supported by the project BIOMED-20 (MIS 5047236) implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the EU (European Regional Development Fund).

P141

Proteogenomics Study of Lung Cancer Patients in Greece

Anna Nefeli Kontouli Pertesi[1], Alexandra Ainatzoglou[1], Olympia Eirini Boulioglou[1], Giannis Vatsellas[2], Ioannis Vamvakaris3, Ioannis Giozos[4], Konstantinos Syrigos[4], Athanasios K. Anagnostopoulos[1]

[1]Department of Biotechnology, Biomedical Research Foundation of the Academy of Athens, Athens 11527, Greece.
[2]Greek Genome Center, Biomedical Research Foundation of the Academy of Athens, 11527 Athens, Greece.
[3]Pathology Department, Sotiria Hospital, National and Kapodistrian University of Athens, 11527 Athens Athens, Greece.
[4]Oncology Unit, 3rd Department of Medicine, "Sotiria" Hospital for Diseases of the Chest, National and Kapodistrian University of Athens, 11527 Athens, Greece.

Introduction: Lung cancer is the leading cause of cancer-related mortality demanding for revolutionary approaches towards ameliorating diagnosis and treatment. The joint characterization of tumor proteomics with genomics and transcriptomics enables proteogenomic analysis. The aim of this prospective study was to apply proteogenomics in lung cancer tumors to unravel molecular mechanisms that drive tumor phenotypes, identify proteome-specific markers of outcome, and identify novel treatment paradigms.

Materials and Methods: Non-small cell lung cancer patients (NSCLC) patients were recruited in the Third Department of Pathology of the "Sotiria" Unirvesity Hospital. Tissue aliquots of tumor and nonneoplastic tissue were obtained from surgical resection specimens along with demographic data. Clinical follow-up data was obtained at 6 months intervals following surgery. Analyses included immunohistochemistry, nanoLC-MS/MS, DNA-seq and RNA-seq of all tumor samples.

Results: It was found that within-tumor correlations of RNA and protein expression associate with tumor purity and immune cell profiles. Expression signatures of RNA and protein that predict patient survival were detected and independently validated. Protein expression was found to be more often associated with patient survival than RNA.

Conclusion: As prevention is the ultimate goal for all cancers, understanding the different molecular subtypes of NSCLC combined with contributing environmental and clinical factors may define specific pathways that could be therapeutically targeted to halt the development of malignancy in pre-cancerous and early-stage NSCLC tumors.

P142

Sharing Genomic and Biomedical data following the FAIR principles in the framework of the UPCAST project

Eleftherios Pilalis[1-2], Konstantinos Voutetakis[1], Georgia Kontogianni[1], Georgia Pyroti[1], Maria Koukovini[3], Eugenia Papagiannakopoulou[3], Georgios Lioudakis[3], Olga Papadodima[1*]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]e-NIOS Applications Private Company, Kallithea, Greece
[3]ICT abovo Information & Communication Technologies, Athens, Greece

Cancer genomics is a rapidly evolving field fueled by the continuously growing amount of generated genomic data. Although data sharing is well recognised as a cornerstone within cancer genomics, several barriers still retain a great amount of data underexploited. Privacy-related concerns, ownership and intellectual property issues, lack of standardisation of analytical pipelines for data generation and interpretation, utilization of different vocabularies for data description, as well as storage of data in diverse file formats, are among the challenges that have to be faced in order to facilitate integration and interpretation of data from different sources. The UPCAST project aims to create a collection of universally applicable, reliable, transparent, and user-friendly plugins, which will promote the adherence to FAIR principles, streamline data sharing, and automate data processing agreements across a broad spectrum of diverse data environments. NHRF is piloting privacy-preserving sharing and seamless integration of genomic and biomedical data in collaboration with technology partner ICT abovo, including the development of semi-automated agreements to facilitate the establishment of multi-disciplinary collaborations between clinicians, biologists and data scientists, ensuring compliance with appropriate standards addressing ethical and legal issues. In parallel, we are developing genomic analysis computational tools and workflows that align with both the FAIR principles and the international standards set forth by the Global Alliance for Genomics and Health (GA4GH) aiming to support harmonization, integration and analysis of multi-modal, heterogeneous data.

This work is supported by the UPCAST project (Universal Platform Components for Safe Fair Interoperable Data Exchange, Monetisation and Trading) which has received funding from the European Union´s Horizon Research and Innovation Actions under Grant Agreement nº 101093216

P143

Proteomics Analysis offers insights on the resilience of the Mediterranean plant Cistus creticus to environmental stress

Elena Sampanai[1], Aggeliki Tsoka[1], Julie Courraud[2,3], Guillaume Médard[3], Constantinos Vorgias[1], Paraskevi Skourou[1*], Jerome Zoidakis[1,4*]

[1]Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, Zografou, 15771, Athens, Greece
[2]Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Alexandra Hospital, Leof. Vasilissis Sofias 80, Athens 11528, Greece
[3]Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimioupolis Zografou, 15771, Athens, Greece
[4]Proteomics Laboratory, Biomedical Research Foundation, Academy of Athens, 11527, Athens, Greece

Cistus creticus is a seasonal dimorphic shrub typical of Mediterranean ecosystems, important for their post-fire recovery and resilience. It produces both hard-coated dormant and non-dormant seeds that can germinate after or in absence of a fire incident, respectively. Plants that grow after forest fires exhibit characteristic phenotypes such as increased leaf surface area compared to plants from mature undisturbed sites.

The aim of the present study was to compare the proteome of dormant and non-dormant seeds of C. creticus and the leaf proteome from plants growing on burnt and undisturbed areas.

Specimens of C. creticus were collected from sites of recent forest fires and from undisturbed areas. Seeds and leaves were homogenized and proteins extracted with appropriate buffers containing chaotropic agents. After protein reduction, alkylation and tryptic digestion the resulting peptides were analyzed by high performance liquid chromatography coupled to mass spectrometry. Statistical comparisons were based on R scripts.

Hard coated seeds contained higher levels of antioxidant enzymes and lower levels of cellulases and pectinases. Moreover, they exhibit significantly increased concentration of proteasomal subunits and ubiquitin ligases. These molecular features provide hints for the elucidation of heat resistance mechanisms of C. creticus seeds.

Leafs collected from plants growing on burnt soil had higher levels of ribsosomal subunits and photosynthetic proteins indicating increased protein synthesis and energy production.

Our future plans are to validate these preliminary findings with enzymatic and biochemical approaches and perform a metabolomics analysis. Thus, we will obtain a global understanding of the biological processes associated with dormancy in seeds and growth on burnt soils.

P144

Study of chitin metabolism of the psychrophilic bacterium Moritella marina by proteomic analysis

Andreas Paliouras[1], Anastasios Georgoulis[1], Aggeliki Tsoka[1], Martina Samiotaki[2], Constantinos Vorgias[1], Jerome Zoidakis[1,3*]

[1]Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, Zografou, 15701, Athens, Greece
[2]Proteomics Facility, Institute for Bioinnovation, Biomedical Sciences Research Center 'Alexander Fleming', 16672, Vari, Greece.
[3]Proteomics Laboratory, Biomedical Research Foundation, Academy of Athens, 11527, Athens, Greece

Chitin metabolism in marine bacteria has not been studied in depth. We used the psychrophilic bacterium Moritella marina to determine protein expression changes associated with the presence of chitin.

M. marina cells were cultured in the presence and absence of chitin, and total cell extracts and secretomes were collected. Proteins were reduced, alkylated and digested with trypsin. The resulting peptides were analysed by liquid chromatography coupled to high resolution mass spectrometer.

Proteomic analysis of total cell extract and secreted proteins revealed numerous proteins involved in chitin degradation and metabolism. The proteomics results were analyzed with bioinformatics tools to place the findings in specific metabolic pathways as well as in the wider context of marine bacterial metabolism.

Our future plans are to validate these preliminary findings with enzymatic and biochemical approaches and perform a metabolomics analysis. Thus, we will obtain a global understanding of chitin metabolism in M. marina.

P145

Divergent Genomic and Transcriptomic Landscapes of MSI and MSS Colorectal Cancer: Implications for Diagnosis and Therapy

Efstathios-Iason Vlachavas[1,2,*], Konstantinos Voutetakis[2,3], Vivian Kosmidou[2,3], Spyridon Tsikalakis[1,2], Spyridon Roditis[4,5], Konstantinos Pateas[4], Georgios N Zografos[4], Ryangguk Kim[6], Kym Pagel[7], Gregor Warsow[8], Alexander Pintzas[2,3] , Johannes Betge[8,9], Olga Papadodima[2,3,*], Stefan Wiemann[1,2,*]

[1]Division of Molecular Genome Analysis, German Cancer Research Center, Heidelberg, Germany
[2]Athens Comprehensive Cancer Center, Athens, Greece
[3]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[4]3rd Surgical Department G.Gennimatas Hospital, Athens, Greece
[5]General Oncology Hospital of Kifisia "Oi agioi Anargyroi", Athens, Greece
[6]Oak Bioinformatics LLC, Vienna, Virginia, USA
[7]Spin Systems, Falls Church, Virginia, USA
[8]Junior Clinical Cooperation Unit Translational Gastrointestinal Oncology and Preclinical Models, German Cancer Research Center, Heidelberg, Germany
[9]Junior Clinical Cooperation Unit Translational Gastrointestinal Oncology and Preclinical Models, Universitätsklinikum Mannheim, Germany

Despite recent advances in new therapeutic combinations, Colorectal Cancer (CRC) remains the second most common cancer killer globally, mainly due to its increasing incidence in developed countries, frequent late diagnosis, and propensity to metastasize (1). The microsatellite (MS) instability status is currently the most pivotal information for CRC from the clinical perspective, as it critically determines treatment decisions. Indeed, MS Instable (MSI) and MS Stable (MSS) CRCs are considered distinct molecular entities, with different therapeutic protocols and patient outcomes (2). The complexity of CRC is further enhanced by the frequent presence of alterations in distinct driver genes, such as RAS and RAF (3). Hence, there is an urgent need for new therapeutic biomarkers and combinatorial treatments. On this premise, we molecularly characterized and stratified CRC patients based on their MS status as well as the presence of KRAS/BRAF somatic variations. To this end, we performed paired-Whole Exome and RNA-Seq analysis of CRC tumors of 28 Greek patients and integrative bioinformatics analysis by introducing a new computational score in combination with OpenCRAVAT web server for cancer variant annotation and prioritization. In parallel, an analytical pipeline for CRC multi-omics datasets was established to leverage high-throughput data from public cancer cohorts. By integrating our molecular data with public datasets, we identified JAK-STAT and MAPK molecular cascades specifically activated in the MSI tumors, whereas TGF-beta was found to be the major signaling pathway activated in the MSS tumors. We further unraveled common mechanisms perturbed in both the transcriptional and mutational circuits and highlighted the RUNX transcription factors as putative novel targets. Additionally, interrelation of RAS/RAF mutations in MSI/MSS CRC tumors revealed an interesting impact of KRAS mutations on the immunogenicity of specific MSS patient subgroups. Finally, we sought to integrate the mutational ranking annotation system with transcriptional regulatory networks to facilitate personalized therapeutic interventions.

[1] Siegel, RL, Wagle, NS, Cercek, A, Smith, RA, Jemal, A. Colorectal cancer statistics, 2023. CA Cancer J Clin. 2023; 73(3): 233-254. doi:10.3322/caac.21772
[2] Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015;372(26):2509-2520. doi:10.1056/NEJMoa1500596
[3] Scharpf RB, Balan A, Ricciuti B, et al. Genomic Landscapes and Hallmarks of Mutant RAS in Human Cancers. Cancer Res. 2022;82(21):4058-4078. doi:10.1158/0008-5472.CAN-22-1731

P146

Evaluation of a protocol for proteomics analysis of biological samples using the organic phase remaining after nucleic acid isolation

Julie Courraud[1,2], Guillaume Médard[2], Fotini Paradisi[3], Sotiris Karapatakis[3], Jerome Zoidakis[3,4*]

[1]Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Alexandra Hospital, Leof. Vasilissis Sofias 80, Athens 11528, Greece
[2]Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimioupolis Zografou, 15771, Athens, Greece
[3]Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, Zografou, 15771, Athens, Greece
[4]Proteomics Laboratory, Biomedical Research Foundation, Academy of Athens, 11527, Athens, Greece

Multiomics analysis of biological specimens can offer unprecedented insights on molecular features of human disease and improve patient outcomes. A significant obstacle for obtaining data from different omics approaches is the limited amount of available biological material. A recent report indicates that it is possible to use a single biological specimen for genomics, transcriptomics and proteomics analysis (1). Our goal was to test this protocol for proteomics analysis of CD138+ cells that are used for diagnostic assessment of Multiple Myeloma patients.

The organic phase containing the protein fraction obtained from the application of a standard RNA/DNA isolation procedure in CD138+ cells was used for proteomics analysis. Proteins were precipitated with acetone, pelleted by centrifugation, and redissolved using Ttrifluoroacetic acid. After neutralization, we proceeded with reduction, alkylation and tryptic digestion. The resulting peptides were analyzed by liquid chromatography coupled to a high-resolution mass spectrometer (Bruker TIMS-TOF Flex).

Close to 4000 proteins were identified with excellent repeatability in technical replicates. Relative quantification data in three replicates indicated that the coefficient of variation was lower than 20% for 69% of the identified proteins. These encouraging results indicate that in-depth proteomics analysis is possible using the leftover organic fraction from nucleic acid isolation.

Our future plans are to validate these preliminary findings using different biological materials and apply this innovative approach for comprehensive multi-omics profiling of CD138+ cells.

(1) Filip Mundt et al. In depth profiling of the cancer proteome from the flowthrough of standard RNA-preparation kits for precision oncology. https://doi.org/10.1101/2023.05.12.540582doi:bioRxiv

Supported by the ELMUMY Horizon Europe program, grant agreement No. 101097094

P147

Study of human glutamate dehydrogenase 2 expression pattern in the hippocampus of transgenic GLUD2 mice

Georgia Sofocleous[1*], Maria Savvaki[1], Kiki Sidiropoulou[2], Cleanthe Spanaki[1], Andreas Plaitakis[1]

[1]Medical School, University of Crete, Heraklion, Crete
[2]Biology Department, University of Crete, Heraklion, Crete

While glutamic acid is the main excitatory neurotransmitter that has been associated with higher human cognitive function, its high levels can result in neurodegeneration through excitotoxicity. In order to maintain glutamate at normal levels, clearance of glutamate from the synaptic cleft and astrocytic metabolism is a very important process. Glutamate dehydrogenase (GDH) is an enzyme with a vital role in glutamate metabolism. While in most organisms there is only one isoform (GDH1), human have recently acquired a new gene, GLUD2, which produces a different isoform of glutamate dehydrogenase, GDH2, that can operate at conditions in which the housekeeping GDH1 is inactive. The origin of GLUD2 coincides with an increase in human brain's size and structural and functional complexity. In order to investigate the role of GDH2 in human brain, we generated a transgenic mouse model that expresses GDH2 together with the housekeeping mouse GDH. Behavioral tests have shown that transgenic mice present better cognitive capabilities related to hippocampal function compared to wild-type mice. Therefore, we studied the expression of the GLUD2 transgene in the mouse hippocampus using western blot and immunofluorescence experiments. We found that GDH2 is expressed in pyramidal cells of both the cerebral cortex and the hippocampus of transgenic mice. GDH2 was also detected in astrocytes of all CA1-3 areas and all layers including the pyramidial. Finally, we found that in the dentate gyrus hGDH2 is expressed in both gray and white matter astrocytes and in the mossy-like neurons of the hilus which are responsible for memory. This expression pattern accords the one observed in human. In conclusion, concomitant expression of GDH2 in glutamatergic pyramidal and mossy-like neurons as well as in astrocytes of mouse hippocampus may account for the enhanced cognition of GLUD2 transgenic mice. The underlying mechanism of this effect is currently under investigation.

References

- *Plaitakis, Andreas, Dimitra Kotzamani, Zoe Petraki, Maria Delidaki, Vagelis Rinotas, Ioannis Zaganas, Eleni Douni, Kyriaki Sidiropoulou, and Cleanthe Spanaki. 2019. "Transgenic Mice Carrying GLUD2 as a Tool for Studying the Expressional and the Functional Adaptation of This Positive Selected Gene in Human Brain Evolution." Neurochemical Research 44 (1): 154–69. https://doi.org/10.1007/s11064-018-2546-3.*
- *Spanaki, Cleanthe, Dimitra Kotzamani, Kleopas Kleopa, and Andreas Plaitakis. 2016. "Evolution of GLUD2 Glutamate Dehydrogenase Allows Expression in Human Cortical Neurons." Molecular Neurobiology 53 (8): 5140–48. https://doi.org/10.1007/s12035-015-9429-2.*

P148

Studying the pathophysiological role of SLC25A46 through proteomic analysis

Vasiliki-Iris Perivolidi[1,2], Pavlina-Ilianna Moutzouri[1,2], MartiOaki[2], George Panayotou[2], Eleni Douni[1,2,*]

[1]Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece
[2]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Fleming 34, 16672, Vari, Greece

SLC25A46, a member of the Solute Carrier 25 (SLC25) family of mitochondrial transporters, has recently been associated with a wide range of human neurological diseases, such as optic atrophy, Charcot-Marie-Tooth type 2, Leigh syndrome, progressive myoclonic ataxia and pontocerebellar hypoplasia. SLC25A46 localizes in the outer mitochondrial membrane and so far there is no evidence for transport activity. Recent studies have shown involvement of SLC25A46 in cristae maintenance through interaction with core MICOS complex subunits MIC60 and MIC19 that are involved in cristae formation. Additionally, SLC25A46 has been found to interact with mitochondrial fusion proteins OPA1, MFN1 and MFN2, suggesting a role for SLC25A46 also in mitochondrial dynamics. Our group has recently identified a non-sense point mutation in the mouse Slc25a46 gene, resulting in a truncated protein, that causes lethal neuropathology in mice ($Slc25a46^{atc/atc}$), characterized by ataxia, optic atrophy and cerebellar hypoplasia, similarly to human pathology.

Our present work focuses on investigating the pathophysiological role of SLC25A46, through comparative proteomic approach in whole-cell extracts isolated from both the cerebellum of $Slc25a46^{atc/atc}$ and WT mice and a shRNA-mediated Slc25a46 knockdown HEK-293 cell line using LC-MS/MS. Proteomic analysis in both $Slc25a46^{atc/atc}$ cerebellum and Slc25a46 knockdown cells revealed a significant decrease in respiratory chain proteins, mainly proteins from complexes I, III and IV. In addition, dysregulated processes related to mitochondrial dynamics and cristae organization, cellular metabolism, lipid and calcium homeostasis were found, suggesting changes in the structure and function of mitochondria.

At the same time, we identified SLC25A46 interactome network in vivo by immunoprecipitation and proteomic analysis with LC-MS/MS. Our analysis revealed that SLC25A46 interacts with a large network of proteins, such as members of oxidative phosphorylation and F_1F_0-ATP synthase, mitochondrial-ER communication proteins, and synaptic vesicle proteins.

The implementation of the doctoral thesis was co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme "Human Resources Development, Education and Lifelong Learning 2014-2020" in the context of the Act "Enhancing Human Resources Research Potential by undertaking a Doctoral Research" Sub-action 2: IKY Scholarship Programme for PhD candidates in the Greek Universities.

P149

Unraveling epitranscriptomic regulation of nuclear chromatin dissociation dynamics

Nikoleta Pateraki[1,2], Nikoletta Triantopoulou[2,3], Maria Vergetaki[1], Christos Katsioulas[2], Marina Vidaki[2,3], Evgenia Ntini[2*]

[1]Department of Biology, University of Crete, Heraklion, Crete, Greece
[2]Institute of Molecular Biology and Biotechnology of the Foundation for Research and Technology Hellas, Heraklion, Greece
[3]Division of Basic Sciences, Medical School, University of Crete, Heraklion, Crete, Greece
** Corresponding author*

The "epitranscriptome" refers to the total of biochemical modifications of RNA within a cell, that do not alter the sequence itself[1].The most abundant RNA modification on RNA transcripts is N^6-methyladenosine (m6A), which plays a regulatory role in many biological processes, including transcription, splicing and RNA metabolism. Recent studies have focused on unraveling potential roles of m6A in response to various stress factors, including those that induce DNA damage. However, the mechanisms underlying the connection between m6A and DNA damage response have not been fully elucidated. In this project, we aim to characterize the potential role of N6-Methyladenosine (m6A) in response to DNA damage and its involvement in regulating R-loop formation and/or resolution.

To resolve this question, our primary focus is on the nuclear m6A reader YTHDC1, which has been shown to localize to double-strand DNA breaks[2]. It is worth noting that this reader also regulates responses to heat stress, suggesting a potential role in DNA damage response as well. In this study we present preliminary data of the role of YTHDC1 in response to UVC-mediated DNA damage. Additionally, we report on the progress in developing the acute and specific protein degradation tag (dTAG) system against YTHDC1 in HCT116 cells. This tool will enable us to better dissect the role of YTHDC1 and m6A in response to UV-induced DNA damage, and uncover epitranscriptomic (m6A-mediated) regulation of R-loop resolution and genomic stability under stress.

[1] Saletore Y et al. Genome Biol. 2012; 13(10):175
[2] Zhang C. et al. Mol.Cell. 2020; 79(3), 425-442.e7.

P150

Characterization of the putative DEAH-box RNA helicase DHX35: Insights into cancer translation deregulation

Nikolaos Kypraios[1], Katerina Gentekaki[1], Eleni G. Kaliatsi[1], Adamantia Kouvela[1], Constantinos Stathopoulos[1], Vassiliki Stamatopoulou[1*]

[1]Department of Biochemistry, School of Medicine, University of Patras, Greece

Helicases are enzymes found universally in all forms of life, playing key roles in various facets of nucleic acid metabolism. They are primarily responsible for unwinding DNA, RNA, DNA-RNA hybrids, and disassembling ribonucleoprotein complexes. Based on our prior research studies, we identified DEAH-box polypeptide 35 (DHX35) as a putative ATP-dependent RNA helicase involved in the synthesis and processing of ribosomal RNA. To further characterize DHX35 we measured its transcription levels by RT-qPCR and we observed a differential DHX35 expression profile among cervical cancer, leukemia, melanoma and lung cancer cell lines, as well as specimens collected from patients. This observation prompted us to elucidate the biological function of DHX35 in cancer and, for this reason we knocked-out the DHX35 gene in cancer cell lines by using the CRISPR/Cas9 genome editing tool. Notably, the edited cell lines were metabolically less active and characterized by G1 arrest. In addition, the knocked-out cells exhibited a significantly decreased rate of protein synthesis by performing the assay of puromycin and a deregulation of crucial translation initiation factors. To gain a deeper understanding of DHX35 role and assess its substrate binding capabilities, we conducted electrophoretic mobility shift assays between DHX35 expressed in a heterologous E. coli system and variable nucleic acids. Of note, DHX35 exhibits a binding specificity only to RNA molecules with 3' overhangs. These findings underscore the importance of further research into DHX35 to unravel its intricate roles in nucleic acid metabolism and its potential significance in cancer biology and therapeutic development.

P151

Regulation of novel Pol III transcribed non-coding RNAs by paralogue ribonuclease genes

Alexandros Maniatis[1], Athanasios-Nasir Shaukat[2], Georgios D. Kefalas[1], Constantinos Stathopoulos[1*]

[1]Department of Biochemistry, School of Medicine, University of Patras, 26504 Patras, Greece
[2]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, 50 South Drive, Bethesda, MD 20892, USA
**cstath@med.upatras.gr*

The regulation of gene expression in eukaryotes is a versatile process primarily governed by the intricate mechanisms of non-coding RNAs (ncRNAs) and it is orchestrated by ribonucleases[1]. Within this context, our investigation focuses on two human paralogue genes of RNase Z, ELAC1 and ELAC2, shedding light, for the first time, on their respective roles in maturation of ncRNAs produced by RNA Polymerase III (Pol III). In mammals, ELAC1 has been shown that plays a vital role in restoring functionality to tRNAs by removing the 2'-3' cyclic phosphate immobilized on ribosomes, while ELAC2 has been acknowledged for its pivotal role of the removal of the 3' trailer from nuclear and mitochondrial tRNA precursors, as well as, in the maturation of long non-coding RNA (lncRNA)[2,3]. The primary objective of this study was to investigate the mechanisms of action of ELAC1 and ELAC2, and study their interactions with pre-tRNAs. Experimental procedures encompassed site directed mutagenesis of D and T loop and alterations of 3' trailer sequence of pre-tRNA^{Arg} and pre-tRNA^{Gly}. Furthermore, recombinant ELAC1 was employed, with its flexible arm removed, to gain insights into its specific recognition regions. In vitro cleavage assays unveiled that tRNA mutants led to reduced enzyme activity and a consequential alteration in cleavage patterns. In addition, our investigation extended to the processing mechanisms of two additional RNA Pol III transcripts, vtRNA1-2 and YRNA4. Interestingly, these transcripts were found to be cleaved exclusively at their 3' ends by ELAC1, with ELAC2 demonstrating no cleavage activity. Kinetic analysis with new substrates showed that ELAC1 displayed a higher affinity for pre-tRNAs in comparison to vtRNA1-2 and YRNA4. This insight signifies that ELAC1, beyond its known role in tRNA processing, can cleave other ncRNAs produced by RNA Pol III, suggesting its potential involvement in diverse cellular processes.

References

1. *Zhang P. et al. (2019) J. Integr. Bioinform. 16*
2. *Yip M.C.J. et al. (2020) Cell Rep. 30:2106-2114.*
3. *Dubrovsky E.B. et al (2004) Nucleic Acids Res. 32:255–262*

P152

The role of eIF6 and eIF3j in melanoma drug resistance

Antonia Petropoulou[1], Angelina Bania[1], Alexandra Anastogianni[1], George C. Kyriakopoulos[1], Vassilis Stamatakis[1], Argyris Alexiou[1], Konstantinos Kotsomitis[1], Katerina Grafanaki[1,2], Constantinos Stathopoulos[1*]

[1]Department of Biochemistry, School of Medicine, University of Patras, Greece
[2]Department of Dermatology, School of Medicine, University of Patras, Greece

Melanoma is the most aggressive type of skin cancer, distinguished by its strong propensity to metastasize. The emergence of melanoma is influenced by a combination of genetic and environmental factors. The prevalent mutation found in melanoma is $BRAF^{V600E}$, which initiates an excessive cascade of downstream signals and disrupts the process of translation. Therapeutic strategies often involve the utilization of specialized small-molecule inhibitors, like vemurafenib, for the management of metastatic melanoma. Nevertheless, malignant cells frequently acquire resistance to these targeted treatment approaches over time. It has been observed that the expression of many eukaryotic initiation factors (eIFs) changes in skin cancers. Two major factors, eIF6 and eIF3j, exhibit multiple roles in cells. On the one hand, eIF6 serves a dual purpose in ribosomal anti-association and 60S biogenesis. On the other hand, eIF3j is one of the thirteen subunits comprising eIF3 and plays a pivotal role in translation initiation, termination, and ribosome recycling. However, the exact role of eIF6 and eIF3j in the development of melanoma cells and their acquisition of resistance is still not fully understood.

Herein, we developed $BRAF^{V600E}$ -mutated vemurafenib resistant (VR) A375 and SK-MEL5 cell lines in which basal levels of eIF6 and eIF3j were checked. The two eIFs were differentially expressed in the VR cells. To further investigate their involvement in melanoma translation regulation and signaling, we created stable cell lines expressing eIF6 and eIF3j. We assessed global translation rates in these cell lines using puromycin staining and polysome profiling via sucrose density gradients. Additionally, we performed Western blot analyses to examine various proteins, including ERK1/2, AKT, and others, which serve as indicators of alterations in cell signaling pathways. These assays were also performed in three melanoma cell lines derived from genetically modified mouse models with specific mutations in key cutaneous melanoma genes, such as BRAF and RAS. Overall, our results suggest a significant role of eIF6 and eIF3j in both vemurafenib-resistant melanoma cells and mouse cells by affecting major signaling events and cap-dependent translation regulation.

P153

Investigating the human epitranscriptome in breast cancer cell lines through nanopore sequencing

Panagiotis G. Adamopoulos, Konstantina Athanasopoulou, Michaela A. Boti, Glykeria N. Daneva, Panagiotis Tsiakanikas, Andreas Scorilas*

Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece.

For decades it has been known that eukaryotic mRNAs undergo extensive post-transcriptional processing, which includes 5 capping, 3 polyadenylation and RNA modifications, to control mRNA stability, RNA-protein interactions, splicing and translation efficiency. Regarding RNA modifications, multiple studies have confirmed the prevalence of chemically modified RNA, but it was only after the revolution in sequencing techniques, the study of epitranscriptomics emerged. N6-methyladenosine (m^6A) stands out as the most abundant internal modification with regulatory roles in cellular processes, including splicing and protein synthesis. Additionally, other modifications within eukaryotic mRNAs include the cytosine methylation to 5-methylcytosine (m^5C) and the conversion of adenosine to inosine (A-to-I). Standard approaches for studying RNA modifications include thin-layer chromatography (TLC), liquid chromatography–mass spectrometry (LC-MS), and next-generation sequencing (NGS). However, despite the availability of detection methods, they have limitations. In contrast, the state-of-the-art nanopore sequencing technology enables the accurate characterization of modified bases at a nucleotide-resolution level. Within the NanoRNAmod framework, our aim is to investigate the extensive array of RNA modifications in apoptosis-related genes using nanopore sequencing approach. Specifically, this project focuses on developing new strategies for profiling major post-transcriptional modifications in breast cancer, such as m^6A and m^5C. Using native and in vitro transcribed mRNA samples, we have designed and implemented long-read RNA sequencing approaches that enable the simultaneous identification of multiple modifications. Our post-processing bioinformatics analysis has demonstrated that nanopore sequencing not only detects the modified bases but also provides quantitative information on m^6A and m^5C. Ultimately, this project seeks to characterize highly significant mRNA modifications with implications for apoptosis, mRNA stability, and translation in breast cancer cells. Although several technical challenges remain, our work illustrates its potential to significantly advance epitranscriptomic research and shed light on the functional and regulatory roles of mRNA modifications.

Acknowledgements

This research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "3rd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers" (Project Number: 07261).

P154

Heterogeneous nuclear ribonucleoprotein A3 is a regulator of intestinal infection

Fotis Ioakeimidis[1#], Panagiotis Mavrommatis-Parasidis[2#*], Sofia Gargani[1,2], Margarita Andreadou[1], Dimitris L. Kontoyiannis[1,2]

[1]Institute of Fundamental Biomedical Research, Biomedical Sciences Research Centre "Alexander Fleming", Vari, Greece
[2]Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece

Gastrointestinal infections world widely, account for a significant burden of acute and chronic diseases and are a severe challenge to healthcare. During gastrointestinal infections, a delicate balance must be maintained between innate immune, adaptive immune and epithelial cells to promote intestinal immunity, preserve barrier integrity, control, and ultimately clear the pathogen. The beneficial responses of innate immune cells necessitate their proper adaptation to infectious and homeostatic signals. Macrophages are central orchestrators of the innate immune response. Post-transcriptional mechanisms controlling mRNA biogenesis and utilization have emerged as central determinants of macrophage functions in the intestinal mucosa. These mechanisms have become central factors in determining the functions of macrophages within the intestinal mucosa and when they malfunction, they become the driving force behind intestinal pathology. However, our knowledge about the roles played by RNA-binding proteins in controlling post-transcriptional programs during innate immune adaptation remains limited. Further exploration in this field is crucial for comprehending the underlying mechanisms of gastrointestinal infections and developing new therapeutic strategies for their management and prevention.

In our study, we provide evidence of the involvement of a nuclear ribonucleoprotein, hnRNPA3, in the adaptation of innate immune cells to gastrointestinal infection caused by the mouse pathogen Citrobacter rodentium, using a novel conditional mouse model. Our findings reveal that mice lacking hnRNPA3 in their myeloid lineage exhibit heightened susceptibility to the infection, characterized by elevated epithelial damage and associated hyperplasia, and increased dysbiosis. Additionally, these changes in susceptibility coincide with alterations of hnRNPA3 deficient macrophage's capacity to respond to danger signals and their support for unresolved Type III cell-mediated immune responses in the intestine. Our data underscore a critical role of RNA regulation mediated by hnRNPA3 in countering the pathological events observed in infections caused by enteropathogenic and enterohaemorrhagic Escherichia coli (EPEC and EHEC), inflammatory bowel diseases, and intestinal cancers.

P155

Exploring m6A epitranscriptome machinery in ovarian cancer: Clinical relevance of FTO m6A RNA "eraser" in early-progression and treatment response

Eleni-Foteini Pasiali[1], Konstantina Panoutsopoulou[1], Eva Obermayr[2], Sven Mahner[3], Toon van Gorp[4], Ioana Braicu[5], Robert Zeillinger[2], Margaritis Avgeris[1,6,*], Andreas Scorilas[1]

[1]Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece
[2]Molecular Oncology Group, Department of Obstetrics and Gynecology, Comprehensive Cancer Center-Gynecologic Cancer Unit, Medical University of Vienna, Vienna, Austria
[3]Department of Gynecology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, AGO; current address: Department of Obstetrics and Gynecology, University Hospital, Ludwig-Maximilians-University Munich, Munich, Germany
[4]Department of Obstetrics and Gynaecology, Division of Gynecologic Oncology, University Hospital Leuven, Leuven Cancer Institute, Leuven, Belgium
[5]Department of Gynecology, Charité University Medicine, Campus Virchow, Berlin, Germany
[6]Laboratory of Clinical Biochemistry - Molecular Diagnostics, Second Department of Pediatrics, School of Medicine, National and Kapodistrian University of Athens, "P. & A. Kyriakou" Children's Hospital, Athens, Greece
[*]margaritis.avgeris@gmail.com; mavgeris@med.uoa.gr

Ovarian cancer (OC) is the most lethal gynecological cancer in developed countries, accounting for approximately 5% of female cancer-related mortality worldwide due to frequent patient's recurrence and chemotherapy resistance. N6-methyladenosine (m6A) is the most prevalent internal mRNA modification, while its dynamic and reversible nature is regulated by m6A methyltransferases ("writers"), m6A demethylases ("erasers"), and m6A binding proteins ("readers"). Within this intricate landscape, FTO m6A RNA demethylase emerges as a pivotal regulator, governing essential pathways implicated in a spectrum of medical conditions, including ovarian carcinoma; however, its potential clinical role has not been deciphered. Herein, we have examined FTO expression in epithelial ovarian cancer (EOC) and assessed its clinical value in EOC patients' prognosis and treatment outcome. Total RNA was extracted from ovarian tumor samples of the OVCAD multicenter study (screening cohort; n=100) and FTO levels were quantified by RT-qPCR. TCGA-OV cohort (n=307) was utilized as validation cohort and RNA-seq dataset was evaluated through UCSC Xena Browser. Survival analysis was performed using disease progression and patients' death as clinical end-points. Kaplan-Meier and Cox regression analyses demonstrated that FTO overexpression is associated with early-progression (PFS: log-rank p=0.012, HR:1.726, 95% CI:1.106-2.695, p=0.016) and poor overall survival (OS: log-rank p<0.001, HR:2.943, 95%CI:1.727-5.018, p<0.001). Importantly, FTO retained its prognostic value once integrated in multivariate models comprising of the most significant clinical variables in EOC prognosis (tumor grade, FIGO stage, residual tumor size, response to chemotherapy and age), being highlighted, thus, as an independent predictor of EOC survival outcome (OS: HR:3.497, 95%CI:1.965-6.225, p<0.001; PFS: HR:1.744, 95%CI:1.084-2.808, p=0.022). Ultimately, TCGA-OV analysis confirmed our findings as FTO levels were strongly associated with poor OS (log-rank p=0.032). Conclusively, FTO has emerged as a potent prognostic indicator in EOC, suggesting that its evaluation could ameliorate modern risk-stratification and benefit prognostication.

P156

A novel RNA-targeted therapeutic approach against Tau-driven neuronal pathology in Alzheimer's disease brain pathology

Anastasia Megalokonomou[1,2 *], Carlos Campos-Marques[3,4], Bruno Godinho[5,6], Jonathan Watts[5,7], Martina Samiotaki[8], George Panayotou[8], Georgia Papadimitriou[1,3,4], Anastasia Vamvaka-Iakovou[1,9], Beatriz Barros dos Santos[3,4], Kalliopi Skourti[1], Filippos Katsaitis[1], Joana Silva[3,4], Ioannis Sotiropoulos[1,3,4]

[1]Institute of Biosciences and Applications (IBA), National Center of Scientific Research "Demokritos", Athens, Greece.
[2]Faculty of Medicine, University of Crete, Heraklion, Greece.
[3]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga 4710-057, Portugal.
[4]ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães 4710-057, Portugal.
[5]RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, Massachusetts, USA
[6]Department of Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA
[7]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts, USA
[8]Institute for Bioinnovation (IBI), Biomedical Sciences Research Center "Alexander Fleming", Athens, Greece
[9]Department of Biological Applications & Technology, University of Ioannina, 45110, Ioannina, Greece

Antisense Oligonucleotides (ASOs) are small synthetic strings of nucleotides that regulate the RNA levels of the protein of interest, they have been proven to be safe for both animal and human use, and they are currently rising as a novel therapeutic tool against various diseases. In light of the emerging focus on the deteriorating role of tau protein in a number of brain pathologies, a number of studies over the last decade are testing the use of ASOs against tau-related neurodegenerative disorders, such as Progressive Supranuclear Palsy and Alzheimer's Disease. In this context, we designed and monitored the efficiency of 22 novel ASOs against total Tau or selectively 4R-Tau in cell lines and primary neurons expressing human Tau, as well as in a pilot in vivo study with Tau transgenic mice. These studies have identified a set of novel and highly efficient ASOs for reducing Tau or modulating 4R/3R isoform ratio as assessed by different types of molecular and cellular, neurostructural and behavioral analysis. Altogether, these data provide in vitro and in vivo evidence of the beneficial use of ASOs against Tau-related neuronal malfunction, supporting ASOs as an innovative RNA-based therapeutic approach in neurodegenerative pathologies of the brain.

P157

PARN-v1, a splice variant that expands the biological roles and reveals an evolutionary switch of Poly(A)-specific ribonuclease

Lampros Balis[1], Dionysios Antonopoulos[1], Athanasios Kyritsis[1], Rafailia A.A. Beta[1], Aikaterini Tsingene[1], George Panayotou[2], Martina Samiotaki[2], Dimitrios Vlachakis[3], Nikolaos A.A. Balatsos[1]

[1]Department of Biochemistry and Biotechnology, University of Thessaly, Viopolis, 415 00 Larissa, Greece
[2]B.S.R.C. "Alexander Fleming", 34 Fleming Street, 166 72 Vari, Greece
[3]Genetics Laboratory, Department of Biotechnology, Agricultural University of Athens, 75 Iera Odos Street, 118 55 Athens, Greece

Poly(A)-specific ribonuclease, PARN, catalyses the shortening of poly(A) tails, the first and rate-limiting step in mRNA degradation. PARN also mediates the maturation of an increasing repertoire of non-coding RNAs, spanning from microRNAs and piRNAs to the telomeric RNA component. We identify a splice variant of PARN, PARN-v1, in pleural malignant mesothelioma (PMM) cell lines and lung fibroblasts. PMM cells express both PARN and PARN-v1, whilst PARN-v1 is hardly observed in benign pleural cells. Moreover, PARN-v1 mRNA levels are significantly higher in the more aggressive PMM subtypes (biphasic and sarcomatoid) than the least aggressive one (epithelioid). This distinct motif is also reflected in the PARN-v1 protein levels. Molecular cloning and sequence analysis revealed that the variant lacks a 61-amino acid sequence from its N-terminus compared to PARN including two residues of the active site. Nevertheless PARN-v1 retains deadenylase activity, while molecular modelling studies and molecular dynamics simulations confirm the folding adopted by the variant as a structurally functional alternative in the 3D conformational space. Phylogenetic analyses have shed light to an across species conservation of the novel variant, suggesting that it may bear a critical biological function and potentially belong to a "core" subclass of PARN genes. To unravel PARN-v1 roles we examined the expression in several cell lines. We find that in MRC5 lung fibroblast cells PARN-v1 protein is abundant, while PARN is barely detectable. Silencing of PARN-v1 in MRC5 cells and subsequent mass spectrometry analysis affected ECM adhesion and cell migration. The investigation of the biological significance of PARN and its variants both at the molecular/cellular and evolutionary standpoints remains an open challenge.

P158

Comparative analysis of telencephalic Tle4/Grg4 expression between domestic cat and mouse embryos reflects on a common repression pattern, with subtle differences

Evangelia Archontoulak[i1], Nikistratos Siskos[1*], Charalampos-Chrysovalantis Chytoudis-Peroudis[1], Andreas-Rafail Vasileiou[1], Charalampos Ververidis[2], George Skavdis3, Maria E. Grigoriou[1]

[1]Laboratory of Developmental Biology & Molecular Neurobiology, Department of Molecular Biology & Genetics, Faculty of Health Sciences, Democritus University of Thrace, Alexandroupolis, Greece
[2]Obstetrics and Surgery Unit, Companion Animal Clinic, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]Laboratory of Molecular Regulation & Diagnostic Technology, Department of Molecular Biology & Genetics, Faculty of Health Sciences, Democritus University of Thrace, Alexandroupolis, Greece
[*]corresponding author

Tle4 is a member of the Groucho family (known as TLE in human or Grg in mouse); Groucho (Gro), the family prototype, encodes a co-repressor that was first identified in Drosophila melanogaster in 1968. Gro co-repressors are involved in several signaling pathways, including BMP, Wnt and Notch and mediate repression either directly, through interaction with transcription factors, or indirectly, through histone acetylation or chromatin modifications. Previous studies of Grg4 expression in the embryonic murine telencephalon, have revealed a dynamic spatiotemporal pattern; functional analysis suggested a role in cell migration mechanisms and in the temporal regulation of neuronal specification. In this work, we have analyzed the spatiotemporal pattern of the Tle4 expression in the embryonic feline telencephalon at E26/27 and E24/25 using in situ hybridization and a battery of subpalial (Lhx6, Lhx7/8, Dlx2, Nkx2-1, Ascl1, Er81) or pallial (Pax6, Emx1, Lhx2, Tbr1, Tbr2) markers. Moreover, the expression pattern of the feline Tle4 was compared to that of Grg4 in E13.5 mouse embryos. Tle4 exhibited a complex expression pattern, overall conserved to that of the mouse, under the transcriptional control of Nkx2-1 and further involved in the regulation of migration of distinct populations of subpallial neuronal progenitors, from the diagonal domain to the anlagen of the basal magnocellular complex and the globus pallidus. Despite the similarities reflecting highly conserved patterning and regional specification mechanisms, careful comparison between the expression patterns of the feline and the murine homologs, revealed subtle differences, possibly associated with the evolutionary emergence of the more elaborate gyrencephalic feline, in contrast with the lissencephalic murine, brain.

References

1. *Agarwal M, Kumar P, Mathew SJ. The Groucho/Transducin-like enhancer of split protein family in animal development. IUBMB Life 2015; 67: 472–481. doi: 10.1002/iub.1395*
2. *Chytoudis-Peroudis CC, Siskos N, Kalyviotis K, Fysekis I, Ypsilantis P, Simopoulos C, Skavdis G, Grigoriou ME. Spatial distribution of the full-length members of the Grg family during embryonic neurogenesis reveals a "Grg-mediated repression map" in the mouse telencephalon. PLOS ONE 2018; 13(12): e0209369. doi: 10.1371/journal.pone.0209369*
3. *Siskos N, Ververidis Ch, Skavdis G, Grigoriou ME. Genoarchitectonic compartmentalization of the embryonic telencephalon: insights from the domestic cat. Frontiers in Neuroanatomy 2021; 15:785541. doi: 10.3389/fnana.2021.785541*

P159

Expression of Neurod1 and Neurod6 in the embryonic pallium of the domestic cat reveals conserved spatiotemporal neurogenic gradients

Alexandra Tsakalidou-Rafailidou[1], Nikistratos Siskos[1*], Andreas-Rafail Vasileiou[1], Charalampos Ververidis[2], George Skavdis[3], Maria E. Grigoriou[1]

[1]Laboratory of Developmental Biology & Molecular Neurobiology, Department of Molecular Biology & Genetics, Faculty of Health Sciences, Democritus University of Thrace, Alexandroupolis, Greece
[2]Obstetrics and Surgery Unit, Companion Animal Clinic, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]Laboratory of Molecular Regulation & Diagnostic Technology, Department of Molecular Biology & Genetics, Faculty of Health Sciences, Democritus University of Thrace, Alexandroupolis, Greece
[*]corresponding author

Basic helix-loop-helix (bHLH) transcription factors Neurod1 and Neurod6 belong to the Neurod family of bHLH neuronal differentiation factors, that bear similarity with the atonal group of Drosophila melanogaster and play pivotal roles in the regulation of differentiation, migration and maturation of the glutamatergic neurons of the embryonic pallium. Neurod1 and Neurod6 present distinct, yet highly overlapping expression patterns in the developing telencephalon of the mouse. Neurod1 is expressed in the subventricular (SVZ) and the adjacent intermediate (IZ) zone of the pallium, regulating the differentiation of intermediate progenitors to postmitotic neurons, downstream of Tbr2 (Eomes) that is expressed by all cycling intermediate progenitors and upstream of the postmitotic glutamatergic marker Tbr1. Neurod6 is expressed by postmitotic pyramidal neurons, all along their radial migration to the cortical plate; moreover, its role in mitochondrial biogenesis, axonal growth and navigation (especially regarding the callosal axons), are considered to correlate with its steady expression in the cortical plate. In this work, the expression patterns of both Neurod1 and Neurod6 were studied in various stages of the developing feline pallium. To this end, in situ hybridization was performed using riboprobes for the aforementioned genes, as well as for well-established markers of the glutamatergic lineage, namely Tbr2, for the SVZ-residing, cycling progenitors, and Tbr1 for the postmitotic cells of the cortical plate (CP). Our results indicate an overall-conserved expression pattern for both Neurod1 and Neurod6 reflecting on common mechanisms underlying neurogenesis. Neurod1 was detected mainly in the SVZ, as well as in the CP, while Neurod6-expressing cells were found in the mantle and the SVZ, but most prominently in the CP. Finally, we observed spatial and temporal gradients in their expression, confirming the gradual progression of neurogenesis across the distinct sectors of the feline pallium, as defined for the mouse.

References

1. *Tutukova S, Tarabykin V, Hernandez-Miranda LR. The Role of Neurod Genes in Brain Development, Function, and Disease. Frontiers in Molecular Neuroscience 2021; 14:662774. doi: 10.3389/fnmol.2021.662774*
2. *Siskos N, Ververidis Ch, Skavdis G, Grigoriou ME. Genoarchitectonic compartmentalization of the embryonic telencephalon: insights from the domestic cat. Frontiers in Neuroanatomy 2021; 15:785541. doi: 10.3389/fnana.2021.785541*

P160

Variability of anthocyanins in Papaver rhoeas L. petals. A column chromatography study

Charilaos Yiotis[1], Orestis Gatsios[1], Ioannis Vrettos[2], Dimitrios Kyrkas[2], Nikolaos Mantzos[2], Paraskevi E. Beza[2*]

[1]Plant Functional Biology Laboratory, Department of Biological Applications & Technologies, University of Ioannina, Ioannina, Greece.
[2]Department of Agriculture, University of Ioannina, Arta, Greece

Papaver rhoeas L. (Papaveraceae), commonly known as corn poppy, is a cosmopolitan weed and an edible plant possessing medicinal properties. It is also known to represent a rich source of anthocyanins. The common poppy flower has four strikingly red petals with a distinctly black area bordered by a thin white line at the petal base, thus creating a color pattern that makes the centre of the flower, where the pollen is located, to visually stand out. Poppy petals are also characterized by color intensity and/or color hue patters, which hint at differences in the anthocyanic content or shifts in the pigments composition along the petals surface. The aim of this paper is to assess the variability in Papaver rhoeas L. petals' color intensity and hue and associate it with corresponding differences in the amount and type of petal pigments. Distribution of pigments in petal epidermis was investigated in different petal segments by column chromatography. Fresh petals were collected during blooming, between April and June 2023, in the region of Epirus. The petals were segmented to separate their peripheral from their inner zones, up to the zone of their black spot and the different zones were analysed separately. 0,2 g of petal segments per examined zone were grounded in a blender and pigments were extracted with water. The chromatography column was prepared with Kieselgel silica gel as the stationary phase whilst 0,1 % HCl acidified methanol was used as elution solvent. UV-visible absorption spectra of the eluted fractions revealed five pigments with varying ratios in petal segments belonging to different zones. Moreover, detected peaks in the region of 330-360 nm revealed the presence of aromatic acylated derivatives of anthocyanins. In the dark/black spots of the petals, anthocyanins coexist with a yellow flavone with a maxima absorption peak at 360 nm, which gives rise to a co-pigmentation effect. We conclude that uneven distribution of floral pigments along the petal epidermis creates a unique color palette, which is key in attracting pollinators responsible for reproduction.

Literature

- *Grauso L., Falco B., Riccardo Motti R., Lanzotti V. 2021. Corn poppy, Papaver rhoeas L.: a critical review of its botany, phytochemistry and pharmacology. Phytochem Rev 20:227–248 https://doi.org/10.1007/s11101-020-09676-7.*
- *Harborne J.B, (1958).The chromatographic identification of anthocyanin pigments. J. Chromatogr., Vol1:pp 473-478.*
- *Mekapogu M., Vasamsetti B., Kwon O., Suk Ahn M., Lim S. Jung J., 2020. Anthocyanins in Floral Colors: Biosynthesis and Regulation in Chrysanthemum Flowers. Int J Mol Sci.; 21(18): 6537.*
- *Trouillas, JC. Sancho-Garcia, V. De Freitas, J. Gierschner, M. Otyepka and O. Dangles (2016). Stabilizing and Modulating Color by Copigmentation: Insights from Theory and Experiment. Chem. Rev. 116, 4937-4982.*
- *Velickovica J., Mitic M., Arsic B., Dusan . PAUNOVIC , Branka T. Stojanovic , Jovana N. Veljkovic, Dimitrijevic D., Stevanovic S., Kostic D. 2019. HPLC analysis of extracts of fresh petals of Papaver Rhoeas L. Studia UBB Chemia, LXIV, 3, 2019 (p. 239-247).*
- *Taniguchi M., LaRocca C., Bernat J., Lindsey J., 2023. Digital Database of Absorption Spectra of Diverse Flavonoids Enables Structural Comparisons and Quantitative Evaluations. J. Nat. Prod. 86, 4, 1087–1119.*

P161

The discovery of novel Autotaxin inhibitors

Elli-Anna Stylianaki[1], Christiana Magkrioti[1], Antreas Afantitis[2], Alexios N Matralis[1], Vassilis Aidinis[1*]

[1]Biomedical Sciences Research Center Alexander Fleming, Athens, Greece
[2]NovaMechanics Ltd, Larnaca, Cyprus

Autotaxin (ATX) is a secreted enzyme, widely present in biological fluids including blood, which catalyzes the conversion of the lipid lysophosphatidylcholine (LPC) to lysophosphatidic acid (LPA). LPA has various functions on almost every cell type given the multitude of its receptors, which are widely expressed. Increased levels of both ATX and LPA have been found in multiple inflammatory and fibroproliferative diseases as well as cancer. Genetic and pharmacological studies in mice have confirmed their pathogenetic role in various disease models [1], such as pulmonary fibrosis [2], providing the proof of principle for subsequent clinical trials. Hence, a range of specific ATX inhibitors have been developed by the pharmaceutical industry and the academia [3]. Our team has also focused on the design and discovery of novel ATX inhibitors. In fact, previously, we have discovered six new chemical classes of ATX inhibitors [4] which were patented in the Hellenic Industrial Property Organization, whereas further ATX inhibitors were applied for an international patent according to the Patent Cooperation Treaty (PCT). Now, we have scanned chemoinformatically a small-molecule database against the ATX crystal structure and found molecules that can bind to ATX. The molecules with the best in silico binding were checked for their inhibitory activity against ATX with an in vitro biochemical assay. Through this process we have discovered two new ATX inhibitors at the low micromolar range. The pipeline that we have established can be used for the further discovery of novel ATX inhibitors, which could be possibly recruited against several pathologies.

References

1. *Magkrioti, C., et al., Autotaxin and chronic inflammatory diseases. J Autoimmun, 2019. 104: p. 102327. doi:10.1016/j.jaut.2019.102327*
2. *Oikonomou, N., et al., Pulmonary Autotaxin Expression Contributes to the Pathogenesis of Pulmonary Fibrosis. Am J Respir Cell Mol Biol, 2012.*
3. *Matralis, A.N., A. Afantitis, and V. Aidinis, Development and therapeutic potential of autotaxin small molecule inhibitors: From bench to advanced clinical trials. Med Res Rev, 2019. 39(3): p. 976-1013. doi:10.1002/med.21551*
4. *Magkrioti, C., et al., Structure-Based Discovery of Novel Chemical Classes of Autotaxin Inhibitors. Int J Mol Sci, 2020. 21(19). doi:10.3390/ijms21197002*

P162

Biocatalytic production of hydroxytyrosol-rich extract from Olea europaea leaf with enhanced biological activity

Alexandra V. Chatzikonstantinou[1,3], Stamatia Spyrou[1], Renia Fotiadou[1], Michaela Patila[1,3], Yannis V. Simos[2,3], Dimitrios Peschos[2,3], Angeliki C. Polydera[1,3] , Haralambos Stamatis[1,3],[*]

[1]Biotechnology Laboratory, Department of Biological Applications and Technologies, University of Ioannina, 45110 Ioannina, Greece
[2]Department of Physiology, Faculty of Medicine, School of Health Sciences, University of Ioannina, 45110 Ioannina, Greece
[3]Nanomedicine and Nanobiotechnology Research Group, University of Ioannina, 45110 Ioannina, Greece

In the present work we report the application of immobilized enzyme batch bioreactors for the biocatalytic treatment of an aqueous Olea europaea leaf extract (OLEx) rich in oleuropein (OL) produce extracts enriched with the bioactive hydroxytyrosol and other compounds derived from the hydrolysis of oleuropein. Firstly, a robust biocatalyst consisting of β-glucosidase from Thermodoga maritima immobilized on chitosan-coated magnetic nanoparticles was developed for the efficient bioconversion of oleuropein and was characterized with respect to its catalytic behavior (activity, thermostability and operational stability). The biocatalyst was successfully used in a rotating bed-reactor for the modification of the olive leaf extract leading to high conversion yields of oleuropein (exceeding 95%), while an up to 5 times enrichment in hydroxytyrosol was achieved. Various chromatographic and spectroscopic techniques were employed for the qualitative and quantitative analysis of the phenolic profile of the modified and non-modified olive leaf extract. Finally, the antioxidant, antibacterial, and cytotoxic activities of the olive leaf extract before and after its modification were investigated Compared to the non-modified extract, the modified one demonstrated 40% higher antioxidant activity, 5-fold higher antibacterial activity, and enhanced cytotoxicity against leiomyosarcoma cells.

Acknowledgments
This study was funded by the European Regional Develop-ment Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call "Aquaculture"- "Industrial Materials"- "Open Innovation In Culture" (project: AntiMicrOxiPack, project code: Τ6ΥΒΠ-00232)

P163

Changes in Biochemical Hematological and Oxidative stress markers after implementation of different therapies in rheumatoid arthritis

Athanasia Tsiakalidou[1], Konstantina Kazeli[1,2], Stavroula Ioannidou[1], Argyrios Ginoudis[3], Evgenia Lymperaki[1*]

[1]International Hellenic University, Department of Biomedical Sciences, Thessaloniki, Greece
[2]School of Physics, Faculty of Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease affecting diarthrodial joints. Inflammation increases the production of ROS, which could elucidate why RA is one of the conditions that induce oxidative stress. This study was conducted to estimate how certain biochemical, hematological and oxidative stress markers differed after different therapeutic approaches. 20 male and 57 female patients aged 34-59 years volunteered in this study. They were divided into two groups; 34 participants were treated with methotrexate (Group A) and 43 who received a combination of a Modifying Antirheumatic Drug (DMARD), such as methotrexate or leflunomide, and biological DMARD (Group B). Erythrocyte sedimentation rate (ESR), platelets (PLT), C reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide (Anti CCP), anti-nuclear antibodies (ANA), reactive oxygen species (ROS), glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), g Glutamyl transferase (γGT), vitamin C (Vit C), vitamin D (Vit D), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alkaline phosphatase (ALP), amylase (AMY), phosphorus (P), magnesium (Mg), calcium (Ca) were measured before the beginning of treatment and in 3 months into treatment in both groups with conventional colorimetric, fluorometric and immunological assays. The statistical analysis was performed by applying the student t-test and Pearson's correlation coefficient p, at 0.0001 and 0.05 level of significance respectively. γGT, RF, and ALP were found to be significantly low, whereas Vit C had significantly higher values after the treatment with methotrexate (Group A). After receiving DMARD (Group B), a significantly reduced average value of TG and ANA was noticed, whereas HDL and Vit D were increased. In both groups ROS values increased and antioxidant markers values (GPx, CAT, SOD) decreased after the amplification of treatment regimens. In conclusion, the two treatment options do not influence redox status parameters differently.

P164

Identification of specific testosterone-like antagonists for the membrane receptor of androgens, OXER1

Athanasios A. Panagiotopoulos[1], Evangelia Konstantinou[1], Konstantina Kalyvianaki[1], Stergios A. Pirintsos[2], Elias Castanas[1*], Marilena Kampa[1*]

[1]Laboratory of Experimental Endocrinology, University of Crete, School of Medicine, Heraklion, Greece
[2]Department of Biology, School of Science and Technology, University of Crete, Heraklion, Greece
**e-mails: Marilena Kampa (kampam@uoc.gr), and Elias Castanas (castanas@uoc.gr)*

Prostate cancer is one of the most common cancers and a hormone-dependent tumor. It is clear that androgens and androgen receptor signaling are crucial for prostate growth and homeostasis. The action of androgens is classically mediated through intracellular androgen receptors, which act as are transcription factors that regulate key cell processes. Recently, we have identified OXER1 as a membrane receptor for androgens in prostate and breast cancer cells. OXER1, is the receptor for the arachidonic acid metabolite 5-oxo- ETE which has a significant role in inflammatory responses, being responsible for leucocyte chemotactic responses. Testosterone action via OXER1 induces specific Ca^{2+} release from intracellular stores, modifies polymerized actin distribution, induces apoptosis and decreases cancer cell migration. These actions are antagonized by 5-oxo- ETE which increases cell growth and migration. Moreover, testosterone antagonizes 5- oxo-ETE cAMP decrease via Gαi protein. In this work, we mined the ZINC15 database, using QSAR, for natural compounds able to signal through Gαi and Gβγ simultaneously, mimicking testosterone actions, as well as for specific G_{α} and $G_{\beta\gamma}$ interactors, inhibiting 5- oxo-ETE tumor promoting actions. We were able to identify nine druggable G_{α}, four $G_{\alpha\beta\gamma}$ and seven $G_{\beta\gamma}$ specific OXER1 interactors. We further confirmed by bio-informatic methods their binding to the 5-oxo-ETE/testosterone binding groove of the receptor, their ADME properties and their possible interaction with other receptor and/or enzyme targets. Three compounds, ZINC15959779, ZINC04017374 (Naphthofluorescein) and ZINC08589130 (Puertogaline A) were purchased, tested in vitro and confirmed their OXER1 G_{α}, $G_{\beta\gamma}$ and $G_{\alpha\beta\gamma}$ activity, respectively. The methodology followed is useful for a better understanding of the mechanism by which OXER1 mediates its actions, it has the potential to provide structural insights, in order to design small molecular specific interactors and ultimately design new anti-inflammatory and anti-cancer agents. Finally, the methodology may also be useful for identifying specific agonists/ antagonists of other GPCRs.

Bibliography

1. *Kalyvianaki K. et. al, Antagonizing effects of membrane-acting androgens on the eicosanoidreceptor OXER1 in prostate cancer, Sci. Rep. 7 (2017) 44418.*
2. *Panagiotopoulos, A. et al., OXER1 mediates testosterone-induced calcium responses in prostate cancer cells. Molecular and Cellular Endocrinology (2022) 539: 111487.*
3. *Panagiotopoulos, A. et al., A simple open source bio-informatic methodology for initial exploration of GPCR ligands' agonistic/antagonistic properties. Pharmacology Research & Perspectives (2020) 8: 1-12.*

This work was partially supported by Greece and the European Union (European Social Fund- ESF) through the Operational Programme (Human Resources Development, Education and Lifelong Learning) in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY) to AP (PhD scholarship), a Special Fund for Research Grants (ELKE) of the University of Crete to MK and KK and by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: 3725 to MK).

P165

Biological activities of Ceratonia siliqua pod and seed extracts towards skin cell protection

Dafni-Alexandra Kavvoura[1], Michalis K. Stefanakis[2], Dimitris Kletsas[1], Haralambos E. Katerinopoulos[2], Harris Pratsinis[1,*]

[1]Laboratory of Cell Proliferation and Ageing, Institute of Biosciences and Applications, NCSR "Demokritos", Athens
[2]Laboratory of Organic Chemistry, Department of Chemistry, University of Crete, Voutes, Heraklion, Crete
** e-mail: hprats@bio.demokritos.gr*

Ceratonia siliqua L., generally known as carob tree, is quite common in most Mediterranean countries, and it can easily grow in arid and semiarid areas. It is also cultivated, usually for harvesting its fruits to be used as food for humans and animals. Gender and cultivar of the tree have been reported to affect carob properties, hence, in the present study we aimed to characterize samples from two common Cretan C. siliqua cultivars in terms of their chemotaxonomic and bioactivity properties. In particular, deseeded pod and seed extracts and fractions were evaluated using chromatographic techniques as to assess their essential oil, fatty acid, and carbohydrate profiles. Moreover, their bioactivities were studied using both cell-free assays, including free-radical scavenging, tyrosinase, collagenase and advanced glycation end product (AGE) formation inhibition, and assays in human skin fibroblast cultures, i.e., reactive oxygen species suppression, glutathione stimulation, and protection from oxidative stress and from ultraviolet (UVB) radiation. Extracts from both cultivars were found to possess antioxidant capacity, tyrosinase- and collagenase-inhibitory activities, an ability to block glucose-induced AGEs, and in certain cases, UVB absorbance and photoprotective activities. Seed extracts were in general more active, while the use of 30% aqueous methanol seemed to be more efficient than n-hexane for extraction. Serial partition of the most active extracts with solvents of increasing polarity resulted in fractions with enriched biological activities, the highest ones being observed with the ethyl acetate fractions. The data presented in this study support a future use of Cretan carob extracts and their fractions in skin care products, while since seeds are regarded by-products of carob powder production, the superior biological activities of seed extracts observed in this study make them even more promising for valorization and upcycled cosmetics.

This study was supported by the Region of Crete through the Program Contract with the University of Crete and the Hellenic Mediterranean University entitled "Actions for the optimal development of the carob tree potential in the Region of Crete" (21SYMV008995374 2021-07-28). The authors would like to acknowledge the support of Ms. Korina Miliaraki, and the Cultural Society of Panormo "EPIMENIDES".
Finally, support was also received by the project "An Open-Access Research Infrastructure of Chemical Biology and Target-Based Screening Technologies for Human and Animal Health, Agriculture and the Environment (OPENSCREEN-GR)" (MIS 5002691) which was implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014–2020) and co-financed by Greece and the European Union (European Regional Development Fund).

P166

Preparation of enzymatically modified oils with enhanced oxidative stability as ingredient of fish feed supplement

Renia Fotiadou[1], Dimitrios Lefas[1], Fountoulaki Eleni[2], Angeliki C. Polydera[1], Haralambos Stamatis[1,*]

[1]Biotechnology Laboratory, Department of Biological Applications and Technologies, University of Ioannina, Greece
[2]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, 46.7 Avenue Athinon-Souniou ,19013 Anavissos, Athens, Greece
**e-mail: hstamati@uoi.gr*

In the present work, we report the exploitation of a green nanobiocatalytic system for the preparation of enzymatically modified pomace olive oil with a scope of fortifying its biological properties1. The green nanobiocatalyst was developed by immobilizing lipase from Thermomyces lanuginosus on the surface of hybrid magnetic nanoparticles derived from a biological and cost-effective methodology. Selected phenolic compounds were esterified with fatty acids present in pomace olive oil enriching the edible oil with lipophilic derivatives. Different reaction parameters were assessed as well as the reusability of the nanobiocatalyst. Furthermore, the antioxidant activity of the modified oils was evaluated in comparison to a control oil which exhibited up to 12-fold increase. Various spectroscopic and chromatographic protocols were employed for the quantitative analysis of the primary and secondary oxidative products providing an overall view on the oxidative stability of the oils. Finally, the effect of the enzymatically modified pomace olive oil on the growth parameters of gilthead sea bream (SBG) and European sea bass (BSS) was also investigated. Feeds supplemented with 1% of modified pomace oil resulted in improved growth rate and feed conversion rate (FCR) in SBG and BSS, respectively.

Acknowledgments
This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call RESEARCH CREATE-INNOVATE (project code: T2EDK-03599).

References
Fotiadou et. al., Biomolecules, (2023), 1034-1050, 13(7).

P167

Establishment of an in vitro MYC-MAX complex formation assay to identify novel inhibitors of the MYC oncoprotein.

Ervelina Dalani[1,2,#], Alexandra Papafotika[1,2], Athina Vasiliki Karra[1,2], Martha Kintostathi[1,2], Savvas Christoforidis[1,2,*]

[1]Biomedical Research Institute, Foundation for Research and Technology, Ioannina, Greece.
[2]Laboratory of Biological Chemistry, Department of Medicine, University of Ioannina, Ioannina, Greece.
#Presenting author: Ervelina Dalani, email: ervelina.dalani@gmail.com
**Corresponding author: Savvas Christoforidis, email: savvas_christoforidis@bri.forth.gr, schristo@uoi.gr*

Expression of the c-MYC protein is tightly controlled in normal cells, but becomes overexpressed in most human cancers, making it one of the most important human oncogenes. Thus, over the years, inhibition of the MYC family has been the goal of intense research. MYC protein initiates transcription of target genes by forming complex with its partner protein MAX. The region of MYC responsible for this interaction lies at the basic helix-loop-helix-leucine zipper (bHLHLZ) domain, making this region of MYC an ideal target for raising new inhibitors of MYC-MAX complex.

Here we report the development of a novel in vitro, cell-free assay, which assesses quantitatively the amount of complex between MYC and MAX and can be used for screening of new inhibitors against MYC. The assay is based on the ELISA principle, using reagents and infrastructure that are common or easily accessible. Specifically, GST-MYC is immobilized on glutathione coated plates, followed by incubation with purified MAX, in the absence or presence of the candidate inhibitors. The amount of bound MAX, which corresponds to the amount of MYC-MAX complex formed at the plate, is quantified using anti-MAX antibodies and appropriate secondary antibodies coupled to HRP. To establish the optimal conditions of the assay, we optimized the amount of the proteins MYC and MAX as well as all parameters of the assay (temperature, time, protein ratio, buffer reagents). To validate the assay, we tested known inhibitors of MYC, thus providing proof-of-principle that the assay can be used for screening of new candidate inhibitors. The established assay prevails to previous methods as it has high signal-to-noise ratio, high dynamic range and throughput capacity. Using this assay, we are currently screening chemical libraries aiming in identifying novel inhibitors of MYC-MAX complex, which could lead to new anticancer drugs that target the protein complex MYC-MAX.

References

1. *Madden, S.K., de Araujo, A.D., Gerhardt, M. et al. Taking the Myc out of cancer: toward therapeutic strategies to directly inhibit c-Myc. Mol Cancer 20, 3 (2021).*
2. *Dang C. V. (2012). MYC on the path to cancer. Cell, 149(1), 22–35.*

Acknowledgment

This research has been co financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T1EDK-03532), title: Innovative Development of Novel Anticancer Drugs for Therapeutic Targeting of the MYC Oncoprotein, acronym: "AKMY".

P168

Natural Product Analogues as Antibacterial Agents: The Case of Cinnamaldehyde and Colupulone

Angeliki Kokkali[1], Anna-Maria Kostaki[2,3], Georgia Athanassopoulou[2,3], Apostolia Makri[4], Marina Sagnou[5], Veroniki P. Vidali[2*], Georgia Kythreoti[1*]

*[1]School of Liberal Arts and Sciences, The American College of Greece, Aghia Paraskevi, Greece. *Email: gkythreoti@acg.edu*
*[2]Institute of Nanoscience & Nanotechnology, National Center for Scientific Research "Demokritos", Aghia Paraskevi, Greece. *Email: v.vidali@inn.demokritos.gr*
[3]Department of Pharmacy, National and Kapodistrian University of Athens, Greece.
[4]Department of Food Science and Human Nutrition, Agricultural University of Athens, Greece.
[5]Institute of Biosciences & Applications, National Centre for Scientific Research "Demokritos", Aghia Paraskevi, Greece.

Alexander Fleming's discovery of the antibacterial properties of penicillin, has undoubtedly revolutionized medicine and resulted in the golden age of antibiotic discovery. A variety of low-cost antibacterial agents, effective against previously life threatening infections were made readily available, resulting though, in overuse and microbial resistance to currently prescribed antibiotics. Consequently, research efforts are once more focusing on the development of novel antibiotics. An attractive strategy is going back to nature in search of leads for new synthetic antibacterial agents or as a source of novel bioactive compounds.

For the purposes of this study, cinnamaldehyde and colupulone were selected as lead compounds. Cinnamaldehyde, a byproduct of the stem bark of Cinnamomum cassia, was isolated in 1834 by Jean-Baptiste Dumas, with uses ranging from the food and cosmetics to pharmaceutical industries. Colupulone, is a known hop -acid found in Humulus lupulus, a plant also used in the pharmaceutical and food industry. Previous studies have shown that both compounds exhibit antibacterial properties.

To investigate essential structures responsible for enhanced action, some functionalities on the selected parental compounds, cinnamaldehyde and colupulone, were preserved while others altered. To this end, short synthetic routes and efficient methods were employed including Wittig reaction, Friedel-Crafts and C-alkylation of phloroglucinol derivatives.

Subsequent testing for their antibacterial activity against gram-positive, Staphylococcus aureus and gram-negative Escherichia coli and Pseudomonas aeruginosa, microorganisms revealed important functionalities required for increased activities. The prospective development of a ligand-based pharmacophore was also investigated, by analyzing the structure-activity relationship of their bacterial growth inhibitory potencies. The para-methoxy substitution of the trans-cinnamaldehyde core resulted in higher growth inhibition activity against E.coli. In addition, all tested colupulone analogues exhibited enhanced activities against both E. coli and S.aureus. These results set the basis for designing new compounds to better understand structure-activity relationship and improve activity and selectivity.

P169

Rational chemoinformatic and in vitro screening approaches revealed novel series of Autotaxin inhibitors binding to allosteric sites

Elli-Anna Stylianaki[1], Antreas Afantitis[2], Alexios Matralis[1], Vassilis Aidinis[1*]

[1]Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece
[2]NovaMechanics Ltd, Larnaca, Cyprus

Autotaxin (ATX) is an ectoenzyme, present in the vast majority of biofluids, which catalyzes Lysophosphatidylcholine (LPC) to Lysophoshatidic acid (LPA). Its biological effect is mediated by the binding of its product to six cognate G-protein coupled receptors (LPAR1-6), which are expressed in nearly all cell types. Pathological increase of ATX levels in various tissues has been shown to be a causal factor for the development and progression of serious autoinflammatory and fibrotic disorders, namely Idiopathic Pulmonary Fibrosis (IPF) and Non-alcoholic Steatohepatitis (NASH). Genetic and pharmacological abrogation of ATX activity, alleviated IPF and NASH symptoms, suggesting ATX as an efficient drug target. This knowledge has been appreciated by both the academic and pharma sector but, despite many efforts, no drug had reached the market. Here, we aimed to discover novel ATX inhibitors that bind to different sites rather than the active site, by employing both chemoinformatic and in vitro screening methods. To do so, we developed a hybrid structure/ligand-based design pipeline at which we initially fed the structure of human ATX co-crystallized with four different compounds (PF-8380, PAT-352, PAT-347, GLPG-1690), each of which represent a different inhibitor type according to their mode of binding. Screening against the HitFinder library yielded a list of 39 initial candidates which showed the best docking scores and similar mode of binding with one, or more of the 4 inhibitors. Subsequent in vitro screening by employing the well established Amplex Red and TOOS assays, revealed that two of the compounds showed an IC50 in the lower μM range. Upon examination of their mode of binding, it was revealed that these compounds, indeed, exhibited a mixed competitive/non-competitive mode of inhibition. The newly discovered compounds consist of two novel classes of ATX inhibitors and could serve as structural starting points for further drug development.

P170

AZD9567, a non-steroidal Selective Glucocorticoid Receptor Agonist with improved therapeutic potential

Dimitra Siakouli[1], Georgios Panagiotou[1], Vassiliki Ganou[1], Eleni-Fani Gkotsi[1], Aristotelis Chatziioannou[2], Olga Papadodima[1], Michael N. Alexis[1], Dimitra J. Mitsiou[1*]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]Center of Systems Biology, Biomedical Research Foundation of the Academy of Athens, Greece
**e-mail: dmitsiou@eie.gr*

Glucocorticoids (GCs) are essential steroid hormones widely used as potent anti-inflammatory drugs; however, their chronic clinical use is often accompanied by adverse side effects. The anti-inflammatory action of GCs is exerted through the glucocorticoid receptor (GR) in part by antagonizing the pro-inflammatory nuclear factor kappa B (NF-kB) whereas the majority of side effects are assumed to be mediated by transactivation of GR target genes. Development of non-steroidal selective GR agonists (SEGRA) favoring transrepression of NF-κB target genes over transactivation of genes associated with undesirable effects could improve the clinical performance of GCs.

Compound AZD9567, a non-steroidal selective GR agonist with improved side effect profile [1], was used in order to study the mechanisms underlying the function of SEGRA. AZD9567 was shown to act as a partial agonist of GR capable of substituting for circulating hydrocortizone (HC, the human GC) at pharmacologically relevant concentrations. Using different cell-based assays we showed that AZD9567 displays full efficacy in transrepression of key pro-inflammatory genes and partial efficacy in transactivation of GR targets genes as compared to dexamethasone (Dex, a classical GR agonist). RNA-sequencing analysis in mouse macrophages revealed that the transcriptomic profile of AZD9567 is distinct than that of Dex. AZD9567 mediates many of the classical actions of glucocorticoids but also different actions. Under conditions that elicit an inflammatory response, the anti-inflammatory action of AZD9567 is mediated, at least in part, by the same principal mechanisms involved in the anti-inflammatory action of classical glucocorticoids. In conclusion, compound AZD9567 mediates common as well as different biological processes compared to classical glucocorticoids both in the absence and presence of an inflammatory response and its distinct transcriptomic profile may account for its improved selective effect. Our data reinforce the importance of the development of selective GR agonists able to confer improved therapeutic potential.

[1] Discovery of a Novel Oral Glucocorticoid Receptor Modulator (AZD9567) with Improved Side Effect Profile. J. Med. Chem. 2018, 61, 5, 1785–1799. DOI: 10.1021/acs.jmedchem.7b01690.

We acknowledge support of this work by the project "STHENOS-b" (MIS 5002398), which is funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the EU (European Regional Development Fund).

P171

In vitro assessment of antioxidant properties and antiproliferative effects of leaf extracts from Stevia rebaudiana Bertoni: a Systematic Review and Meta-Analysis

Maria Papaefthimiou[1], Anastasia Tsiarsioti[1], Panagiota I. Kontou[2], Pantelis G. Bagos[1], Georgia G. Braliou[1*]

[1]Department of Computer Science and Biomedical Informatics, University of Thessaly, Lamia, Greece
[2]Department of Mathematics, University of Thessaly, Lamia, Greece
**Correspondence to: gbraliou@dib.uth.gr*

Stevia rebaudiana Bertoni, is an aromatic plant with high sweetening capacity due to steviol glycosides. Recent studies have shown benefits of stevia consumption on human health due to several bioactive compounds. In this study, a systematic review and meta-analysis was performed to investigate antioxidant and antiproliferative activities of stevia leaf extracts based on data from ABTS and DPPH assays. A total of 39 articles, out of 138 articles that were initially retrieved, included in the meta-analysis, containing 83 individual studies. Meta-analysis of antioxidant capacity values (given in Trolox equivalents) indicated that the aqueous extracts exhibited the highest antioxidant activity with both DPPH (92.80 mg/g) and ABTS (680 μmol/g) assays. Hydroalcoholic extracts showed intermediate levels of activity (DPPH: 48.35 mg/g and ABTS: 581 μmol/g), while organic extracts displayed the lowest activity of 8.46 mg/g for DPPH and 313.64 μmol/g for ABTS assays. In addition, radical scavenging activity assays against DPPH• indicated that aqueous extract exhibited the highest inhibition 73.32%, whereas hydroalcoholic and organic extracts displayed 57.09% and 57.22% inhibition, respectively. Antiproliferative effect was also investigated enrolling meta-analysis approach, across 40 studies, considering five cancer types, various time points, and stratified by compound or extract. As effect estimate the half maximal inhibitory concentration (IC_{50}) measured by MTT assay, on various cancer cell lines, was recorded. Our results suggest that stevia biocompounds exert an overall IC_{50} of 152.5 μg/ml (for 48 hours of incubation) which is mostly attributed to stevia phenolic compounds (IC_{50}: 86.0 μg/ml) and not to glycosides (IC_{50}: 229.7 μg/ml) or diterpenes (IC_{50}: 167.3 μg/ml). Moreover, a very high inhibitory activity was observed on neuronal cancer cells (IC_{50}: 21.0 μg/ml). Our study synthesizes all available data and quantitatively assesses antioxidant and antiproliferative activity of Stevia rebaudiana compounds from leaf extracts, thus, illustrating stevia as a potent source for developing novel nutraceuticals.

Acknowledgement
We acknowledge support for this work by the project "GENOMIC-OASIS: GENOMIC Analysis of Organisms of Agricultural and Livestock Interest in Sterea", Grant Number (MIS) 5045902.

P172

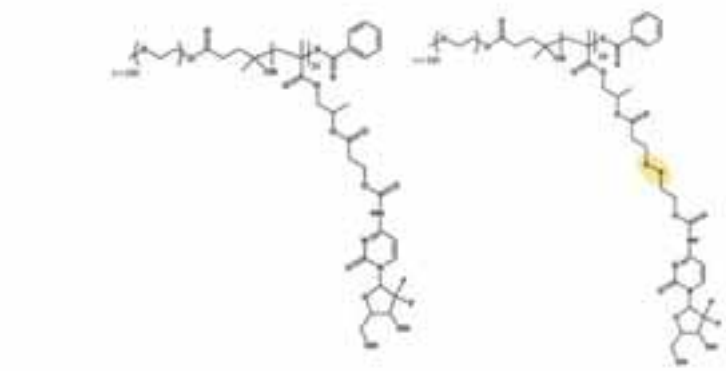
ULTRASONIC ACTIVATION OF POLYMER-GEMCITABINE CONJUGATES FOR TARGETED CANCER THERAPY

Toumpa Dimitra*, **Angelopoulou Athina, Pasparakis George**

Department of Chemical Engineering, University of Patras, 26504 Patras, Greece

Breast cancer is a significant malignancy that disproportionately affects women. Despite notable advancements in the fields of surgery, chemotherapy, and radiotherapy, the quest for a definitive cure remains an ongoing challenge. In recent times, polymer drug conjugates (PDCs) have attracted substantial attention within the pharmaceutical research community owing to their ability to facilitate controlled and precisely targeted drug release, which can be triggered by specific stimuli, using hydrolyzable linkers. This research delves into synthetic methods to generate polymer-gemcitabine conjugates from polymerizable monomer- prodrugs. These conjugates are created through reversible addition-fragmentation chain transfer (RAFT) polymerization, resulting well-defining gemcitabine-rich conjugates (see Scheme 1). When co-polymerized with a poly(ethylene glycol) macro-RAFT chain transfer agent, these constructs form self-assembled PDCs with a remarkably narrow dispersity index (Đ<1.2) and a low critical micelle concentration (CMC) ranging from 0.08 to 0.61 g/mL. One particularly intriguing finding from this study is the bond dissociation rate of the disulfide linkers, which was observed to increase proportionally with the intensity of ultrasound dosage. This phenomenon, in turn, led to higher rates of gemcitabine liberation when exposed to ultrasound radiation. In vitro experiments conducted on breast cancer cells (MCF-7), showed a substantial improvement of IC_{50} when compared to the parent drug. In summary, these findings offer new insights into the intricate relationship between polymer-drug linker chemistry, ultrasound dosage, and their collective impact on therapeutic effectiveness.

Keywords: *polymer drug conjugates, gemcitabine, nanomedicines, ultrasound therapeutics*



Scheme 1. Model polymer-gemcitabine conjugates with and without disulfide linkers.

Acknowledgements
This project was funded by the Hellenic Foundation for Research and Innovation (H.F.R.I. project No.81439).

References

1. Pasparakis, G. et al., Nature Communications 5, 3623 (2014).
2. Barnett C. et al., Molecular Pharmaceutics 20 (3), 1818-1841 (2023).
3. Emamzadeh M. et al., Journal of Materials Chemistry B 6, 2230-2239 (2018).
4. ACS Macro Letters 6, 535-540 (2017).

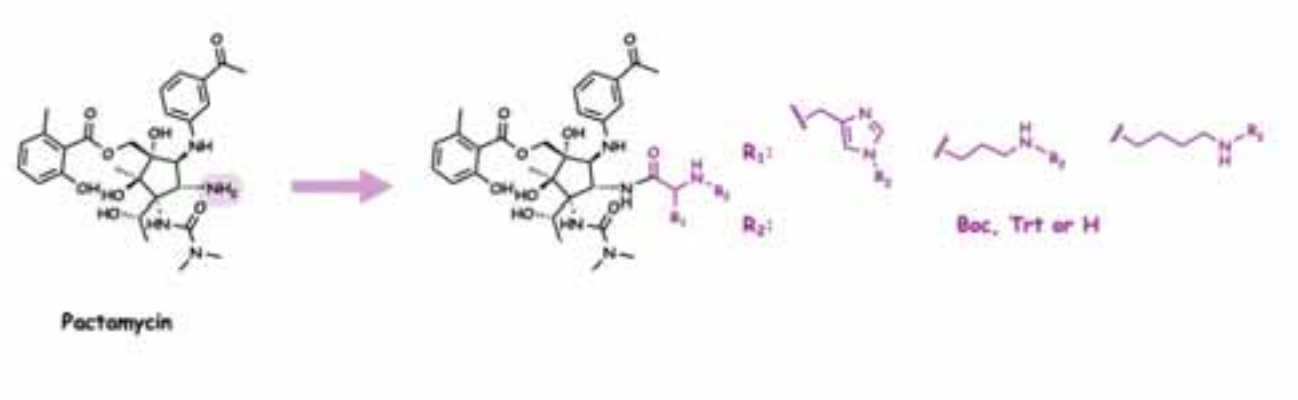
P173

New pactamycin derivatives: design, synthesis, and biological assessment

Artemis Tsirogianni[1*], Maria Mpogiatzoglou[2], George Dinos[2], Georgia G. Kournoutou[2], Constantinos M. Athanassopoulos[1]

[1]Synthetic Organic Chemistry Laboratory, Department of Chemistry, University of Patras, GR-26504 Patras, Greece;
[2]Department of Biochemistry, School of Medicine, University of Patras, 26504 Patras, Greece;

Pactamycin an antibiotic produced by Streptomyces pactum is a five-membered ring aminocyclitol, that is active against a variety of Gram-positive and Gram-negative microorganisms, and against several animal tumor lines in culture or in vivo. Pactamycin is presently employed exclusively as a biochemical research instrument due to its toxicity and its ability to block protein synthesis in bacteria, archaea, and eukaryotes by attaching to the small ribosomal subunit. Following here the successfully established procedure for new antibiotics production, known as the derivatization of antibiotics, we modified pactamycin by tethering basic acids to its free prime amino group of the aminocyclitol ring. We linked the basic amino acids lysine, ornithine, and histidine via an amide bond and the antimicrobial activity of all compounds was evaluated both in vivo and in vitro. According to the results, the antimicrobial activity was equally kept while their toxicity was reduced suggesting that our new compounds could be considered as potential antimicrobial agents to be further improved in order to combat resistant pathogens.



P174

Effect of cannabidiol on behavior and biochemical markers in aged male mice

Konstantinos Mesiakaris[1], Korina Atsopardi[1,2], Konstantinos Kanellopoulos Kotsonis[2], Masinga Maria Foka[1], Athina Kleopatra Galani[2], Nikolaos T. Panagopoulos[2], Marigoula Margarity[2], Konstantinos Poulas[1]

[1]Laboratory of Molecular Biology and Immunology, Department of Pharmacy, University of Patras, Greece
[2]Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, Greece

Age-related changes have been associated with behavioral manifestations and cholinergic system alterations. Due to its safety profile and the absence of psychotropic action, cannabidiol (CBD) is a cannabinoid of interest, with many reports of pharmacological effects in various pathological models. These models range from inflammatory and neurodegenerative diseases to polycystic arthritis, epilepsy, and autoimmune diseases, among others. The aims of the present study were to investigate the effect of cannabidiol treatment on anxiety-like behavior, redox status (in liver and brain regions), and on the activity of two isoforms of acetylcholinesterase (in brain regions) in aged male mice. Mice were divided into two groups: the CBD group (CBD 10mg/kg in saline with 10% DMSO and 2% tween-80) and the Control group (saline, 10% DMSO, 2% tween-80). CBD was administered for 10 days, and 24 hours after the last administration, behavioral analysis was performed. Anxiety-like behavior was assessed using the open field test in a 10-minute task. Antioxidant analysis was performed by determining the GSH (Glutathione) levels. Moreover, acetylcholinesterase activity was determined in both salt-soluble and detergent-soluble fractions in the brain using Ellman's colorimetric method. Behavioral studies revealed an anxiolytic-like effect and an increase in mobility after CBD treatment. Furthermore, CBD treatment appears to exhibit antioxidant activity while reducing acetylcholinesterase activity in both brain fractions.

P175

A study of antimicrobial and antiproliferative activity of indigenous Greek hop (Hmulus Lupulus) plants from Central Greece

Elisavet M. Andronidou[1], Gregoria Mitropoulou[3], Anastasios Nikolaou[3], Vasileios Gkalpinos[5], Panagiota I. Kontou[2], Konstantinos Tegopoulos[3], Ioannis Tamposis[1], Panagiotis Pergantas[4], George Skavdis[3], Pantelis G. Bagos[1], Maria E. Grigoriou[3], Andreas G Tzakos[5], Yiannis Kourkoutas[3], Georgia G. Braliou[1*]

[1]Department of Computer Science and Biomedical Informatics, University of Thessaly, Lamia, Greece
[2]Department of Mathematics, University of Thessaly, Lamia, Greece
[3]Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, Greece
[4]Bioapplications Ltd., Levadia, Greece
[5]Department of Chemistry, University of Ioannina, Ioannina, Greece

Hop (Humulus Lupulus) is a main ingredient of beer and is responsible for the plethora of aromas, flavors, and colors of the beer. Hop is a natural product of economic importance and recently has been extensively investigated for different biological properties of female flower (cones) extracts. In this work, we investigate the antimicrobial and antiproliferative properties of cone extracts from native Greek hop wild cultivars, from three different regions in Central Greece, and a commercially available variety Zeus from the USA. Aqueous and ethanolic extracts were produced and their profiles of volatile and non-volatile compounds were created with GC/MS analysis. LC/MS analysis was used to identify their content of chalcones, flavones, and bitter acids. Antimicrobial activity was assessed with minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) on beer spoilage bacteria. MIC and MLC values revealed that ethanolic extracts were significantly more potent than aqueous ones. Crystal violet was the assay that was used on the Hela cancer cell line to measure antiproliferative activity and IC50 values were estimated for 24, 48, and 72 hours of treatment. The values of IC50 (and SD) for 24 hours were 45.6 (20), 76.3 (20), 45.15(20) μg/ml, for 48 hours were 38.15 (20), 28.15 (15), 20.3 (6) μg/ml, and for 72 hours were 2.99 (1.5), 6.5 (1) and 3.8(0.2) μg/ml for Livadia, Mavrilo and USA respectively. Our results suggest that the extracts from different indigenous hop cultivars consist of different bioactive compounds, concomitant with differences in their antimicrobial and antiproliferative activities, suggesting that investigation of hop phytogenetic capital may uncover important health-beneficial properties.

Acknowledgements
"Molecular identification and exploitation of in-digenous hop varieties for the production of high-quality beers with designation of origin-PAZL (Production Analysis of Hop Beers)", Grant Number STER1-0026139, funded by the Operational Program "STEREA ELLADA" 2014 - 2020 (Strengthening of Research Projects, Technological Devel- opment and Innovation in the fields of RIS3).

P176

A study on the structure, optical properties and cellular localization of novel 1,3-benzothiazole-substituted BODIPYs

Olga Kirkilessi[1], Christina Arapatzi[2], Heribert Reis[1], Vassiliki Kostourou[2], Kyriakos C. Prousis[1*], Theodora Calogeropoulou[1*]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]Institute for Bioinnovation, "Alexander Fleming" Biomedical Sciences Research Center, Athens, Greece

Borondipyrromethenes (BODIPYs) constitute a privileged class of organic fluorophores with applications in various biomedical fields, including fluorescence sensing, optical imaging, photoacoustic imaging in addition to photodynamic and photothermal therapy.[1] Despite the synthesis of various BODIPY derivatives that address specific bioimaging requirements a number of drawbacks, characteristic of these dyes, have not been resolved satisfactorily to date. Therefore, there is a continuous need for custom made dyes with fine-tuned properties that meet specific demands for bioimaging applications in vitro and in vivo.

In the context of our studies on the synthesis of new fluorophores[2] recognizing the need for improved BODIPY derivatives for biomedical applications and intrigued by the interesting properties endowed in fluorescent probes by the 1,3-benzothiazole (BZT) moiety, we set out to perform a more detailed study on BZT-substituted BODIPYs.

Thus, a library of novel 1,3-benzothiazole-substituted BODIPY derivatives with tunable optical properties were synthesized. The new fluorescent dyes exhibited bathochromically shifted absorptions and emissions centered in the red and near-infrared spectral region. (TD)DFT calculations were performed to rationalize the spectroscopic properties of the new BODIPYs. The cellular biodistribution of the new dyes, their fluorescence stability and toxicity were investigated in both living and fixed fibroblasts using time-lapse fluorescent imaging and confocal microscopy.

Reference

1 *Cheng, H.-B., Cao, X., Zhang, S., Zhang, K., Cheng, Y., Wang, J., Zhao, J., Zhou, L., Liang, X.-J., Yoon, J., BODIPY as a Multifunctional Theranostic Reagent in Biomedicine: Self-Assembly, Properties, and Applications. Adv. Mater. 2023, 35, 2207546. https://doi.org/10.1002/adma.202207546*

2 *Prousis, K.C.; Canton-Vitoria, R.; Pagona, G.; Goulielmaki, M.; Zoumpourlis, V.; Tagmatarchis, N.; Calogeropoulou, T. New cationic heptamethinecyanine-graphene hybrid materials Dyes Pigm. 2020, 17, 108047. https://doi.org/10.1016/j.dyepig.2019.108047*

Acknowledgements

This work was supported by the project BIOIMAGING-GR (Grant number MIS 5002755) implemented under "Action for Strengthening Research and Innovation Infrastructures,", funded by the Operational Programme "Competitiveness Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

P177

A high-throughput screening of Greek plants for the discovery of novel compounds with anti-ageing and skin-protective properties

Asimina Fotopoulou[1], Sentiljana Gumeni[2], Adamantia Agalou[3], Gabriela Belen Lemus Ringele[4], Adamantia Papadopoulou[1], Maria S. Manola[2], Konstantina Karamanou[1], Georgios Stavropoulos[5], Zoi Evangelakou[2], Antonia Theodoridi[3], Konstantina Angeli[5], Despoina D. Gianniou[2], Xanthippi P. Louka[2], Eirini Gkogkou[2], Eleni Mavrogonatou[1], Aikaterini Argyropoulou[4,6], Harris Pratsinis[1], Alexios-Leandros Skaltsounis[4], Maria Halabalaki[4], Dimitris Beis[3], Ioannis P. Trougakos[2], Dimitris Kletsas[1]

[1]Laboratory of Cell Proliferation and Ageing, Institute of Biosciences and Applications, NCSR "Demokritos", Attiki, Greece
[2]Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, 15784 Athens, Greece
[3]Biomedical Research Foundation Academy of Athens, Athens, Greece
[4]Department of Pharmacy, Division of Pharmacognosy and Natural Products Chemistry, National and Kapodistrian University of Athens, 15784 Athens, Greece
[5]Korres S.A. Natural Products, 57th Athens-Lamia National Road, Inofyta, 32011, Greece
[6]PharmaGnose S.A., 57th km Athens-Lamia National Road, Inofyta, 32011, Greece

The natural environment, and especially the plants have long been considered as rich sources of novel bioactive compounds or composite Natural Products (NPs) with cosmeceutical, pharmacological and/or medicinal properties. Aim of the CosmAGE project was the extensive high-throughput screening of extracts from various plants of the Greek flora, the final goal being the discovery of novel NPs with potential anti-ageing and/or cosmeceutical properties. Specifically, 52 plant species and organs from different genera, such as Abies sp., Achillea sp., Arbutus sp., Cistus sp., Epilobium sp., Pistacia sp., and Juniperus sp., were extracted using two different techniques, the Supercritical Fluid Extraction (SFE) and the Accelerated Solvent Extraction (ASE). Composition of the extracts was revealed using LC-HRMS-based profiling, while all extracts were evaluated regarding their effects on a broad range of biological activities. A variety of methodologies was employed, ranging from cell-free systems to normal human skin cell-assay systems, for the assessment of the extracts' antioxidant capacity and of their ability to activate cytoprotective modules of the proteostasis network. Moreover, the extracts' interference with the activity of enzymes such as collagenase, elastase and tyrosinase was also studied, since these enzymic activities are closely related with skin ageing features. Finally, the extracts were also tested in zebrafish embryos – a model allowing the in vivo monitoring of complex cell behavior and physiological parameters – for their capacity to inhibit melanogenesis and/or promote wound healing. The results of CosmAGE up to now have led to the identification of promising extracts with anti-ageing and/or skin-protective properties (with IC_{50} values in the range of 1-10 μg/ml) that could be used for the future development of novel cosmeceuticals and/or pharmaceuticals.

Acknowledgments
This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH-CREATE-INNOVATE (project code: T2EDK-02583, CosmAGE).

P178

Repurposing Extracellular Vesicles: Tiny Messengers with a Big Impact on Advanced Materials

Vivi Bafiti1, Sotiris Ouzounis[1], Theodora Katsila[1*]

[1]National Hellenic Research Foundation, Athens, Greece

Extracellular vesicles have emerged as key players in intercellular communication. These tiny membrane-bound structures, secreted by various cell types, are known to transport a diverse cargo of biomolecules, including proteins, nucleic acids, and metabolites. In addition to their biological roles, recent technological advances have unveiled their fascinating potential in the field of advanced materials.

Herein, we explore the significant impact of extracellular vesicles on advanced materials. Extracellular vesicles have been found to possess unique properties that make them promising tools for material scientists and engineers. Firstly, their nanoscale size and membrane composition enable efficient interactions with various advanced materials, including nanoparticles, nanocomposites, and biomaterials. This enables precise manipulation and modification of material properties at the nanoscale. Furthermore, the cargo carried by exosomes, such as signaling molecules, enzymes, and genetic material, can influence the behavior and performance of advanced materials. By harnessing the specific molecular content of exosomes, researchers can engineer material properties, enhance functionality, access biocompatibility and induce desired responses, opening up new possibilities for the design and development of advanced materials with tailored properties.

We also perform structural analysis as it plays a vital role in understanding the interactions between extracellular vesicles and advanced materials. Techniques such as mass spectrometry, advanced imaging methods and image processing pipelines allow for the visualization and characterization of extracellular vesicles and provide valuable insights into the structural organization, surface properties, and interfacial interactions of extracellular vesicles with advanced materials, enabling a deeper understanding of their impact and potential applications.

We showcase recent advancements in the field and discuss key applications in areas such as drug delivery systems, tissue engineering, and sensors. Additionally, we highlight the challenges and future directions for utilizing extracellular vesicles as versatile messengers for advancing materials science.

Acknowledgements
The work is supported by the Key Digital Technologies Joint Undertaking and co-funded by the European Union under Grant Agreement nº 101112347 (NerveRepack). We acknowledge PRACE for awarding access to the Fenix Infrastructure resources at CINECA and Jülich hosting sites, which are partially funded from the European Union's Horizon 2020 research and innovation programme through the ICEI project under the grant agreement No. 800858

P179

A New Strategy in the Development of Mitochondrial Targeting Chemotherapeutics

Christina N. Banti, Sotiris K. Hadjikakou

University of Ioannina, Department of Chemistry, 45110, Ioannina Greece
Email: cbanti@uoi.gr, shadjika@uoi.gr

Positively charged compounds are accumulated in the mitochondrial matrix as a result of negative inner membrane potential. Mitochondriotropic lipophilic cations such as trialkyl derivatives of pnictogens are directed to mitochondria, causing loss of mitochondrial membrane permeabilization. Nonsteroidal anti-inflammatory drugs (NSAIDs), on the other hand, are accumulated in the mitochondria where the inflammation mechanism occurs, such as mefenamic acid (MefH). Therefore, the combination of a mitochondriotropic agent with an NSAID such as MefH via a metal ion might provide a new more effective targeted cancer chemotherapeutic [1].

Silver(I) metallodrugs were obtained by the conjugation of mefenamic acid with a mitochondriotropic derivative of pnictogens through silver(I). Their hydrophilicity was adjusted by their dispersion into Sodium Lauryl Sulphate (SLS) forming micelles. The conjugations inhibit the proliferation of human breast adenocarcinoma cells: MCF-7 (hormone depended (HD)) and MDA-MB-231 (hormone independent (HI)). X-ray fluorescence reveals the Ag cellular uptake. The in vitro and in vivo non-genotoxicity was confirmed with micronucleus (MN), Artemia salina and Allium cepa assays. Their mechanism of action was studied by cell morphology, DNA fragmentation, Acridine Orange/Ethidium Bromide (AO/EB) Staining, cell cycle arrest, mitochondrial membrane permeabilization tests, DNA binding affinity, LOX inhibitory activity and was rationalized by regression analysis [1].

Reference

[1] Christina N. Banti, Constantina Papatriantafyllopoulou, Christina Papachristodoulou, Antonios G. Hatzidimitriou, Sotiris K. Hadjikakou, New Apoptosis Inducers Containing Anti-inflammatory Drugs and Pnictogen Derivatives: A New Strategy in the Development of Mitochondrial Targeting Chemotherapeutics, J. Med. Chem. 2023, 66, 4131 4149

Acknowledgments

This program is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning 2014-2020» in the context of the project "Sub-project 6 ("Biological Inorganic Chemistry (BIC)" (MIS 5162213)."

P180

Association between GALANIN gene rs4432027 SNP and variations in depressive and anxiety symptoms, sense of coherence and vital exhaustion in the real-life setting of mandatory basic military training

Maria Toptsi[1], Polychronis Economou[2], Panagiotis Alexopoulos[3], Ioannis K. Zarkadis[1*]

[1]Laboratory of General Biology, Department of Medicine, School of Health Sciences, University of Patras, 26504, Rion, Patras, Greece
[2]Department of Civil Engineering (Statistics), School of Engineering, University of Patras, 26504, Rion Patras, Greece
[3]Department of Psychiatry, Faculty of Medicine, School of Health Sciences, University of Patras, University Hospital of Patras, 26504, Rion, Patras, Greece

Depression and anxiety are complex multifactorial disorders that affect a large part of the world's population. These disorders exhibit overlapping phenotypes and usually occur as a response to environmental stress. However, their genetic and molecular etiology is still not fully understood. Previous studies have shown that the galanin neuropeptide regulates mood disorders. Single nucleotide polymorphisms (SNP) in the regulatory region of the GALANIN gene have been associated with variations in symptom severity of these disorders.

In the present work, the SNP rs4432027 (C>T), located in the 5' region, 2kb upstream of the GALANIN gene was studied. RFLP genotyping was performed on 149 healthy male recruits. Additionally, neuropsychiatric evaluations were conducted at three intervals during their 19-day basic military training, to observe possible variations in depression and anxiety symptoms, as well as their sense of coherence and vital exhaustion, based on their SNP status. Results indicated that T-allele carriers experienced a statistically significant decrease in the severity of depression symptoms (Skillings-Mack p-value= 0.04862<0.05), compared to C-allele carriers. Moreover, both T-allele homozygous individuals and T-allele-carriers, in general, exhibited increased levels of vital exhaustion during the observation period (Skillings-Mack p-value= 0.04584 and 0.03334 respectively), in contrast to C-allele carriers, for whom no statistically significant change was observed. No association of SNP rs4432027 with change in anxiety symptoms or sense of coherence was observed. These findings suggest a possible association between the T-allele and lower severity of depressive symptoms and higher severity of vital exhaustion symptoms, following exposure to environmental stressors and stressful life experiences.

However, it is important to note that, due to the small number of study subjects and the linkage disequilibrium between rs4432027 and rs948854 polymorphisms but also with other SNPs of the regulatory region of GALANIN gene, additional studies are needed, to confirm the above results.

P181

The lipid metabolism regulator Angiopoietin-like-3 protein (ANGPTL-3) modulates cholesterol biosynthesis in Hepatitis C virus (HCV)-infected hepatocytes

Athanassios Batsilas, Despoina Olga Papaggeli, Georgia Papadopoulou, Vaia Valiakou, Danai Damda, Eirini Karamichali, Urania Georgopoulou, Pelagia Foka*

Molecular Virology Laboratory, Hellenic Pasteur Institute, Athens, Greece

The lipid metabolism regulator ANGPTL-3 increases plasma lipids through inhibition of lipoprotein and hepatic lipases, thereby reducing lipoprotein clearance and causing intracellular lipid shortage. HCV-orchestrated reprograming of host lipid metabolism is vital for the establishment of HCV infection. It often leads to hepatic fibrosis and steatosis that could progress to late-stage hepatocarcinogenesis, even after virus eradication with direct-acting antivirals. Previous work from our group has shown that HCV down-regulates ANGPTL-3 at the early stages of infection. This may be due to the fact that high levels of ANGPTL-3 reduce intracellular triglycerides and lipid droplets, which are both crucial for completion of the viral life cycle, and effectively inhibits HCV replication. The present study aimed to investigate whether ANGPTL-3 could attenuate intracellular cholesterol biosynthesis as a complementary antiviral mechanism. Thus, we examined the expression of the rate-limiting enzyme of cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), in the permissive to HCV infection Huh7.5 hepatoma cells and daughter cell lines, G4 and E4, with high (over 10-fold) and low (5–10–fold) ANGPTL-3 overexpression levels, respectively. ANGPTL-3 negatively regulated HMGCR expression, regardless of its own overexpression levels, but depending on culture age and feeding frequency. Notably, HCV infection of Huh7.5 cells over 96h also decreased HMGCR expression compared to mock-infected controls. However, the HCV-mediated effect on HMGCR was abolished in infected G4 cells. Such regulation patterns were not observed for mevalonate kinase (MVK), the next in line enzyme of the cholesterol biosynthesis pathway, indicating that alterations of the rate-limiting step of the pathway were enough to confer ANGPTL-3- or HCV-induced changes in intracellular cholesterol. Taken together, our results demonstrate for the first time that ANGPTL-3 is implicated in the modulation of key reactions in cholesterol biosynthesis. This ANGPTL-3 function may have profound effects in the establishment of viral infection.

Acknowledgements
The work was funded by the Askepios Gilead Hellas Grants.

P182

Food restriction in high anxiety: focus on brain mitochondrial dynamics

Maria P. Papageorgiou[1,2], Markus Nussbaumer[1,2], Afroditi Divane[1,2], Jinqiu Xiao[3], Marianthi Firoglani Moschi[1,2], Chris W. Turck[3], Michaela D. Filiou[1,2]

[1]Laboratory of Biochemistry, Department of Biological Applications and Technology, School of Health Sciences, University of Ioannina, Ioannina, Greece
[2]Biomedical Research Institute, Foundation for Research and Technology-Hellas (BRI-FORTH), Ioannina, Greece
[3]Proteomics and Biomarkers, Max Planck Institute of Psychiatry, Munich, Germany

Our modern, stressful, fast-paced lifestyle affects our eating patterns and poses a great risk for developing eating disorders. Eating disorders are highly comorbid with anxiety disorders, with half of eating disorder patients showing a concurrent mood disorder. Mitochondrial pathways have been found to modulate anxiety phenotypes and be affected in disordered eating behaviors. To date, how the crosstalk of disordered eating patterns in highly anxious populations is mediated by mitochondria and mitochondrial dynamics mechanisms remains elusive.

Here, we exposed female high anxiety-related behavior (HAB) mice to temporal food restriction, using a limited food access (LFA) protocol, according to which mice had 2h/day ad libitum access to food, while the control HAB group had ad libitum diet 24h/day. We then investigated the LFA effects on (a) mouse behavior, (b) brain/plasma metabolome and total antioxidant capacity levels, (c) brain mitochondrial dynamics mechanisms. At the behavioral level, we found that HAB LFA mice showed increased depression-like behavior compared to HAB controls. At the molecular level, diverse metabolomic signatures were observed between the two groups and total antioxidant capacity was increased in the plasma of HAB LFA vs. HAB controls. Furthermore, LFA significantly reduced hypothalamic mitochondrial dynamics gene expression of fission markers in HAB LFA vs. HAB controls.

Taken together, our findings indicate that mitochondrial dynamics pathways are involved in the regulation of co-occurring mood and eating disorders and may act as potential targets for therapy for pertinent pathologies.

Acknowledgments
The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 4th Call for HFRI PhD Fellowships (Fellowship Number: 9431).

P183

Monocarboxylate transporters 1 and 4 on Circulating Tumor Cells (CTCs) in Patients with Non-Small Cell Lung Cancer

Karolina Mangan[1*], Evangelia Pantazaka[1*], Athina Markou[2], Athina Christopoulou[3], Athanasios Kotsakis[4], Vassilis Georgoulias[5], Galatea Kallergi[1]

[1]Laboratory of Biochemistry and Metastatic Signaling, Division of Genetics, Cell and Developmental Biology, Department of Biology, University of Patras, 26504 Patras, Greece
[2]Analysis of Circulating Tumour Cells Lab, Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, 15771 Athens, Greece
[3]Oncology Unit, ST Andrews General Hospital of Patras, 26332 Patras, Greece
[4]Department of Medical Oncology, University General Hospital of Larissa, 41334 Larissa, Greece
[5]Hellenic Oncology Research Group (HORG), 11526 Athens, Greece
**These authors contributed equally to this work.*
Correspondence: gkallergi@upatras.gr

Non-small cell lung cancer (NSCLC) is the most common lung cancer subtype (85% of lung cancer cases). Since most patients are usually diagnosed at advanced disease stages, there is an urgent need to identify new biomarkers, to achieve disease prognosis, early diagnosis and personalized treatment. Liquid biopsy offers valuable real-time insights into disease progression through repetitive blood sampling. Circulating tumor cells (CTCs) are a useful liquid biopsy diagnostic tool. Both CTCs' enumeration and phenotypic characterization can provide important information on the disease progression, treatment response and metastasis probability. Monocarboxylate transporters 1 and 4 (MCT1-MCT4) are responsible for the transportation of lactate and they play a crucial role in the survival and proliferation of cancer cells, inducing metastasis. Moreover, their expression has been correlated with disease prognosis, suggesting their potential significance in clinical practice. We aimed to investigate the expression of these biomarkers on CTCs isolated from NSCLC patients and their potential association with patients' clinical outcome.

This study enrolled fifty-three NSCLC patients at baseline. CTCs were isolated using the ISET system and triple immunofluorescence experiments were performed, using cytokeratin (CK), MCT1/MCT4, and CD45 antibodies. MCT1 and MCT4 expression was assessed by confocal laser scanning microscopy, followed by statistical analysis.

CK expression was characterized as high or low. Among CK-positive patients, the predominant phenotypes were the CK_{low}/MCT1+/CD45- or CK_{low}/MCT4+/CD45- (52 and 64%, respectively), followed by the phenotypes CK_{high}/MCT1+/CD45- or CK_{high}/MCT4+/CD45- (48 and 36%, respectively) and CK_{low}/MCT1-/CD45- or CK_{low}/MCT4-/CD45- (33 and 27%, respectively). The phenotypes CK_{high}/MCT1-/CD45- or CK_{high}/MCT4-/CD45- had the lowest percentage (4 and 0%, respectively). MCT1 and MCT4 expression were related to poorer overall survival (OS_{12m}) ($p<0.001$ and $p=0.031$) and progression-free survival (PFS_{12m}) ($p=0.025$), respectively.

In conclusion, MCT1 and MCT4 may constitute interesting biomarkers relevant to cancer progression in NSCLC patients, indicating their potential as prognostic and diagnostic factors.

P184

Brain mitochondria at the interface of early handling and anxiety-related behavior

Afroditi Divane[1,2], Christina Thomou[1,2,#], Markus Nussbaumer[1,2,#], Elena Grammenou[1,2,#], Maria P. Papageorgiou[1,2], Eirini Panteli[1,2], Michaela D. Filiou[1,2,*]

[1]Laboratory of Biochemistry, Department of Biological Applications and Technology, University of Ioannina, Ioannina, Greece
[2]Biomedical Research Institute, Foundation for Research and Technology-Hellas (BRI-FORTH), Ioannina, Greece
[#]equal contribution
**Correspondence: mfiliou@uoi.gr*

Early postnatal experiences modulate adult behavior. Early handling (EH), an early life intervention applied in rodents, refers to the brief and repeated separation of pups from their dam during their first days of life. Here, we used an EH protocol for postnatal days 1-14, during which EH pups are taken away from their dam for 15 min/day, while non-handled (NH) pups are only subjected to animal facility rearing.

We applied this protocol to high (HAB) and normal (NAB) anxiety-related behavior mice. Maternal behavior was observed for postnatal days 2-7 and a behavioral test battery assessing sociability, anxiety- and depression-like behavior was conducted to investigate EH-induced behavioral changes in male and female adult offspring. We then looked for EH-induced brain molecular changes in HAB mice by western blots, biochemical assays and real-time qPCRs.

Our results demonstrate that EH does not affect maternal behavior. However, it exerts an anxiolytic effect in HAB male and female offspring. This EH-induced effect is mediated by altered levels of key mitochondrial brain proteins and oxidative stress-related readouts in male HAB mice, as well as reduced blood mitochondrial DNA copy number in female HAB mice. Taken together, our results show that mitochondria may regulate the molecular and metabolic impact of postnatal experiences on adult life outcomes in a high anxiety background and they constitute attractive therapeutic targets for the amelioration of anxiety disorders.

We acknowledge funding from BRI-FORTH and Fondation Santé. The research work was also supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 4th Call for HFRI PhD Fellowships (Fellowship Number: 9431).

P185

The role of glucocorticoid receptor signaling in stress-induced disruption of dentate gyrus plasticity in Tau pathology

Zoe Kotsikou[1,2 #], Eirini Gratsia[1,3 #], Nikolina Ntinou[1], Anastasia Megalokonomou[1,4,5], Chrysoula Dioli[1,4,5], Ioannis Sotiropoulos[1,4,5]

[1]Institute of Biosciences and Applications, NCSR Demokritos, Athens, Greece
[2]Athens International Master's Programme in Neurosciences, Department of Biology, National & Kapodistrian University of Athens, Athens, Greece
[3]Master in Neurosciences, Medical School, University of Crete, Heraklion, Greece
[4]Life and Health Sciences Research Institute (ICVS), School Medicine, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[5]ICVS/3B's- PT Government Associate Laboratory, Braga/ Guimarães, Portugal
#Contributed equally

Chronic stress and inflammation are increasingly recognized to be involved in the precipitation of Alzheimer's disease (AD). One of the primary brain areas affected in AD is the hippocampus with the dentate gyrus (DG), the input subarea of the hippocampus, being the only one that combines newly-born neurons, pre-existing neurons, and microglia. However, the exact role of chronic stress and glucocorticoid receptor (GR) signaling in stress-driven DG damage of AD brain remains unclarified. Our study aims to unravel the interplay of chronic stress and the aforementioned DG cell types and how this interplay precipitates DG dysfunction in Tau pathology, using P301L-Tau transgenic mice with specific deletion of GR in forebrain neurons or microglia. We found that chronic stress suppresses neurogenesis causing a decrease of newly-born neurons and this effect isn't altered by microglial GR deletion, whereas stress-induced spine loss in pre-existing neurons is blocked in microglial GR-depleted mice. In addition, stress-driven microglial hyper-ramification is blocked by microglial GR deletion in the DG hippocampal area, whereas these microglia plasticity changes also depend on neuronal GR signaling. Together, these findings provide novel insights into the impact of chronic stress on the complex neuron-microglia interplay that damages DG plasticity in Tau-related AD pathology.

P186

Regulation of the expression of the transcription factor Ets-2 in activated T cells

Vasiliki Theodoraki, Anastasios Georgakopoulos, Athanasia Mouzaki

School of Medicine, Patras, Greece.

Th (T helper) cells play one of the most key roles in immunity. During their induction from naive to activated they remain in a transient but very important state (Th0) before they engage to a Th effector cell fate (Th1, Th2, Th9, Tregs and more). In this transient state they produce their primary cytocine, IL-2 that is crucial for their proliferation and maturation. IL-2 production is regulated by the Ets-2 transcription inhibitor during pre-induction. Ets-2 right after activation decreases and allows IL-2 to be synthesized. Meanwhile, IL-2, due to her receptor on T cells, has the ability to induce signaling cascades and work her own regulatory plan. One of these signaling paths downstream to IL-2 includes the JAK/STAT molecules. In the end of this path among other activational cascades, the STAT3 molecule after phosphorylation can be activated and work as a transcriptional factor. Then it can regulate gene transcription related to T cell function and IL-2 production.

In our work we used Jurkat cells as a CD4+CD25- cell model in order to study the effect of IL-2 on Ets-2 expression with the induction or blockage of IL-2 signaling in their environment. Also we tried to identify if the STAT3 downstream molecule can participate in this effect. STAT3 overexpression and silencing experiments proved that STAT3 affects the expression of Ets-2 factor not necessarily via the IL-2 pathway but through other possible activational paths during T cell activation. Our observations led us to the conclusion that IL-2 signaling reduces the transcription of the pre-activation inhibitor Ets-2 and the downstream STAT3 molecule could be involved in this regulation.

References

1. *Panagoulias, I., Georgakopoulos, T., Aggeletopoulou, I., Agelopoulos, M., Thanos, D., & Mouzaki, A. (2016). Transcription factor ets-2 acts as a Preinduction repressor of interleukin-2 (IL-2) transcription in naive T helper lymphocytes. Journal of Biological Chemistry, 291(52), 26707-26721. https://doi.org/10.1074/jbc.m116.762179*
2. *Egwuagu, C. E. (2009). STAT3 in CD4+ T helper cell differentiation and inflammatory diseases. Cytokine, 47(3), 149-156. https://doi.org/10.1016/j.cyto.2009.07.003*
3. *Ng, J., & Cantrell, D. (1997). undefined. Journal of Biological Chemistry, 272(39), 24542-24549. https://doi.org/10.1074/jbc.272.39.24542*
4. *Jones, D. M., Read, K. A., & Oestreich, K. J. (2020). Dynamic roles for IL-2–stat5 signaling in effector and regulatory CD4+ T cell populations. The Journal of Immunology, 205(7), 1721-1730. https://doi.org/10.4049/jimmunol.2000612*

P187

Emerging roles of SLITRK family members in αSyn- p.A53T mediated synaptic dysfunction

P. Fourtini[1,2], E. K. Akrioti[1], K. Segklia[1], R. Matsas1, E.Taoufik[1]

[1]Laboratory of Cellular & Molecular Neurobiology - Stem Cells, Hellenic Pasteur Institute, Athens, Greece, 2 Agricultural University of Athens, Greece

Parkinson's disease (PD) is a common neurodegenerative disease associated with α-synuclein (aSyn). Its pathological form aggregates in protein inclusions called Lewy Bodies. The G209A mutation in the αSyn SNCA gene leads to the pathological p.A53T-αSyn protein, responsible for familial PD cases. Previous studies in preclinical p.A53T models showed dysregulation of the expression level of several proteins, including three members of the SLITRK (1,2,4) family (Kouroupi et al., 2017). SLITRKs are group of 6 adhesion molecules that interact with the presynaptic proteins LAR-RPTPs (Won et al., 2019) and are responsible for neuronal growth and synapse formation (Proenca et al., 2011). Mutations or polymorphisms within this gene family have been associated with neuropsychiatric syndromes and neurodevelopmental disorders. However, the link between SLITRKs and PD is not yet known. The purpose of this work is to study how dysregulated and aggregated aSyn affects the expression and localization of selected SLITRK members and overall the trans-synaptic adhesion processes. To address this, we examine the subcellular localization of SLITRK 1, 2, 4, the quantification at the mRNA and protein levels in p.A53T experimental systems. Experiments were performed in a transgenic mouse model expressing human p.A53T-αSyn (Prnp-SNCA*A53T) (Giasson et al., 2001) and in pluripotent stem cells (hiPSC) from patients carrying the mutation. This work focuses on the human neuroblastoma cell line SH-SY5Y expressing human p.A53T α-synuclein. Preliminary results show endogenous expression of SLITRKs in SH-SY5Y. Their subcellular localization is significantly altered in the presence of p.A53T-aSyn in SH-SY5Y cells, indicating dysfunction in the cellular trafficking mechanisms. Additionally, in the mouse and hiPSCs models a downregulation of SLITRK 1, 2, 4 expression was observed as well as weaknesses in the formation and function of synapses.Altogether, our work aims to identify the link between SLITRKs dysregulation and p.A53T-αSyn induced synaptopathy and characterize the molecular and cellular mechanisms.

References

1. *Kouroupi, G., Taoufik, E., Vlachos, I. S., Tsioras, K., Antoniou, N., Papastefanaki, F., Chroni-Tzartou, D., Wrasidlo, W., Bohl, D., Stellas, D., Politis, P. K., Vekrellis, K., Papadimitriou, D., Stefanis, L., Bregestovski, P., Hatzigeorgiou, A. G., Masliah, E., & Matsas, R. (2017). Defective synaptic connectivity and axonal neuropathology in a human iPSC-based model of familial Parkinson's disease. Proceedings of the National Academy of Sciences, 114(18).*
2. *Won, S. Y., Lee, P., & Kim, H. M. (2019). Synaptic organizer: Slitrks and type IIa receptor protein tyrosine phosphatases. Current Opinion in Structural Biology, 54, 95–103.*
3. *Giasson, B.I., Duda, J.E., Quinn, S.M., Zhang, B., Trojanowski, J.Q., and Lee, V.M. (2002). Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha synuclein. Neuron 34, 521-533.*
4. *Proenca, C.C., Gao, K.P., Shmelkov, S.V., Rafii, S., and Lee, F.S. (2011). Slitrks as emerging candidate genes involved in neuropsychiatric disorders. Trends in neurosciences 34, 143-153.*

Acknowledgements and Funding

Funded by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment" (Project Number: 1019; to RM); the Hellenic Pasteur Institute Excellence Scholarship awarded to E. Akrioti- Nostos Foundation.

P188

Whole-genome profile of Greek patients with asthenozoospermia: Identification of candidate variants and genes

Maria-Anna Kyrgiafini[1,*], Chrysi Kontse[1], Themistoklis Giannoulis[2], Alexia Chatziparasidou[3], Nikolaos Christoforidis[3], Zissis Mamuris[1]

[1]Laboratory of Genetics, Comparative and Evolutionary Biology, Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece
[2]Laboratory of Biology, Genetics and Bioinformatics, Department of Animal Sciences, University of Thessaly, Larissa, Greece
[3]Embryolab IVF Unit, Thessaloniki, Greece

Nowadays, infertility represents a multifaceted health issue that significantly impacts numerous couples, giving rise to notable psychological and social complexities. Notably, one in six couples experience infertility, with approximately 50% of cases attributed to male factors. Male infertility, a complex disorder influenced by both environmental factors and genetic predisposition, involves the interplay of various genes contributing to its manifestation. It can be categorized into specific subtypes, including asthenozoospermia. The primary aim of this study was to identify novel variants associated with asthenozoospermia within the Greek population and to elucidate the roles of the genes involved. To achieve this, whole-genome sequencing (WGS) was conducted on both normozoospermic and asthenozoospermic individuals. Following the identification of variants exclusively present in asthenozoospermic men, an extensive range of tools, functional assessments, and predictive algorithms were employed to prioritize these variants. The investigation unveiled numerous polymorphisms, comprising 155 classified as high impact and 715 as moderate impact. While several of these variants were found within genes previously linked to male infertility, a notable subset was associated with asthenozoospermia for the first time. Furthermore, pathway enrichment analysis and Gene ontology (GO) analyses revealed polymorphisms on genes implicated in teratozoospermia through various mechanisms and pathways. Therefore, this study reaffirms the involvement of previously studied genes in male infertility, while also shedding light on novel molecular mechanisms. By providing a comprehensive list of variants and candidate genes associated with asthenozoospermia within the Greek population, this research contributes significantly to our understanding of male infertility and paves the road for future studies.

P189

Investigation of antibody responses against selected SARS-CoV-2 accessory proteins

Eleni Tryfonopoulou[1], Konstantina Gkopi[1], Eugenia Moschogianni[1], Theocharis Konstantinidis[2], Maria Panopoulou[3], Penelope Mavromara[1], Katerina Chlichlia[1*]

[1]Department of Molecular Biology and Genetics, Democritus University of Thrace, University Campus-Dragana, 68100 Alexandroupolis, Greece
[2]Blood Transfusion Center, University General Hospital of Alexandroupolis, University Campus-Dragana, 68100 Alexandroupolis, Greece
[3]Department of Medicine, Democritus University of Thrace, University Campus-Dragana Campus, 68100 Alexandroupolis, Greece
** Correspondence: achlichl@mbg.duth.gr*

Although intensive vaccinations against SARS-CoV-2 managed to control the virus spread during the worldwide pandemic of COVID-19, the ongoing infections pose an alarming reminder of the need to develop more effective vaccines, surveillance systems and therapeutic approaches. Additionally, the broad spectrum of SARS-CoV-2 clinical manifestations and the underlying immunological mechanism involved are still poorly defined. SARS-CoV-2 acquires in its arsenal a group of auxiliary open reading frames, named 'accessory proteins'. These proteins are dispensable for the viral life cycle, however they appear to be crucial immunogenic factors involved in various viral pathogenesis mechanisms, like immune bypassing. As a result, a deep understanding of humoral responses specific to accessory proteins might be an essential step to achieve the aforementioned goals. Here, we developed recombinant accessory proteins to serve as antigens to detect IgG anti-SARS-CoV-2 antibody responses. Initially, BepiPred-2.0 was employed to determine predicted linear B-cell epitopes on SARS-CoV-2 accessory proteins. In order to detect antibody responses of SARS-CoV-2 accessory proteins, recombinant His-tagged proteins expressed in E. coli were produced and purified. The purified accessory proteins were used as antigens in Western blotting to test the reactivity of sera from a cohort of COVID-19 patients. Our future goal is to examine the presence of antibody responses in a wide range of infected individuals, in order to assess their utility as serological indicators for disease monitoring.

P190

Unraveling behavioral deficits in neurofibromatosis type 1: insights from drosophila models

Eirini-Maria Georganta[1], Kalliopi Atsoniou[1*], Kyriaki Foka[1], Eleni Giannopoulou[1], Panagiotis Giannousas[1], Ourania Semelidou[1], Maritina Zerva[1], Martina Samiotaki[2], Efthimios M.C. Skoulakis[1]

[1]Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece, [2]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece

Neurofibromatosis type 1 (NF1) is an autosomal dominant multi-systemic disorder, affecting 1 in 2000-3000 individuals worldwide. It results from mutations in the Nf1 tumor suppressor gene, leading to a wide array of symptoms. Nf1 encodes Neurofibromin (Nf1), a large multifunctional protein, preferentially expressed in the central and peripheral nervous system, crucial for regulating multiple signaling pathways. Although typically considered a tumor predisposition syndrome, it is also associated with skeletal and skin pigmentation abnormalities, short stature and broad cognitive/behavioral presentations, including impaired learning, attention deficit hyperactivity disorder, autism spectrum disorder, social/communicative disabilities and disturbed sleep. Progress towards amelioration of these behavioral deficits requires understanding the cellular and molecular impact of particular Nf1 mutations that govern such behaviors. For this reason, appropriate animal models emulating human phenotypes are necessary. Loss of the highly conserved Drosophila dNf1 ortholog mimics human NF1 pathology, causing reduced size, impaired learning, synaptic defects, behavioral inflexibility, and abnormal activity and sleep patterns. Furthermore, particular Nf1 point mutations are associated with specific behavioral deficits that implicate distinct molecular mechanisms than those affected upon total dNf1 loss. Our evidence thus far, suggests that different Nf1 mutations may impact differentially both established and previously unidentified functions of the protein, possibly in a cell-type-specific manner. These allele-specific effects could be a contributing factor to the variability observed in NF1-related pathologies. Elucidating the determinants of these phenotypes will contribute significantly to the development of novel, potentially personalized ameliorative strategies for these defects.

Supported by
Department of Defense (USA), Neurofibromatosis 1 Program W81XWH-20-1-0255W81XWH-Children's Tumor Foundation CTF-2022-05-004

P191

Effects of Chios Mastic extracts on the cellular proteostatic modules

Despoina D Gianniou[1], Eirini Gkogkou[1], Xanthippi P Louka[1], Sentiljana Gumeni[1], Zoi Evangelakou[1], Eleni V Mikropoulou[2], Panagiotis Efentakis[3], Ilias Smyrnioudis[4], Kimon Stamatelopoulos[5], George Dedoussis[6], Ioanna Andreadou[3], Maria Halabalaki[2], Alexios-Leandros Skaltsounis[2], Ioannis P Trougakos[1*]

[1]Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, 15784 Athens, Greece.
[2]Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece
[3]Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, 15771 Athens, Greece.
[4]The Chios Mastiha Growers Association, K. Monomachou 1, Chios, Greece
[5]Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece
[6]Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University of Athens, Athens, Greece
[*]e-mail: itrougakos@biol.uoa.gr

Chios Mastic Gum (CMG) is an aromatic resin secreted by the evergreen shrub Pistacia lentiscus var. Chia (Anacardiaceae). CMG is produced only by the mastic trees of the Greek island of Chios and consists of several bioactive compounds, especially triterpenes. CMG is characterized by a long-standing history of medical applications as a traditional herbal product with many beneficial properties including antibacterial, antifungal, anticancer, and anti-inflammatory activities. Therefore, the aim of our study was to investigate how CMG extracts affect the proteostasis network (PN), the functionality of which declines during aging and age-related diseases. We exploited different biological platforms, starting from CMG extracts effect(s) on human umbilical vein endothelial cells (HUVEC), as well as on Peripheral Blood Mononuclear Cells (PBMCs) and Red Blood Cells (RBCs) isolated from donors treated with different doses of CMG as part of an on-going clinical trial. In HUVEC cells CMG-based extracts activated proteostatic responses as they enhanced the Ubiquitin-Proteasome and Autophagy-Lysosome pathways; they also suppressed cell oxidative load (i.e., ROS levels). The anti-oxidative and proteasomal activities were further evaluated in PBMCs and RBCs from patients with similar findings. Overall, these data suggest that CMG enhance anti-aging proteostatic mechanisms and exert antioxidant effects, both in vitro and in vivo. These findings provide further mechanistic details to the noted cardiovascular protective action of CMG in patients with hypertension (on-going clinical trial).

Acknowledgment
This research has been co financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call RESEARCH –CREATE –INNOVATE [project code T2EΔK-00547 (KE 18120) HYPER-MASTIC]

P192

Lysine-specific demethylase 1 (LSD1) is a key player against the establishment of Hepatitis C Virus (HCV) infection

Georgia Papadopoulou, Eirini Karamichali, Alfonsos Eleftherios Delis, Athanassios Batsilas, Stavroula Petroulia, Alexios Dimitriadis, Pelagia Foka, Urania Georgopoulou*

Molecular Virology Laboratory, Hellenic Pasteur Institute, Athens, Greece

Epigenetic changes that occur in HCV infection often lead to severe hepatic dysfunction, thus, despite antiviral therapy and viral clearance, hepatitis C is still a major cause of liver disease and hepatocellular carcinoma. HCV enters host cells via endocytosis. Low pH triggers fusion of the virion envelope with endosomal membranes and release of HCV RNA, which is translated into HCV proteins. Cellular lipids are vital for HCV propagation, as viral replication is carried out onto cellular lipid droplets (LDs) and are found increased in HCV infection. LSD1 is an epigenetic factor that participates in many cellular processes, with interferon-induced antiviral mechanisms and regulation of lipid metabolism being among them. Lately, LSD1 has been shown to possess antiviral properties against RNA viruses, such as Influenza A Virus and Vesicular Stomatitis Virus. Our aim was to investigate whether LSD1 is a component of host cell defense mechanisms against HCV. Thus, we tested the effect of overexpression or silencing of LSD1 on HCV propagation, revealing an important role of LSD1 in HCV infection. Electroporation of the full-length HCV genome and a subgenomic replicon showed that inhibition of HCV replication occurs at the early steps of infection and RNA replication. Immunoprecipitation experiments revealed a direct interaction of LSD1 with Interferon-induced transmembrane protein 3 (IFITM3), an antiviral protein located on endosomal and lysosomal membranes which, upon demethylation, leads endocytosed viral particles to lysosomal degradation. Lastly, confocal microscopy experiments showed that LDs were reduced in LSD1 overexpressing cells compared to paternal cells, infected or not, possibly reducing the efficiency of HCV RNA replication. Taken together, this is the first time that an inhibitory role of LSD1 in HCV infection is described. The activity of LSD1 that leads to IFITM3-mediated endolysosomal formation and LDs depletion renders LSD1 an essential host factor against the establishment of HCV infection.

P193

The neuroprotective role of NR5A2 under oxidative stress

Angeliki Nomikou, Ismini Rozani, Valeria Kaltezioti, Efstathia Tetringa, Katerina Dimitropoulou, Dimitrios Gkikas, Panagiotis K. Politis

Biomedical Research Foundation of the Academy of Athens, Athens, Greece

Neurodegenerative diseases are characterized by the loss of structural and functional properties of groups of neurons and their subsequent death. Oxidative stress is implicated in the most prevalent neurodegenerative disorders leading to neuronal dysregulation and death. The development of neuroprotective therapies that promote neuronal survival by reversing the damage of oxidative stress could provide novel therapeutic insights for nervous system-related diseases. Towards this direction, we focused on the orphan nuclear receptor NR5A2, which is known for inducing neurogenesis during development and maintaining neuronal properties in the adult brain. In this study, we investigated the potential neuroprotective effect of this receptor on neuronal cells that undergo oxidative stress. We demonstrate that the adenoviral overexpression of NR5A2 in ex vivo cultured murine cortical neurons promotes their survival under oxidative stress conditions, whereas knockdown of NR5A2 has the opposite effect on neuronal survival. Most importantly, dilauroyl phosphatidylcholine (DLPC), a phospholipidic agonist of NR5A2, recapitulated the effect of the overexpression of NR5A2 in decreasing neuronal apoptosis under oxidative stress. RNA-seq analysis of DLPC treated neurons, unravels a panel of significant genes and pathways that are upregulated and downregulated and are linked with neuroprotective processes. We validated which of these genes are altered the most by DLPC and could reveal the pathway(s) by which NR5A2 inhibits neuronal apoptosis in oxidative stress conditions. These findings suggest that NR5A2 promotes neuronal survival under oxidative stress conditions and its agonist DLPC could be used as a neuroprotective treatment in neurodegenerative disorders.

This work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: 1782) and Fondation Santé Biomedical Research grants.

P194

Altered Gut Microbiota is Associated with Reduced Weight Gain and Extended Life Span in Canagliflozin-treated Male C57BL6 Mice

Evagelia E. Habeos[1], Fotini Filippopoulou[1], Menelaos Kanakis[2], George I. Habeos[1], Naima Belmokhtar[3], Panagiota Stathopoulou[3], George Tsiamis[3], Stavros Taraviras[1], Dionysios V Chartoumpekis[1], George Lagoumintzis[4*]

[1]University of Patras, Department of Medicine, 26504 Patras, Greece
[2]University of Patras, General Hospital of Patras, Department of Ophthalmology, 26504 Patras, Greece
[3]University of Patras, Department of Sustainable Agriculture, 30100, Agrinio, Greece
[4]University of Patras, Department of Pharmacy, 26504, Patras, Greece

To control their blood sugar levels, people with diabetes are routinely prescribed sodium-glucose cotransporter-2 inhibitors (SGLT2i). SGLT2i's beneficial health effects appear to be pleiotropic and are not limited to the amelioration of glycemic profile. It is assumed that SGLT2i-induced glycosuria triggers a metabolic shift comparable to fasting. Since calorie restriction extends the lifespan of mice, we hypothesized that administering SGLT2i to mice would also extend their lives. To understand the underlying mechanisms of these functions, 4-months male C57BL6 mice were fed with a standard chow diet (Control, n=83) or a diet supplemented with 200 mg/kg canagliflozin (Cana, n=92) and were followed until they died naturally. Also, as the gut microbiome is known to change with age and be associated with the development of age-related diseases, feces were collected from 17-month mice (n=10 per group), and analysis of 16S rRNA sequences was performed. We found that canagliflozin-treated mice gained less weight over time. Notably, at 10 months of age, the body weight of control mice was higher (means SEM: 33.1 0.26g) than that of canagliflozin-treated mice (means SEM: 31.4 0.21g; p<0.01). We also examined age-related cataract progression and severity at 14 months. However, there was no statistically significant difference between the two groups. The survival curves of the control and Cana mice differed significantly, with the control group having a median survival of 107.5 weeks versus 112.5 weeks (p=0.011). We also identified differences in alpha- and beta-diversity and bacterial abundance between the Control and Cana groups. Herein, we investigated the impact of SGLT2i on longevity using a well-utilized mouse model. Several microbiome changes associated with SGLT2i treatment, as seen in other mouse models that exhibit greater longevity, warrant further research even though the exact underlying signaling mechanisms supporting the longer lifespan are yet to be elucidated.

References

• *Zannad F, Ferreira JP, Pocock SJ, et al. Lancet. 2020;396(10254):819-829.*
• *Hoong CWS, Chua MWJ. Endocrinology. 2021;162(8):bqab079.*
• *Most J, Tosti V, Redman LM, Fontana L. Ageing Res Rev. 2017;39:36-45.*
• *Kern L, Kviatcovsky D, He Y, Elinav E. Curr Opin Microbiol. 2023;73:102287.*
• *Shintani T, Shintani H, Sato M, Ashida H. Geroscience. 2023;10.1007/s11357-023-00851-0.*
• *Deng L, Yang Y, Xu G. Biochim Biophys Acta Mol Cell Biol Lipids. 2022;1867(12):159234.*

P195

Neurological biomarker detection in blood using the ultrasensitive SIMOA technology. Validation of pTau181 and pTau217 in the context of Alzheimer's disease

Christos Stergiou[1], Manos Koutsos[1], John Tzartos[2], Socrates Tzartos[1]

[1]Tzartos NeuroDiagnostics, Athens, Greece,
[2]2nd Department of Neurology, School of Medicine, NKUA, "Attikon" General University Hospital, Athens, Greece

Introduction: The emergence of ultra-sensitive assays pioneered by the single molecule array **(SIMOA - Quanterix®)**, has enabled the precise measurement of neurological biomarkers in blood (overcoming the need for cerebrospinal-fluid, CSF). Tzartos NeuroDiagnostics is already utilizing this technology to measure NFL, GFAP, and phosphorylated-Tau (pTau) levels in blood, as preliminary prognostic and diagnostic biomarkers of multiple sclerosis and Alzheimer's disease (AD). In the context of early detection of Alzheimer's disease (AD) the plasma pTau protein has been shown as very promising biomarker. Plasma pTau at threonine181 (pTau181) distinguishes AD from other neurodegenerative disorders (NDD) and healthy controls, and correlates with CSF pTau181 and Amyloid- and Tau-PET imaging scores[1-4]. Also, plasma pTau217 was shown to discriminate AD from other NDD with higher accuracy and was recently proposed to be incorporated into a diagnostic workflow to detect AD in memory clinic settings[5].

Goal: The independent validation of the diagnostic accuracy of SIMOA pTau181 and pTau217 plasma assays for AD patients, using the SIMOA HD-X platform in **"Tzartos NeuroDiagnostics"**.

Method: Established AD CSF biomarkers (Aβ42/40, pTau, and tTau) were measured with Lumipulse immunoassays (Fujirebio). 26 participants were classified as AD_{CSF} if their Aβ42/40 ratio was below 0.063 (N=12 for AD_{CSF} and N=14 for non- AD_{CSF}). Plasma pTau181 and pTau217 were measured using the SIMOA HD-X instrument (Cutoff values: 32pg/ml for pTau181, 0.42pg/ml for pTau217). To evaluate the efficacy of pT181 and pT217 we computed areas under the curve (AUCs), Pearson correlation coefficient (r), Sensitivity and Specificity.

Results: Each plasma pTau181 and pTau217 was significantly higher in the AD_{CSF} group compared to the non-AD_{CSF} group and significantly discriminated abnormal CSF Aβ42/40 ratio. Combination of both plasma biomarkers further increased sensitivity of the approach.

References:

1. Thijssen, E. et al. Nat Med 26 (2020),
2. Janelidze, S. et al. Nat Med 26 (2020),
3. Tzartos, J. S. et al. Biomolecules 12 (2022),
4. Karikari, T. et al. Lancet Neurol 19 (2020)
5. Brum, W. S. et al. Nat Aging 3 (2023)

P196

Study of the relationship between mitochondrial function and dysregulation of significant signaling pathways in Parkinson's disease through comparative proteomics analysis

Sofia Ioannidou[1,2], Konstantina Psatha[1,2,3], Sofia Notopoulou[4], Martina Samiotaki[5], Nikolaos Grigoriadis6, Spyros Petrakis[4], Michalis Aivaliotis[1,2,7]

[1]Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece
[2]Functional Proteomics and Systems Biology (FunPATh), Center for Interdisciplinary research and Innovation (CIRI), Aristotle University of Thessaloniki, Greece
[3]Laboratory of Medical Biology and Genetics, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece
[4]Institute of Applied Biosciences (INAB), Center for Research and Technology Hellas, Thessaloniki, Greece
[5]Biomedical Sciences Research Center "ALEXANDROS FLEMING", Athens, Greece
[6]Laboratory of Experimental Neurology and Neuroimmunology, School of Medicine, Aristotle University of Thessaloniki, Greece
[7]Basic and Translational Research Unit, Special Unit for Biomedical Research and Education, School of Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by progressive loss of dopaminergic neurons in the substantia nigra and aggregation of a-synuclein, forming cytoplasmic Lewy bodies. Although several factors are implicated in PD pathophysiology, disruption of mitochondrial function is associated with neurodegeneration, since neuronal integrity depends on the function and dynamic balance of mitochondria. Considering the important role of mitochondria in neurodegeneration, the present study aims to investigate the relationship between mitochondrial function and the deregulation of significant pathways in PD, conducting comparative proteomics analysis. To simulate the pathological phenotype, TetOn YFP-SNCA A53T smNPCs cell model was utilized, which consists of human, neuronal stem cells that are genetically modified to overexpress the mutated form of SNCA, compared to control, in which SNCA expression is absent. Both conditions underwent proteomics analysis through bottom-up label free relative quantitation, while immunoblotting was performed, investigating specific proteins of interest that contribute to mitochondrial homeostasis. Our study indicated that during PD, proteins involved in mitochondrial fitness, such as COXIV and DJ-1, are deregulated, suggesting potential disruption of mitochondrial integrity. This can be interpreted by an increase in the signal for mitophagy, since PINK1, PARKIN and MIRO1,2 have been detected to be enriched in mitochondria of diseased condition. However, autophagic process is deficient, since the membrane-bound LC3-II is reduced in diseased, limiting the formation of autophagosomes, thus, rendering more acute the disruption of mitochondria. In addition to mitochondrial impairment, several pathways were found to be dysregulated during PD that are implicated in cell growth and survival, epigenetic regulation, hormone regulation and neuroinflammatory response. As a conclusion, the study of mitochondrial proteome may render possible the understanding of the molecular fingerprint of PD, since the latter is reflected through dysregulation of signaling pathways and alteration in the profile and subcellular topology of various proteins.

P197

Prolonged adrenergic stress disrupts mitochondrial homeostasis and primes the innate immune response in salivary gland epithelium

Maria Filika[1*], Chrysafenia Papavissarion[1], Maria Skoufou[1], Kalliopi Moustaka[2], Roxanne Tenta[2], Martina Samiotaki[3], George Stamatakis G[3], Fotini Skopouli[2], Stergios Katsiougiannis[1]

[1]Biomedical Research Foundation Academy of Athens, Greece
[2]Department of Nutrition and Dietetics, Harokopio University, Athens, Greece
[3]Biomedical Sciences Research Center Alexander Fleming, Athens, Greece

Stress is a normal response of the body to a challenging or a difficult circumstance. When the duration of stress is long, the concept of stress is defined as chronic stress, characterized amongst others, by prolonged activation of the sympathetic nervous system and secretion of the hormones epinephrine and norepinephrine. Chronic stress is an important regulator of immunity; however, the molecular mechanisms are poorly understood. Mitochondrial damage is connected to innate immunity since mitochondrial stress can lead to the release of mtDNA in the cytosol and trigger proinflammatory and type I interferon (IFN) response. Type I IFN signature is a hallmark of Sjögren's syndrome (SS) and other autoimmune diseases such as Systemic Lupus Erythematosus. SS is a rather common autoimmune disease, the most prominent feature of which are the salivary gland infiltrations by T and B lymphocytes in situ. Salivary gland epithelial cells (SGEC) are active participants in the induction and perpetuation of the inflammatory process and their phenotype in SS is mainly characterized by increased adrenergic signaling resembling chronic stress conditions. Thus, SS-derived SGEC provide a robust and credible model to characterize cellular responses triggered by chronic stress.

Here we show that prolonged adrenergic stress (PAS) elicited by epinephrine, affects mitochondria homeostasis and engages type I IFN response conferring autoimmune milieu. We found that PAS reprograms mitochondria in SS-derived SGEC but not in control SGEC, as mitochondrial proteins show non-canonical response to it. Furthermore, PAS induces mitochondrial stress as seen by aberrant mitochondrial morphology, loss of electron density, reduction of membrane potential and escape of mtDNA in the cytosol, accompanied by the induction of interferon-beta 1 (IFNb1) and increased expression of interferon-stimulated genes, such as OAS1 and OAS3. These data demonstrate that PAS compromises mitochondria machinery and primes innate immune responses in SS-derived SGEC.

P198

The effect of Nutlin-3a-induced activation of the p53 signaling pathway on mitochondrial proteome in a human mantle cell lymphoma model

Alexandros Syllas[1,2], Martina Samiotaki[3], Konstantina Psatha[1,2,4], Michalis Aivaliotis[1,2,5]

[1]Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece
[2]Functional Proteomics and Systems Biology (FunPATh), Center for Interdisciplinary research and Innovation (CIRI), Aristotle University of Thessaloniki, Greece
[3]Biomedical Sciences Research Center "ALEXANDROS FLEMING", Athens, Greece
[4]Laboratory of Medical Biology and Genetics, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece
[5]Basic and Translational Research Unit, Special Unit for Biomedical Research and Education, School of Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

Mantle cell lymphoma (MCL) is a non-Hodgkin lymphoma of B-cell origin. Mutations in the TP53 gene occur with low frequency, mainly in the most aggressive forms of the disease, thus, enabling the application of potentially therapeutic molecules, which activate the p53 signaling pathway. Considering the important role of mitochondria in lymphomas, the present study aims to shed light on the effect of Nutlin-3a (N3a) on the destruction of cancer cells through activation of the p53 signaling pathway, focusing on the analysis of changes in mitochondrial proteome in a human MCL cell model. Moreover, it is feasible to investigate both the topology of the proteins in subcellular compartments and the changes in their relative abundance levels. In the present study mitochondrial fractions were separated from MCL with and without N3a-induced activation of wt p53 leading to apoptosis. Subsequently, protein extracts from both fractions and conditions were subjected to comparative proteomics analysis. The comparative proteomic analysis, which focuses on the relative abundance of proteins between the total proteome and the proteins in the mitochondrial extract, indicates a plethora of mitochondrial proteins affected by the N3a treatment including proteins involved in metabolism and signaling pathways. Both proteomics and western-blotting demonstrate the accumulation of the p53 protein in mitochondria, while it confirms its already known role as a transcription factor, activating p21 and mitochondrial pro-apoptotic proteins, such as BAX, associated with cell cycle arrest and apoptosis induction, respectively. In conclusion, our study sheds light on the role of specific mitochondrial proteins in the N3a-induced apoptosis in MCL and the translocation of p53 and other non-mitochondrial proteins to the mitochondrion during p53 signaling pathway activation.

P199

Sequencing-based mutational screening of the β-globin gene and pipeline development for variant calling in β-thalassemia modifier loci from Whole Exome Sequencing data

Sotiris Mavromatis[1], Eirini Veltsou[1], A. Symeonidis[2], A. Kourakli[2], Zoi Lygerou[1]

[1]Molecular Genetics Unit, Department of General Biology, School of Medicine, University of Patras, Greece.
[2]Thalassemia Center, Haematology Unit, University Hospital of Patras, School of Medicine, University of Patras, Greece

Beta-thalassemia is a hereditary blood disorder, characterized by abnormal synthesis of hemoglobin beta chains, due to mutations within the beta globin gene (HBB) that either block or reduce gene expression. More than 300 pathogenic mutations have been recorded, with specific mutations associated with different disease severity. However, multiple additional genomic modifier loci have been identified, affecting disease severity. Mediterranean countries are a hotspot for the thalassemia trait, with a recorded incidence ~ 7-8%. Molecular diagnosis of thalassemia allows accurate identification of asymptomatic carriers, prenatal testing and accurate prognosis, guiding early prevention and clinical management. We developed an assay for the mutational analysis of β-thalassemia patients, following amplification of HBB and detection of pathogenic variants. HBB exonic, intronic and promoter regions, containing all the currently known pathogenic/likely pathogenic SNV and indel variants in the HBB gene, are amplified by PCR and subjected to Sanger sequencing, followed by variant calling and annotation. We genotyped 32 beta-thalassemia patients. Variants identified include c.93-21G>A (VAF 0,26 in our patient cohort), c.118C>T (VAF 0,20) and c.92+6T>C (VAF 0,13). Allele frequencies are consistent with earlier analyses in Western Greece area. Most patients were compound-heterozygotes (19/32) while 9 patients were homozygotes. Other loci associated with beta-thalassemia disease severity, include both copy number variations (ie in the alpha-globin gene locus) and SNVs/indels in modifier genes. Towards a prognostic tool which would integrate major mutations in the HBB locus with variants in modifier loci, we are developing a bioinformatics pipeline to extract variant information from Whole exome data. CNV calling from exome data relies on baseline estimations from hundreds of Greek population subjects. This work will pave the way for analyses aiming to combine highly penetrant major variants to susceptibility/modifier loci for correct estimation of risk and prognosis for individual patients, applicable to different monogenic as well as multi-factorial diseases.

P200

Importance of Biobanks for Translational Cancer Research in Serbia

Ana Djuric[1], Miljana Tanic[1], Snezana Bjelogrlic[1], Mladen Marinkovic[2,3], Aleksandra Stanojevic[1], Jelena Spasic[4], Suzana Stojanovic-Rundic[2,3], Radmila Jankovic[1], Ana Krivokuca[5], Katarina Mirjacic Martinovic[1], Ana Vuletic[1], Marija Djordjic Crnogorac[5], Ana Damjanovic[1], Marko Radulovic[1], Remond Fijneman[6], Jerome Zoidakis[7,8], Sergi Castellví-Bel[9], Milena Cavic[1]

[1]Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia
[2]Clinic for Radiation Oncology and Diagnostics, Department of Radiation Oncology, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia
[3]Faculty of Medicine, University of Belgrade, Belgrade, Serbia
[4]Clinic for Medical Oncology, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia
[5]Department of Genetic Counseling for Hereditary Cancers, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia
[6]Department of Pathology, The Netherlands Cancer Institute - Antoni van Leeuwenhoekziekenhuis, Amsterdam, the Netherlands
[7]Department of Biotechnology, Biomedical Research Foundation, Academy of Athens, Athens, Greece
[8]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece
[9]Gastroenterology Department, Fundació de Recerca Clínic Barcelona-Institut d'Investigacions Biomèdiques August Pi i Sunyer, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas, Hospital Clínic, University of Barcelona, Barcelona, Spain

Introduction: Implementing good biobanking practice (GBP) and promoting interdisciplinary collaborations between healthcare professionals, scientists, patients, and industry stakeholders might play a pivotal role in the rapid translation of cutting-edge scientific discoveries into clinical practice. Following the GBP principles, we aimed to establish a workflow for the first Rectal Cancer Biobank (RCB) at the Institute for Oncology and Radiology of Serbia (IORS) within the framework of the Horizon Europe STEPUPIORS project.

Material and Methods: The workflow for the RCB was set up according to guidelines provided by relevant international authorities and national and EU regulations and monitored by higher partner institutions from Spain, the Netherlands and Greece. Biobank equipment and Laboratory Information Management Software (LIMS) were procured to ensure infrastructural, storage and data protection requirements. Human capacities were built through relevant training and expert visits to partner institutions. All decisions were approved by a consensus of the partner consortium.

Results: Within the first year, the STEPUPIORS team established the procedures for the first RCB of around 100 patients with locally advanced rectal cancer. Fifteen IORS personnel (5 physicians, 3 biochemists, 4 molecular biologists, 3 pharmacists) were successfully trained in biobanking. Thirteen standard operating procedures (SOPs) were implemented to comply with GBP. Scientific and management oversight committees comprised of members of all participating institutions were formed. A strengths, weaknesses, opportunities and threats (SWOT) analysis was performed as a starting point for developing sustainable biobanks at IORS and Serbia in the future. The IORS RCB was fully operational on October 01, 2023, when the prospective collection of tissue, plasma and sample derivatives was initiated for the current and three new planned projects.

Conclusions: Implementing the principles of GBP and promoting interdisciplinary collaborations is expected to ensure that innovative diagnostic tools and therapies reach patients more rapidly, contributing to the principles of circular bioeconomy.

Key words: biobank, rectal cancer, translational cancer research.

Acknowledgements
This study was funded by the Horizon Europe Twinning Project STEPUPIORS (Agreement No. 101079217) and the Ministry of Education and Science of the Republic of Serbia (Agreement No. 451-03-47/2023-01/200043).

P201

SAP as a possible therapeutic target in Alzheimer's Disease

Dimitra G. Velentza[1], Georgia I. Nasi[1], Anastasios Routsis[2], Vassiliki Magafa[2], Ioannis P. Trougakos[1], Vassiliki A. Iconomidou[1*]

[1]Section of Cell Biology and Biophysics, Department of Biology, School of Science, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 157 01, Greece,
[2]Laboratory of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, University of Patras, Patras 26504, Greece
**email: veconom@biol.uoa.gr*

Alzheimer's Disease (AD) is the most common form of dementia, currently affecting 47 million people worldwide, expecting that the number of cases will triple by the end of 2050. One of AD neuropathological hallmarks is the formation of extracellular amyloid plaques, mainly composed of amyloid beta (Aβ) peptides, alongside other components and proteins, with the latter referred to as co-deposited proteins. Serum Amyloid P component (SAP) is one such co-deposited protein, constituting 14% of the deposits' dry mass. SAP, a serum glycoprotein with pentameric organization plays a role in facilitating the phagocytosis of amyloid fibrils by binding to them. However, this binding leads to SAP getting trapped within the deposits. Moreover, this binding protects amyloid fibrils from being recognized by other components of the immune system, resulting in their maintenance and further aggregation. Our study employs a therapeutic approach focusing on the inhibition of Aβ peptide aggregation from SAP peptides-analogues. A consensus algorithm, named AMYLPRED 2 was used to locate protein regions in the SAP sequence that exhibit increased aggregation propensity. The predicted regions were chemically synthesized and co-incubated with the Aβ peptide in vitro. Afterward, molecular biophysical techniques were applied, such as Transmission Electron Microscopy after negative staining, Congo Red birefringence and Thioflavin T (ThT) fluorescence assays, to determine whether the peptide analogues had any inhibitory effects on Aβ aggregation. Our findings suggest that some of the peptide-analogues exhibit the potential to inhibit and/or delay Aβ peptide aggregation, offering promise for therapeutic intervention.

The present work was co financed by the European Union and Greek national funds through the Operational Program "Competitiveness, Entrepreneurship and Innovation", under the call "RESEARCH-CREATE-INNOVATE" (project code: T1EDK-00353).

P202

Comparative study of key epigenetic protein regulators in different subtypes of human lymphoma after Nutlin-3a-induced p53 activation

Stergiani Telliou[1,2], Paschalina Tangili[1,2], Stefania Maniatsi[2,3], Georgia Orfanoudaki[2], Konstantina Psatha[2,3,4], Michalis Aivaliotis[2,3,5]

[1]School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece
[2]Functional Proteomics and Systems Biology (FunPATh), Center for Interdisciplinary research and Innovation (CIRI), Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[4]Laboratory of Medical Biology and Genetics, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[5]Basic and Translational Research Unit, Special Unit for Biomedical Research and Education, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

Dysregulation of key-enzymes of epigenetic regulation has been shown to be associated with various types of hematological cancers, such as lymphoma[1]. Polycomb group proteins (PcG) are highly conserved controllers of gene suppression that play crucial roles in maintaining the integrity of stem cell potential, cellular growth, and inactivation of the X chromosome. PcG proteins assemble into multi-component units (PRC1, PRC2). Notable constituents of PRC2 include EZH2, EED, SUZ12, and RbAp46/48[2]. In this research, our objective is to study five epigenetic proteins, namely EZH2, SUZ12, EED, DNMT1, and SIRT1, in lymphoma cell lines after Nutlin 3a(N3a)-induced wt p53 activation that leads to apoptosis. We aimed to assess the impact of N3a-treatment on these epigenetic regulators, which led to a significant increase in the expression of both p53 and MDM2[3]. Systematic literature and datasets searches were performed combined with comparative proteomics analysis. We utilized three human lymphoma cell lines with wild-type p53 (cHL, MCL, ALCL) in our study. Comparative proteomics and western immunoblotting analysis were performed. Literature study revealed that protein expression level of EZH2 is higher in lymphoma cell lines (DLBCL, FL)[4]. Our results showed that the abundance of the five proteins after the N3a-treatment does not follow the same pattern. For the SIRT1 and DNMT1 proteins, there was a slight decrease in their abundances, as for the EED protein and in particular in MCL, while for the EZH2 protein, we did not observe any noticeable change in comparison to the control samples. The expression level of SUZ12 protein was higher in MCL than that in the other two lymphoma types. This research project, also, maximized our interest for the role of EZH2 enzyme in hematological malignancies, so we are going to explore further the role of EZH2 and its isoforms in human cell lines of MCL, cHL, CLL and ALCL.

Bibliography

1. *Ntziachristos P, Mullenders J, Trimarchi T, Aifantis I. Mechanisms of epigenetic regulation of leukemia onset and progression. Adv Immunol. 2013;117:1-38. doi: 10.1016/B978-0-12-410524-9.00001-3. PMID: 23611284; PMCID: PMC3830954.*
2. *Martinez-Baquero D, Sakhdari A, Mo H, Kim DH, Kanagal-Shamanna R, Li S, Young KH, O'Malley DP, Dogan A, Jain P, Wang ML, McDonnell TJ, Miranda RN, Vega F, Medeiros LJ, Ok CY. EZH2 expression is associated with inferior overall survival in mantle cell lymphoma. Mod Pathol. 2021 Dec;34(12):2183-2191. doi: 10.1038/s41379-021-00885-9. Epub 2021 Aug 10. PMID: 34376807; PMCID: PMC10563799.*
3. *Psatha, K.; Kollipara, L.; Drakos, E.; Deligianni, E.; Brintakis, K.; Patsouris, E.; Sickmann, A.; Rassidakis, G.Z.; Aivaliotis, M. Interruption of p53-MDM2 Interaction by Nutlin-3a in Human Lymphoma Cell Models Initiates a Cell-Dependent Global Effect on Transcriptome and Proteome Level. Cancers 2023, 15, 3903. https://doi.org/10.3390/cancers15153903*
4. *Hao B, Sun M, Zhang M, Zhao X, Zhao L, Li B, Zhai L, Liu P, Hu H, Xu JY, Tan M. Global characterization of proteome and lysine methylome features in EZH2 wild-type and mutant lymphoma cell lines. J Proteomics. 2020 Feb 20;213:103614. doi: 10.1016/j.jprot.2019.103614. Epub 2019 Dec 14. PMID: 31846764.*

P203

A Novel Mutation in the CYCS Gene Alters Apoptosis in a Family with Thrombocytopenia

Konstantina Giavi[1], Stavros Glentis[2], Anthi Bouchla[3], Anastasia Apostolidou[4], Nikolaos M. Marinakis[5], Antonis Kattamis[2], Eleni Katsantoni[1*], Vasiliki Pappa3

[1]Basic Research Center, Biomedical Research Foundation, Academy of Athens, Athens, Greece
[2]Division of Pediatric Hematology-Oncology, First Department of Pediatrics, "Aghia Sophia" Children's Hospital, National and Kapodistrian University of Athens, Athens, Greece
[3]Department of Internal Medicine - Propaedeutic and Research Unit, National and Kapodistrian University of Athens, Medical School, University General Hospital "Attikon", Athens, Greece
[4]Clinical, Experimental Surgery and Translational Research Center, Biomedical Research Foundation, Academy of Athens, Athens, Greece
[5]Laboratory of Medical Genetics, "Aghia Sophia" Children's Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece
**ekatsantoni@bioacademy.gr*

A novel mutation in the CYCS gene (CYCS (NM_018947.6):c.292T>C (p.Tyr98His)) has been identified using whole exome sequencing in a Greek family with thrombocytopenia. The mutation has been found to be heterozygous in all six members of the family tested. In humans, the CYCS gene encodes Cytochrome c, a small protein associated with the inner membrane of the mitochondrion, which constitutes an essential component of the respiratory electron transport chain in mitochondria and plays an important role for apoptosis. The clinical features of known CYCS variants have been reported to be linked with thrombocytopenia. To investigate the functional role of the mutation and its potential link to the thrombocytopenic phenotype, Florescence Activated Cell Sorting (FACS) has been performed in bone marrow mononuclear cells of members of the family and healthy controls. The antibody panel used included anti-human CD42a, CD42b, CD31 and CD71 antibodies. The positive for CD42a, CD42b, CD31 and medium positive for CD71 sorted cell population has included immature and mature megakaryocytes. Subsequently, the sorted cells have been stained with Annexin V/7-AAD, to allow the combined detection of early-stage cell apoptosis and late-stage cell apoptosis / necrosis. Cells undergoing early apoptosis have stained positive for Annexin V, while late apoptotic cells have been characterized by positive staining for both Annexin V and 7-AAD. Our preliminary results show increased numbers of apoptotic cells in the family members tested in comparison to the healthy controls. This indicates a potential premature pro-platelet release, leading to ineffective platelet production and low platelet counts, explaining the thrombocytopenic phenotype. We have then inserted the CYCS gene mutation in the megakaryocytic cell line DAMI, using the CRISPR-Cas9 system, and we are currently performing functional experiments. This study delineates the molecular mechanisms underlying thrombocytopenia in the family with the mutation and it is expected that the findings will be translated in novel therapeutic management strategies for patients with thrombocytopenic related pathologies.

P204

Deciphering the role of autophagy and exosomes in human lymphoma and leukemia using systems biology approaches

Konstantina Psatha[1,2,3], Angeliki Christidou[1,4], Aikaterini Kalantidou[5], Anna-Aspasia Karkavitsa[5], Eirini Papadaki[6], Georgia Orfanoudaki[1], Laxmikanth Kollipara[7], Albert Sickmann[7,8], George Rassidakis[9], Elias Drakos[10], Michalis Aivaliotis[1,2,11]

[1]Functional Proteomics and Systems Biology (FunPATh), Center for Interdisciplinary research and Innovation (CIRI), Aristotle University of Thessaloniki, Thessaloniki, Greece
[2]Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece
[3]Laboratory of Medical Biology and Genetics, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[4]School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece
[5]Department of Biology, University of Crete, Heraklion, Greece
[6]Department of Chemistry, University of Crete, Heraklion, Greece
[7]Leibniz-Institut fór Analytische Wissenschaften–ISAS–e.V., Dortmund, Germany
[8]Medizinische Fakultδt, Medizinische Proteom-Center (MPC), Ruhr-Universitδt Bochum, Bochum, Germany
[9]Department of Oncology and Pathology, Cancer Centrum, Karolinska Institutet,Stockholm, Sweden
[10]School of Medicine, University of Crete, Heraklion, Greece
[11]Basic and Translational Research Unit, Special Unit for Biomedical Research and Education, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

The orchestrated homeostatic action of autophagy and exosome biogenesis provides a potential causal relation with lymphomagenesis. This study aims to characterize the differently expressed proteins associated with both above-mentioned pathways, as well as to explore their potential implication in therapeutic response controlling lymphomagenesis via N3a-induced re-activated p53 in different lymphoma subtypes. In vitro cell lines that were used as model system of three lymphoma subtypes (Hodgkin lymphoma-HL, mantle cell lymphoma-MCL, anaplastic large cell lymphoma-ALCL), were screened for the antiproliferative effect of N3a related to p53's restoration, exhibiting enhanced apoptotic death. Total proteome corresponding to the three lymphoma subtypes before and after N3a-treatment was subjected to comparative proteomic analyses, and selected proteins were confirmed by immunoblotting. Moreover, a systematic literature and dataset review was performed to collect and integrate all the currently available information and data. Global proteomic analysis detected more than 4000 proteins, while functional pathway analysis revealed more than 32 proteins to be related to autophagy and 77 to be related to exosomal pathways. N3a-induced autophagic stimulation in HL, MCL, ALCL, in a different, cell-type-dependent manner, involving increased levels of well-known p53 targets, such as DRAM and PRKAB1. DRAM, a p53-mediated modulator of autophagy, holds a critical role for programmed cell death, linking autophagy to p53 signaling pathway and apoptosis. The identified PRKAB1 is the beta subunit of AMPKb, a central energy sensor. AMPK activates serine/threonine kinase ULK1, playing a key-role in autophagy promotion. Moreover, N3a-treatment was associated, among other proteins, with the deregulation of several Rab GTPases, known to play a direct and significant role in the endocytic and exosome secretion pathways (Rab3, Rab13, Rab27). The resulting data indicate that endosome-lysosome fusion is possibly increased in all lymphoma cell lines after treatment, as well as exosome secretion. Vesicle fusion is suggested to be higher in ALCL and HL cell line, whose trafficking may be regulated by Rab13.

P205

Indirubin derivative effects on STAT3 and STAT5 in AML cells

Konstantina Giavi[1], Eirini Sofia Fasouli[1], Konstantina Vougogiannopoulou[2], Alexios-Leandros Skaltsounis[2], Eleni Katsantoni[1*]

[1]Basic Research Center, Biomedical Research Foundation, Academy of Athens, Athens, Greece
[2]Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens, Greece
**ekatsantoni@bioacademy.gr*

Signal transducers and activators of transcription (STATs) are involved in many physiological cell functions, including hematopoiesis, differentiation, proliferation, apoptosis and survival. STATs contribute to signal transduction and regulate transcription of target genes. Their abnormal activation is linked to solid and hematologic malignancies. For this reason, the study of indirect or direct inhibition of STATs is of great interest for therapeutic purposes. Indirubins are characterized by anti-cancer / anti-metastatic properties, they inhibit various kinases and have been used to treat chronic myeloid leukemia. Here we tested three indirubin derivatives for their effects on STAT3 and STAT5 action in acute myeloid leukemia (AML) cells. The derivative 6BIO has been found to influence viability of leukemic cells in a dose dependent manner and to decrease STAT3 and STAT5 phosphorylation. RNA-seq experiments in AML cells treated with 6BIO, have defined specific expression signatures showing the effects of the compound on the whole transcriptome and changes on STAT3 and STAT5 target genes expression levels. It is expected that this work will delineate the molecular mechanisms of 6BIO action and will provide insights on novel therapeutic strategies for AML.

P206

Elucidation of the relative abundance of p53 isoforms and protein complexes in human lymphoma and leukemia cell line models

Anastasia Theodosiadou[1,2], Athina Kyriazi[2,3], Ilektra Mavroudi[1,2], Stefanos Polychronis[4], Stefania Maniatsi[2,5], Elias Drakos[6], Martina Samiotaki[7], Konstantina Psatha[2,5,8], Michalis Aivaliotis[1,2,9]

[1]School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece
[2]Functional Proteomics and Systems Biology (FunPATh), Center for Interdisciplinary research and Innovation (CIRI), Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]Department of Biology, University of Crete, Heraklion, Greece
[4]Department of Biochemistry, Eberhard Karls University of Tuebingen, Tuebingen, Germany
[5]Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece
[6]School of Medicine, University of Crete, Heraklion, Greece
[7]Biomedical Sciences Research Center "ALEXANDROS FLEMING", Athens, Greece
[8]Laboratory of Medical Biology and Genetics, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[9]Basic and Translational Research Unit, Special Unit for Biomedical Research and Education, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

There are at least 12 protein isoforms of TP53, and their stoichiometry leads to different cellular effects, as is the case of the overexpression of the isoform Δ133p53 involved in different cancers. The purpose of the present study is to investigate the presence and relative abundance of p53 isoforms and protein complexes formed in different human lymphoma subtypes and the effect of Nutlin 3a (N3a)-induced p53 activation that leads the cells to apoptosis. The detailed understanding of the p53 mechanisms involved in the development and progression of lymphoma and leukemia, will significantly contribute to the clinical research and therapeutic strategies. We conducted systematic literature and public data search related to our aim, in combination with comparative mass spectrometry (MS)-based proteomic analysis, for the identification and quantification of proteins, the detection of protein modifications and mapping of protein interactions. Results were further validated by western-blot and the relative quantitation of the mRNA levels using RT-PCR. Our results confirmed p53 oligomerization after N3a-treatment and alteration on p53 isoforms stoichiometry. The composition of p53 oligomers was elucidated by proteomics analysis and showed lymphoma-specific interactors. mRNA levels of wt p53, Δ133p53, MDM2, and p21 were detected and relatively quantified, showing lymphoma-dependent abundancies. Δ133p53 found in all lymphoma subtypes more abundant than wt p53. N3a-induced activation enhanced the abundance of wild type p53 and in all lymphoma subtypes on both transcript and protein level. The detailed study of p53 isoforms stoichiometry and interactors will shed light in lymphomatogenesis required for precise and personalized diagnosis and therapy.

P207

Organoids as model to study the development of colon adenocarcinoma

Dimitris Koutsoumparis[1], Elena Constantinou[1], Vasileios Papadopoulos[2], Eudoxia Chatzivasileiou[1*]

[1]School of Health Sciences, Department of Medicine, Aristotle University , Thessaloniki Greece
[2]A' Surgical Clinic of the University of Athens, Papageorgiou Hospital, Thessaloniki, Greece
**corresponding author eudoxiah@auth.gr*

Organoids are three-dimensional cellular structures grown long-term ex vivo from patient stem cells that mimic the structural and functional properties of the organ from which they were derived. In the present study, organoid or short-term three-dimensional (3D) cultures were established and grown from colorectal adenocarcinoma (CAC) patient samples. Such cultures will be used as models for specific neoplasias and will contribute a) to the development of new therapeutic protocols and b) to the understanding of the mechanisms underlying the pathogenesis of the neoplasias. For this purpose, 3D cell cultures were established from three different patients with CAC, followed by the administration of chemotherapeutic drugs (oxaloplatin and fluorouracil). Using a cell viability test (Cell Titer-GLO, Promega) the response of the cultured cells to the above chemotherapeutics was measured and sensitivity to fluorouracil or oxaloplatin was established. This study will be expanded with the establishment of more organoids and the administration of new drugs or a combination of known drugs. At the same time, it will be tested whether the expression of the tumor suppressor protein CYLD is related to sensitivity or resistance to chemotherapy, such as taxanes or platinum respectively. This study builds on previous data showing a correlation of CYLD expression with chemotherapeutic sensitivity in different types of neoplasias. These results will contribute to the development of personalized therapeutic protocols for CAC.

P208

SayWES4Life: Whole exome sequencing for diagnosis of rare genetic diseases for 400 patients in Greece reveals ultra-rare entities

E. Veltsou[1,2], V. Tzimogianni[1,2], O. Preza[1,2], S. Mavromatis[1,2], N. Georgakopoulou[1,2], D. Veltista[3], E. Chroni[3], E. Kostopoulou[4], G. Dimitriou[4], A. Katerelos[5], M.Iliopoulou[5], P. Tsoumpos[5], L. Lykopoulou[6], M. R. Pons[6], A. Kattamis[6], R. Koros[7], G. Tsigkas[7], P. Davlouros[7], V. Panagiotopoulos[8], G. Karadima[9], G. Koutsis[9], G. Papadimas[9], L. Stefanis[9], V. Giannatos[12], A. Konstantopoulou[5], K. Loritis[10], A. Briasoulis[10], G. Georgiopoulos[10], R. Seung Woo[11], K. JiHye[11], S. Taraviras[12], Z. Lygerou[1,2]

[1]Institute of Precision Medicine, University Research and Innovation Center, University of Patras.
[2]Molecular Genetics Unit, General Biology Department, Medical School, University of Patras, Greece.
[3]Neuromuscular Center, University General Hospital of Patras and Medical School, University of Patras,
[4]Paediatric Clinic, University General Hospital of Patras and Medical School, University of Patras,
[5]Karamandaneio Childrens' Hospital, Patras,
[6]Ag. Sofia Children's Hospital, Athens,
[7]Cardiology Unit, Pathology Clinic, University General Hospital of Patras and Medical School, University of Patras,
[8]Neurosurgery Unit, Surgery Clinic, University General Hospital of Patras and Medical School, University of Patras,
[9]Neurology Clinic, Eginition Hospital, Athens,9Neurology Clinic, Eginition Hospital, Athens,
[10]Department of Clinical Therapeutics, National and Kapodistrian University of Athens,
[11]3billion Inc., Seoul, South Korea
[12]Department of Physiology, School of Medicine University of Patras, Greece.

Rare diseases (RDs) are disease entities that affect less than 1 in 2000 people. To date, there are over 7,000 known RDs, with new entities regularly emerging. Nearly 400 million people worldwide are affected by RDs, with an estimated 600,000 individuals impacted in Greece. Due to their high phenotypic and genetic heterogeneity, accurate diagnosis of RDs is challenging, and many patients remain undiagnosed. As the majority of RDs have a genetic etiology, Whole-Exome Sequencing (WES) has proven valuable in the identification of underlying genetic defects and definitive diagnoses. Here, 400 patients treated in public hospitals in Greece which remained undiagnosed at the time of analysis were enrolled in a multi-site study for pathogenic variant identification through WES. The patient cohort consisted of pediatric and adult patients exhibiting diverse phenotypes, including neurodevelopmental, neurological/neuromuscular, growth, metabolic, cardiovascular, skeletal, hematological and vascular abnormalities. WES identified the responsible genetic etiology in 32% of the cases analysed (positive diagnosis), while for a further 18% of cases a possible, though not certain, underlying genetic cause was identified (inconclusive). Identified variants were primarily single nucleotide variants (75%), followed by indels (18%) and chromosomal aberrations (6%), while trinucleotide repeat expansions and mitochondrial mutations were also identified. Dominant mode of inheritance prevailed (51%), with 38% recessive autosomal and 11% X-linked. Amongst the novel diagnoses, ultra-rare genetic diseases were identified, including Fibrodysplasia Ossificans Progressiva, with ~900 cases identified worldwide, Beck-Fahrner syndrome, a chromatinopathy with 14 reported cases worldwide and TERT-related pulmonary fibrosis. Genetic diagnosis enables precise clinical management, facilitates inclusion in clinical trials and permits family analysis and genetic counseling. Follow-up segregation analysis and functional analyses of Variants of Uncertain Significance in inconclusive cases is expected to lead to further diagnoses, as well as provide novel insight into the underlying molecular pathology of RDs.

P209

Reconstructing the extracellular matrix using innovative 3D cell culture systems

Zoi Piperigkou[1,2*], Christos Koutsakis[1], Sylvia Mangani[1], Nikos K. Karamanos[1,2*]

[1]Department of Chemistry, Biochemistry, Biochemical Analysis and Matrix Pathobiology Res. Group, Laboratory of Biochemistry, University of Patras, Greece.
[2]Foundation for Research and Technology-Hellas (FORTH)/Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece.

Cancer initiation and progression are heavily influenced by microenvironmental signals originating from various components of the niche, including the extracellular matrix (ECM). The ECM, a complex macromolecular network, plays a pivotal role in regulating cellular function. While traditional two-dimensional (2D) cell culture systems have been valuable for molecular-level insights and preclinical testing, they fall short in accurately replicating the intricate architectural features of the in vivo matrix microenvironment. Consequently, it is not surprising that over the past decade, researchers have redirected their efforts towards the development of innovative in vitro culture models that closely mimic the unique environments and interactions found in tumor and tissue-specific niches. 3D cell culture systems provide researchers with enhanced tools to investigate cancer progression and explore novel therapeutic avenues. The main goal of this study is the development of state-of-the-art 3D cell culture platforms, including cancer-cell derived spheroids. Specifically, 3D spheroids were developed from different breast cancer cell lines, including MCF-7 (luminal A), MDA-MB-231, Hs574T (triple negative) and shERβ MDA-MB-231 (ERβ-suppressed MDA-MB-231). 3D spheroids have been characterized in terms of morphological characteristics and ECM composition and the results highlighted major alterations in the formation of 3D spheroids derived from different breast cancer cell lines. Conclusively, the results of this study suggest the promising role of these advanced breast cancer cell culture platforms in filling the gap between pre-clinical evaluation of potential drug targets, improving the personalized treatment approaches for breast cancer management.

Reference

- *Kyriakopoulou K, Koutsakis C, Piperigkou Z, Karamanos NK. (2023) Recreating the extracellular matrix: novel 3D cell culture platforms in cancer research. FEBS J. In Press*

P210

Regulation by ERK1/2-mediated phosphorylation is a conserved mechanism in both human and mouse HIF-1

Dimitrios - Foivos Thanos, Kreon Koukoulas, George Simos, Ilias Mylonis

Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Larissa, Greece

In cells deprived of oxygen, Hypoxia Inducible Factor-1 (HIF-1) is the main transcriptional activator that facilitates cell adaptation and survival under hypoxia. Functional HIF-1 heterodimers consist of HIF-1α and ARNT and induce the expression of target genes involved in processes such as oxygen delivery, metabolism and cell migration. The oxygen-regulated HIF-1α subunit, is also controlled by signaling pathways leading to its direct phosphorylation. Modification of human HIF-1α by ERK1/2 occurs at Ser641/643, promotes its nuclear accumulation, by masking an adjacent CRM1-dependent NES[1], and stimulates HIF-1 transcriptional activity, by stabilizing the association of HIF-1α with histone chaperone NPM1[2]. To establish whether the role of HIF-1α phosphorylation by ERK1/2 is conserved among mammalian species, we examined the murine homologue of HIF-1α (mHIF-1α). Sequence alignment suggested that one of the ERK1/2 phosphorylation sites in human HIF-1α (Ser641) is also present in mHIF-1α (Ser652). To test this, we introduced point mutations in a stable form of mHIF-1α that either abrogate (S652A) or mimic (S652D) phosphorylation of Ser652 and tested the localization and functionality of the mHIF-1α forms ectopically expressed in HeLa cells. Immunofluorescence microscopy demonstrated that, in contrast to the wild-type or the phosphomimetic S652D mutant that were almost exclusively nuclear, a significant proportion of the phospho-deficient S652A mHIF1α mutant form was mislocalized in the cytoplasm. In support, reporter gene assays showed that the transcriptional activity of mHIF-1 depended on the phosphorylation state of mHIF-1α Ser652. Although the defective phenotype of the mHIF-1α S652A mutant is weaker than the phenotype of its phosphodeficient human homologue, these data support that the basic regulation of human HIF-1 by ERK1/2-mediated phosphorylation is also present in mice, underlie its importance for cancer cell adaptation to hypoxia and allow testing of our previously developed[3] peptide inhibitors, that target the phosphorylation-dependent interactions of HIF-1α, in in vivo mouse cancer models.

1. Mylonis et al. (2008) J Biol Chem: 283, 27620-27627.
2. Koukoulas et al. (2021) Mol Oncol: 15, 3468-3489.
3. Karagiota et al. (2019) CMLS: 76, 809-825.

This work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: HFRI-FM17-2132 to I.M.).

P211

Deciphering the role of TFAP2A in the hypoxic transcriptional control

Amalia Kanoura[1], Antonis Giakountis[2], Angeliki Karagiota[1], Chrysa Filippopoulou[1], George Stamatakis[3], Martina Samiotaki[3], George Panayotou[3], George Simos[1], Georgia Chachami[1]*

[1]Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Biopolis 41500, Larissa, Greece,
[2]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa 41500, Greece
[3]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", 16672, Vari, Attica, Greece
**Email: ghah@med.uth.gr*

Hypoxia can be established under either physiological or pathological conditions, such as cancer, due to the imbalance between oxygen supply and consumption. Hypoxia Inducible Transcription Factors (HIFs) are the master regulators of the cellular response to hypoxia and are responsible for reprogramming gene expression under low oxygen conditions. Recent studies have identified several HIF and chromatin associated co-regulators as important players in the transcriptional response to hypoxia. We have recently shown that TFAP2A physically interacts with HIF-1 and hypoxia-dependent deSUMOylation of TFAP2A positively affects HIF-1 activity1. TFAP2A is a general transcription factor orchestrating a variety of cell processes including cell growth, differentiation and apoptosis. In order to understand the involvement of TFAP2A in the regulation of hypoxia-inducible genes, ChIP-seq analysis was performed in HeLa cells. We could show that TFAP2A resides together with HIF-1α in the promoters of a set of hypoxia-regulated genes. Moreover, silencing of TFAP2A down-regulated their mRNA expression under hypoxia in both HeLa and MCF7 cells. Interestingly, silencing or CRISPR-mediated knockdown of TFAP2A expression under hypoxia also decreased the occupancy of HIF-1α on these promoters. Furthermore, immunoprecipitation of TFAP2A forms that either lack or constitutively carry a SUMO modification, followed by proteomic analysis, revealed a network of proteins that interact with TFAP2A in a SUMO-dependent manner under hypoxia and may also participate in HIF-dependent gene expression. Overall, our data suggest that TFAP2A is an important co-regulator of the transcriptional response to hypoxia and, as both TFAP2A and HIFs play critical roles in cancer progression, characterization of their crosstalk may lead to novel strategies for targeting and killing cancer cells in hypoxic tumors.

1. Chachami et al. (2019) Mol Cell Prot 18, 11971209

The research work is supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: 1460 to G.C.).

P212

Epigenetic deregulation in the development of acquired chemoresistance in TNBC

Dimitris Kordias[1,2 +], **Spyros Foutadakis**[3 +], **Giannis Vatsellas**[4], **Angeliki Magklara**[1,2,5 *]

[1]*Biomedical Research Institute-Foundation for Research and Technology, 45110 Ioannina, Greece*
[2]*Department of Clinical Chemistry, Faculty of Medicine, University of Ioannina, 45110 Ioannina, Greece*
[3]*Biomedical Research Foundation, Academy of Athens, Athens, Greece*
[4]*Center of Basic Research, Biomedical Research Foundation, Academy of Athens, 4 Soranou Ephessiou St., 11527 Athens, Greece.*
[5]*Institute of Biosciences, University Research Center of Ioannina (URCI), 45110 Ioannina, Greece*
[+]*equal contribution*
[*]*email: magklara@uoi.gr*

Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer and it is characterized by the lack of estrogen, progesterone and human epidermal growth factor receptors. Chemotherapy is the standard treatment for TNBC, but, despite a good initial response, many patients will develop resistance and tumor recurrence. In the present study, we have employed two paclitaxel-resistant TNBC cell lines (SUM159 and BT-549) to investigate epigenetic mechanisms that may be implicated in the development of chemoresistance and identify potential targets. Analysis of chromatin accessibility by ATAC-sequencing demonstrated significant differences between parental and resistant cells. Integrated analysis of ATAC-seq and RNA-seq data showed that induced open and closed chromatin sites were associated with up- and down- regulated genes, respectively, in the chemoresistant cells. In the SUM159 resistant cells, chromatin opening or closing was accompanied by an enrichment or loss in H3K27ac, a mark of active regulatory sites, further supporting our hypothesis that the expression of the associated genes was under epigenetic regulation. Pathway enrichment analysis of the up-regulated genes showed that the epithelial-to mesenchymal transition (EMT) was one of the most overrepresented biological processes in the chemoresistant cells. CREMA (Cis-Regulatory Element Motif Activities) analysis of the chromatin accessibility data of the SUM159 resistant cells revealed that the p63 binding motif was the most overrepresented in the closed chromatin sites, compared to the parental cells. Western blot experiments showed that p63 was strongly down-regulated in the resistant cells and ChIP-seq experiments confirmed the collapse of the p63 cistrome in these cells, probably leading to loss of an epithelial program and acquisition of a more aggressive mesenchymal phenotype. Our results strongly suggest that an epigenetically regulated program of EMT, probably driven by the repression of p63, plays an important role in the development of chemoresistance in TNBC.

P213

Exploring the regulatory impact of 3 -tRF-CysGCA on gene expression in HEK-293 cells

Paraskevi Karousi[1], Martina Samiotaki[2], Manousos Makridakis[3], Jerome Zoidakis[1,3], Diamantis C. Sideris[1], Andreas Scorilas[1], Thomas Carell[4], Christos K. Kontos[1]

[1]Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Panepistimiopolis, Athens, Greece;
[2]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece;
[3]Center of Systems Biology, Biomedical Research Foundation of the Academy of Athens, Greece;
[4]Department for Chemistry, Institute for Chemical Epigenetics, Ludwig Maximilian University of Munich, Munich, Germany.

tRNA fragments (tRFs) constitute a class of small non-coding RNA molecules formed through tRNA cleavage, participating in diverse biological phenomena. Among these, 3'-tRFs have garnered substantial scientific attention for their gene expression regulatory functions. We embarked on an exploration of 3'-tRF-Cys^{GCA}, originating from T-loop cleavage of tRNACys^{GCA}, aiming to unveil its role in gene expression control within HEK-293 cells. While prior research has implied the integration of 3 -tRF-Cys^{GCA} into the RISC complex, where it interacts with AGO proteins, suggesting its influence on gene expression, the broader implications and consequences of altering 3'-tRF-Cys^{GCA} levels in human cells remain uncharted territory. To bridge this knowledge gap, we established stable overexpression of 3'-tRF-Cys^{GCA} in HEK-293 cells, thus creating 3 HEK-293 clones. Subsequently, we conducted comprehensive transcriptomics and proteomics experiments, as well as extensive bioinformatics analyses to discover putative 3'-tRF-Cys^{GCA} direct targets. After analyzing and integrating all aforementioned data, we noticed that 3'-tRF-Cys^{GCA} overexpression leads to the alteration of various genes, in both RNA and protein level, in HEK-293 clones compared to the parental cell line. We validated the interactions between this tRF and putative target molecules, witnessing their altered abundance post-3'-tRF-Cys^{GCA} elevation. Notably, we delved into the involvement of 3'-tRF-Cys^{GCA} in diverse cellular pathways through extensive bioinformatic analyses. Our findings unequivocally demonstrate that 3'-tRF-Cys^{GCA} overexpression induces a shift in mRNA and protein expression profiles. We pinpointed several pathways impacted by the perturbation of this tRF, such as organelle dynamics and cell cycle regulation. Additionally, our reporter assays substantiated direct interactions between 3'-tRF-Cys^{GCA} and TMPO as well as ERGIC1, resulting in altered expression levels of these targets. Cumulatively, our findings underscore the pivotal role of 3'-tRF-Cys^{GCA} in gene expression regulation and underscore its potential significance in various cellular processes.

Acknowledgement
Part of this work was supported by an EMBO Scientific Exchange Grant to Mrs. Paraskevi Karousi. The Authors wish to thank Dr. Vasiliki Lygirou for her advice regarding the proteomics data analysis. The Authors also acknowledge support in the proteomics experiments by the project "The Greek Research Infrastructure for Personalised Medicine (pMED-GR)" (MIS 5002802), which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

P214

Treatment with ascorbic acid normalizes the aerobic capacity, antioxidant defence, and cell death pathways on Mytilus galloprovincialis exposed to increased temperature

Konstantinos Feidantsis[1*], Ioannis Georgoulis[2], Ioannis A. Giantsis[3],Basile Michaelidis[2]

[1]Department of Fisheries and Aquaculture, University of Patras, Mesolonghi, Greece
[2]Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]Faculty of Agricultural Sciences, University of Western Macedonia, Florina, Greece

Increase of sea temperature due to climate change, provokes molecular, biochemical, and physiological changes which can, in turn, result in changes at the biogeographical, population, and ecosystem levels of biological organization. Considering temperature's upcoming increase due to climate change, combined with the fact that Mediterranean mussels Mytilus galloprovincialis (Lamarck, 1819) live at their upper limits [critical temperatures (Tc) beyond 25oC], we cannot be sure of this species' sustainable future in the Mediterranean Sea. Deviation from optimum temperatures leads to cellular damage due to oxidative stress. Although ascorbic acid (AA) is a major scavenger of reactive oxygen species (ROS), its capacity to minimize oxidative stress effects is scarcely studied in aquatic organisms. Thus, treatment with 5 mM and 10 mM AA of thermally stressed molluscs had been employed in order to examine its antioxidant capacity. While 5 mM had no effect, 10 mM normalized COX1 and ND2 relative mRNA levels, and superoxide dismutase (SOD), catalase, and glutathione reductase (GR) enzymatic activity levels in both examined tissues: posterior adductor muscle (PAM) and mantle. ATP levels, probably providing the adequate energy for antioxidant defence in thermally stressed mussels, is also normalized under 10 mM AA treatment. Moreover, autophagic indicators such as LC3 II/I and SQSTM1/p62 levels are normalized, indicating autophagy amelioration. Apoptosis also seems to be inhibited since both Bax/Bcl-2 and cleaved caspase substrate levels decrease with 10 mM AA treatment. Therefore, treatment of mussels with AA seems to produce threshold effects, although the precise underlying mechanisms must be elucidated in future studies. These findings show that treatment of mussels with effective antioxidants can be useful as a strategic approach for the reduction of the deleterious effects on mussels' summer mortality in aquaculture zones.

P215

Modulation of neuronal and astroglial gene expression markers in European Sea Bass (Dicentrarchus labrax) infected with nervous necrosis virus

Antonia Efstathiou, Dimitra K. Toubanaki, Odysseas P. Tzortzatos, Evdokia Karagouni*

Hellenic Pasteur Institute, Department of Microbiology, Vas. Sofias 127, 11521, Athens, Greece
**e-mail: ekaragouni@pasteur.gr*

Aquaculture is essential to cover fish-product demands, providing seafood in high quantities, and covering the half amount of fish consumed worldwide. Greece is one of the leading producers of sea bream (Sparus aurata) and European sea bass (D. labrax) in the Mediterranean. As fish health and welfare is a prerequisite for sustainable and profitable production in the Mediterranean area, combating diseases is highlighted as priority for the development and improvement of aquaculture sector. The most significant disease in terms of severity, economic impact and spread, is viral nervous necrosis (VNN). VNN is a devastating disease, which induces cell necrosis accompanied by vacuolation in fish retina and brain. The disease is caused by nervous necrosis virus (NNV), affecting more than 30 different fish species, worldwide. The first step to move forward on the battle against the NVV disease is to fully understand its progression and its effect on the host. Aim of the present study is to identify which cell brain population is attacked by the NNV virus and how it affects the neuronal and astroglial gene expression during the progression of the disease. Hence, we studied the gene expression of neuronal and astroglial markers in brain tissues of experimentally infected D. labrax upon 0 and 3 hours of infection, 2 and 14 days of infection and upon 7 days upon re-infection of the fishes that survived the virus. Moreover, in brain primary cell cultures derived from uninfected D. labrax, we evaluated the expression of neuronal and astroglial markers using specific antibodies in an immunofluorescence study. Overall, the results of the present study will provide a better understanding of the impact of NNV in the fish brain cell populations and it will give us the tools to further investigate potential signaling pathways involved in the progression of a nervous necrosis infection.

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: 03990)

P216

Physiological response to probiotics (Lactobacillus plantarum) administration in the diet of land snails Cornu aspersum

Efstratios Efstratiou[1], Konstantinos Feidantsis[2], Alexandra Staikou[3], Vasiliki Makri[1,3], Ioannis A. Giantsis[1*]

[1]Faculty of Agricultural Sciences, University of Western Macedonia, Florina, Greece
[2]Department of Fisheries and Aquaculture, University of Patras, Mesolonghi, Greece
[3]Department of Zoology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece

Nutrition is one of the most important factors for the growth and welfare of farmed animals. Many of the microorganisms ingested with food settle in the intestinal epithelium of animals, constituting their intestinal microflora. The set of interactions between the intestinal microflora and the host systems provide a key mechanism for enhancing and maintaining homeostasis. The administration of rations enriched with probiotics is recommended as a mean to prevent unwanted conditions. Research is so far limited to large productive animals, leaving unanswered questions about the effect of probiotics on the growth rate, stress responses and energy metabolism of invertebrates such as the land snail Cornu aspersum. In this research juvenile snails were fed probiotic-enriched ration in two different proportions and their growth rate was monitored over a period of three months. Additionally, the RNA/DNA ratio, the Hsp70 and Hsp90 genes expression, the respective protein levels and NADH expression were measured in the intestine, digestive gland and mantle of the snails. Our results demonstrate that snails' growth rate was not affected throughout the experiment both in terms of shell size and body mass. Response was initially measured by RNA/DNA ratio resulting in an increase in the RNA in various tissues, indicating intense physiological response. Hsps' levels were higher in the presence of the probiotic, but probably also as a preparation of the animal to face potentially stressful situations. NADH expression levels in the hepatopancreas indicate the intense metabolic and antioxidant activity of the specific tissue. In conclusion, although no direct effects on the growth rate were observed, RNA/DNA ratio as well as Hsp and NADH expression indicated a general trend due to probiotics administration. Further studies and markers are needed at the molecular, biochemical, and genetic level to better evaluate the benefits of probiotics in the diet of terrestrial gastropods.

P217

Modulation of Immune Response and Gene Expression Profiling of European Sea Bass (Dicentrarchus labrax, L.) Challenged with bacterial and viral infection mimics

Odysseas P. Tzortzatos[1], Dimitra K. Toubanaki[1], Akindynos Palaiologos[2], Michail-Aggelos Valsamidis[2], Leonidas Papaharisis[3], Vasileios Bakopoulos[2], Evdokia Karagouni[1*]

[1]Hellenic Pasteur Institute, Department of Microbiology, Vas. Sofias 127, 11521, Athens, Greece;
[2]University of the Aegean, Department of Marine Sciences, 81100 Mytilene, Greece;
[3]Nireus Aquaculture S.A., 1st klm. Koropiou-Varis Avenue, 19400 Koropi, Greece.
**e-mail: ekaragouni@pasteur.gr*

Widespread aquaculture losses attributed to pathogen infections are frequently observed in fish farms, especially in the Mediterranean area, with high economic damage. In order to establish a sustainable aquaculture sector and reduce disease outbreaks, it is essential to understand the immune mechanisms in fish during infection. Aim of the present study was to evaluate immune response and gene expression of European sea bass (Dicentrarchus labrax, L.) challenged with bacterial (Lipopolysaccharide – LPS) and viral (polyinosinic:polycytidylic acid - poly(I:C)) infection mimics. Fish were challenged with the mimics and blood and tissues were collected in specific time points for an experimental period of 28 days. Serum samples were used for immunological parameters assessment. Non-specific immune parameters (i.e. nitric oxide, myeloperoxidase, lysozyme, proteases and anti-proteases) were determined according to well established protocols and total antibodies levels were determined by ELISA. Head kidney tissue samples were subjected to qPCR to assess the fold change of genes related to interferon pathway (MxA, IRF7 and ISG12), cytokines (Il-1b, Il10, STAT3 and TNFa), immunoglobulin (IgHM) and T-cell markers (CD8a and CD4). The analyzed immune parameters did not change significantly during the experiment. Proteases and anti-proteases of control group slightly increased at the initial time-points, whereas NO values of control and poly(I:C) groups increased on 12hpi and of LPS on 14dpi. Both LPS and poly(I:C) induced expression of most genes between 3-12 hpi. Some of the analysed genes of poly(I:C) group showed a second increase at 7dpi indicating a second reaction of immune system. In poly(I:C) challenged fish total antibody levels were slightly increased 3-4 dpi whereas in LPS challenged fish antibody levels were slightly increased 7-21 dpi. In the present study, we attempted to investigate and compare the levels of serum parameters and immune related genes that are expressed during bacterial and viral infections. The resulting datasets will be useful for preparation of future studies in aquaculture species, which can further deepen our understanding of specific fish immune functions against pathogens.

This work was supported by European Maritime and Fisheries Fund and Hellenic Ministry of Rural Development and Food in the frame of O.P. Maritime & Fisheries 2014-2020.

P218

Impact and functional role of KMT2B in pediatric astrocytomas

Angeliki-Ioanna Giannopoulou[1], Alexia Klonou[1], Penelope Korkolopoulou[2], Andromachi Pampalou[2], Dimitrios S. Kanakoglou[2], Andreas Mitsios[3], Spyros Sgouros[3], Athanasios G. Papavassiliou[1], Christina Piperi[1]

[1]Department of Biological Chemistry, National and Kapodistrian University of Athens Medical School, Greece
[2]Department of Pathology, National and Kapodistrian University of Athens Medical School, Greece
[3]Department of Pediatric Neurosurgery, IASO Children's Hospital, Athens, Greece

Histone modifications coordinate gene expression by regulating transcription factor binding. Although the role of the epigenome is clear in a variety of cancers, in pediatric astrocytomas still remains ambiguous. The Mixed Lineage Leukemia 2 (MLL2/KMT2B) protein, responsible for H3K4 trimethylation (H3K4me3), was shown to mediate pro-oncogenic effects in several cancers. Here, we aimed to investigate the functional role of histone KMT2B and KMT2B-regulated signaling pathways in pediatric gliomas. We first performed in silico analysis of a publicly available microarray dataset of 49 pediatric astrocytoma samples using the R2: Genomic Analysis. Then, we evaluated the mRNA and protein expression levels of KMT2B by qRT-PCR, immunohistochemistry, and Western immunoblotting in 38 archival pediatric astrocytoma tissues (Grade I-IV) and normal brain samples. We proceeded to evaluate the functional role of KMT2B silencing (using siRNA) in pediatric glioblastoma cell lines (SJGBM2, CHLA-200) proliferation by XTT, as well as in cell migration by scratch assay.

In silico analysis revealed that pediatric astrocytomas exhibit significantly increased KMT2B expression levels compared to normal brain tissues, which was further confirmed by qRT-PCR, and Western immunoblotting in tissue samples. Immunohistochemical analysis showed significantly lower KMT2B immunoreactivity in grade I pilocytic astrocytomas compared to grade III-IV diffusely infiltrating tumors. Accordingly, H3K4me3 protein levels were detected significantly lower in normal brain compared to astrocytomas grade I and grade II-IV. The univariate survival analysis of the entire cohort showed correlation of reduced patient's survival with increased KMT2B expression, indicating a significant clinical impact. To this end, silencing of KMT2B in pediatric astrocytoma cell lines showed a significant reduction in cell proliferation and p53 expression, as well as in cell migration and at mesenchymal marker vimentin levels.

Taken together, our data show a potential oncogenic role of KMT2B in pediatric astrocytomas, correlating to tumor progression and inferior patients' survival that needs further investigation.

P219

"Endonucleosis": an unusual autophagy process in senescent cells

Ourania Galanopoulou[1,2], Evangelia C. Tachmatzidi[1,2], Elena Deligianni[1], Dimitris Botskaris[1,2], Konstantinos C. Nikolaou[3], Sofia Gargani[3], Yannis Dalezios[4], Georges Chalepakis[2], Iannis Talianidis[1]

[1]Institute of Molecular Biology & Biotechnology - Foundation for Research and Technology, Hellas (IMBB-FORTH), Heraklion, Crete, Greece
[2]Dept. of Biology, University of Crete, Heraklion, Greece
[3]Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece
[4]Dept. of Medicine, University of Crete, Heraklion, Greece

Setd8 catalyzes H4K20 monomethylation, which regulates genome integrity, DNA damage response and replication licensing. Previous studies have shown that hepatocyte-specific inactivation of Setd8, display cell division-dependent DNA damage and cell death. Here we show, that shortly after massive hepatocyte death in the livers of Setd8-KO mice, a significant number of cells undergo G2 arrest and enter into a senescent stage. Unexpectedly, we observed dramatic enlargement of nuclei and an "endoreduplication" activity, which leads to chromosomal polyploidy in vast majority of the cells. Closer examination of the enlarged nuclei revealed multiple nuclear engulfments and nuclear vacuoles surrounded by nuclear lamina. These vacuoles had externally-positioned electron-dense membrane-associated chromatin and contained glycogen, various cytoplasmic proteins and even whole organelles, like ER, mitochondria or peroxisomes. In analogy to endocytosis, we propose the term "endonucleosis" for this process. Hepatocytes in Setd8/Atg5 double knockout mice had somewhat enlarged nuclei but lacked intra-nuclear lamina-coated vacuoles, suggesting that the canonical autophagy machinery is required for endonucleosis. This notion was further supported by the detection of LC3 inside of a large number of vacuoles. Taken together, the results demonstrate that Setd8-mediated chromatin modifications play an important role in preventing endoreduplication, cellular senescence and in safeguarding nuclear lamina integrity.

P220

3D Genome re-organization during liver development

Evangelia Tachmatzidi[1,2*], Dimitris Botskaris[1,2], Ourania Galanopoulou[1,2], Haroula Kontaki[1], Argyris Papantonis[3], Iannis Talianidis[1]

[1]Institute of Molecular Biology & Biotechnology, Foundation for Research and Technology, Hellas (IMBB-FORTH)
[2]Department of Biology, University of Crete, Heraklion, Greece
[3]Translational Epigenetics Group, Institute of Pathology, University Medical Center Gottingen, Germany

Cell-specific gene expression programs are established by the temporal activation and selective repression of genes during development. In a previous study, we have shown that stable gene expression patterns in the liver are generated by the combinatorial activity of multiple transcription factors, which mark regulatory regions long before activation and promote progressive broadening of active chromatin domains. Both temporally stable and dynamic, short-lived binding events contribute to the developmental maturation of active promoter configurations.

To obtain further insights into the mechanism of developmental gene activation, we performed Hi-C and Promoter Capture Hi-C experiments and generated high-resolution 3D organization maps at different stages of liver-development. Our results show that genome compartments are largely established already at the hepatoblast stage and remain stable during liver development. On the other hand, dynamic changes were observed in the organization of specific TADs, sub-TADs and the emergence of chromatin loops. Comparative analyses of 3D genome architectures with transcription factor binding patterns, histone modification and ATAC-seq profiles revealed that dynamic chromatin rewiring plays an important role in orchestrating transcriptome changes during development.

P221

Unraveling Transcriptomic Signatures in Milk-derived Exosomes: A Comparative Study Across Mammalian Species

Margaritis Tsifintaris[1], Androniki Tasiopoulou[1], Antonis Giannakakis[1]

[1]Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, Greece

Milk exosomes are key players in intercellular communication, delivering a diverse array of functional biomolecules. Understanding their intricate composition and regulatory dynamics across species is crucial for unlocking their multifaceted contributions to health and development. This study presents a transcriptomic analysis of milk exosomes from three mammal species: human, cow, and goat. Sample collection included 12 human breast milk samples obtained from mothers who had given birth to both preterm and full-term infants, allowing for a deep dive into the temporal dynamics of exosomal RNA content within the same lactating individuals. In addition to human milk samples, 3 samples each of cow and goat milk exosomes were incorporated for comprehensive comparative analysis. Leveraging cutting-edge RNA sequencing (RNA-seq) technology, we isolated and meticulously characterized exosomes from these milk samples. Our investigation unveiled distinct transcriptional landscapes within milk exosomes of each species, comprising of protein-coding mRNAs and long non-coding RNAs (lncRNAs). Comparative analysis revealed both conserved and species-specific RNA profiles, hinting at unique functional roles across evolutionary lines. Several enriched pathways within the exosomal cargo, including those related to immune modulation, cellular growth, and development, displayed conservation across species, underscoring the fundamental role of milk exosomes in neonatal health and immune priming. Moreover, species-specific differences in exosomal RNA content may offer insights into distinct developmental and health outcomes among humans, cows, and goats. These findings significantly contribute to expanding the knowledge of transcriptomic diversity in milk exosomes across species, shedding light on exosome evolution and functional relevance, with potential applications spanning from optimizing neonatal nutrition to innovative therapeutic development

Acknowledgment

The study has been supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship Number: 05704) and by the project "The Greek Research Infrastructure for Personalised Medicine (pMedGR)" (MIS 5002802) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

P222

Bioinformatics approach to endogenous retroviruses expression detection through Nanopore sequencing

Mariana Polychronopoulou, Eleni Kyriakou, Filiana Sotiriadou, Magda Bletsa, Gkikas Magiorkinis

Medical School, National and Kapodistrian University of Athens, Greece

Retroviruses infiltrate the animal genomes either horizontally, by infecting somatic cells and leaving their genetic trace in the form of the provirus or vertically, by infecting germline cells, in which proviral integrations may lead to endogenization. In humans, endogenous retroviruses (HERVs) occupy approximately 8% of the genome. Although due to stochastic mutations HERVs have lost their ability to replicate and infect, some HERV genes encode for functional proteins which are indispensable for the human host. However, HERVs are also implicated in many pathological conditions, such as multiple types of cancer, autoimmune and neurodegenerative disorders. The most recently integrated HERV family, whose members are less degenerated by mutations, is the HERV-K HML-2 (HK2) group. Our aim is to study HK2 expression in diseases caused by exogenous viruses, such as HIV and SARS-CoV-2. Nanopore sequencing is a great tool for gene expression studies, as its main advantage is the read length that it can generate. By recovering long reads of unaltered transcripts through the Direct RNA Sequencing (DRS) protocol, we will be able to determine the length distribution of HK2-related mRNAs and assess which specific HK2 loci are expressed in a certain condition. To this end, we have sequenced native RNA of the teratocarcinoma cell line (NCCIT), where HK2 is upregulated. Our bioinformatics protocol, following the acquisition of the produced data, is aimed at mapping such long reads to the human reference genome, extracting the transcriptionally active HK2 loci and quantifying their expression levels. Standardization of this scalable protocol could be further employed to determine the transcriptome profile of endogenous retroviruses in various pathogenic conditions.

P223

Phytochemicals as bone remodelers for the development of nutraceutical for osteoporosis

Alexandros-Timotheos Loukas[1,2], Michail Papadourakis[1], Vasilis Panagiotopoulos[1,3], Apostolia Zarmpala[1], Sofia-Antigoni Tsatsouli[1], Minos-Timotheos Matsoukas[1,3], Panagiotis Zoumpoulakis[2,4]

[1]Cloudpharm PC, Monumental Plaza, Building C, Kifissias Avenue, 44, 15125 Marousi, Greece
[2]Department of Food Science and Technology, University of West Attica, Egaleo Park Campus, Agiou Spyridonos, 28, 12243 Egaleo, Greece
[3]Department of Biomedical Engineering, University of West Attica, Egaleo Park Campus, Agiou Spyridonos, 28, 12243 Egaleo, Greece
[4]Institute of Chemical Biology, National Hellenic Research Foundation, Vasileos Konstantinou, 48, 11635, Athens, Greece

Osteoporosis is becoming a serious healthcare issue due to the high morbidity, mortality and significant healthcare cost involved and is characterised by skeletal fragility and susceptibility to fractures.1 It is a metabolic bone disease occurring mostly in postmenopausal women and is caused by the disruption of the cooperative balance between osteoblasts and osteoclasts.2 The object of the proposed work is the design and development of an innovative dietary supplement for osteoporosis focusing on the selection of natural ingredients through in vitro and in silico experiments taking into account their effect on the regulation pathways of osteoclasts and osteoblasts. Mitogen-Activated Protein Kinases (MAPK) have been established as key regulators of osteoclast differentiation and activation [3] and they thus offer a promising therapeutic approach for osteoporosis. For this purpose, the inhibition potential of several classes of phytochemicals collected from open access chemical libraries was examined against five MAPK related with bone metabolism and osteoporosis focusing on p38b and ERK1. Virtual screening was performed for the selected protein targets and the top-ranked compounds were further subjected to 100 ns Molecular Dynamics (MD) simulations in triplicates to unravel the structural dynamics, conformational behavior and the stability of the protein-ligand complexes. Finally, the binding free energy of each protein-ligand complex was assessed using the Molecular Mechanics Poisson- Boltzmann surface area (MM-PBSA) method.

[1] Wong, S. K. et al. Nutrients 2016, 8 (6), 347.
[2] Zhang, W. et al. Am. J. Clin. Exp. Immunol. 2018, 7 (2), 40.
[3] Lee, K.; Seo, I.; Choi, M.H.; Jeong, D. Int. J. Mol. Sci. 2018, 19, 3004.

Acknowledgments
This research has been co financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE - INNOVATE (project code: T2EDK-03847)

P224

Meta-analysis of candidate gene association studies in Atopic Dermatitis in European and Asian population

Alexandros Pontikas[1], Charalabos Antonatos[1], Evangelos Evangelou[2,3,4,†], Yiannis Vasilopoulos[1*]

[1]Laboratory of Genetics, Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, 26504 Patras, Greece,
[2]Department of Hygiene and Epidemiology, Medical School, University of Ioannina, 45110 Ioannina, Greece,
[3]Department of Biomedical Research, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, 45510 Ioannina, Greece,
[4]Department of Epidemiology and Biostatistics, MRC Centre for Environment and Health, Imperial College London, London W2 1PG, UK,
[†]Deceased [*]e-mail: iovasilop@upatras.gr

Atopic Dermatitis (AD) is a common autoimmune inflammatory skin disorder with a lifetime prevalence of 10-20%. AD is characterized by a compromised epidermal barrier, attributed to FLG mutations, an altered skin microbiome favoring Staphylococcus aureus, and a predominantly type-2-skewed immune dysregulation. Candidate gene association studies have been pivotal in exploring the genetic predisposition of AD, but they have yielded conflicting results. Here, we conducted a random-effects meta-analysis utilizing candidate-gene approaches in patients of European and Asian ancestry. A systematic literature search was performed in the Medline database for peer-reviewed case-control candidate-gene studies, while effect sizes and standard errors were calculated for the allelic model of inheritance. Given the crucial role of FLG loss of function (LOF) mutations in AD predisposition, these LOF variants were further evaluated under a combined genotype. Statistical analyses were conducted using Stata 13.1 software, with a P-value significance level of 0.05 for meta-analysis results and 0.1 for heterogeneity, that was assessed by Harbord's Modified test. Out of 3772 studies evaluated, 99 candidate gene studies were included (53 in European populations and 46 in Asian populations) and examined 32 different polymorphisms in 17 genes in Europeans and 30 polymorphisms in 14 candidate genes in Asians. Apart from validating prior reports regarding significant associations between FLG, SPINK5, IL4, IL9, IL13 and 11q13.5 loci and AD, we report four novel, ancestry-specific loci that have not yet been identified by genome-wide scans. Particularly, IL18 rs187238 (P-value<0.001) and TGFB1 rs1800471 (P-value<0.001) variants were associated in the European population, while IL12RB1 rs393548 and rs436857 (P-value=0.017; P-value=0.015 respectively) and MIF rs755622 (P-value=0.002) showed significant hits in AD patients of Asian ancestry. By leveraging traditional candidate gene approaches, our cumulative results contribute to the complex genetic profile of AD, providing valuable insights into specific genetic variants associated with the disease in an ancestry-specific manner.

P225

Bioinformatic analysis of the mitochondrial carrier SLC25A46: evolution, mutations, interactions & structure

Nikolaos Nodarakis, Vassiliki Lila Koumandou*

Department of Biotechnology, Agricultural University of Athens, Greece

SLC25A46 is a mitochondrial outer membrane protein, encoded by a nuclear gene and consisting of 418 amino acids. Although the protein belongs to the SLC25 mitochondrial solute carrier family, its precise function has not yet been determined. It appears to play a crucial role in maintaining the balance between mitochondrial fusion and fission affecting the internal architecture of mitochondria. Mutations in the SLC25A46 gene have been identified in patients with neurological disorders, such as axonal neuropathy and optic or cerebellar atrophy. In this study, a bioinformatic analysis of the human SLC25A46 protein was performed to get further insights into its function. Specifically, the pathogenic mutations, the common polymorphisms in the human Slc25a46 gene and the post-translational modifications of the protein were recorded and mapped onto the predicted 3D protein structure. The protein interaction network of SLC25A46 and UGO1, a putative yeast ortholog, were compared, as well as the conservation of these two proteins and their interactors. Our structural analysis indicates that the N-terminus of the protein may localize to the intermembrane space and that SLC25A46 is missing two of the three conserved domains of mitochondrial transporters, suggesting that it may not function as a carrier. 27 pathogenic mutations were analyzed, causing diseases that usually develop at a young age, some of which are fatal. In addition, 18 post-translational modifications were analyzed that could affect important features of the protein. Most of the proteins interacting with SLC25A46 are located or function in the mitochondrion and play a role in mitochondrial dynamics, internal architecture and protein translocation. SLC25A46 appears to be conserved only in metazoans, whereas UGO1 is conserved in fungi. This may suggest that SLC25A46 is not truly orthologue of UGO1, but more work needs to be done to confirm the evolutionary relationship of these proteins.

P226

A novel network-based approach for studying WD40 domain sequences

Vrazas V.[1], Promponas V.J.[2], Boulougouris G.[3], Katsani K.R.[1,*]

[1]Laboratory of Biochemistry and Molecular Virology, Department of Molecular Biology and Genetics, Democritus University of Thrace, 68100 Alexandroupolis, Greece
[2]Bioinformatics Research Laboratory, Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus
[3]Laboratory of Computational Physical Chemistry, Department of Molecular Biology and Genetics, Democritus University of Thrace, 68100 Alexandroupolis, Greece

WD40 domains are central actors in biological processes acting as hubs in cellular networks, and thus are a potential drug target[1]. Despite their ubiquity in the human proteome (~1% of human proteins carry WD40 domains), they are not as well studied as other domains (e.g. PDZ or SH3)[2]. We present the development of an integrative network-based approach for reavealing common interaction patterns among human WD40 repeat-containing proteins (WDRPs).

Specifically, we compiled a comprehensive dataset of high-confidence human WDR protein sequences based on curated UniProt entries, organized by the number of their (predicted) WD40 units. In addition, we constructed an all-inclusive protein-protein interaction (PPI) network for human WDRPs from a wide wide collection of PPI databases. WDRP sequence were then all-vs-all aligned to compute a sequence similarity-based network. Alignment of these independent networks allows us to select groups of WDRPs with higher expectation of possessing common binding interfaces with (some of) their interactors.

Work in progress includes the downstream analysis of the selected WDRPs with respect to common sequence patterns (on their WD40s) which in combination with structural data (including AlphaFold predictions) can provide input patterns for building machine-/deep-learning classifiers for predicting –yet unknown– WD40 binding interfaces.

1. *Schapira, Matthieu, Mike Tyers, Maricel Torrent, and Cheryl H Arrowsmith. "WD40 Repeat Domain Proteins: A Novel Target Class?" Nature Reviews. Drug Discovery 16, no. 11 (November 2017): 773–86. https://doi.org/10.1038/nrd.2017.179.*
2. *Stirnimann, Christian U, Evangelia Petsalaki, Robert B Russell, and Christoph W Müller. "WD40 Proteins Propel Cellular Networks." Trends in Biochemical Sciences 35, no. 10 (October 2010): 565–74. https://doi.org/10.1016/j.tibs.2010.04.003.*

P227

The Notch protein family and Neurodegenerative Diseases; A mutation analysis reveals new insights in CADASIL syndrome.

Louis Papageorgiou[1,2], Lefteria Papa[1], Elias Eliopoulos[1], and Dimitrios Vlachakis[1,*]

[1]Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Athens 11855, Greece;
[2]Department of Biomedical Sciences, School of Health and Care Sciences, University of West Attica, Agioy Spyridonos, 12243, Egaleo, Greece;

Neurodegenerative diseases are incurable disorders of the central nervous system that cause progressive degeneration and/or death of brain nerve cells, affecting both mental function and movement (ataxia). The evolutionary conserved Notch signaling pathway functions as a mediator of direct cell-cell communication between neighboring cells during development. Notch plays a crucial role in various fundamental biological processes in a wide range of tissues. Accordingly, aberrant signaling of this pathway underlies multiple genetic pathologies, such as developmental syndromes, congenital disorders, neurodegenerative diseases and cancer. Over the last two decades, biological data has shown that the Notch signaling pathway displays a significant function in the mature brain of vertebrates and invertebrates, beyond neuronal development and specialization during embryonic development. Neuronal connection, synaptic plasticity, learning, and memory appear to be regulated by this pathway. Specific mutations in human Notch family proteins have been linked to several neurodegenerative diseases, including Alzheimer's disease, ischemic injury, and CADASIL. Several on-going studies are focusing to understand the molecular mechanisms by which Notch proteins play an essential role in the mature brain. In this study, an in silico analysis of polymorphisms and mutations in human Notch family members that lead to neurodegenerative diseases was performed. Particular emphasis was placed on the study of mutations in the NOTCH3 protein and the structure analysis of mutant NOTCH3 protein family, in order to identify possible conserved mutations and interpret their effect in the NOTCH3 protein structure. This leads to the manifestation of the CADASIL syndrome. Based on the results of the present study, conserved mutations of cysteine residues may be candidate pharmacological targets for potential therapeutic suggestions for the CADASIL syndrome.

Keywords
Bioinformatics, Biotechnology; Computational Biology; Genetics; Proteomics; Notch; neurodegenerative diseases; CADASIL;

P228

Characterizing the genetic architecture of psoriasis in microRNA binding sites

Foteini Papageorgiou[1], Panagiotis Xiropotamos[1], Evangelos Evangelou[2,3,4,†], Georgios K. Georgakilas[1,5], Yiannis Vasilopoulos[1,*]

[1]Laboratory of Genetics, Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, 26504 Patras, Greece,
[2]Department of Hygiene and Epidemiology, Medical School, University of Ioannina, 45110 Ioannina, Greece,
[3]Department of Epidemiology & Biostatistics, MRC Centre for Environment and Health, Imperial College London, London W2 1PG, UK,
[4]Department of Biomedical Research, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, 45510 Ioannina, Greece,
[†]Deceased,
[5]Laboratory of Hygiene and Epidemiology, Department of Clinical and Laboratory Research, Faculty of Medicine, University of Thessaly, 41222 Larissa, Greece,
[*]e-mail: iovasilop@upatras.gr

Psoriasis, a chronic inflammatory skin condition affecting approximately 2% of the global population, results from a complex interplay between genetic and environmental factors. Genetic predisposition plays a significant role, accounting for around 70% of the total variability. Genome-wide association studies (GWAS) have identified numerous single nucleotide polymorphisms (SNPs) associated with psoriasis across different populations. These SNPs are typically mapped to regulatory regions, implicated in gene expression and post-transcriptional modifications. A characteristic example includes variants in microRNA (miRNA) binding regions, which contribute to the mRNA degradation, destabilization, or translational repression of the target gene. In this study, we explored SNPs mapped in miRNA binding regions by employing the largest psoriasis GWAS in patients of European ancestry1. The genetic coordinates of the GWAS SNPs were mapped to the Grch37/hg19 genome assembly, while TargetScanHuman 8.02 was used to retrieve miRNA target sites in the reference human genome. To unveil the appropriate significance threshold in miRNA binding sites variants, we utilized various statistical thresholds and counted the number of significant hits. We reported 99 statistically significant polymorphisms associated with psoriasis across 31 genetic loci at a P-value threshold of 5 10-6. These loci encompass critical psoriasis mediators, with the exemplars of HLA-C and IL23R. Gene ontology enrichment analysis highlighted their established role in the disease pathogenesis by providing key pathways like the regulation of leukocyte-mediated immunity, by using the clusterProfiler version 4.6.23 package. These variations within miRNA binding sites may disrupt miRNA:mRNA interactions, potentially influencing disease development and progression. Future studies should investigate the functional consequences of these identified SNPs in miRNA binding sites and their precise role in the pathogenesis of psoriasis. Understanding how these genetic variations impact miRNA-mediated regulation of gene expression is crucial for unraveling the molecular mechanisms underlying psoriasis and potentially leading to the development of more targeted and effective therapies.

1 *Tsoi LC, Stuart PE, Tian C, et al. Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants. Nat Commun. 2017;8(1):15382.*
2 *McGeary SE, Lin KS, Shi CY, et al. The biochemical basis of microRNA targeting efficacy. Science. 2019;366(6472):eaav1741.*
3 *Wu T, Hu E, Xu S, et al. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. The Innovation. 2021;2(3):100141.*

P229

Deciphering TF dynamics during T-cell differentiation: Transcription factor motif identification through deep learning interpretation

Panagiotis Xiropotamos[1], Yiannis Vasilopoulos[1], Georgios K. Georgakilas[1,2*]

[1]Laboratory of Genetics, Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, 26504 Patras, Greece,
[2]Information Management Systems Institute, "ATHENA" Research and Innovation Center in Information, Communication and Knowledge Technologies
[*]e-mail: ggeorgakila@upnet.gr

Mammalian T-cell development require a differential activation of transcription factors (TFs) to establish lineage discriminating gene expression. Hematopoietic stem cells originating from the bone marrow, migrate to the thymus and undergo intensive gene regulation through a seven-stage differentiation process, mainly controlled by phase specific TFs. While some of them have been characterized as pioneer regulators from previous studies[1,2], comprehensive understanding of the dynamic TF interactions during each developmental stage remains elusive. In our study we utilized open chromatin data via ATAC-seq[3] protocol, which encompasses to regulatory regions such as enhancers and promoters, where TFs bind and regulate gene expression, on a genome-wide scale for all differentiation stages and generated a chromatin accessibility profile for each regulatory region. The chromatin profile along with the corresponding sequence and nucleotide conservation were used as input to an in-house developed Convolutional Neural Network (CNN) model, trained to distinguish the development phases where chromatin is accessible and thus TFs can bind and regulate gene expression. Our hypothesis posited that transcription factor motifs, pivotal in specific developmental stages, underlie the key distinguishing features among diverse regulatory elements and developmental stages. Interpreting the first convolutional layers and especially the filter kernels provided us with insights into what the model grasped during training, to associate DNA sequences to differentiation stages. Among the motifs derived from the interpretation module, we uncovered several TFs with an established role in T-cell development including GATA3[4] which regulates the expression of a pioneer TF, TCF-1, in early stage and CD4, CD8 receptors in the latent stages, and BCL11B[5] which is implicated to the T-cell lineage commitment. Further explanation of the knowledge acquired by the deep learning model aspects and deciphering the associations between learned motifs and developmental stages will facilitate the construction of a TF regulation atlas across the T-cell differentiation process.

1 *Hosokawa H, Rothenberg EV. How transcription factors drive choice of the T cell fate. Nat Rev Immunol. 2021;21(3):162-176. doi:10.1038/s41577-020-00426-6*
2 *Johnson JL, Georgakilas G, Petrovic J, et al. Lineage-Determining Transcription Factor TCF-1 Initiates the Epigenetic Identity of T Cells. Immunity. 2018;48(2):243-257.e10. doi:10.1016/j.immuni.2018.01.012*
3 *Buenrostro JD, Wu B, Chang HY, Greenleaf WJ. ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. Curr Protoc Mol Biol. 2015;109:21.29.1-21.29.9. Published 2015 Jan 5. doi:10.1002/0471142727.mb2129s109*
4 *Rothenberg EV. The chromatin landscape and transcription factors in T cell programming. Trends Immunol. 2014;35(5):195-204. doi:10.1016/j.it.2014.03.001*
5 *Kueh HY, Yui MA, Ng KK, et al. Asynchronous combinatorial action of four regulatory factors activates Bcl11b for T cell commitment. Nat Immunol. 2016;17(8):956-965. doi:10.1038/ni.3514*

P230

Evidence for nuclear translocation of the membrane androgen receptor, OXER1 through its interaction with karyopherins systems

Panagiotis Malamos[1], Athanasios A. Panagiotopoulos[1], Konstantina Kalyvianaki[1], Amalia Vogiatzoglou[1], Artemis Tsitsikalaki[1], Panayiotis A. Theodoropoulos[2], Christos A. Panagiotidis[3], George Notas[1], Elias Castanas[1*], Marilena Kampa[1*]

[1]Laboratory of Experimental Endocrinology, University of Crete, School of Medicine, Heraklion, Greece
[2]Department of Biochemistry, School of Medicine, University of Crete, Heraklion, Greece
[3]Laboratory of Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece
[*]e-mails: Marilena Kampa (kampam@uoc.gr), and Elias Castanas (castanas@uoc.gr)

Protein shuttling among cellular compartments has evolved in eucaryotic cells. An elegant system is responsible for the cytoplasmic-nuclear transport, involving specialized transporters named collectively karyopherins. Cargo proteins express specific motifs named Nuclear Localization Signal (NLS), responsible and necessary for the identification and the binding of importins. Until recently, few NLS motifs were recognized only for importin α (IMPOα), with an increasing number of proteins being identified to express this sequence. OXER1 is the GPCR receptor for the 5-oxo-ETE metabolic product of arachidonic acid produced by the action of 5-lipoxygenase (5-LOX) and peroxidase. It is particularly expressed in inflammatory cells, liver, kidney, spleen and pulmonary tissue, and cancer cells including prostate and breast. A few years ago we have reported that OXER1 is a membrane receptor for androgens and we found that it can also be detected to the nucleus. In attempt to decipher the role and the mechanism for its nuclear translocation we further explored this initial observation by cell fractionation, western blot and immunoprecipitation. We show that its presence in the nucleus is ligand dependent, with agonists (5-oxo-ETE) enhancing, and antagonists (testosterone) impairing nuclear localization. In addition by using a bio-informatics approach, based on bibliographic and simulation data and experimental in vitro validation we identified new recognition motifs for binding with importin 7,4 and 5. Further more, in silico and in vitro data support the association of importins with OXER1 receptor and their involvement in its transportation to the nucleus of prostate cancer cells. Our findings provide new evidence on the mechanism for the cytoplasmic-nuclear trafficking of proteins including GPCRS like OXER1, and reveal an alternative pharmaceutic target.

Bibliography

1. *Panagiotopoulos, A. et. al, Recognition motifs for importin 4 [(L)PPRS(G/P)P] and importin 5 [KP(K/Y)LV] binding, identified by bio-informatic simulation and experimental in vitro validation, Computational and Structural Biotechnology Journal. (2022) 20: 5952.*
2. *Panagiotopoulos, A. et al., The sequence [EKRKI(E/R)(K/L/R/S/T)] is a nuclear localization signal for importin 7 binding (NLS7) (2021) 5: 129851.*
3. *Kalyvianaki K. et. al, Antagonizing effects of membrane-acting androgens on the eicosanoidreceptor OXER1 in prostate cancer, Sci. Rep. (2017) 7: 44418.*

This work was partially supported by Greece and the European Union (European Social Fund- ESF) through the Operational Programme (Human Resources Development, Education and Lifelong Learning) in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY) to AP (PhD scholarship), a Special Fund for Research Grants (ELKE) of the University of Crete to MK and KK and by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: 3725 to MK).

P231

Assessment of salinity and drought-responsive pathways in rice (Oryza sativa) using meta-analysis of RNA-seq data

Maria Kampa[1], Konstantinos-Andreas Tzovaras-Mitroglou[1], Konstantinos Makropoulos[1], Elisavet Andronidou[1], Panagiota Kontou[2], Pantelis Bagos[1], Georgia Braliou[1*]

[1]Department of Computer Science and Biomedical Informatics, University of Thessaly, Lamia, Greece,
[2]Department of Mathematics, University of Thessaly, Lamia, Greece
[*]Correspondence to: gbraliou@uth.gr

Rice is the most widely consumed staple food for more than half of the world's population, particularly in Asia and Africa. Soil drought and salinity are two major threats for rice crop yields since they impose severe stress on metabolic homeostasis of the plants [1]. Identifying genes involved in drought and salinity response is critical for planning feasible agriculture strategies for rice to ensure food security and nutrition. In the present work we investigated the impact of drought- and salinity-stress on rice gene expression profiles by conducting a meta-analysis of RNA-Seq data from two major rice subspecies, Japonica and Indica. Using related keywords and abiding to PRISMA guidelines, Next Generation Sequencing (NGS) data were retrieved from Gene Expression Omnibus (GEO) public repository. Nine studies regarding seedling/shoot and seven on root tissue of two rice subspecies, encompassing case and control groups, were recorded. Four separate meta-analysis tests were conducted using the bioinformatics online tool MAGE [2] at a 0.05% significance level (FDR). Meta-analysis retrieved 829 differentially expressed genes (DEGs) for drought-, and 902 DEGs for salinity-conditioned seedling/shoot tissues. Meta-analysis of root tissue data yielded only 531 and 251 DEGs for drought and salinity conditions, respectively. Enrichment analysis using STRING, PANTHER and gProfiler tools revealed that response to drought in seedling/shoot tissues is highly related to genes involved in photosynthesis and carbon metabolism pathways mainly associated with membrane of chloroplast/organelle functions. Salinity-stress signaling mechanisms in rice seedling/shoot tissues involve response to chemical signaling, protein folding and function of highly structured proteins, as well as pathways regulating developmental processes of multicellular organisms. No enriched pathways for root tissue were identified for either stress conditions. Our results uncover possible pathways that should be experimentally further investigated for the establishment of salinity- and drought-tolerant rice genotypes.

Acknowledgement
"GENOMIC-OASIS: GENOMIC Analysis of Organisms of Agricultural and Livestock Interest in Sterea", Grant Number (MIS) 5045902.

References

[1] M. Ahmad, "Genomics and transcriptomics to protect rice (Oryza sativa. L.) from abiotic stressors:-pathways to achieving zero hunger," Front Plant Sci, vol. 13, p. 1002596, 2022.
[2] I. A. Tamposis, G. A. Manios, T. Charitou, K. E. Vennou, P. I. Kontou, and P. G. Bagos, "MAGE: An Open-Source Tool for Meta-Analysis of Gene Expression Studies," Biology (Basel), vol. 11, no. 6, p. 895, 2022.

P232

Utilizing ^{1}H NMR based metabolomics to associate metabolism alterations with COVID-19 severity.

Panagiota D. Georgiopoulou[1], Georgios Schinas[2], Styliani A. Chasapi[1], Eleni Polyzou[2], Karolina Akinosoglou[2*], Georgios A. Spyroulias[1*]

[1]Department of Pharmacy, University of Patras, 26504 Patras, Greece
[2]Department of Medicine, University of Patras, 26504 Patras, Greece
[*]Equal co-authorship

COVID-19 disease, the outcome of SARS-CoV-2 virus cellular activity, has adversely impacted on public health and global economy over the past few years. Still, it remains a persistent threat, as the number of deaths per day has not yet been eliminated. COVID-19 clinical manifestations range from asymptomatic to severe pneumonia and even acute respiratory distress syndrome (ARDS). Most of the diseased individuals recover without hospital care, while others experience mild to moderate symptoms that require hospitalization, facing the risk of death. Utilizing NMR-based untargeted analysis, we aim to characterize the metabolic response of COVID-19 patients of different clinical severity. Serum samples collected on the first day of hospitalization, from 77 patients requiring respiratory support for COVID-19 were analyzed in total. Severity was judged by the eventual need for non-invasive ventilatory (NIV) support. Multivariate statistical analysis on 1D ^{1}H NMR data was implemented to detect differences in metabolite levels between severe and non-severe groups. Our results indicated alterations in metabolic processes as reflected by elevated levels of glucose, various amino acids, 3-Hydroxybutyrate, and lipid dysregulation in the group requiring NIV. As NIV and control group revealed a distinctive profile, which is primarily attributed to glucose and lipids, we intended to examine, if these metabolic alterations, appear also within different levels of severity patients of NIV group. Consequently, the NIV group was first segmented into three subgroups, based on the values of P/F ratio which indicates different oxygen status. Also, the metabolic status was examined in relation to the outcome of the NIV patients. Indeed, the metabolites characteristic of the NIV group exhibited varying levels among these subgroups, reflecting the association of metabolic profile with the disease severity and outcome.

Acknowledgments
We acknowledge support of this work by the R-NMR project, funded from the European Union's Horizon Europe research and innovation programme (2021-2027), under grant agreement 101058595.

P233

Unveiling the Metabolomic Profile of Growth Hormone Deficient Children Using NMR Spectroscopy

Eftychia A. Aggelaki[1], Alexandra Eythymiadou[2], Panagiota D. Georgiopoulou[1], Styliani A. Chasapi[1], Aristeidis Giannakopoulos[2], Dionysios Chrysis[2*], Georgios A. Spyroulias[1*]

[1]Department of Pharmacy, School of Health Sciences, University of Patras, 26504, Rio, Greece
[2]Division of Endocrinology Department of Pediatrics, Medical School, University of Patras, 26504, Rio , Greece
[*]Corresponding authors: Dionysios Chrysis (dchrysis@upatras.gr) and Georgios A. Spyroulias (G.A.Spyroulias@upatras.gr)

Growth Hormone Deficiency (GHD) is a disorder caused by inadequate synthesis and/or secretion of growth hormone (GH) from the anterior pituitary gland. GHD, approximately affects one in every 4.000 to 10.000 children and includes a group of disorders with various etiologies. Until the present moment, diagnosis of the disease is characterized as a complicated procedure based on laboratory and invasive tests, without ideal reproducibility and sensitivity. Hence, there is a need for new biomarkers discovery, which will be able to establish a valid diagnostic signature for GHD. Metabolomics is considered one of the most developing fields of omic-sciences over the last years. It is an ideal approach for in-depth understanding of biological mechanism of diseases, specifically in combination with Nuclear Magnetic Resonance (NMR) spectroscopy, which has been proven a powerful analytical technique for characterizing complex biological samples. This work aims to explore serum and plasma metabolomic profiles of growth hormone deficient children by NMR based metabolomics and to investigate the metabolic differences 3 months after the initiation of treatment with recombinant growth hormone. Experimental data were processed by both multivariate and univariate statistical analysis. The results of this pilot study revealed a clear different metabolic fingerprint of children with GHD in comparison to age matched healthy individuals. However, the detected alterations in the metabolite patterns before and after GH treatment exhibited relatively smooth and of minor discriminative statistical power.

Acknowledgments
R-NMR project, funded from the European Union's Horizon Europe research and innovation programme (2021-2027), under grant agreement 101058595.

P234

Exploring viral Sirtuins as key drivers of continuous evolution across diverse domains of life: An in silico evolutionary study of Sirtuin family

Dimitrios Skliros[1*+], Louis Papageorgiou[2,3+], Athina Marougka[2], Dimitrios Vlachakis[2], Emmanouil Flemetakis[1*]

[1]Laboratory of Molecular Biology, Department of Biotechnology, Agricultural University of Athens, Athens, Greece;
[2]Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Athens, Greece;
[3]Department of Biomedical Sciences, School of Health and Care Sciences, University of West Attica, Egaleo, Greece
[+]Authors that equally contributed to the study
[*]Authors to whom any correspondence should be addressed.
DS: dskliros@aua.gr, EF: mflem@aua.gr
Agricultural University of Athens, Iera Odos street, 75, 11855, Votanikos, Athens, Greece

Post-translational modifications (PTMs) of proteins are highly prevalent across all domains of life. Sirtuins are primarily recognized as protein deacylating enzymes capable of utilizing acyl-lysine as a substrate, which is commonly found in critical proteins like histones and acyl-coenzyme synthetases. They are known to be NAD+ dependent, emphasizing the crucial role of NAD+ as a co-substrate for their function. The Sirtuin family have been identified in various taxa in the tree of life, but also in several viruses, including bacteriophages. Their functions span across a wide range of biological processes related to energy production and utilization, including fatty acid metabolism, the tricarboxylic acid (TCA) cycle, glycolysis, scavenging of reactive oxygen species (ROS), oxidative phosphorylation, and regulation of the urea cycle. Furthermore, their subcellular localization varies. Bacteriophages are known to possess molecular tools that serve their self-interest by manipulating the metabolic machinery of bacterial cells, enabling quicker and more efficient replication. In the past, bacteriophages have been shown to carry PTM-related tools. More than 100 viral genomes belonging to the classes of Caudoviricetes and Megaviricetes have been identified carrying Sirtuins, as per GenBank records. The intriguing presence of Sirtuins in viruses with large genome sizes and their genomic and mitochondrial localization in photosynthetic organisms and animals has prompted us to investigate evolutionary events among prokaryotes and eukaryotes, considering their common domain structure and relevance to cellular energy availability. Various evolutionary scenarios were formulated and explored with selected representatives, yielding insights into their evolution, function, and acquisition events. The results indicate specific acquisition events involving viral proteins that play a pivotal role in their evolution. This study lays the groundwork for a comprehensive evolutionary analysis of all known Sirtuins, including detailed classification and the identification of structural 'hotspots' directly related to their function.

Keywords: *sirtuin family; viral Sirtuins; evolution; phylogenetic analyses; classification; bioinformatics, genetics; proteomics;*

P235

Urine metabolome profiling in CKD patients using NMR spectroscopy.

Effrosini Papazacharia[1], Styliani A. Chasapi[1], Panagiota D. Georgiopoulou[1], Sotiris S. Vamvakas[2], Dimitra Kalavrizioti[2], Dimitrios S. Goumenos[2], Georgios A. Spyroulias[1*]

[1]Department of Pharmacy, University of Patras, GR-26504, Patras Greece
[2]Nephrology and Transplant Center, University General Hospital, Patras Greece
[*]email:G.A.Spyroulias@upatras.gr

Chronic kidney disease (CKD) is considered a significant public health problem, characterized by progressive loss of renal function, leading to end-stage kidney failure. CKD is divided into five stages according to the estimated glomerular filtration rate (eGFR), which is calculated from serum creatinine or urinary albumin excretion. The complex pathogenesis of CKD and its influence by both genetic and environmental factors, complicates the understanding of the underlying pathomechanisms and optimal patient treatment [1]. Also, the estimation of GFR has limitations, due to its influence by biological factors such as age, sex and muscle mass. In that context, metabolome profiling, can provide information to examine the biochemical background, establish prompt and accurate diagnosis and treatment of CKD [2]. After conducting untargeted NMR-based analysis, our aim was to characterize the metabolic profile of patients with chronic glomerulonephritis and capture the course of the patients' urine metabolic profile, as a function of time. Thirty-nine patients, diagnosed with chronic glomerulonephritis and admitted to the General University Hospital of Patras, were divided into five groups, depending on the stage of the disease. NMR data from urine samples of the participants, collected at various time points, underwent numerical transformation. Subsequently, multivariate statistical analysis was carried out to identify metabolites exhibiting significant differences among the five groups. The initial results demonstrate a significant impact of glucose urine excretion for the 2nd CKD stage group.

References

[1] Schultheiss, U. T., Kosch, R., Kotsis, F., Altenbuchinger, M., & Zacharias, H. U. (2021). Chronic kidney disease cohort studies: A guide to metabolome analyses. Metabolites, 11(7), 460
[2] Chasapi, S. A., Karagkouni, E., Kalavrizioti, D., Vamvakas, S., Zompra, A., Takis, P. G., Goumenos, D. S. & Spyroulias, G. A. (2022). NMR-based metabolomics in differential diagnosis of chronic kidney disease (CKD) subtypes. Metabolites, 12(6), 490.

Acknowledgments

R-NMR project, funded from the European Union's Horizon Europe research and innovation programme (2021-2027), under grant agreement 101058595.

P236

Analyzing the microbial community diversity of 10 different terroirs of the Drama region by shotgun NGS

Konstantinos Tegopoulos[1*], Anastasios Nikolaou[1], Anastasia Voumvouraki[1], Christos Stekas[1], Ioanna Christoforou[1], Kisara Giolena[1], George Skavdis[1], Yannis Kourkoutas[1], Petros Kolovos[1], Maria Grigoriou[1]

Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, Greece

The French term "terroir," is widely used to convey the uniqueness of the wine of a region reflecting both the impact of abiotic factors (soil, climate, etc.) as well as human factors (cultivation practices, wine making tradition, etc.). In the past decade a number of studies have pointed to another factor, namely the microbial community of the grapes (known also as the "microbial terroir"), as a factor with a significant role in both shaping the quality of the wine as well as in providing source for characterizing novel strains that can be used to produce wines with unique organoleptic characteristics. During the implementation of the "DRAMA terroir" project a variety of methods was used to identify different terroirs within the Drama prefecture, one of the most important wine producing areas in Greece. In this work we have studied the microbial community diversity in 10 terroirs using shotgun metagenomics: we developed a comprehensive (meta)genomics methodology that encompasses shotgun sequencing on the ION Torrent platform, followed by bioinformatic analysis for the identification of microbial communities' structure. Furthermore, we have isolated novel yeast strains from these terroirs that will be further evaluated in experimental fermentations.

P237

Clustering of eukaryotic genes in one and three dimensions reveals distinct genomic territories with characteristic functional and regulatory properties

Athanasia Stavropoulou[1,2], Michalis Georgoulopoulos[1], Antonis Klonizakis[3], Vassiliki Varamogianni-Mamatsi[4], Argyris Papantonis[4], Christoforos Nikolaou[1]

[1]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Athens, Vari, Greece
[2]Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center "Alexander Fleming", Athens, Vari, Greece
[3]Centre for Genome Regulation (CRG), Barcelona, Catalunya, Spain
[4]Institute of Pathology, University Medical Center Göttingen, 37075, Göttingen, Germany

A number of studies have pointed out spatial associations of genes at various levels including co-expression, epigenetic modifications and functional properties. In this work we build on previous results from our lab[1,2] and present a versatile, robust and powerful computational framework that enables the discovery of hitherto unknown spatial-functional preferences in the genomes of eukaryotes.

We have devised two distinct strategies for the assessment of gene clustering that may be applied on any given categorization of genes. At the linear level we employ a permutation test strategy to evaluate gene clustering based on the linear distances between consecutive genes of the same type. Starting from a given subset S of genes, the pipeline returns a set of gene subclusters, which enclose a significantly higher proportion of S than expected by chance. At the three-dimensional level, we make use of either publicly available three-dimensional genome models, or we proceed to create our own using Hi-C data and the implementation of Chrom3D[3]. Genes of a given subset S are mapped onto randomly sampled 3D-spheres and spheres with a statistically significant proportion of S genes are merged into three-dimensional clusters. Downstream analyses further enable the detailed description of the discovered self-contained gene clusters in terms of function, regulation, spatial and radial positioning etc.

We have applied both the linear and 3D approaches on a large compendium of yeast gene categorizations which include transcription factor gene targets, gene ontology terms, histone modifications and co-expression patterns. We have also implemented our 3D approach on a multi-omics profiling of the process of trans-differentiation of human macrophages into B-cells. We showcase our methods' ability to identify gene co-expression domains in yeast and to monitor changes in the radial positioning of key deregulated genes in human cell trans-differentiation.

Bibliography

1. *Tsochatzidou M, Malliarou M, Papanikolaou N, Roca J and Nikolaou C. (2017). Genome urbanization: Clusters of topologically co-regulated genes delineate functional compartments in the genome of S. cerevisiae. Nucleic Acids Research, 45 (10), 5818-5828.*
2. *Stavropoulou A, Tassios E, Kalyva M, Georgoulopoulos M, Vakirlis N, Iliopoulos I and Nikolaou C. (2022). Distinct chromosomal "niches" in the genome of Saccharomyces cerevisiae provide the background for genomic innovation and shape the fate of gene duplicates, NAR Genomics and Bioinformatics, 4 (4): lqac086.*
3. *Paulsen, J., Sekelja, M., Oldenburg, A.R. et al. Chrom3D: three-dimensional genome modeling from Hi-C and nuclear lamin-genome contacts. Genome Biol 18, 21 (2017).*

P238

Kinetoplastea PX domain containing proteins: an in-silico study uncovering unique architectures and evolutionary conservation

Marina Petsana[1,2], Ahmed F Roumia[1,3], Pantelis G Bagos[1], Haralabia Boleti[2*], Georgia G Braliou[1*]

[1]Department of Computer Science and Biomedical Informatics, University of Thessaly, Lamia, Greece.
[2]Intracellular Parasitism Laboratory, Department of Microbiology, Hellenic Pasteur Institute, Athens, Greece.
[3]Department of Agricultural Biochemistry, Faculty of Agriculture, Menoufia University, Egypt.

Kinetoplastea are flagellated ancient eukaryotes encompassing known human pathogens, such as Leishmania and Trypanosoma. To exert their virulence, these parasites undergo extensive cytoskeleton and membrane remodeling. Eucaryotic phosphoinositides play pivotal roles in membrane reformation processes, protein transport, sorting, and signal transduction. PX domain is a key feature of proteins that bind to phosphoinositide (PI) phospholipids. PX domain structures of various organisms have been solved, revealing a conserved structural organization comprising three alpha helices and three beta sheets. Recently, PX proteins in eukaryotic parasites have gained attention as promising drug target candidates. The objective of this study entails retrieval of all PX domain containing Kinetoplastea protein sequences available, evolutionary relationship analysis, identification of structural characteristics and conservation traits. Based on PFam's pHMM for PX domain (PF00787) and using both the HMMER (v.3.3) packet in Linux environment and UniProt (v.2021_03), a total of 149 protein sequences were retrieved from two different searches. These 149 sequences were aligned and subsequently used to construct a novel Kinetoplastea_PX pHMM. This comprehensive search process led to the retrieval of 170 PX protein sequences. Sequence alignment and phylogenetic analysis, in combination with structural insights unveiled three additional distinct structural domains. The organization of these domains within proteins led to the identification of five distinct subfamilies of PX domain containing proteins that embrace: a) one PX, b) two PX, c) PX and Pkinase, d) PX, Pkinase and Lipocalin_5, and e) PX and Vps5/BAR3-WASP domains. Their evolutionary relationship can help predict structural components of yet unidentified Kinetoplastea proteins. A high level of conservation of PX domains in residues crucial for PtdIns3P recognition between Kinetoplastea spp. and Homo sapiens was also observed. Our findings provide valuable insights into the evolutionary relationships and structural characteristics of these proteins laying the ground for potential anti-parasitic drug development.

Acknowledgements
We acknowledge support for this work by the project "GENOMIC-OASIS: GENOMIC Analysis of Organisms of Agricultural and Livestock Interest in Sterea", Grant Number (MIS) 5045902.

P239

Genomic investigation and taxonomic analysis of the alleged alien mummies in Mexico

Paraskevi-Maria Moysi[1], Maria Papavasileiou[2],Christina Pertsali[3], Fotios Tsetsos[1]

[1]Department of Food Science and Nutrition, University of the Aegean, Myrina, Greece
[2]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupoli, Greece
[3]Department of Medicine, University of Ioannina, Ioannina, Greece

In a startling revelation, researchers affiliated with the Mexican government have unearthed a cache of mysterious mummies referred to as "alien mummies", because of their seemingly extraterrestrial features. These enigmatic specimens, discovered beneath the Mexican soil, have ignited fervent debates in the scientific and layman sphere. In this study, we employ state-of-art genomic analysis methods for ancient DNA to explore the genomic components of the mummies. The samples were sequenced on the Illumina HiSEQX10, using 150bp paired-end read sequencing. We analyzed the data using established analytic pipelines for ancient DNA (EAGER, ATLAS), while also employing our in-house ancient DNA pipeline. Taxonomic analysis was performed using Kraken2, while also employing BLAST. The samples presented with severe contamination, hampering the process to distinguish authentic biological material from extraneous contaminants. In two mummies the human derived material amounts to 3-5% of the total genomic content, while in one of them the human-derived material reaches up to 30%. We present a comprehensive genomic investigation and taxonomic analysis of these mysterious specimens, employing an innovative approach rooted in toric geometry. By integrating advanced sequencing technologies and computational methods, we elucidate the genetic composition of the alleged alien mummies, exploring their relationship with terrestrial life forms. Finally, the study contributes to the broader scientific discourse on ancient DNA and population genetic analysis while delving into the realm of unconventional taxonomic categorization.

P240

Cutting edge insights into extracellular vesicle engineering; Seeing is believing

Sotiris Ouzounis[1], **Theodora Katsila**[1, *]

[1]*National Hellenic Research Foundation, Athens, Greece*

Extracellular vesicles (EVs) are nanoscale entities that are considered circulating translational biomarkers of choice when inter-individual variability, drug resistance, and adverse drug reactions are considered, while they are becoming key actors in bio-engineering and biomaterials science. Several microscopy approaches have been employed to study EVs, each with advantages and disadvantages, while a gold-standard method is still missing. To that end, standardization upon high-yield EV manufacturing remains a key challenge.

To enable the democratization of EV applications, herein, we introduce a multitask deep learning model for the optimal segmentation of EVs in publicly available transmission electron microscopy (TEM) images. We aim to overcome current limitations such as the error of the predicted masks, which affects all other measurements and provide a unified and robust solution for the automated detection, quantification, and characterization of EVs in TEM images.

The proposed multitask deep learning framework utilizes a total of 260 TEM images of more than 2,500 annotated EVs from publicly available data used in previous studies. Data augmentation is implemented for data enrichment, empowering the training and generalization abilities of the model. The proposed methodology is based on instance segmentation and the Mask R-CNN model. The backbone of the Mask R-CNN follows a dilated convolution scheme and residual attention blocks are added to solve the information loss problem. The network has five output heads: a) mask generator; b) bounding box generator; c) classification head; d) structural annotation generator; and e) annotation score head. The model is designed to discriminate, count and annotate both EVs and none EVs per image.

The proposed end-to-end deep learning framework enables the fully automated annotation and analysis of EVs in TEM images. Application wise, our model empowers digital biomarker identification, tracking in EV-based delivery systems and profiling the kinetics of bioengineered EVs.

Acknowledgements
The work is supported by the Key Digital Technologies Joint Undertaking and co-funded by the European Union under Grant Agreement nº 101112347 (NerveRepack). We acknowledge PRACE for awarding access to the Fenix Infrastructure resources at CINECA and JURECA hosting sites, which are partially funded from the European Union's Horizon 2020 research and innovation program through the ICEI project under the grant agreement No. 800858.

P241

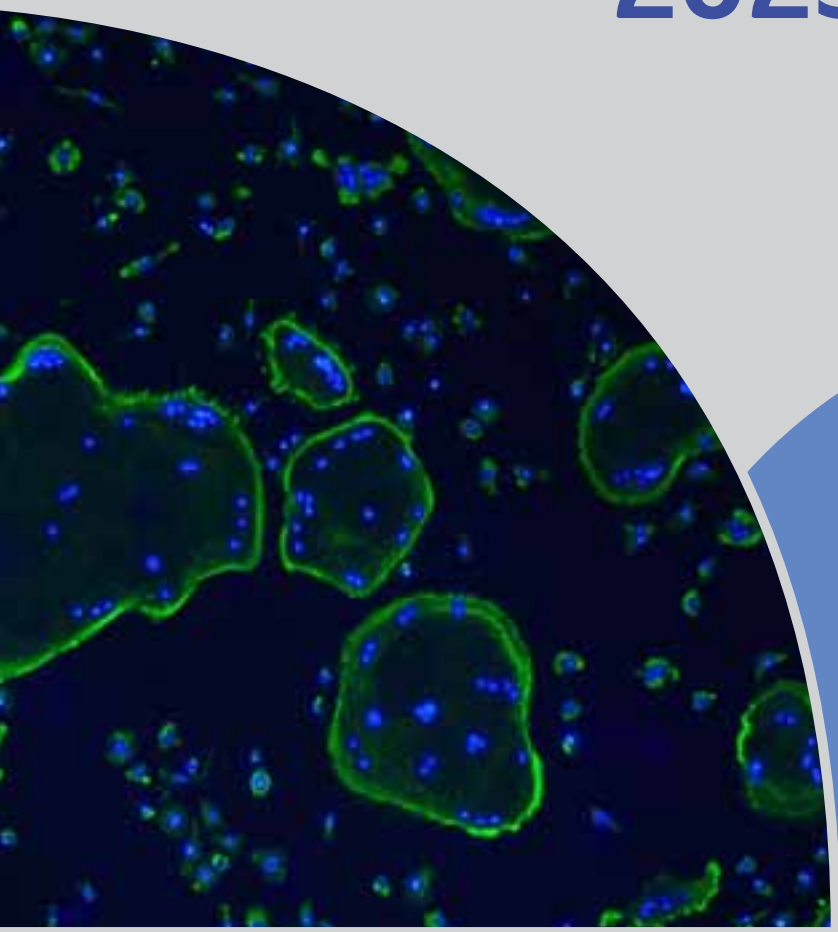
VaccineDesigner: A Web application for Integrative Epitope-based Vaccine Design

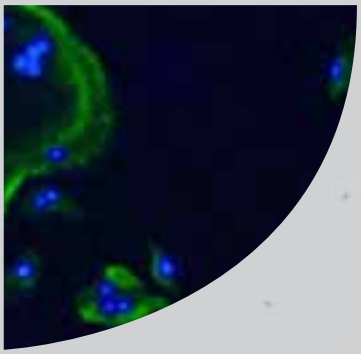
Dimitrios Trygoniaris[1,*], Anna Korda[2], Georgios Tzimagiorgis[1], Minas Yiangou[2], Charalampos Kotzamanidis[3], Andigoni Malousi[1,*]

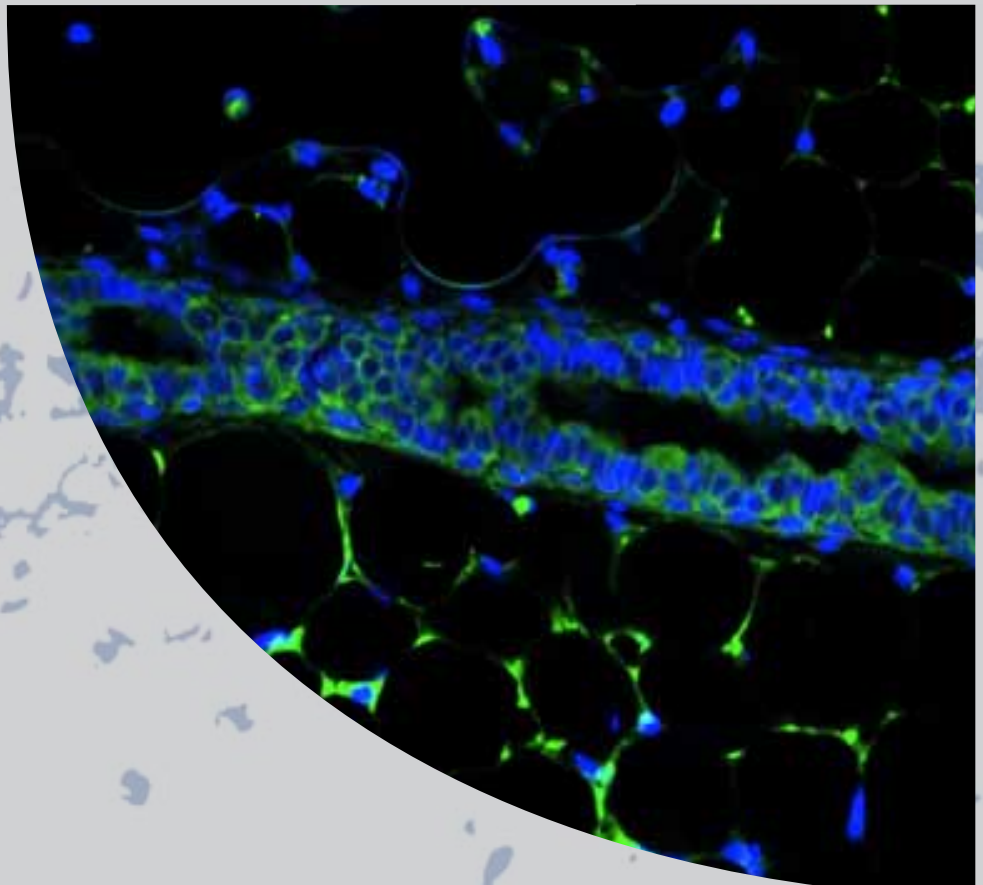
[1]Lab of Biological Chemistry, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece
[2]School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]Veterinary Research Institute of Thessaloniki, Campus of Thermi, Greece

Vaccination, the cornerstone of preventive medicine, has historically transformed global health care, reduced disease burden, and saved lives. Conventional vaccine development relies on live attenuated pathogens, inactivated agents, subunit formulations, or recombinant protein antigens, facing challenges such as pathogen cultivation and safety concerns. Epitope-based vaccine design presents a promising alternative from conventional methods, focusing on selected antigenic epitopes and molecular fragments that may activate appropriate immune responses. These epitopes encompass B-cell epitopes for humoral responses, cytotoxic T lymphocyte epitopes for cellular immunity, and helper T lymphocyte epitopes for immune regulation. The computational workflow for epitope-based vaccine design incorporates software applications starting with the prediction of B-cell/CTL and HTL epitope sequences based on a user-defined protein sequence. These computational predictions are filtered based on quality criteria including epitope toxicity, antigenicity and allergenicity, aiming to construct a multi-epitope vaccine sequence library. Epitopes with the desired properties, are linked with linker sequences to form a multi-epitope vaccine sequence. Various combinations of epitopes result in the creation a set of high-quality vaccine sequences, and this compilation serves as a library. To be deemed as potential vaccine candidates, the elements within this library need to be evaluated based on specific properties. VaccineDesigner addresses this complexity by providing a user-friendly platform that integrates B-cell, CTL, and HTL epitope prediction and evaluation methods, along with a methodology for epitope combinations and the construction of a library with multiple multi-epitope vaccine sequences. Its modular architecture enables the vaccine sequences formation from combination of epitopes of different sequences. The Web-based interface facilitates integrative analyses, empowering researchers, from novices to experts, for fast, cost-effective, and rationalized multi-epitope vaccine design. Availability: http://155.207.86.162:3838/vaccineDesigner

HSBMB 2023







HSBMB