SEBBM'18
SANTANDER

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41 Congreso de la Sociedad Española de Bioquímica y Biología Molecular

10 - 13 septiembre 2018

ORGANIZA:

SEBBM

LIBRO DE RESÚMENES

2

CONTENIDOS

BIENVENIDA

Queridas amigas, queridos amigos:

En nombre del Comité Organizador os damos la bienvenida y os animamos a participar activamente en el 41 Congreso de la SEBBM. La sede, el Hotel Santemar ****, está situada en el Sardinero, un lugar privilegiado dentro de la ciudad. El acto inaugural (lunes 10 de septiembre, a las 18:30 h) contará con la presencia del Prof. Miguel Beato del Rosal. El congreso terminará con la conferencia de clausura a cargo del Prof. David Sherratt, de la Universidad de Oxford (jueves 13 a las 12:30 h), sobre el mantenimiento del cromosoma bacteriano.

Durante los dos días y medio del congreso, disfrutaremos de un amplio abanico de Conferencias Plenarias, Simposios, Reuniones de Grupo y Sesiones de poster. Podremos también disfrutar con las actividades satélite, como el Curso de Iniciación a la Investigación en Bioquímica y Biología Molecular, el Foro del emprendedor, la Reunión de Coordinadores de Grado, la Bioquímica en la Ciudad, y los Talleres. Animamos también a los congresistas a interesarse por los stands de las empresas patrocinadoras. Desde aquí nos gustaría agradecer a estas empresas por su constante apoyo, sin el cual este Congreso no podría celebrarse.

Fernando de la Cruz Calahorra
Presidente del Comité Organizador

Félix M. Goñi
Presidente de la SEBBM

SEBBM
Sociedad Española de Bioquímica y Biología Molecular

D. Federico Mayor Zaragoza

Será nombrado Presidente de Honor de la SEBBM en reconocimiento a su excepcional labor de apoyo a la Sociedad, así como a su incansable promoción de la investigación española en Bioquímica y Biología Molecular. El acto tendrá lugar el Jueves 13 de Septiembre, en la Ceremonia de Clausura del 41 Congreso de la SEBBM

BIENVENIDA

Nuestro agradecimiento a las entidades públicas y privadas que han colaborado económicamente en la realización del **41 Congreso de la SEBBM**

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UC UNIVERSIDAD DE CANTABRIA

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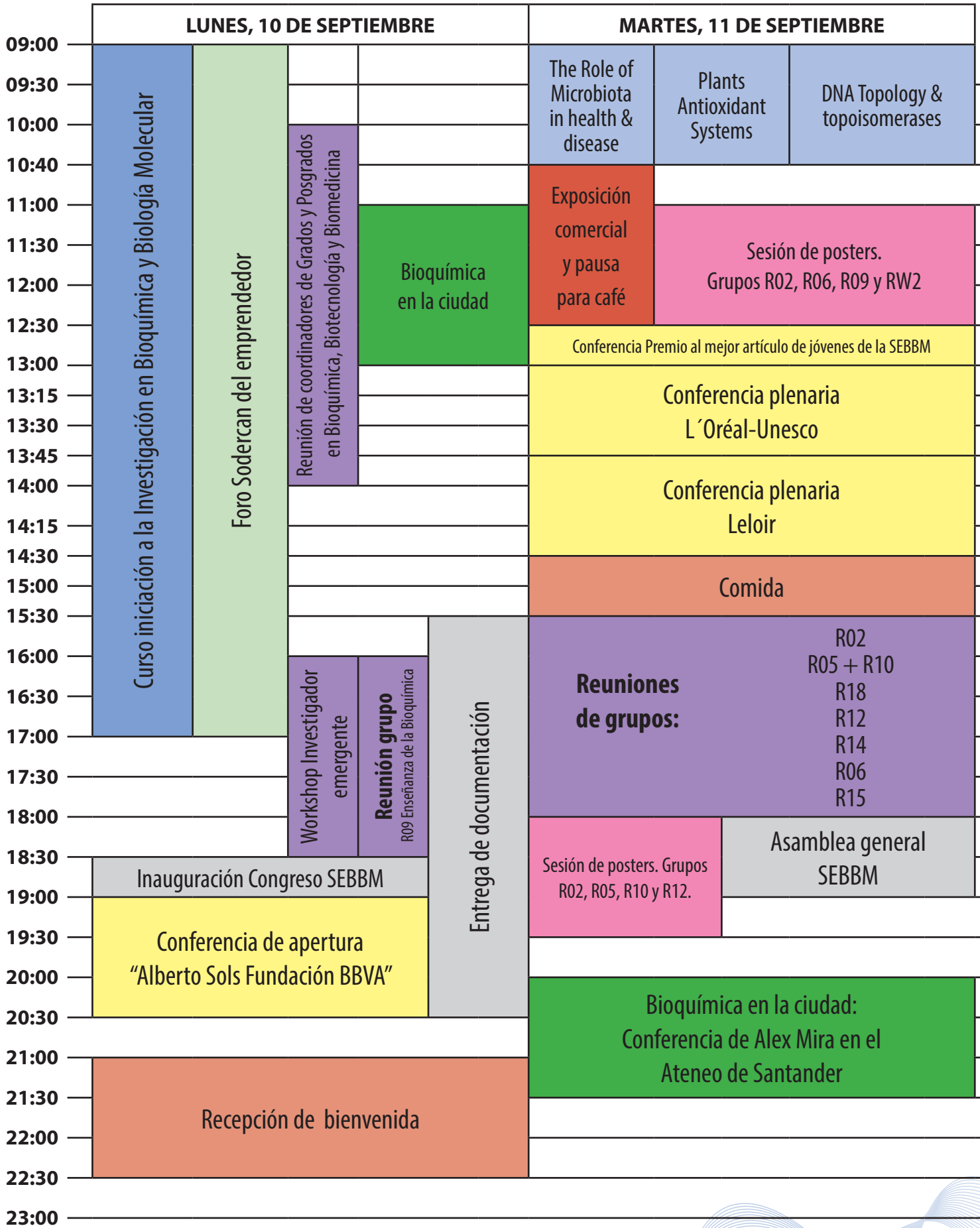
SEBBM'18 SANTANDER

PROGRAMA GENERAL



11:00 a 13:00 y 18:00 a 20:00 Bioquímica en la ciudad: Exposición
"Las moléculas que nos comemos" en sala de exposiciones Universidad de la Escuela Técnica Superior de Náutica de la Universidad de Cantabria

MIÉRCOLES, 12 DE SEPTIEMBRE

- Cellular Senescence in physiology and Pathology
- MicroRNAs in health & disease
- Genomic Engineering with CRISPR
- Exposición comercial y pausa para café
- Sesión de posters. Grupos R03, R04, R14, R15, R16 y R18
- Conferencia Joven Investigador SEBBM
- Conferencia Científico Margarita Lorenzo (Fundación Lilly)
- Conferencia plenaria Niemeyer
- Comida
- **Reuniones de grupos:** R19, R08, R04 + R11, R17, R03, R01, R16
- Sesión de posters. Grupos R07, R08, R11, R13 y R17
- Bioquímica en la ciudad: Conferencia de Lluis Montoliú en IES Santa Clara
- Cena del Congreso

JUEVES, 13 DE SEPTIEMBRE

- Cancer Genomics
- Topological Control of Gene Expression
- Nanomaterials for biomedical applications
- Exposición comercial y pausa para café
- Sesión de posters. Grupos R02 y R19
- Conferencia de clausura Fundación Ramón Areces
- Entrega de premios. Homenaje al Profesor Ortíz Melón
- Nombramiento del Prof. D. Federico Mayor Zaragoza como Presidente de Honor de la SEBBM y Clausura del 41 Congreso de la SEBBM
- Bioquímica en la ciudad: en Enclave Pronillo

09:00 · 09:30 · 10:00 · 10:40 · 11:00 · 11:30 · 12:00 · 12:30 · 13:00 · 13:15 · 13:30 · 13:45 · 14:00 · 14:15 · 14:30 · 15:00 · 15:30 · 16:00 · 16:30 · 17:00 · 17:30 · 18:00 · 18:30 · 19:00 · 19:30 · 20:00 · 20:30 · 21:00 · 21:30 · 22:00 · 22:30 · 23:00

11:00 a 13:00 y 18:00 a 20:00 Bioquímica en la ciudad: Exposición
"Las moléculas que nos comemos" en sala de exposiciones Universidad de la Escuela Técnica Superior de Náutica de la Universidad de Cantabria

CONFERENCIAS PLENARIAS

Conferencia de apertura "Alberto Sols-Fundación BBVA"

0384

Unexpected complexity in hormonal gene regulation

Miguel Beato

CRG, BARCELONA

Steroid hormones regulate gene expression in cells equippe with the appropriate receptors. While studying this process in breast cancer cells we found in the eighties that the hormone receptors act as ligand activated transcription factors that bind to specific DNA sequences (hormone response elements or HREs) in the vicinity of regulated genes (Scheidereit et al Nature 1984). HREs are recognized by the receptors even when they are included in nucleosomes, provided they are properly exposed in the surface of the histone octamer (Piña et al Cell 1990). Later, we found that a small proportion of the receptors are attached to the cell membrane via palmitoylation and upon hormone binding activate kinases, which phosphorylate the receptors and accompany them to the genome where they participate in chromatin remodeling (Vicent et al MolCell 20066). Analyzing this process, we discovered its unexpected complexity. The receptors use an extensive enzymatic machinery for modifying chromatin to enable changes in gene expression. In addition to multiple modifications of the core and linker histones, and several ATP-dependent chromatin remodeling multisubunit enzymes, gene regulation requires the synthesis of polyADP-Ribose (PAR) for the displacement of histone H1 (Wright et al GenesDev 2012). Not only PAR needs to be synthesized by PARP1, but its subsequent hydrolysis to ADP-Ribose (ADPR) by PARG is also required for a proper hormonal response. This led us to discover that the pyrophosphorylase NUDIX5 in the presence of pyrophosphate can convert ADPR to ATP in the cell nucleus (Wright et al Science 2016). In fact, this enzymatic reaction is essential for the proliferation of breast cancer cells in response to hormone and for the repair of DNA damage. Thus, NUDIX5 is an attractive target for cancer management. Another pot-translational histone modification that influences de hormonal response is the deimination of arginine and its conversion to citrulline, which weakens the histone-DNA interaction. While studying this reaction that is catalyzed by the PADI (Peptidyl Arginine De-Iminases) enzymes, we discovered that PADI2 deiminiates arginine 1810 in the C-terminal domain of RNA Polymerase II. This modification is essential for transcriptional elongation and for the proliferation of breast cancer cells in response to hormones. For the above studies, we used cells synchronized in G1 by serum deprivation. Exploring the effect of this condition, we found that serum deprivation remodels chromatin architecture by inducing the binding of the architectural protein TFIIIC to Alu/SINE repeats. Alu-bound TFIIIC interacts with distant CTCF, another architectural protein, bound near cell cycle regulating genes. This interaction is essential for the initiation of the cell growth upon re-exposure to serum. These three examples show how exploring in depth a particular cell response can lead to the discovery of unexpected basic mechanisms of cell function that have a much wider general function.

Conferencia Premio al mejor artículo de jóvenes de la SEBBM

0354

The spindle-stabilizing function of Cdc14 is required to promote recombinational DNA repair

Facundo Ramos, María Teresa Villoria, Encarnación Dueñas, Peter Faull, Pedro Rodríguez Cutillas, Andrés Clemente-Blanco

Instituto de Biología Funcional y Genómica (IBFG), Salamanca. España. Cell Cycle and Genome Stability Group

Endogenous metabolic products, such as reactive oxygen species, and exogenous genotoxic stress constantly assault the genetic material of cells. In response to these sources of DNA damage, eukaryotic cells have developed a coordinated signalling network known as the DDR (DNA damage response), which coordinates cell cycle progression with DNA repair. While the biochemical mechanisms behind this response are well understood, little is known about the spatial regulation of the components involved in the DDR and specifically in DNA repair. Recently, it has been demonstrated that DNA double-strand breaks (DSBs) are re-localized from the nucleoplasm to the nuclear periphery [1]. However, whether the nuclear periphery harbours an environment that is permissive for DNA repair and its implications in maintaining genome integrity is a subject that remains unclear. Strikingly, we have demonstrated that the serine/threonine phosphatase Cdc14 is involved in DNA repair by controlling the tethering of a DNA lesion into the spindle pole body (SPB) proximity in the budding yeast [2]. This function is attained by preserving the integrity of the metaphase spindle which is crucial to stimulate DSB-SPB interaction and DNA repair. Accordingly, disruption of the spindle stability impairs both DSB-SPB interaction and DNA repair by homologous recombination [2]. These observations directly connect spindle integrity with DNA repair and reveal that DSBs are preferentially tethered to the SPBs to be restored. Importantly, this new function of Cdc14 could provide a physiological mechanism that spatially regulates the DDR and therefore the fate of the DNA repair process.

1.Horigome C, Oma Y, Konishi T, Schmid R, Marcomini I, Hauer MH, Dion V, Harata M, Gasser SM: SWR1 and INO80 chromatin remodelers contribute to DNA double-strand break perinuclear anchorage site choice. Mol Cell 2014, 55:626-639.
2.Villoria MT, Ramos F, Duenas E, Faull P, Cutillas PR, Clemente-Blanco A: Stabilization of the metaphase spindle by Cdc14 is required for recombinational DNA repair. EMBO J 2017, 36:79-101.

Conferencia plenaria L'oréal-UNESCO For Women in Science

0387

The Balbiani Body: A super-organelle held together by amyloid-like assembly

Elvan Boke

CENTRE FOR GENOMIC REGULATION (CRG) BARCELONA

Most vertebrate oocytes contain a Balbiani body, a large, non-membrane-bound compartment packed with RNA, mitochondria, ER and Golgi. Little is known about this compartment, though it specifies germline identity in many non-mammalian vertebrates. It is recently shown by our laboratory that the Balbiani body is held together by a physiological amyloid network, which disperses upon maturation in Xenopus laevis oocytes. Velo1, a disordered protein with an N-terminal prion-like domain, is an abundant constituent of Xenopus Balbiani bodies. Disruption of the prion-like domain of Velo1, or substitution with a prion-like domain from an unrelated protein, interferes with its incorporation into Balbiani bodies in vivo. Recombinant Velo1 forms amyloid-like networks in vitro. Amyloid-like assemblies of Velo1 recruit both RNA and mitochondria in cell-free assays. We proposed that Xenopus Balbiani bodies form by amyloid-like assembly of Velo1, accompanied by co-recruitment of mitochondria and RNA. However, how the Balbiani body disassembles, and how the amyloid network of Velo1 gets solubilised remain unknown. I will present recent unpublished data from my group on different factors involved in Velo1 solubilisation and Balbiani body disassembly. Prion-like domains are found in germ plasm organizing proteins in other species, suggesting that Balbiani body formation, and disassembly could be a conserved mechanism that helps oocytes function as long-lived germ cells. We also expect that understanding the disassembly behaviour of physiological amyloids could be useful to give clues for the behaviour of pathological forms that are associated with devastating diseases

Conferencia plenaria Leloir

0388

MOLECULAR MECHANISM INVOLVED IN POLAR GROWTH AND ROS-HOMEOSTASIS IN SINGLE PLANTS CELLS IN ARABIDOPSIS

José Manuel Estévez

FUNDACIÓN INSTITUTO LELOIR AND INSTITUTO DE INVESTIGACIONES BIOQUÍMICAS DE BUENOS AIRES

Tip-growing root hairs are excellent model systems to decipher the molecular mechanism underlying reactive oxygen species (ROS)-mediated cell elongation. Root hairs are able to expand in response to external signals, increasing several hundred-fold their original size, which is important for survival of the plant. Although their final cell size is of fundamental importance, the molecular mechanisms that control it remain largely unknown. Root hair polar growth is endogenously controlled by auxin and sustained by oscillating levels of reactive oxygen species (ROS). The speed at which they grow is determined both by cell-intrinsic factors like hormones (e.g., auxin) and external environmental signals like nutrient availability in the soil (e.g., phosphate). Overall root hair growth is controlled by the transcription factors RSL4 and RSL2. While high levels of auxin promote root hair growth, high levels of inorganic phosphate (Pi) in the media are able to strongly repress RSL4 and RSL2 expression linked to a decreased polar growth. In this work, we inquired the mechanism used by root hairs to integrate conflicting growth signals like the repressive signal of high Pi levels and a concomitant high auxin exposure that promotes growth and questioned whether these complex signals might activate known molecular players in root hair polar growth. Under these conditions, RSL2 expression (but not RSL4) is activated linked to ROS production and root hair growth. On the other hand, by blocking ROS production derived from the NADPH Oxidase C (or RBOHC for RESPIRATORY BURST OXIDASE HOMOLOG C) and ROS production from Secreted type-III Peroxidases (PERs), it was possible to repress the auxin growth-promoting effect. This study identifies a new layer of complexity between auxin, Pi nutrient availability and RSL2/RSL4 transcription factors all acting on ROS homeostasis and growth at the root hair level.

Conferencia Premio Joven investigador SEBBM

0353

MOLECULAR TIPS AND TRICKS TO DECONSTRUCT THE BIOLOGY OF TUMORS

Arkaitz Carracedo

CIC bioGUNE NA

Cancer cells live and grow in aggressive competition with the environment within an organism. Indeed, the Darwinian laws of evolution are reproduced at large in tumors, and these principles are at the core of disease progression and therapy resistance. The heterogeneity between and within tumors has been the driver of precision medicine, which aims at stratifying patients based on the molecular characteristics of the tumor and to assign the most efficacious therapy in an individualized manner. In the lab, we focus on understanding the molecular routes that are central to prostate and breast cancer progression. Our research endeavor has motivated us to develop bioinformatics tools to achieve our goals, which we combine with the study of cell signaling and metabolism in vitro, in mouse models and human specimens. In this lecture, I will comment on the ideas that have driven our research, and present a perspective of how we envision the future prevention, detection and therapy in cancer.

1. Arruabarrena-Aristorena A, et al. Oil for the cancer engine: The cross-talk between oncogenic signaling and polyamine metabolism. Sci Adv. 2018;4:eaar2606. 2. Zabala-Letona A, et al. mTORC1-dependent AMD1 regulation sustains polyamine metabolism in prostate cancer. Nature. 2017;547:109-13. 3. Valcarcel-Jimenez L, et al. Trends Endocrinol Metab. 2017;28:748-57. 4. Torrano V, et al. The metabolic co-regulator PGC1alpha suppresses prostate cancer metastasis. Nat Cell Biol. 2016;18:645-56.

Conferencia Premio Científico Margarita Lorenzo (Fundación Lilly)

0055-M

Role of PKD1 in the control of liver endoplasmic reticulum stress and insulin signaling responses during non-alcoholic fatty liver disease progression

Patricia Rada, Alejandra Mosquera, Carmelo García-Monzón, Teresa Iglesias, Ángela M. Valverde

Instituto de Investigaciones Biomédicas Alberto Sols and CIBERDEM Metabolismo y señalización celular

Protein kinase D1 (PKD1) is a ubiquitous Ser/Thr kinase belonging to the CAMK family. It is increasingly implicated in the regulation of fundamental biological processes such as apoptosis, cell proliferation, trafficking and oxidative stress. It has been previously reported that PKD1 plays a role in different tissues including immune cells, cardiac myocytes and pancreas. However, its role in liver metabolism remains unclear. To this end, a mouse model that lacks PKD1 in hepatocytes was generated by using the Cre-loxP system (PKD1ΔHep and PKD1fl/fl as control mice).

Primary hepatocytes isolated from PKD1ΔHep mice showed higher levels of ER stress markers and signature of a blockade of autophagic flux in parallel with an increase in PKD2 phosphorylation. Interestingly, in palmitic-acid stimulated hepatocytes lacking PKD1 further enhanced PKD2 phosphorylation was detected above basal in addition to an increased and sustained molecular signature of ER stress, blockade of autophagy flux and lipoapoptosis. Importantly, PKD1-deficient primary hepatocytes showed a reduced sensitivity upon insulin stimulation.

Since obesity induced by high fat diet (HFD) has been shown to induce ER stress response and insulin resistance, mice from both strains were fed a HFD for 20 weeks. After this period, PKD1ΔHep mice exhibited higher body weight gain compared to PKD1fl/fl mice. Moreover, pyruvate and insulin tolerance tests revealed that HFD-fed PKD1ΔHep mice presented increased insulin resistance than control mice. These results were also confirmed by decreased phosphorylation levels of IR and AKT. Likewise, the histological analysis of liver sections showed a more elevated NAS score than comprises steatosis, inflammation and ballooning in the PKD1ΔHep group, suggesting that PKD1 deficiency worsens the histological course of non-alcoholic fatty liver disease (NAFLD). In addition, electronic microscopy images revealed that livers from PKD1ΔHep mice presented a pronounced dilation of the ER lumen compared to PKD1fl/fl control mice.

Taken together, our results strongly suggest that PKD1 might restrain ER stress signature and, therefore, its deficiency in hepatocytes accelerates the course of metabolic liver pathologies associated to excessive ER stress such as insulin resistance and NAFLD

Conferencia plenaria NIemeyer

0390

Synthetic Biology, Optogenetics and Eidetic Memory: Developing Biotechnological Solutions and Pushing the Boundaries between Science and Art.

Luis F. Larrondo

MILLENNIUM INSTITUTE FOR INTEGRATIVE BIOLOGY (IBIO) , DEPARTAMENTO DE GENÉTICA MOLECULAR Y MICROBIOLOGIA

The filamentous fungus Neurospora crassa perceives and responds to blue light through a transcriptional heterodimer named White Collar Complex (WCC), which contains a LOV (Light Oxygen Voltage) domain capable of detecting blue wavelengths, which promotes a conformational change that leads to dimerization, resulting in strong transcriptional activation, in a light-intensity dependent manner. Thus, through the development of Neurospora-based optogenetic switches we have successfully implemented a blue-light responding transcriptional system in Saccharomyces cerevisiae. Therefore, in yeast, now we can implement synthetic biology circuits to efficiently induce gene expression over 1000-fold and control biotechnological relevant phenotypes such as flocculation, by switching on/off the lights. We have also adopted optogenetic approaches to further delve into Neurospora's light-responses. In doing so, we have been able to genetically program 2D-images in this organism. Thus, we can project a photograph on top of a Neurospora carrying a luciferase reporter under the control of a light responsive promoter, obtaining back a bioluminescent pattern mimicking the original image: a live canvas in which images are genetically processed and reconstituted with real-time dynamics. Such technology not only allows studying light-responses with great resolution, but is also provides a powerful artistic substrate. As this live canvas system can be easily integrated in the Neurospora circadian regulatory network, the fungus reproduces on subsequent days -in a circadian manner- the image that it had originally "seen", creating an eidetic (photographic) memory effect. Such phenomenon, based on local discrete phase changes, not only will provide new insights on phase responses, but it also allows for the opportunity to ponder on concepts such as vision and transcriptional memory. iBIO, FONDECYT 1171151 and HHMI International Research Scholar grant.

Conferencia de clausura Fundación Ramón Areces

0389

Managing a chromosome (The journey of a Molecular Detective)

David Sherratt

DEPT BIOCHEMISTRY UNIVERSITY OF OXFORD

DNA is compacted >1000-fold into chromosomes from bacteria to Man. How is this DNA organised and compacted in order that it can be replicated, repaired, transcribed and unlinked in an orderly fashion? We use interdisciplinary approaches to understand how the bacterial chromosome can be 'managed' in a way that allows function. Increasingly, we use quantitative single-molecule and single-cell methods to avoid the averaging of ensemble studies. We have shown that E. coli genes have specific addresses, that change predictably as the cell proceeds through the cell cycle. Replication initiates at the replication origin independently of where it is placed in a cell, and the two replication forks proceeding from a given replication event track independently around the chromosome, with replisome components exchanging every few seconds. The two replication forks merge at the termination of replication close to where the division septum will form. Type II topoisomerases and SMC complexes have inter-related key roles in chromosome organisation and management; with the topoisomerases removing the ~200, 000 links that entwine the two strands of duplex DNA and the E. coli SMC complex, MukBEF, acting to organize chromosomal DNA and to facilitate chromosome segregatio

SIMPOSIOS

Simposio 1. BIOMEDICINA MOLECULAR

Simposio 1.1 The Role of Microbiota in health & disease

0381

The role of microbiota in aging and progeria

PEDRO M QUIROS

Recent works have shown that the gut microbiome is a key regulator of several metabolic, immune and neuroendocrine pathways. Gut microbiome deregulation has been implicated in multiple pathologies such as obesity, type 2 diabetes, cardiovascular disease, non-alcoholic fatty acid liver disease or cancer, but its precise role in aging remains to be elucidated. Here, we characterize the gut microbiome profile of accelerate aging and show that its external modulation is sufficient to extend healthspan and lifespan. These results suggest the existence of a link between gut ageing and the microbiota, and lay the foundations for the future use of microbiome-based interventions against age-related diseases.

0379

Maternal microbiome and its role in infant health

Maria Carmen Callado

NSTITUTE OF AGROCHEMISTRY AND FOOD TECHNOLOGY-NATIONAL RESEARCH COUNCIL (IATA-CSIC), VALENCIA, SPAIN

The advances in the understanding of the host–microbes interactions suggest that maternal microbiota plays a crucial role in infant health. Early microbial colonization is essential for the immune system development and function and also, for other physiological functions. Accumulating evidence

suggest that the human microbial contact starts during gestation "in utero" and later, at birth, it is shaped by perinatal factors including mode of delivery, antibiotic use and breastfeeding practices. This initial microbiota evolves in composition and diversity towards an adult-like microbiota by the end of the first years of life. Then, maternal microbiota forms the first and unique microbial inoculums, and factors affecting maternal microbiota would affect the microbial transference from mothers to infants. Early microbiota alterations have been linked to higher risk for the development of non-communicable diseases as allergies, obesity, etc.. later in life. In this scenario, it is needed increase our knowledge on maternal-infant microbiota as it would help us to identify and to develop personalized dietary strategies targeted to microbial modulation, including probiotics and prebiotics intervention, during first 1000 days of life.

0383

Human Microbiome Studies: Clinical Implications

Alex Mira

Next-generation sequencing and metagenomic technologies have bursted the study of commensal microorganisms that live together with human beings, generally known as the microbiome. Most of these microorganisms have not been cultured in vitro until now and DNA/RNA studies on clinical samples show a huge microbiological diversity adapted to each body niche. In our group, we have developed second-generation molecular techniques to study microbiological communities in the mouth, stomach, respiratory track, maternal milk and intestine both at health and disease. These studies have allowed us firstly, to determine the polymicrobial etiology of diseases like dental caries or periodontitis and secondly, to identify those bacteria that work as biomarkers for colorectal cancer, to determine the complete compositions of regular samples in the clinical setting like sputum, to identify bacteria with potential pro-biotic use in different diseases or to characterize stomach microbiota in the presence or absence of Helicobacter pylori. Additionally, the combination of fluorescence-based flow cytometry and sorting in bacteria with next-generation sequencing technologies have allowed us to quantify the degree of microorganisms opsonization with different antibodies as well as to identify bacteria recognized by IgA, IgG and IgM together to those able to evade antibody recognition. The application of this technology to fecal samples of one-month old children allows the prediction, for example, of asthma development years before the disease onset, fostering the design of new preventive tools in high-risk children. All these reinforces the diagnostic value of microbiome studies. Among the different techniques recently applied to study human microbiome we can find : massive sequencing of the bacterial 16S rRNA gene (18S or ITS in fungi), metagenomics (total DNA sequencing of a sample, showing the genetic potential of the whole microbial community), metatranscriptomics (total RNA sequencing of a sample showing the expressed genes and active microorganisms in a specific moment) o massive cloning of microbial DNA in a vector followed by its insertion in E. coli which allows the access to genes of uncultured bacteria. All these is seeding light on the total diversity and functionality of the human-associated microorganisms as well as their potential for preventive strategies. One example of this has been the comparative metagenomics study of oral samples in patients with or without caries, which revealed a high frequency of a new Streptococcus species, subsequently isolated and cultured in vitro. This new bacterium, denominated Streptococcus dentisani, produces bacteriocins that inhibit caries causing bacteria and buffer extracellular pH, reducing caries risk. This bacterium is being tested as anti-caries pro-biotic in clinical trials. Consequently, there are multiple clinical applications of these metagenomics studies and, likely, in the near future human microbiota studies will be regularly performed in hospitals with diagnostic and preventive applications once the technical problems associated with next-generation sequencing data analysis in the clinical setting are solved.

Simposio 1.2 Cellular senescence in physiology and pathology

0373

The two faces of cellular senescence in tissue repair and in tissue dysfunction

MANUEL serrano

INSTITUTE FOR RESEARCH IN BIOMEDICINE (IRB BARCELONA), BARCELONA, SPAIN

A major advance in the field of ageing research has been the demonstration that senescent cells play a key role in aging and, even more importantly, the discovery of small pharmacological compounds that can kill senescent cells within the organism resulting in improved health. Upon tissue damage or stress, a substantial fraction of cells respond by adopting a cellular state known as "senescence". Regardless of their initial cell identity, senescent cells share key properties; namely, global chromatin remodelling, robust proliferation blockade, and a massive pro-inflammatory secretome. The initial biological purpose of senescent cells is to orchestrate tissue repair, ultimately leading to their own disposal by the immune system and to their replacement by new, functional cells. This is the favorable, beneficial, face of cellular senescence. However, in certain contexts that are generally associated with chronic damage, degenerative processes, or organismal ageing, tissue repair is inefficient and senescent cells are not cleared. Indeed, senescent cells accumulate in many human pathologies including various fibrotic diseases, atherosclerosis, and neurodegenerative diseases. This is the detrimental, pathological, face of cellular senescence. Importantly, the last few years have witnessed the identification of small compounds that preferentially kill senescent cells, termed senolytic drugs. Such senolytic treatments in mice show an unprecedented therapeutic effect on the aforementioned diseases including lung fibrosis, atherosclerosis, and neurodegenerative diseases. I will present our contributions to the understanding of cellular senescence both in tissue repair and in pathological contexts.

0385

Cellular senescence during tumor progression and treatment

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VALL D'HEBRON INSITUTE OF ONCOLOGY (VHIO) AND CIBERONC

Cellular senescence is a terminal cell cycle arrest that can be triggered by a variety of stressors including perturbations in signaling pathways and DNA damaging agents. Senescent cells remain metabolically active and display a series of distinct features including enlarged lysosomes and a secretome completely different to that of the cell of origin known as SASP (Senescence-Associated Secretory Phenotype). It has been recently realized that many anti-tumor therapies, including conventional chemotherapy (such as DNA-damaging agents) and some targeted therapies (such as inhibitors of the cyclin-dependent kinases cdk4/6) induce senescence, rather than apoptosis, in target cells. We have previously shown that, under certain circumstances, cellular senescence contributes to tumor progression and invasion (1-3). However, the role of senescence during the different stages of the malignant progression is far from clear and how senescence affects different anti-tumor therapies is not understood. We have developed an approach to label in vivo senescent cells by detecting those cells expres-

sing high levels of the cyclin dependent kinase inhibitors p16 or p21 and the prototypical component of the SASP interleukin 6 (IL6). This approach allows us to monitor the presence of senescent cells in tumor cells derived from patients under a variety of conditions, including treatment with anti-tumor therapies. We will present data showing how induction of senescence can be used to improve some of these therapies.

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0339

Senescencia celular en cáncer y desarrollo

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La senescencia celular juega un papel clave en el control del balance celular en diferentes contextos, bloqueando la proliferación y promoviendo la eliminación inmunitaria de células dañadas o no necesarias. Evidencias recientes indican la importancia de senescencia en patologías diversas, como cáncer, fibrosis o diabetes, pero también en procesos fisiológicos como el desarrollo embrionario. Resultados recientes de nuestro grupo han identificado a la homeoproteína SIX1 como un nuevo regulador negativo de senescencia celular. Previamente a esta conexión con senescencia, SIX1 se ha implicado en cáncer y en desarrollo embrionario. Su activación aberrante es frecuente en diversos tumores humanos, mientras que su pérdida de función está asociada a patologías del desarrollo tanto en modelos animales como en humanos. Con estos antecedentes, hemos explorado el papel de la senescencia celular tanto en la actividad oncogénica de SIX1 como en su papel como regulador del desarrollo. Nuestros resultados indican que la acción protumoral de SIX1 está asociada a la represión de senescencia y la activación de plasticidad celular, mientras que los defectos de desarrollo asociados a deficiencias en SIX1 podrían estar debidos a activación aberrante de senescencia. Mediante el estudio de la conexión de SIX1 con senescencia, nuestros resultados subrayan la relevancia de la senescencia celular en contextos tan diversos como el cáncer y el desarrollo embrionario.

Simposio 1.3 Cancer Genomics

0376

Identification of new driver mutations in cancer

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The application of next generation sequencing to cancer has resulted in the identification of a large number of cancer driver genes. This has improved our understanding of the molecular mechanisms underlying the transformation process, and is helping the development of novel diagnostic tools and therapeutic approaches. Nonetheless, most of these discoveries have relied in our incomplete ability to interpret the effect of somatic mutations, a process that is almost exclusively limited to those affecting the protein-coding region. We are trying to understand the importance of mutations in non-coding sequences or with different effects of those predicted by the simple interpretation of the genetic code. Thus, by combining different layers of genomic information, including whole genome sequencing, RNA-seq, long-insert mate-pair sequencing and functional validation studies, we have identified novel mutations involved in the tumorigenic process. Those include mutations in non-coding regions that have an impact on the functional activity of different genes by affecting its expression due to alterations involving enhancer elements, or by affecting the protein activity, stability or translation efficiency due to mutations in untranslated regions. Other mutations outside of splicing sites have a major impact on RNA maturation, either by generating novel splicing acceptor or donor sites. Many of these mutations that affect RNA maturation, including synonymous or non-synonymous mutations, affect tumor suppressors as well as novel genes, reinforcing the hypothesis that silent mutations may frequently contribute to human cancer, and highlighting the relevance of these mutations to properly interpret the results derived from cancer genome sequencing studies.

0370

The noncoding genome in cancer: a long story not so short (Functional long noncoding RNAs as cancer drivers)

Maite Huarte

UNIVERSITY OF NAVARRA CIMA

A major shift in our conception of genome regulation has emerged in recent years. It is now obvious that the majority of cellular transcripts do not code for proteins, and a significant subset of them are long RNAs (lncRNAs). Moreover many lncRNAs have been shown to be functional, and are emerging as important regulatory molecules in tumor-suppressor and oncogenic pathways. Supporting this idea, we found that the transcription factor p53, which is crucial for the maintenance of cellular homeostasis, specifically regulates the expression of dozens of lncRNAs, and constitute active components of this important tumour suppressor pathway. In contrast, other lncRNAs can promote the malignant phenotype of cancer cells, acting as oncogenes. We will present our findings implicating alterations in copy number or expression levels of lncRNAs in the regulation of the transformed phenotype of cancer cells, with particular attention to the molecular mechanisms that underlie their function.

0386

New oncogenic roles of retrotransposons found in cancer

JOSE MANUEL TUBÍO

U. SANTIAGO DE COMPOSTELA

About half of all cancers have somatic integrations of retrotransposons. To characterize their role in oncogenesis, we analyzed the patterns and mechanisms of somatic retrotransposition in 2,954 cancer genomes from 37 histological cancer subtypes. We identified 19,166 somatically acquired retrotransposition events, affecting 35% of samples, and spanning a range of event types. L1 insertions emerged as the first most frequent type of somatic structural variation in esophageal adenocarcinoma, and the second most frequent in head-and-neck and colorectal cancers. Aberrant L1 integrations can delete megabase-scale regions of a chromosome, sometimes removing tumour suppressor genes, as well as inducing complex translocations and large-scale duplications. Somatic retrotranspositions can also initiate breakage-fusion-bridge cycles, leading to high-level amplification of oncogenes. These observations illuminate a relevant role of L1 retrotrans-

position in remodeling the cancer genome, with potential implications in the development of human tumours.

Simposio 2. REGULACIÓN GÉNICA Y COMUNICACIÓN CELULAR

Simposio 2.1 Plant Antioxidant Systems

0324

Reactive oxygen species regulate organelle dynamics: Implications for signaling and inter-organelle communication

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Basal levels of reactive oxygen species (ROS) are produced in different cell compartments and play a key role as potent signaling molecules in the regulation of most metabolic pathways and cell responses to biotic and abiotic stress factors. Differential ROS production and the regulation of ROS accumulation by antioxidants enables cells to perceive and identify specific stimuli, thus triggering specific cell responses (1). ROS and redox-dependent gene hubs and transcription factors have been identified using transcriptomic analysis. However, ROS sensors and the mechanism involved in directing the ROS-dependent signal to the nucleus have not been clearly identified. Although this process involves the trafficking at the subcellular level of redox metabolites, such as H2O2, ascorbate and glutathione, our knowledge in this field is still rather limited. Technical advances in cell biology have led to the incorporation of new concepts, such as organelle dynamics, into the study of cell responses to stimuli. Thus, protrusion formation, as well as changes in organelle motility, position and quantity have gained importance with respect to understanding cell responses to stress conditions (2). Apart from physical organelle-nucleus and interorganelle contact, communication between organelles can take place through the formation of the following dynamic extensions: chloroplast stromules, mitochondrial matrixules and peroxisomal peroxules. The formation of these structures in chloroplasts and peroxisomes is associated with oxidative stress, although the regulatory mechanism involved and their function have not been clearly identified (2, 3). Even less information is available on the regulation and functionality of mitochondrial extension. In this study, we will focus on the role played by ROS in regulating the dynamics of organelles (mainly peroxisomes) and on the function of these changes in ROS-dependent signaling and inter-organelle communication.

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0202

The function of hydrogen peroxide scavenging systems in plant chloroplast redox homeostasis

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Light is the most important environmental factor modulating chloroplast metabolism, which shows an extraordinary capacity to respond to unpredictable changes in light intensity. Central to this capacity is thiol-dependent redox regulation, in which the disulfide reductase activity of thioredoxins (Trxs) plays a key role. Chloroplast Trxs use photosynthetically reduced ferredoxin (Fd) as source of reducing power, which is transferred via a Fd-dependent Trx reductase (FTR). Thus, the so-called Fd-FTR-Trxs system links redox regulation to light (1). More recently our group identified a novel plastid-localized NADPH-dependent Trx reductase (termed NTRC) with a joint Trx domain at the C-terminus. NTRC allows the use of NADPH for the efficient reduction of 2-Cys peroxiredoxins (2-Cys Prxs), thus having an antioxidant function (2). The identification of two redox systems in plant chloroplasts raised the question of whether these redox systems act concertedly. We have addressed this issue by analyzing *Arabidopsis thaliana* mutants simultaneously deficient in NTRC and 2-Cys Prxs. Based on these results, our group has recently proposed a novel model of chloroplast redox regulation, according to which the function of the Fd-FTR-Trx and NTRC redox systems is coordinated by the redox balance of 2-Cys Prxs (3). Thus hydrogen peroxide scavenging systems play a crucial role in the regulation of chloroplast photosynthetic metabolism and its adaptation to environmental changes in light intensity.

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0356-R/M

Reversible molecular signalling of nitric oxide (NO) during plant development.

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Nitric oxide (NO) has evolved from a gaseous free radical to become a key gasotransmitter involved in a plethora of plant developmental and stress processes1. In most cases, NO function is achieved through its interaction with phytohormones2,3. Additionally, NO reacts mainly with cysteines to form S-nitrosothiols, actively regulated by the action of redoxin like enzymes. Thus, S-nitrosylation has been described as a reversible modification by an enzymatic process called denitrosylation. We identified NO targets related to redox control of seed bZIP transcription factors, essential to define the molecular mechanisms underlying the switch from stress to developmental issues. Our current research deepens on the relevance of reversible S-nitrosylation of seed bZIP master regulators by enzymes related to the redox control, commonly implied in cellular stresses. We deciphered the

crosstalk between abscisic acid (ABA), a key hormone involved in stress conditions, and NO during seed development and germination focusing on S-nitrosylation and trans-denitrosylation redox mechanisms. This work is financed by grants: ERC.KBBE.2012.1.1-01 (EcoSeed-311840). MINECO (BIO2017-85758-R), CONSOLIDER (CSD2007-00057). Junta de Castilla y León (SA093U16). Fundación Solórzano (FS/26-2017).

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Simposio 2.2 MicroRNAs in health & disease

0374

microRNAs in B cell Lymphomagenesis

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Most of the lymphomas arise from the transformation of B lymphocytes, and within those, the vast majority originate from mature B lymphocytes that have germinal center (GC) experience. GCs are transient microstructures that develop in the context of the antibody immune response. In GCs, B cells that are activated in secondary lymphoid organs by T cell–dependent antigens engage in intense proliferation and diversify their antibody genes, which ultimately results in the generation of high-affinity plasma cells and long-lived memory B cells. Thus, a highly sophisticated form of immune protection is at the same time a permissive environment for B cell transformation. microRNAs are short RNA molecules that regulate gene expression at a posttranscriptional level and are key regulators of multiple biological processes in health and disease. We have identified miR-28 as a microRNA that is very frequently lost in B cell neoplasia. We found that in normal B cells, miR-28 is specifically upregulated during the GC reaction, and regulates B cell proliferation and survival. Further, we show that reintroduction of miR-28 in B cell lymphoma cells interferes with tumor growth in vivo. We will discuss the potential of microRNAs as therapeutic tools for Non-Hodgkin Lymphoma

0375

Non-coding RNA in lipid metabolism and cardiovascular disease

Carlos Fernandez-Hernando

Cellular and plasma cholesterol levels are maintained through tightly controlled mechanisms, which regulate key metabolic genes both at the transcriptional and post-transcriptional level. Alterations in the control of cholesterol homeostasis can lead to pathological processes, including atherosclerosis, the most common cause of mortality in Western societies. To date, most lipid and lipoprotein research has focused on alterations of protein coding genes, whereas the functions of non-coding RNAs remain largely unknown. Our group originally identified miRNA-33, an intronic miRNA encoded within the intronic sequences of SREBP genes, the master transcriptional regulators that control lipid metabolism. In a number of relevant studies, we were able to demonstrate that miRNA-33 provides a critical link between the regulation of cholesterol biosynthesis by SREBP2 and cholesterol efflux pathways mediated by ABCA1, a transporter that controls cellular cholesterol efflux and high-density lipoprotein (HDL) biogenesis. Most importantly, we found that pharmacological inhibition or genetic ablation of miR-33 increases hepatic ABCA1 expression, circulating HDL-C and attenuates the progression of atherosclerosis. These findings have been reproduced by numerous groups and the application of miR-33 inhibitors for reducing heart disease has been widely recognized and is, in fact, being commercially developed by drug companies. I will be discussing the novel approaches and mouse models that we are using for assessing the specific role of miR-33 in different tissues, identify physiologically relevant miRNA/target interactions under different physiologic conditions, and directly determine the relative importance of specific miRNA target interactions by genetically disrupting miRNA binding sites within individual target genes in vivo using CRISPR-Cas9 genome editing. Furthermore, we will discuss the development of novel therapeutic approaches for targeting of miR-33 in specific tissues including establishing a system to direct anti-miR-33 therapeutics to atherosclerotic plaques and employing techniques for disrupting specific miRNA/target interactions in vivo.

0323

The role of micro RNAs in the metabolic basis of renal fibrosis

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Fibrosis is a process related to abnormal scarring in response to inflammatory stimuli or environmental aggressions. In the kidney, fibrosis is the final common pathway of several highly prevalent clinical conditions, such as diabetes or arterial hypertension, leading to chronic renal failure. MiRNAs play a role in kidney development, homeostasis of renal function and in different renal pathologies, including renal fibrosis. Recent work has evidenced that the progression of renal fibrosis is related to an alteration in fatty acid metabolism and more precisely to a reduction of fatty acid oxidation (FAO). We have previously demonstrated an anti-fibrotic role of miR-9-5p in pulmonary, peritoneal and skin fibrosis. Our data point to a similar behavior of miR-9-5p in renal fibrosis. Histological analysis showed a significant attenuation of fibrosis in the kidneys of mice that over-express miR-9-5p after unilateral ureteral ligation (UUO), a murine model widely used in the study of renal fibrosis. In addition, the induction of fibrotic markers α-SMA, fibronectin and collagen1α1, was also lower in the kidneys of these mice. RNA-sequencing (RNA-seq) analysis of kidney samples from the UUO model indicated that some of the miR-9-5p-regulated pathways are related to oxidative phosphorylation, ATP synthesis, glycolysis and FAO. We have demonstrated that gain-of-function of FAO in the kidney by using conditional genetic models is able to improve the outcome of renal fibrosis in the UUO model. Studies in miR-33a-deficient mice (KO) subjected to UUO and folic acid-induced injury showed decreased kidney fibrosis and lipid accumulation compared with wild type animals. MiR-33 analogs reduced FAO-related oxygen consumption, while miR-33 antagonists increased it in HKC-8 cells. Overall, we propose that strategies based on the modulation of FAO in the kidney may prove useful in therapies for renal fibrosis.

Simposio 2.3 Topological Control of Gene Expression

0382

High-resolution regulatory maps connect cardiovascular risk variants to disease related pathways

Pelin Sahlen

Genetic variant landscape of cardiovascular disease (CVD) is dominated by non-coding variants among which many occur within putative enhancers regulating the expression levels of relevant genes. It is crucial to assign the

genetic variants to their correct gene both to gain insights into perturbed functions and better assess the risk of disease. We generated high-resolution genomic interaction maps (~750 bases) in aortic endothelial, smooth muscle and THP-1 macrophages using Hi-C coupled with sequence capture targeting 25,429 features including variants associated with CVD. We detected interactions for many CVD risk variants obtained by genome-wide association studies (GWAS) and identified novel as well as established functions associated with CVD. We were able to fine-map hundreds of GWAS variants using interaction networks, thereby identifying additional genes and functions. We also discovered a subset of risk variants interacting with multiple promoters and the expression levels of such genes were correlated. In this talk, I will describe the resource that enables functional studies for cardiovascular disease to provide novel approaches for its diagnosis and treatment.

0372

Genome's architecture surprising role in cell fate decisions

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In the past few years studies using HiC revealed that the mammalian genome is not chaotically intermingled in the nucleus but shows a specific configuration. Although by and large, the spatial organization of the genome is similar between say neurons and stem cells, there are also cell type specific differences. This raises the question: Are these topological differences a mere side effect, or do they carry information required for the expression of cell type specific genes? In other words, what comes first: architecture or transcription? To address this question we performed HiC at different times during reprogramming from pre-B cells to iPS cells, using a highly efficient reprogramming method developed in our lab. The results were surprising: although in large proportion of the genome the changes in architecture (compartments, TAD borders) and transcription occurred simultaneously, in an equally large proportion topological changes happened before the genes' expression. Our findings indicate that genome topology has an informational value for gene expression during cell fate changes and explain the observation that some pluripotency genes only become activated at late stages of reprogramming.

0355

Structural Variation in the 3D Genomic Era

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Berlin Institute for Medical Systems Biology (BIMSB). Max Delbrueck Center for Molecular Medicine (M "Epigenetics and Sex Development" Group

Structural variants (SVs) largely contribute to genomic variability, being highly relevant for human disease and evolutionary genetics. Such rearrangements cannot only affect gene dosage, but also influence regulatory mechanisms by altering the copy number of regulatory elements or disrupting 3D chromatin organization. In this talk, I will show examples at different genomic loci, highlighting the potential of SVs to induce developmental disease by distinct pathomechanisms. Furthermore, I will present the Iberian mole Talpa occidentalis, a unique case of true XX mammalian hermaphroditism, and a case study of how SVs can also be a force of evolutionary innovation.

Simposio 3 ESTRUCTURA Y FUNCIÓN DE BIOMOLECULAS

Simposio 3.1 DNA Topology & topoisomerases

0337

DNA Topology and Chromatin Shape

Joaquim Roca

CSIC IBMB-SBU

Following a general introduction on the relevance of DNA topology and topoisomerase activities in genome transactions, I will summarize main insights and recent findings of my laboratory. I will show how eukaryotic chromatin structure adapts to the high levels of positive and negative DNA supercoiling generated during gene transcription, and high degree of DNA catenation produced during chromosome replication. In addition to supercoiling and catenation, I will show that intracellular DNA undergoes also significant levels of knotting. Since DNA knots footprint the spatial trajectory of DNA, I will show how their analysis let us to uncover novel aspects of the local architecture and dynamics of intracellular chromatin.

0013

THE TRANSCRIPTOME OF THE BACTERIAL PATHOGEN *STREPTOCOCCUS PNEUMONIAE* UNDER LOCAL AND GLOBAL TOPOLOGICAL CHANGES

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The bacterial chromosome is compacted in a manner optimal for DNA transactions to occur. The degree of compaction results from the level of DNA-supercoiling (SC) and the presence of nucleoid-binding proteins. SC is homeostatically maintained by the opposing activities of relaxing DNA topoisomerases and negative supercoil-inducing gyrase. SC acts as a *cis* regulator of transcription. Transcriptomic studies on *S. pneumoniae* have been performed under conditions of local and global changes in SC. Local SC changes induced by fluoroquinolone antibiotics, which target gyrase subunit A and/or topoisomerase IV, trigger an increase in oxygen radicals which reduces cell viability, while the induction of global SC changes by novobiocin (a gyrase subunit B inhibitor), or by seconeolitsine (a topoisomerase I inhibitor), has revealed the existence of topological domains that specifically respond to such changes. The control of SC in *S. pneumoniae* occurs *via* the regulation of topoisomerase gene transcription: relaxation triggers up-regulation of gyrase and down-regulation of topoisomerases I and IV, while hypernegative supercoiling down-regulates topoisomerase I. Relaxation affects 13% of the genome, with the majority of the genes affected

located in 15 domains. Hypernegative supercoiling affects 10% of the genome, with one quarter of the genes affected located in 12 domains**.** However, all the above domains overlap, suggesting that the chromosome is organized into topological domains with fixed locations. Based on its response to relaxation, the pneumococcal chromosome can be said to be organized into 5 types of domain: up-regulated, down-regulated, position-conserved non-regulated, position-variable non-regulated, and AT-rich. Genes within the different domains share structural and functional characteristics. A topology-driven selection pressure has defined the chromosomal location of the metabolism, virulence and competence genes, which suggests the existence of topological rules that aim to improve bacterial fitness.

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0391

Roles of DNA topoisomerase II in genome organization, expression and stabilit

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It is becoming more and more apparent that all processes of genome dynamics occur within a topological context. DNA topoisomerase II (TOP2) is a cornerstone in these DNA transactions, and as such, it can have a profound impact in the organization, expression and stability of the genome. An imbalance in these processes can lead to serious health issues, such as developmental and degenerative problems, and very importantly, trigger or favour neoplastic transformation. Furthermore, TOP2 activity can lead to the formation of highly cytotoxic and clastogenic DNA breaks, either spontaneously or as a consequence of chemotherapeutic treatment. We will present and discuss our latest results regarding the function of TOP2 in regulating gene expression, how this integrates within the context of 3D chromosome organization, and its implications for the maintenance of genome integrity.

Simposio 3.2 Genomic Engineering with CRISPR

0371

Synthetic toxin-intein combinations as novel genetic weapons for specific killing of pathogenic bacteria.

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Antibiotic resistance is becoming a major concern worldwide. The development of new antimicrobials is urgently needed. We constructed a synthetic system, which can perform killing of specific pathogenic bacteria within mixed populations. Our system is based on the coupling of artificially split toxins (non-functional) with split intein technology. Inteins are protein sequences embedded inside a host protein (called extein) from where they can be self excised in a process called protein splicing. Naturally, inteins exist also as split modules. Each half of toxin-intein fusion was cloned in two different plasmids that, separately, do not kill the cell. Together, intein halves recognize each other in the cytoplasm and, after protein splicing, the toxin is reconstituted, killing the bacterium. Cholera is a severe watery diarrhea caused by the Gram-negative bacterium Vibrio cholerae. We cloned our toxin-intein combinations under promoter sequences controlled by specific transcriptional regulators from the genome of V. cholerae and involved in its pathogenicity. We then engineered a plasmid -the genetic weapon- to spread through conjugation into mixed population of bacteria, identifying and killing specifically V. cholerae. We tested the in vivo potential of this approach in zebrafish larvae infected by V. cholerae and showed that conjugation of our genetic system and the specific killing of the targeted bacteria take place in this context. Our system precisely recognizes and kills bacteria in a natural complex community that could help in the resolution of main problems of classical antibiotic treatments: indiscriminate killing of beneficial bacteria and the emergence of resistance.

0360

Uso del sistema CRISPr/Cas para el estudio del cáncer.

Jorge Melendez

Instituto Nacional de Medicina Genómica Subdirección de Investigación Médica

El análisis genómico del ADN de pacientes con diversas enfermedades incluyendo el cáncer, ha destacado la presencia de diversas alteraciones en este tipo de patologías. Sin embargo, en la gran mayoría de los casos no se conoce el mecanismo mediante el cual estas alteraciones causan o participan en estas enfermedades. El conocer estos mecanismos, independientemente de la importancia en la elucidación de los procesos fisiopatológicos involucrados, ayudará a crear nuevas herramientas diagnósticas, pronósticas y señalar nuevos blancos terapéuticos. Para este fin es indispensable la recapitulación de la variación o mutación genómica en un modelo in vitro o in vivo con el fin de reproducir su participación en la enfermedad estudiada. Por su modularidad y versatilidad, el sistema CRISPr/Cas se ha posicionado como la herramienta ideal para este tipo de ensayos. Con ella es posible no solo realizar cambios de un solo nucleótido, sino también producir modificaciones genómicas complejas como fusiones génicas o deleciones mayores. Asimismo, la versatilidad de esta herramienta permite también regular genes de manera epigenética e incluso detectar de manera ultrasensible secuencias génicas específicas. Aprovechando estas ventajas, en nuestro laboratorio hemos creado alteraciones puntuales e inactivación de genes codificantes y no codificantes con el fin de elucidar el mecanismo de acción de estos en la iniciación y progresión del cáncer en humanos. Esta aproximación permite tener modelos mucho más cercanos a la realidad, así como acercarnos a su posible uso en la clínica.

0377

Heterochromatin stability and transcription.

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In our group we are interested in understand how heterochromatin maintains the stability of the chromosomes and how transcription modulates chromatin structure. Two different parts concerning these two subjects will be presented. First, the role of the ADD1 factor in the localization and

maintenance of the Heterochromatin Protein 1 (HP1) at the telomeres in D. melagostaer as well as its role in chromosome stability will be presented. Intriguingly, the chromatin distribution of ADD1 that is not located at the telomeres and the chromocenter suggest that it may also participate in chromosome conformation. On the other hand, an old controversy that still is subject of discussion is if the chromatin conformation dictates transcription or if transcription dictates chromatin structure. In order to answer this question we are using a dead Cas9 fused to transcription activation domains, in order to determine if the presence of an artificial transcription factor is enough to activate transcription in any kind of heterochromatin. Our results indicate that using this CRISPR-Cas9 modified system we are able to activate transcription in either, facultative heterochromatin and constitutive heterochromatin in the fly. These results have important implications in how the transcriptional machinery modulates the chromatin conformation.

Simposio 3.3 Nanomaterials for biomedical applications

0338

Potential of inorganic magnetic and/or plasmonic nanohybrids for magneto-photo-thermal cancer therapy and for magnetically-guided tissue engineering.

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To overcome some of the limitations of current therapies, new strategies have emerged since the advent of nanotechnology in medicine.

In cancer therapy, thermal treatments (magnetic hyperthermia or photothermal therapy mediated by magnetic or plasmonic nanoparticles) have provided noninvasive means of heating and killing cancer cells. In such case, the ultimate target is the cancer cell, so that heating must be generated and measured inside the cells. We provided thermal measurements mediated by magnetic [1] or plasmonic [2] nanoparticles inside cancer cells, in vitro or in vivo in the tumor environment [3]. The ultimate goal of nanotherapies is anyway to improve the efficacy and combat the tumour from within. We proposed combined nanotherapeutic concepts [4-7] based on magneto-photo-thermal, therapies which led to complete cancer cell destruction in vitro and complete tumor ablation in vivo.

While magnetic nanoparticles are increasingly used as clinical agents for imaging and therapy, their use as a tool for tissue engineering opens up challenging perspectives that have rarely been explored. Our strategy has been to take advantage of magnetic nanoparticles internalization to create thick, organized, purely cellular 3D tissue structures [8,9], that can be stimulated on demand [10,11]. This magnetic tissue engineering strategy is an alternative to current popular development of tissue bio-printing.

The use of nanoparticles for cancer cell therapies or tissue engineering raise more general issues of nanoparticles biosafety, once internalized in cells. Yet the nanoparticles long-term tissular fate is poorly documented. We have developed original magnetic and thermal techniques to follow the fate of magnetic and plasmonic nanoparticles and their assimilation within a living tissue, and reveal the massive biotransformations experienced by magnetic nanoparticles [12,13], together with the remarkable shielding potential of gold shells [13,14].

[1] Biomaterials 24, 6400 (2014)[2] Advanced HC Materials, 5, 1040 (2016)[3] Advanced Funct Materials (2018)[4] Journal of Controlled Release 279, 271 (2018) [5] ACS Nano, 10, 2436 (2016)[6] Nanoscale, 7, 18872 (2015)[7] ACS Nano, 9, 2904 (2015)[8] Advanced Materials, 25, 2611 (2013)[9] Integrative Biology, 7, 170 (2015)[10] Phys Rev Lett, 114, 098105 (2015)[11] Nature comm 8, 400 (2017)[12] ACS nano, 10, 7627 (2016)[13] ACS nano, 8b00482 (2018)[14] Advanced Funct Materials 27, 9 (2017)

0332

Magnetic Nanoparticles for NMR imaging: Controlling T1 and T2 from the synthesis

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Magnetic nanoparticles are playing an important role in NMR imaging for the detection of different diseases. These contrast agents consist of a central core made of iron oxide coated with an inorganic or organic shell. The chemical and physical properties of the nanoparticles determine not only the MRI relaxivity properties but also its pharmacokinetics.

Here we will show how the control of the synthesis enables the production of highly reproducible nanoparticles showing a "spectrum" of relaxivities, from T1 contrast to T2 contrast capabilities [1]. The very good T1 contrast capabilities are further demonstrated by in vivo magnetic resonance angiography in large magnetic field equipment while the T2 contrast agent is used for in vivo liver imaging.

Moreover, the development of dual probes allows the combination of complementary imaging techniques, like hybrid PET/MRI [2] or CT/MRI [3]. On this sense the combination of iron oxide nanoparticles and 68Ga isotope or bismuth compound gave rise to a new generation of hybrid systems and multimodality approaches. We evaluated these dual targeting nanoparticles in an murine model by PET/MRI and CT/MR.

[1] Langmuir, 33, 10239-10247, 2017 [2] Contrast Media Mol. Imaging 11, 203–210, 2016. [3] Nanotechnology 26(13),135101, 2015.

0334

Nanoparticles for Point-of-Care diagnostic tests

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Great efforts have been made in recent years in order to develop novel and improved diagnostic techniques. Some of them are based on the use of functional nanoparticles to report the presence of the targeted biomarkers. The World Health Organization has given some indications for the "ideal" diagnostic test1. It should combine good detection properties (sensitivity and specificity) with attractive handling features, such as user-friendly and portability. We use the words "Point-of-Care" (POC) to refer to the devices meeting those requirements. Since a central laboratories or specialized training are not needed, they would allow doctors to take fast decisions regarding diagnostics or therapy monitoring. They would also empower the patients with health monitoring tools. All of this would have a great impact on the health national systems and the society well-being.

One of the most representative examples of this new generation of devices is the pregnancy test, which detects the hCG hormone. This system is based on lateral flow immunoassay technology, and can be designed for other biomarkers. However, very often quantification is required for diagnostics. This step can be overcome by using different nanoparticles and me-

asuring a physical property related to them. In this work, examples will be given for the design of POC devices for cardiac, tumor and infectious diseases biomarkers2. The platform has also been adapted for the detection of small biological vesicles (exosomes)3, which are attracting lot of attention in the biomedical field as emerging source of biomarkers for liquid biopsy.

Acknowledgements: Projects MINECO MAT2017-84959-C2-1-R y MAT2016-81955-REDT

1. R.W. Peeling, D. mabey, Clin Microbiol Infect 16: 1062–1069. 2. D. Lago-Cachón, M. Oliveira-Rodríguez, M.C. Blanco-López, M. Rivas, J. C. Martínez-García, A. Moyano, M. Salvador, J.A. García, IEEE Magnetics Letters, Vol 8 (2017), 1506305. 3. Myriam Oliveira-Rodríguez, Esther Serrano-Pertierra, Agustín Costa García, Soraya López Martín, María Yáñez Mo, Eva Cernuda-Morollón, M.C. Blanco-López, Biosensors and Bioelectronics 87(2017)38–45.

POSTERS

R01 Apoptosis y estrés celular

0019-R/M

La carencia de DNA mitocondrial en células de cáncer hepático impide la respuesta autofágica a la privación de nutrientes y a la acidificación del microambiente tumoral

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Antecedentes: La pérdida de DNA mitocondrial (DNAmt) y las alteraciones en la autofagia son frecuentes en células tumorales. La autofagia juega un papel dual en las células cancerosas, pudiendo actuar como mecanismo de resistencia/adaptación o como proceso desencadenante de muerte celular. **Objetivo:** Estudiar el efecto de la pérdida del DNAmt sobre la autofagia inducida por factores del microambiente tumoral en células SKHep1 de cáncer hepático humano. **Métodos:** Para la simulación de privación de nutrientes y acidosis se utilizaron medios de cultivo carentes de aminoácidos (AA) y con valores de pH de 7,4, 6,8 y 5,8, sin bicarbonato, en los que se utilizó HEPES como amortiguador del pH. **Resultados:** Comparadas con las células silvestres (WT), las carentes de DNAmt (Rho) presentaban mayor resistencia a la muerte inducida por privación de AA pero mayor sensibilidad a la inducida por acidosis. Ambos estímulos aumentaron la expresión del mediador de parada del ciclo celular p21, en células WT y Rho. En ambos tipos celulares, la acidosis aumentó el marcador de susceptibilidad a la apoptosis Bax/Bcl2. Sin embargo, la privación de AA aumentó marcadamente la expresión de la proteína anti-apoptótica Bcl2. Las dos condiciones de estrés metabólico elevaron el número de autofagosomas, pero solo en células WT. El estrés del retículo endoplásmico (ERE), involucrado en la activación de la autofagia, se indujo con tapsigargina, lo que aumentó los niveles de LC3-II en las células WT pero no en las Rho. Sin embargo, la expresión de CHOP y GRP78, marcadores de ERE, aumentaron por la privación de AA, pero no por la acidosis en las células WT. **Conclusión:** La pérdida del genoma mitocondrial en las células de cáncer hepático altera su sensibilidad a los factores del microambiente tumoral debido al bloqueo de la inducción de autofagia, proceso que está presente en células WT y que conlleva muerte celular y supresión tumoral, involucrando al ERE en condiciones de privación de nutrientes pero que actuaría como mecanismo de supervivencia frente a la muerte celular inducida por acidosis.

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0285-R/M

MECANISMOS DE MUERTE E INMUNOGENICIDAD EN EL TRATAMIENTO DEL MIELOMA MÚLTIPLE.

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Entender los mecanismos de muerte celular, como la apoptosis o la muerte celular inmunogénica, es clave para diseñar estrategias más efectivas en el tratamiento del cáncer. En particular, en el mieloma múltiple (MM) que representa el 10% de las neoplasias hematológicas, sigue habiendo un porcentaje importante de pacientes que responden mal a la terapia. La reciente introducción, con un cierto éxito, de la inmunoterapia en el MM, ha puesto de manifiesto la necesidad de identificar fármacos solos o en combinación para transformar modelos de muerte tolerogénica en muerte inmunogénica. Datos recientes sugieren que el estrés en el retículo endoplásmico (ER), tiene un papel fundamental en la inducción de señales inmunogénicas.

Por ello, en el presente trabajo hemos estudiado el mecanismo de muerte y el potencial inmunogénico de nuevos fármacos anti-mieloma, como el inhibidor del proteasoma carfilzomib, así como combinaciones con fármacos que induzcan estrés en el ER (cloroquina, DBeQ). La combinación de carfilzomib pero no bortezomib, con cloroquina o DBeQ, potencian notablemente la muerte inducida respecto a los tratamientos individuales en diversas líneas de MM. Además, dicha muerte se induce de manera más rápida tal y como revelan los experimentos de análisis temporal de la toxicidad. Esto se traduce también en el aumento de la activación de la caspasa-3 en los tratamientos combinados. Dicha potenciación, también se observa en células de mieloma aisladas de pacientes con esta enfermedad. Como marcador de inmunogenicidad, se ha medido la exposición de calreticulina, cuyo nivel de expresión aumenta en los tratamientos combinados. En cuanto al mecanismo de muerte, se ha estudiado la expresión de diversas proteínas implicadas en el estrés del ER (PERK, BIP, eIF2α, CHOP y XBP1) así como diversas proteínas de la familia Bcl-2 (Puma, Noxa, Bcl-2, etc).

Estos resultados parecen indicar que los tratamientos estudiados activan la respuesta a estrés en el ER, correlacionando con la exposición de señales inmunogénicas. Sin embargo, el nexo con los efectores reales de la muerte celular queda aún por elucidar.

0289

Estudio de la apoptosis inducida por el inhibidor del proteasoma ixazomib en células de mieloma.

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El mieloma múltiple (MM) representa el 10% de las neoplasias hematológicas y se caracteriza por la proliferación y acumulación de células B plasmáticas en la médula ósea. Los inhibidores de proteasoma bortezomib y carfilzomib son la piedra angular de los mejores tratamientos actuales para el MM. Recientemente, se ha aprobado para el tratamiento de pacientes con MM un tercer inhibidor, el ixazomib, que se puede administrar por vía oral y presenta pocos efectos secundarios. En el presente estudio hemos analizado el mecanismo de la apoptosis inducida por ixazomib en distintas líneas de MM humano, así como su posible efecto potenciador por parte de miméticos de BH3, inhibidores de autofagia e inhibidores de la AAA+

ATPasa VCP (p97). La proliferación celular se evaluó por análisis de la reducción del MTT. La apoptosis midiendo la caída de potencial mitocondrial con TMRE y la exposición de fosfatidilserina con anexina V, por citometría de flujo. Se estableció una dosis subletal de ixazomib y se estudió el efecto de las combinaciones de ixazomib con distintos miméticos de BH3, S63845 (anti-Mcl-1), A-1155463 (anti-Bcl-xL) y ABT-199 (anti-Bcl-2), inhibidores de autofagia (cloroquina) e inhibidores de p97 (CB5083, DBeQ). Los resultados obtenidos indican que las líneas de MM H929, MM.1S, OPM-2, RPMI 8226 y U266 son sensibles a ixazomib con una IC50 creciente. La combinación de ixazomib con miméticos de BH3 reveló un efecto sinérgico con S63845 (inhibidor de Mcl-1). La combinación de ixazomib con inhibidores de autofagia potenció la apoptosis, por lo que la inhibición de la autofagia podría ser una estrategia para mejorar el índice terapéutico del ixazomib, como se demostró en estudios anteriores con el inhibidor del proteasoma carfilzomib< ![endif]-->. En conclusión, el inhibidor de proteasoma ixazomib, sólo o en combinación con miméticos BH3, inhibidores de autofagia o de p97, es un eficaz inductor de apoptosis en las células de MM. Estos resultados preliminares deberán verificarse en un estudio más detallado para poder formular recomendaciones para la posible mejora de la terapia del mieloma múltiple.

0292

Mechanisms of programmed necrosis in kidney diseases

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Acute kidney injury (AKI) implies that kidney damage results in an acute and usually transient decrease in renal function. The incidence of AKI is increasing as an aging population is subjected to complex medical procedures. AKI increases mortality and chronic kidney disease progression. However, therapy is only symptomatic. Pathogenesis-based therapy is needed. Inflammation and, parenchymal cell death are key features of AKI loss and nephron loss are features of AKI that may eventually lead to tubulointerstitial fibrosis. In recent the last years, our understanding ofabout the mechanisms of that lead to tubular cell death has been improved but there are ongoing discussions on the timing or molecular regulators involved. Morphologically, both Aapoptosis and necrosis were observedas the most common form of tubular cell death from a morphological point of view; although necrosis was also present, indeed in the la. Apoptosis, as a regulated form of cell death, was an attractive therapeutic target. However, no therapy targeting apoptosis is in clinical use. More recently, interventional evidence of the involvement of different forms of regulated necrosis in experimental AKI has emergedst years different pathways of regulated necrosis has been observed in AKI. Contrary to apoptosis, regulated necrosis is more immunogenic and pro-inflammatory because plasma membrane integrity is lost andas dying cells release DAMPs, stimulating and amplifying sterile inflammatory responses., and this could explain the sterile inflammation that occurs in AKI. Between Of the different pathways of regulated necrosis pathways potentially involved in AKI (are necroptosis, ferroptosis, pyroptosis, or mitochondria permeability transition-regulated necrosis (MPT-RN), there is strong evidence for the involvement of ferroptosis and ferroptosis. Now, we review the specific role of different cell death pathways during AKI and their contribution to renal inflammation with emphasis on the timing of event, a key feature that will influence potential diagnostic and therapeutic implications. Experimental AKI is characterized by an early wave of ferroptosis and it is prevented by pre-treatment with ferroptosis inhibitors. Ferroptosis triggers local inflammation and an inflammation-dependent secondary wave of necroptosis.

0331-R/M

Personalizing cancer treatment using cell-based functional assays

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Most of anti-cancer agents now used in the clinic induce apoptotic programmed cell death, and include conventional chemotherapy (DNA damaging agents, anti-metabolites, plant alkaloids and others that target different cellular components), targeted therapies (like kinase inhibitors that target mutated or highly expressed proteins responsible for tumour growth and survival) and the promising immunotherapies (like checkpoint inhibitors that target cancers' suppression on the immune system). Apoptosis is regulated by the BCL-2 family of proteins that controls the mitochondrial outer membrane permeabilization (MOMP) that triggers this type of cell death. When a cancer cell is effectively treated, early changes in the BCL-2 family of proteins can be rapidly detected preceding activation of apoptosis and cancer cell's commitment to die. Over the past years, I pioneered the development of a revolutionary functional test: **dynamic BH3 profiling (DBP).** This test monitors mitochondrial health in cancer cells after a short therapeutic agents' incubation using titrated doses of pro-apoptotic synthetic BH3 peptides, like BIM, that act as pro-death signals to induce MOMP. By performing a BIM titration and comparing treated cells with untreated cells, DBP measures how much a treatment primes cancer cells for apoptosis (expressed as delta% priming) in less than 24 hours, which is predictive of later cell death; allowing a rapid analysis of many samples and treatments at once. DBP represents an enormous technical improvement compared to existing laboratory procedures, because it allows a rapid functional therapy testing in patient-isolated tumour cells, avoiding sample deterioration due to the short *ex vivo* cancer cells' incubation. In the last years, I successfully tested DBP predictive capacity *in vitro*, on murine models and on patient samples (Montero et al., Cell 2015), accurately determining therapy response ; and I even used this technology to find a novel treatment to an rare aggressive hematological malignancy called BPDCN (Montero et al., Cancer Discovery 2017), that will be evaluated as clinical trial. DBP represents a breakthrough in personalizing cancer treatment to find new therapies, stratify patients in the clinic and choose the best treatment among several to guide precision medicine.

Ramon y Cajal Senior researcher at IBEC. He has a PhD in Biomedicine and worked as a postdoctoral fellow at Dana-Faber Cancer Institute / Harvard Medical School. He published in high-impact journals, including a Cell and Cancer Discovery publications as first author, achieved a remarkable impact (h-index: 14 and 1208 citations) and secured funding as PI from very competitive sources to work in personalized cancer treatment and he is coinventor in the Dynamic BH3 Profiling patent.

0362

MCJ, a new therapeutic target for liver Disease

Maria Luz Martinez-Chantar

Rolf Luft described the first mitochondrial dysfunction in 1962 in a woman with hypermetabolism. Since then, the role of mitochondria in health and disease has been broadly studied. Due to the tremendous metabolic activity of the liver, hepatocytes are one of the cell types with the highest density of mitochondria, which make it very susceptible to disorders that affect mitochondrial functions. Mitochondrial hepatopathies are classified depending on the cause of the liver disorder. Defects in the respiratory chain are the most frequent mitochondrial primary disorders (1 in 5,000 births) and present as ALF in the first weeks of life. In the secondary disorders, the mitochondrial damage is caused by genetic defects of nonmitochondrial proteins or by exogenous insults such as viruses, alcohol, toxins, drugs or other factors8. Importantly, mitochondrial dysfunction has been described as a common mechanism in the pathogenesis of several acute and chronic human liver diseases, including DILI, NAFLD, cholestatic liver diseases and HCC. Methylation-controlled J protein (MCJ), also known as DnaJC15,

is a small protein that belongs to the DnaJ family of co-chaperones. Unlike other DnaJ proteins, MCJ is not soluble since it contains a transmembrane domain and localizes in the inner mitochondrial membrane (IMM). Importantly, MCJ interacts with and represses the function of complex I of the ETC, making it the first endogenous inhibitor of complex I. MCJ deletion in vivo results in increased complex I activity, MMP and ATP production, without affecting mitochondrial mass. Additionally, it has been shown that MCJ interferes with the formation of respiratory SC, which facilitate an efficient transfer of electrons and minimize ROS production. These findings point out the important role of MCJ as an essential negative regulator of mitochondrial metabolism and provide new basis and tools for the study and discovery of new mechanisms regulating mitochondrial liver diseases along with therapies to treat them

R02 Bases moleculares de la patología

0006

CYCLOOXYGENASE-2 EXPRESSION IN HEPATOCYTES PROTECTS AGAINST HEPATIC ISCHEMIA-REPERFUSION INJURY IN MICE

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Liver ischemia and reperfusion injury (IRI) remains a serious clinical problem affecting liver transplantation outcomes. IRI causes up to 10% of early organ failure and predisposes to chronic rejection. Cyclooxygenase-2 (COX-2) is involved in different liver diseases but the significance of COX-2 in liver IRI is a matter of controversy. This study was designed to elucidate the role of COX-2 expression in hepatocytes in the pathogenesis of liver IRI. In the present work, hepatocyte-specific COX-2 transgenic mice (hCOX-2-Tg) and their wild-type (Wt) littermates were subjected to partial IRI and the results show that hCOX-2-Tg exhibited lower grades of necrosis and inflammation than Wt mice, in part by reduced recruitment and infiltration of hepatic macrophages and netrophils, with a corresponding decrease in serum levels of pro-inflammatory cytokines. Moreover, hCOX-2-Tg mice showed a significant attenuation of the IRI-induced increase in oxidative stress and hepatic apoptosis, an increase in autophagic flux and a decrease in endoplasmic reticulum (ER) stress comparing with that observed in Wt mice. Preconditioning of Wt mice resembles the beneficial effects of hCOX-2-Tg mice in IRI due to an increase in endogenous COX-2 expression. Furthermore, measurement of PGE2 levels in plasma from patients who underwent orthotopic liver transplantation revealed a significantly negative correlation between PGE2 levels and graft function, and time of ischemia. Overall, the data support the view of the beneficial effects of hepatic COX-2 dependent prostaglandins after liver IRI.

0026-R

A viral interference assay for the discovery of bacterial effector proteins

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Viruses are microorganism that absolutely depend of host cell for their replication. A particularly interesting group of viruses is the *Flaviviridae* family. This family contains four genus of (+) RNA genome viruses that are principally transmitted by arthropod vectors like mosquitoes and ticks. *Flaviviridae* are enveloped viruses that use specialized machinery to fuse viral and host cell membranes after internalization through the endosomal pathway. After internalization, their replicate and exit from the cell manipulating host membranes, mainly ER, Golgi and autophagic vesicles. Their intimate dependence to the host membranes recalls the intracellular cycle of several pathogenic bacteria, such as *Brucella*, they are able to survive and then replicate in host cells. In particular, *Brucella spp*. through its Type 4 Secretion System (T4SS) can regulate the inflammatory response and manipulate vesicular trafficking of cells, stablishing a favorable growth niche for themselves. This process involves the utilization of the endocytic pathway (early and late endosomes and lysosomes), the endoplasmic reticulum (ER) and autophagic vesicles. However, there are few effector proteins of the T4SS identified so far. No single strategy has been able to identify a majority of the known effectors, so it is reasonable to think that new strategies to detect *Brucella* effector proteins can identify new subsets of real effector proteins.

This similarity in the ecological niche among *Flaviviridae and Brucella* made us hypothesize the presence in *Brucella* of bacterial effector proteins with the potential of interfering with the virus replication cycle. And therefore, the use of a new screening method based on this interference could reveal new *Brucella* effector proteins. We are using a novel interference system, in which the ectopic expression of putative *Brucella* effector proteins takes place simultaneously to the infection of a flavivirus in the same cell. This interaction is followed by flow cytometry, measuring the fluorescence associated with the viral replication on one hand, and the expression of the putative effector protein on the other. Changes in the viral fluorescence levels may identify new effector proteins that will be studied to determine their role in *Brucella* pathogenicity.

0028-R

La deficiencia de Igf1r atenúa la implantación y progresión tumoral en un modelo murino de cáncer de pulmón

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El cáncer de pulmón es la neoplasia con mayor mortalidad en nuestro entorno sociosanitario pero poco conocida molecularmente. En los tumores, además de las células cancerosas, es de gran relevancia el microambiente tumoral, que contribuye al establecimiento de la metástasis y a la progresión tumoral. En él participan de forma destacada fibroblastos, células del sistema inmune y vasos sanguíneos asociados al tumor. IGF1R (*Insulin-like Growth Factor type 1 Receptor*) es un receptor transmembrana de expresión ubicua con funciones biológicas esenciales como la supervivencia, proliferación y diferenciación celular. Existen evidencias de que los IGFs y su receptor IGF1R, podrían modular la metástasis y progresión neoplásica desde la zona no tumoral durante el establecimiento del tumor. Para determinar la implicación de IGF1R en el microambiente tumoral del cáncer de pulmón, se ha generado un modelo murino usando células de carcinoma de Lewis (LLC1) que se instilaron por vía intratraqueal a ratones deficientes de *Igf1r* (*UBC-CreERT2; Igf1rΔ/Δ*) y a sus controles (*Igf1rfl/fl*) **a**. Tras 21 días, los pulmones de los ratones deficientes de *Igf1r* presentaron una reducción en el número de focos tumorales por inspección *de visu* respecto a los controles. Además, mostraron menor pérdida de peso y reducción en el recuento de células totales y neutrófilos en la médula ósea. Se presentarán datos histológicos para confirmar el impacto de *Igf1r* sobre la carga tumoral y sobre la respuesta inmune y vascularización asociada al tumor. Estos resultados preliminares apuntan a que la señalización por *Igf1r* en el microambiente tumoral condiciona la implantación y progresión del cáncer de pulmón.

a López et al. Transgenic Res 2015; 24(2):279-294.

0029

EL PAPEL DE LOS RECEPTORES NOTCH Y LAS PROTEÍNAS DLK EN EL FENOTIPO ADIPOGÉNICO DE LAS CÉLULAS MESENQUIMÁTICAS MULTIPOTENTES C3H10T1/2

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La familia de receptores NOTCH y sus ligandos participa en el desarrollo embrionario, la homeostasis de los tejidos adultos de mamíferos y aves, y en numerosos procesos de diferenciación celular, como la adipogénesis. El papel de la señalización de los receptores NOTCH en la adipogénesis es controvertido. Los datos publicados indican tanto efectos nulos, como positivos y/o negativos en este proceso. Anteriormente, demostramos que las proteínas de la familia NOTCH, DLK1 y DLK2, que funcionan como ligandos inhibidores no canónicos de los cuatro receptores NOTCH de mamíferos, también modulan el proceso de adipogénesis de los preadipocitos 3T3-L1 y de las células mesenquimáticas multipotentes C3H10T1/2. Tanto las variantes DLK1 y DLK2 de membrana, como las solubles, activan la adipogénesis de las células C3H10T1/2, un efecto contrario al producido en las células 3T3-L1, y esto a pesar de actuar como inhibidoras de la señal de los receptores NOTCH en ambas líneas celulares. Recientemente, hemos demostrado que la sobreexpresión de cada uno de los cuatro receptores NOTCH es capaz de potenciar el proceso adipogénico de preadipocitos 3T3-L1 y modular la adquisición de un fenotipo blanco o marrón de los adipocitos. En este trabajo demostramos que, como ocurre en las células 3T3-L1, la sobreexpresión de cada uno de los cuatro receptores NOTCH también activa el proceso de adipogénesis de las células C3H10T1/2 y afecta a la adquisición del fenotipo final de los adipocitos. Además, la adición al medio de cultivo de ligandos solubles de NOTCH de tipo canónico, como DLL4 y JAG1, ejerce igualmente un efecto potenciador de la adipogénesis en estas células. En conclusión, los receptores NOTCH y las proteínas DLK potencian la adipogénesis de células C3H10T1/2 y parecen modular el proceso de diferenciación de estas células hacia un fenotipo blanco o marrón. Los resultados obtenidos sugieren que es necesario un nivel preciso de señalización global de NOTCH, regulado por sus ligandos canónicos y no canónicos, para determinar la capacidad adipogénica y el fenotipo final de los adipocitos C3H10T1/2.

0038

Effects of hyperglycaemia in SIRT6

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URJC Ciencias básicas de la Salud

Introduction: Hyperglycaemia favours tumour development through both systemic and intracellular effects (REF). We have previously shown that high glucose concentrations enhance Wnt/b-catenin signalling in cancer cells though the unbalanced acetylation of β-catenin, the effector of the pathway. SIRT6 presents deacetylase and ADP-ribosyl-transferase activities, both of which play important roles in tumorigenesis. Depletion of this sirtuin [CG1] provokes hypoglycaemia in mice and a more frequent appearance of tumours than WT mice[CG2] . Since SIRT6 is known to be required for DNA double strand breaks (DSB) repair by activating PARP and to stabilize H2AX in DSB foci, enhanced tumorigenesis could be explained by an inability to repair DA damage.

Objective: We aimed to analyse whether the concentration of glucose regulates SIRT6 at any level in healthy vs colon cancer cells and evaluate whether SIRT6 is required for DNA damage repair induced by hyperglycaemia.

Methods: HCT116 (colon cancer) and CCD18-Co (healthy colon) cells were exposed to different concentrations of glucose. After manipulating SIRT6 levels, HCT116 were compared to healthy cells in their capacity to repair DNA damage. ROS were measured by FC, DSB repair foci were revealed by IF and the levels of critical DSB repair markers were analyzed by WB.

Results: Hyperglycaemia increased both cellular ROS and SIRT6 levels in the nuclei of colon cancer cells. Likewise, SIRT6 overexpression correlated with increased p53 and H2AX[CG1] levels. Preliminary results indicate that hyperglycaemia reduces the levels of total p53 and H2Ax while increasing SIRT6 levels. Ongoing experiments are trying to elucidate the mechanisms that underpin these complex SIRT6-p53 interactions under normo or hyperglycaemia.

Conclusions: Increased SIRT6 levels in response to hyperglycaemia may represent a cellular defense against the DNA damage induced by increased ROS production.

Future perspectives: We will try to elucidate the mechanisms that ensure increased levels of SIRT6 under hyperglycemia as well as how this affects its enzymatic capabilities or its capacity to induce DNA repair.

0050-R/M

NOD1 aggravates atherogenic inflammatory processes by triggering haematopoiesis and vascular recruitment of myeloid progenitors

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Nucleotide-Binding Oligomerization Domain-1 (NOD1) is a pattern recognition receptor (PRR) of the innate immunity known to be activated by infections. PRRs are also involved in highly prevalent and mortal diseases, such as in cardiovascular diseases. Atherosclerosis, the underlying cause of most of them, is characterized by the accumulation of lipids and fibrotic materials in the arteries as well as by an exacerbated inflammatory response. Not only this, but one of its main risk factors, hypercholesterolemia, also promotes haematopoiesis that further aggravates the disease. The objective of this project was to determine the role of NOD1 in the regulation of the immune response in atherogenesis. To this aim, phenotypic and physiological characterization of *Apoe-/-Nod1-/-* against *Apoe-/-* mice was made under a high fat diet regimen. Our results show that NOD1 is induced in atherosclerotic and hematopoietic tissues, and it contributes to the mobilization of myeloid progenitors to the atheromatous plaque thanks to the induction of certain chemokines. Actually, the spleen is an important extramedullar hematopoietic organ that upregulates NOD1 in the context of hypercholesterolemia. While spleen weights' did not differ between both mice genotypes, its leukocyte content in *Apoe-/-Nod1-/-* mice was highly reduced as it did in these mice aortas. As consequence, NOD1 inactivation in *Apoe-/-* mice reduced atheroma buildup. In conclusion, here we demonstrate that NOD1 plays an active role in leukocyte ontogeny and mobilisation in response to hypercholesterolemia, promoting vascular recruitment and subsequent inflammation and atherogenesis. Its study as a pharmacological target could contribute to the control and eradication of athero diseases.

Caruso, et al. 2014. Lusis, 2000. Soehnlein and Swirski, 2013.

0055-M

Role of PKD1 in the control of liver endoplasmic reticulum stress and insulin signaling responses during non-alcoholic fatty liver disease progression

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Protein kinase D1 (PKD1) is a ubiquitous Ser/Thr kinase belonging to the CAMK family. It is increasingly implicated in the regulation of fundamental biological processes such as apoptosis, cell proliferation, trafficking and oxidative stress. It has been previously reported that PKD1 plays a role in different tissues including immune cells, cardiac myocytes and pancreas. However, its role in liver metabolism remains unclear. To this end, a mouse model that lacks PKD1 in hepatocytes was generated by using the Cre-loxP system (PKD1ΔHep and PKD1fl/fl as control mice).

Primary hepatocytes isolated from PKD1ΔHep mice showed higher levels of ER stress markers and signature of a blockade of autophagic flux in parallel with an increase in PKD2 phosphorylation. Interestingly, in palmitic-acid stimulated hepatocytes lacking PKD1 further enhanced PKD2 phosphorylation was detected above basal in addition to an increased and sustained molecular signature of ER stress, blockade of autophagy flux and lipoapoptosis. Importantly, PKD1-deficient primary hepatocytes showed a reduced sensitivity upon insulin stimulation.

Since obesity induced by high fat diet (HFD) has been shown to induce ER stress response and insulin resistance, mice from both strains were fed a HFD for 20 weeks. After this period, PKD1ΔHep mice exhibited higher body weight gain compared to PKD1fl/fl mice. Moreover, pyruvate and insulin tolerance tests revealed that HFD-fed PKD1ΔHep mice presented increased insulin resistance than control mice. These results were also confirmed by decreased phosphorylation levels of IR and AKT. Likewise, the histological analysis of liver sections showed a more elevated NAS score than comprises steatosis, inflammation and ballooning in the PKD1ΔHep group, suggesting that PKD1 deficiency worsens the histological course of non-alcoholic fatty liver disease (NAFLD). In addition, electronic microscopy images revealed that livers from PKD1ΔHep mice presented a pronounced dilation of the ER lumen compared to PKD1fl/fl control mice.

Taken together, our results strongly suggest that PKD1 might restrain ER stress signature and, therefore, its deficiency in hepatocytes accelerates the course of metabolic liver pathologies associated to excessive ER stress such as insulin resistance and NAFLD.

0061-R/M

Matriptase-2 deficiency protects from obesity by modulating iron homeostasis

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The role of iron and its main regulatory hormone hepcidin in obesity is still unclear. On the one hand, epidemiological studies have associated elevated hepcidin levels and iron deficiency with obesity, suggesting that increased hepcidin concentrations occur as a result of the chronic inflammatory condition linked to obese individuals. On the other hand, iron overload, which also triggers hepcidin up-regulation, has been linked with fatty-liver disease, diabetes and insulin resistance, three common conditions frequently associated with obesity. In this work, we investigated whether the *in vivo* deletion of a negative regulator of hepcidin, matriptase-2 (encoded by *Tmprss6*), contributes to the development of obesity and its pathogenic features. Thus, we challenged mice deficient in matriptase-2, which phenocopy the human disease iron-refractory iron deficiency anemia (IRIDA) and develop hypochromic microcytic anemia caused by the inadequate hepcidin up-regulation, with a protocol of diet-induced obesity. In this regard, *Tmprss6–/–* mice show a significant decrease in body fat, improved glucose tolerance and insulin sensitivity, and are protected against hepatic steatosis. Moreover, these mice exhibit a significant increase in fat lipolysis, consistent with their dramatic reduction in adiposity. In addition, rescue experiments that block hepcidin up-regulation and restore iron levels in *Tmprss6–/–* mice via anti-hemojuvelin (HJV) therapy, revert the obesity-resistant phenotype of *Tmprss6–/–* mice. Overall, our findings provide new insights regarding the role of hepcidin and iron regulation in metabolic homeostasis and adipocyte function, and suggest new strategies based on matriptase-2 inhibition for the treatment of obesity.

Folgueras, A. R. et al. Matriptase-2 deficiency protects from obesity by modulating iron homeostasis. Nat. Commun 9, 1350 (2018).

0062-R/M

Essential roles of USP36 deubiquitinase in cell and organism viability

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Protein ubiquitination status is tightly controlled by deubiquitinases or DUBs, a large group of proteases with a wide functional diversity and a profound impact on the regulation of multiple biological processes. Among them, the ubiquitin-specific protease 36 (USP36) has been implicated in the regulatory mechanisms underlying ribosomal RNA and protein synthesis. However, its functional relevance has not yet been fully described. We have shown that Usp36 deficiency is lethal in preimplantation mouse embryos, preventing the transition from morula to blastocyst through the induction of an apoptotic response. Through the molecular characterization of *Usp36* haploinsufficient cells and embryos we have demonstrated that USP36 carries out its functions in part by regulating the stability of the DEAH-box RNA helicase DHX33, which is also critically involved in the control of nucleolar activity. In agreement with these findings, we have demonstrated the role of USP36 in ribosomal RNA and protein synthesis and we have shown that USP36 downregulation in human cancer cells alters their proliferation by inducing both apoptosis and cell cycle arrest. In this work, we have explored the implications of USP36 in cancer biology, with the finding of apparently paradoxical effects in different tumor types. Likewise, we have observed that oncogenic transformation involves a complex mechanism of regulation of USP36 protein levels. These results support the relevance of this deubiquitinating enzyme in cancer biology as well as in physiological processes.

0067

IMPAIRED EXPRESSION IN LIVER AND PLACENTA OF KEY GENES INVOLVED IN PROGESTERONE METABOLISM DURING CHOLESTASIS OF PREGNANCY

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Intrahepatic cholestasis of pregnancy (ICP) is characterized by hypercholanemia accompanied by changes associated with high serum levels of progesterone and some of its metabolites due to an abnormal metabolism of sex hormones. Enzymes belonging to HSDs family are involved in the production of these metabolites catalyzing biotransformations of active hormones into their inactive derivatives.

The aim was to investigate the effect of maternal cholestasis on placental and hepatic expression of key genes involved in progesterone metabolism.

As model of hypercholanemia during pregnancy in rats, complete obstructive cholestasis from day 14 of gestation was used. Placenta and liver samples were collected at term to measure mRNA and protein levels of main enzymes involved in metabolism of sex hormones by RT-qPCR and western blot, and the catalytic activity of Akr1c2 by measuring the rate of 5α-DHP disappearance by HPLC-MS/MS.

In maternal liver, cholestasis did not affect the expression levels of proteins involved in the metabolism of progesterone, except for Srd5a1 and Akr1c2, that convert progesterone into their inactive metabolites 5α-DHP and PM4, respectively, which were down-regulated. This was accompanied by a similar decrease in protein levels and enzymatic activity of Akr1c2. In contrast, in placenta, Srd5a1, Akr1c2 and other enzymes involved in progesterone catabolism such as Cyp17a1 and Cyp21a1, were up-regulated. 3β-Hsd, involved in the last step of progesterone biosynthesis whose expression showed a trend to increase in liver, was significantly reduced in placenta. To elucidate whether changes found might be under Fxr control, their expression was determined in the liver of cholestatic Fxr-/- mice. No significant differences between WT and Fxr-/- mice were found, suggesting that mechanisms other than Fxr pathway may be involved in the regulation of these enzymes during cholestasis.

In conclusion, through an Fxr-independent mechanism, ICP induces in the placenta up-regulation of enzymes involved in the inactivation of progesterone, together with decreased expression and catalytic activity of hepatic enzymes. These changes can act as an adaptive mechanism to counterbalance the accumulation of progesterone, thus protecting the fetus from high concentrations of this hormone during ICP.

0074-R

Nanovacunas para prevenir listeriosis neonatal y adulta

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Introducción: Los casos clínicos de listeriosis en adultos se asocian con meningitis y septicemia y en neonatos con abortos y afectaciones neurológicas. Los casos severos de listeriosis han aumentado en España, siendo urgente disponer de vacunas seguras. Nuestro grupo ha preparado formulaciones de nanovacunas para *Listeria monocytogenes* (LM) con dos adyuvantes diferentes.

Resultados: Los ratones adultos vacunados con gliconanopartículas de oro conjugadas a péptidos cortos de LM, resultan protegidos de forma eficiente con las formulaciones con nanovacunas que utilizan ambos adyuvantes y con buena inducción de respuestas inmunológicas tanto CD4 como CD8 (1). Sin embargo, los neonatos nacidos de gestantes vacunadas con dichas formulaciones, solo resultan protegidos con formulaciones de nanovacunas que incluyen adyuvantes que induzcan respuestas inmunológicas CD8 (2). Los casos clínicos de listeriosis entre 2013–2015 y los sueros de los ratones vacunados e infectados permiten definer dos biomarcadores de pronóstico de listeriosis, útiles en el diseño de vacunas: los títulos altos de anticuerpos y los ratios altos de factor de necrosis tumoral (TNF)/interleukina (IL)-6 (3).

Discusión: Vacunas de gliconanopartículas de oro conjugadas a péptidos cortos de LM y formuladas con un adyuvante que se dirige a los receptores Toll-like 2/4, protege de forma segura en listeriosis neonatal y adulta. Los ratones vacunados con estas nanovacunas no desarrollan listeriosis ni co-morbilidades cerebrales y presentan un cambio en las respuestas inmunológicas hacia perfiles pro-inflamatorios del tipo Th1/IL-12; así como una alta producción de anticuerpos anti-LM, lo que sugiere una buena estimulación de la respuesta inmunológica de memoria. Además, estas nanovacunas son capaces de activar la producción de citocinas Th1 en MoDC de pacientes con listeriosis, lo que sugiere que pudieran ser una buena formulación de nanovacunas que pudiera beneficiar y proteger a individuos mayores, mejorando su respuesta inmune específica para LM.

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0079

El sitio sinérgico de la fibronectina; su papel en la regeneración muscular

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La Fibronectina (FN) es una proteína de la Matriz Extracelular (MEC) muy abundante en mamíferos y fundamental en embriogénesis y regeneración. Tras una lesión tisular se produce un proceso inflamatorio y de fibrillogénesis en el cual la FN forma una red fibrilar para que migren las células adyacentes a la herida hasta rellenar y regenerar el hueco de la lesión. Se ha sugerido que la FN puede tener un papel fundamental en los cambios que sufren las células satélite cuando se produce una lesión muscular, incluida la migración y diferenciación de estas células hasta formar miofibras. Esto es posible gracias a la interacción que las células hacen con la FN mediante receptores, como las integrinas y sindecanos.

La FN tiene estructura modular formada por repeticiones de tres tipos, módulos de tipo I, II y III. La región que más integrinas es capaz de unir es la repetición 10 del dominio III (FNIII-10), con un motivo RGD, que une tanto integrinas α5β1 como αv. En el dominio colindante, FNIII-9, existe un motivo DRVPPSRN o *sitio sinérgico* que actúa como cinturón de seguridad cuando las tensiones entre las uniones integrina a5β1-FN son muy grandes y el motivo RGD por sí mismo no bastaría para garantizar un agarre con las células.

Nuestro laboratorio generó una cepa de ratones con un sitio sinérgico mutado (DAVPPSAN) que no es funcional (FNsyn/syn) y está estudiando en estos ratones la regeneración muscular tras inducir lesiones con cardiotoxina. Pudimos comprobar que en las regiones lesionadas de los ratones FNsyn/syn la población de células *Pax7*+ estaba significativamente disminuida, comparando con ratones control FNwt/wt. Para analizar estos aspectos, derivamos e inmortalizamos una línea de células satélite a partir de ratones FNsyn/syn y estamos analizando su proliferación y diferenciación sobre sustratos de FN.

0097

The mitochondrial protein GDAP1 participates in the autophagic pathway

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GDAP1 is located in both the outer mitochondrial membrane (OMM) and the mitochondrial-associated membranes (MAMs) [1]. MAMs are specialized subdomains of the endoplasmic reticulum (ER) that participates in the cross-talk between the ER and mitochondria. GDAP1 depletion causes mitochondrial dysfunction and reduces the juxtaposition between ER and mitochondria [1,2], possibly affecting the functionality of the MAM. In addition, GDAP1 mutations cause Charcot-Marie-Tooth (CMT) neuropathy.

MAMs function is involved in the autophagy pathway. MAMs, and more specifically the OMM, supply the membrane for autophagosome formation. The autophagosomes will fuse with the lysosomes to degrade and recycle the cytoplasmic material and organelles targeted to degradation.

Recent findings of our group reported an increment of autophagic vesicles by electronic microscopy analysis of *Gdap1-/-* mouse embryonic motor neurons [2], thus suggesting the autophagy involvement in the pathophysiology of CMT caused by *GDAP1* mutations.

Here, we found that silencing of GDAP1 in neuroblastoma SH-SY5Y cells and absence of GDAP1 in mouse motor neurons induce accumulation of autophagic vesicles and LC3-II protein levels. The cells also present accumulation of p62 cytosolic aggregates, indicating impairment in the autophagic flux. Accordingly, we also found downregulation of the Akt/mTOR pathway. PLA and co-IP experiments showed the constitutive interaction between GDAP1 and Syntaxin 17 and the autophagic marker LC3. In addition, we observed accumulation of aberrant perinuclear lysosomes that suggests a new implication of GDAP1 in the functionality of these organelles. Altogether these results are positioning GDAP1 in the autophagy pathway and the relationship between mitochondria and lysosomes.

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0098

Study of copper transporters ATP7A and ATP7B in human brain endothelial cell line (HBEC-5i)

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Copper is an essential dietary element with a high redox potential, which makes it an important enzyme cofactor for different biological processes and crucial for the nervous system function. An impairment of copper homeostasis drives to different pathologies with neurological affectation like Menkes Disease (MD) caused by mutations in the *ATP7A* gene (Xq21.1) characterized by a systemic copper deficiency. In the opposite case, Wilson Disease (WD) caused by mutations in the *ATP7B* gene (13q14.3) is a disorder where there is a toxic accumulation of copper in the organism. In both cases severe neurological alterations are described proving the significance of copper balance for neurological correct function.

ATP7A and ATP7B (P-type ATPases) are the two main proteins implicated in the systemic and cellular homeostasis of copper. Focusing on nervous system, the blood-brain barrier (BBB) regulates the copper transport between blood and cerebral parenchyma and the endothelial cells of brain capillaries of BBB express ATP7A and ATP7B; thus, they have been proposed as the main regulators for the entrance of copper into the brain. For this reason the study of the BBB and how these proteins are implicated in the copper flow into/out the brain arises as a necessity to understand how the copper dyshomeostasis is involved in neurodegenerative processes.

Here we present the characterization of the endogenous ATP7A and ATP7B expression in HBEC-5i cell line (as a BBB cell model) and the generation of HBEC-5i stable cell lines expressing either ATP7A or ATP7B wild type and mutants (mutations found in MD and WD patients) to study and compare their biology related to the copper transport in brain.

0108-R/M

Identification of MicroRNAs involved in the metabolic basis of renal fibrogenesis

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Excessive accumulation of extracellular matrix (ECM) is the hallmark of fibrotic diseases. In the kidney, fibrosis is the final outcome of highly prevalent clinical conditions [1]. While TGF-β plays a fundamental role in myofibroblast generation, a reduction in fatty acid oxidation (FAO) is an important pathogenetic contributor [2]. MicroRNAs (miRNAs) regulate gene expression post-transcriptionally and control diverse biological processes, including fibrogenesis [3]. To identify miRNAs involved in the metabolic regulation of renal fibrosis, we performed an array screening in the renal fibrosis mouse model of unilateral ureteral obstruction (UUO). Three miRNAs, miR-150-5p, miR-495-5p and miR-33-5p were selected for study due to their link to human pathology, their role in mitochondrial metabolism and their potential targeting of the fatty acid shuttling enzyme CPT1a. We found a 4- and 2-fold upregulation of miR-495-5p and miR-150-5p respectively in both the UUO and folic acid nephropathy (FAN) models while TGF-β1 upregulated their expressions in the human epithelial renal cell line HKC-8. Both miRNAs synergized with TGFβ regarding its pro-fibrotic effects and its ability to supress CPT1a expression and reduced FAO-associated oxygen consumption rate (OCR) in HKC-8 cells. In the case of miR-33-5p, its expression was downregulated in the UUO and FAN models by 40% and 50%, respectively. Studies in miR-33a-deficient mice (KO) subjected to UUO and FAN showed decreased kidney fibrosis and lipid accumulation compared with wild type animals. MiR-33 analogs reduced FAO-related OCR while miR-33 antagonists increased it in HKC-8 cells. To establish the role of FAO in the pathogenesis of kidney fibrosis, we generated a conditional transgenic mouse model with specific CPT1a overexpression in renal tubular epithelial cells. We found that kidneys from transgenic mice subjected to 7 days UUO exhibited decreased expression of fibrotic markers compared with those from wild type animals. Overall, we propose that strategies based on the modulation of FAO in the kidney, either by identifying mechanistic culprits such as miRNAs, or by genetically based gain-of-function approaches, may prove useful in therapies directed towards the prevention or reversion of renal fibrosis.

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0109

Developing novel strategies to block melanoma metastasis.

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Metastasis is the deadliest phase of cancer progression and responsible of 90% of all cancer deaths in melanoma patients. Although numerous studies have identified genes and mechanisms that control this process, our knowledge about how cancer cells initiate metastasis remains limited. Evidence accumulated in the past decade supports a crucial role for cells in the surrounding tumor microenvironment and their crosstalk with cancer cells as critical components for metastasis. In addition to soluble factors secreted from primary tumors, recent research has shown that exosomes (vesicles ≈30-100nm that contains both genetic material and proteins) can also influence pre-metastatic niche formation, as well as the cellular response and trafficking of immune cells and fibroblasts during tumor progression. Here, we propose for the first time that Nerve growth factor receptor (NGFR, also known as p75NTR or CD271) is secreted in melanoma-derived exosomes and promote changes in the tumor microenvironment favoring metastasis. NGFR-expressing exosomes induce NGFR expression in lymphatic endothelial cells (LECs) as the first hallmark of pre-metastatic niche formation at the Lymph nodes, usually the initial place for the metastatic colonization of melanoma patients. Moreover, NGFR overexpression in exosomes foster homing of melanoma cells in the lymph nodes and increase spontaneous lung metastasis in orthotropic models. Interestingly, inhibition of NGFR by CRISPR technology and, by the use of NGFR small molecule inhibitor, results in the blockage of tumor growth, local and distal metastasis. Since microenvironment dependent regulation of NGFR in particular subsets of melanoma has been associated with inflammation and resistance to current melanoma therapies, we are currently investigating the use of specific targeted therapies against NGFR in melanoma, understanding the molecular mechanisms involved. We believe that this approach could benefit melanoma therapeutic landscape and open new opportunities for metastatic patients.

0119-R/M

microRNAS AS PROGNOSIS BIOMARKERS OF RESPONSE TO ANTIANGIOGENIC DRUG BEVACIZUMAB IN METASTATIC COLORECTAL CANCER

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Colorectal cancer (CRC) is the third most important type of cancer causing cancer-related mortality worldwide (1). Most deaths occurs as result of the development of metastatic CRC (mCRC). Bevacizumab (BVZ) is a monoclonal antibody that binds the ligand 'vascular endothelial growth factor' (VEGF-A) inhibiting the ability of tumors to produce new blood vessels from existing vessels (angiogenesis). BVZ has demonstrated efficacy in some patients with mCRC (2, 3), however many patients develop resistance to VEGF inhibition through changes in expression of other genes involved in angiogenesis or cell proliferation. Therefore, the identification of predictive biomarkers of prognosis for bevacizumab has become a major goal with clinical interest. MicroRNAs (miRNAs) are small non-coding RNAs that work as post-transcriptional regulators of gene expression and play important roles in CRC where are important regulators of carcinogenesis, progression, invasion, angiogenesis and metastases in CRC (4, 5, 6, 7). Thus, miRNAs might serve as potential predictive and prognostic factors of mCRC. We tested the hypothesis that expression of miRNAs could be used as biomarkers of response to BVZ in patients with mCRC. In this study, the expression profiles of 14 miRNAs in two groups of patients treated with BVZ (Good response and No response to BVZ) have been analyzed using quantitative qRT-PCR. The screening analysis revealed that hsa-miR-497-5p was overexpressed in patients that show a good response to BVZ. This is consistent with previous studies showing that miR497 acts as tumor suppressor gene in different type of cancers (targeting IRS1) and negative regulator of angiogenesis (targeting VEGF-A). Despite the number of samples analyzed, miR-497 is proposed as a potential biomarker of good response to BVZ.

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0123-R/M

Study of potential genetic biomarkers of the eficacy of Bevacizumab in patients with metastatic colorectal cancer

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Metastatic colorectal cancer (mCRC) is one of the most common carcinomas (the first one in Spain) and it is responsible of a high number of deaths in the world1,2. One of the used drugs added to the previous line is Bevacizumab (BVZ), monoclonal antibody directed to VEGF, impairing the binding to its receptors and neutralizing its proangiogenic activity. However, many patients develop resistance to VEGF inhibition activating the expression of other factors3,4. Because response to BVZ is related to expression level of some genes5, the objective of this project was deepening into interindividual efficacy of BVZ in mCRC patients.

In this way, 89 angiogenesis related genes were studied in 60 mCRC patients treated with BVZ, from tumors or biopsies extracted in Hospital de Fuenlabrada (Madrid). Patients were classified in good and bad responders with respect to tumor progression after 6 months of treatment, in radiologic imaging.

Differences in the expression of 10 genes between the two patients groups has been reported. BAI1 and EFNB2 genes present a possible relationship with good response to BVZ, while ANGPT2, EDN1, IL6, PDGFA, PTGS1, HGF, PLAU and NOTCH4 are related to a bad prognosis in mCRC and a lower response to the treatment. These differential genes expression among good responders and bad responders patients could be used as biomarkers of efficacy and prognosis in mCCR patients with BVZ treatment. Additional studies are needed to further examine the role of gene expression in the optimization of the efficacy of BVZ, going ahead with precision medicine

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0132-R

Curative action of hyperbaric oxygen therapy in chronic wounds: molecular mechanisms

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Hyperbaric oxygen therapy (HBOT) is the clinical utilization of the oxygen at pressures higher than atmospheric pressure, HBOT increases the capacity of blood plasma to transport oxygen and has been successfully employed to manage diverse clinical diseases. The HBOT participation in the recovery of chronic non-healing wounds can be associated with increase the production of reactive oxygen species (ROS), which may act as cellular messengers in many signal transduction pathways. The aim of this study was to define the time course of various inflammatory mediators and grow factors in response to HBOT

in chronic wounded patients in which conventional treatments have been reported to be ineffective. The patients received 20 HBOT sessions (five sessions/week), and blood samples were obtained after the sessions 1, 5 and 20 and an additional blood sample was collected 1 month after wound recovery. The results evidenced a progressive and significant decrease in the levels of TNFα and IL-1β with the different treatments indicating a decrease in the degree of inflammation. The levels of pro-inflammatory enzymes xanthine oxidase and myeloperoxidase continue to suffer from change pattern diminishing the levels as the sessions progress. The levels of the growth factors (PDGF, TGF-1β and HIF-1α) progressively increased between the first session and the 5th and 20th sessions, being significantly higher in this last point respect the first day. A month after the recovery the levels began to reduce but without reaching the initial levels. HBOT enhanced the healing resolution reducing the plasma levels of pro-inflammatory mediators and increasing the levels of grow factors.

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0134

Desarrollo de un cultivo organotípico de leiomiomas uterinos como nuevo modelo pre-clínico

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Los leiomiomas uterinos, también llamados miomas o fibromas, son tumores monoclonales de la musculatura lisa del útero y dependientes de hormonas ováricas. Alteraciones genéticas o epigenéticas en células madre somáticas (SSC) parecen ser las responsables del origen de estos tumores. Dos genes frecuentemente alterados y considerados promotores del inicio y desarrollo de los miomas son *HMGA2* y *MED12*.

Actualmente no existen cultivos *in vitro* adecuados para estudiar estos tumores. Nuestro principal objetivo ha consistido en desarrollar cultivos de láminas finas de miomas sobre una matriz de alginato. Este polímero incorpora microesferas biodegradables que permiten encapsular fármacos o diferentes compuestos cuyo efecto puede ser determinado en los explantes de las pacientes. Además, este modelo de cultivo permitiría estudiar los mecanismos moleculares implicados en el desarrollo de estos tumores y especialmente determinar la relación existente entre las células musculares tumorales, las SSC y la matriz extracelular que las rodea.

El análisis histológico de láminas finas de mioma (500 µm) cortadas con vibratomo demuestra que el tejido se mantiene por un periodo de 2 semanas sin que exista pérdida de viabilidad. En comparación con el tumor original y a diferencia de los cultivos tradicionales 2D, las láminas en cultivo mantienen los niveles de expresión, medidos mediante PCR a tiempo real, de los receptores de estrógenos (ERa) y progesterona (PGR). Otros marcadores analizados que permanecen desregulados en las láminas de cultivo al igual que el tumor original incluyen factores de crecimiento (IGF1, TGFβ3) y componentes de la matriz extracelular (Col1A1, VCAN y FN1). A nivel inmunohistoquímico, la tinción con a-actina y desmina demuestra la abundante presencia de células de la musculatura lisa que expresan ERa y PGR. Finalmente, las láminas en cultivo mantienen las mutaciones en *MED12* o la desregulación de *HMGA2* durante las dos semanas de cultivo, alteraciones que suelen perderse en los cultivos 2D.

Aquellas moléculas que muestren un claro efecto antitumoral en las láminas de cultivo organotípico serían candidatas para una terapia local alternativa basada en inyectar los sistemas microparticulados en el tumor, provocando la reducción del mismo y evitando los efectos sistémicos adversos.

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0137-R/M

El efecto del Paclitaxel en la expresión de los genes HMGB1 y HMGB2 en células cancerosas SKOV-3

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La quimioterapia utilizada frente a cánceres de ovario ejerce un papel fundamental en su tratamiento, permitiendo en muchos casos que la enfermedad se cronifique o pueda erradicarse. Un incremento en la expresión de los genes que codifican para proteínas HMGB se ha asociado con cáncer de ovario y se conoce que las proteínas HMGB intervienen en procesos de resistencia a cisplatino, pero no se conoce la respuesta de estos genes frente a otros tratamientos que se usan en quimioterapia. En este estudio hemos evaluado la respuesta de los marcadores HMGB1 y HMGB2 al tratamiento de las células cancerosas SKOV-3 Paclitaxel, fármaco utilizado habitualmente en quimioterapia contra el cáncer de ovario. Además, se incluyeron en este estudio los genes *MIEN1* y *NOP53*, también relacionados con cáncer de ovario y que codifican proteínas capaces de interactuar con las proteínas HMGB, según hemos podido demostrar mediante la técnica de doble híbrido. Los resultados indican una variación en la expresión de *MIEN1* y *HMGB1* en respuesta al tratamiendo con Paclitaxel.

0141

Evaluation of the anti-angiogenic potential of hydroxytyrosol derivatives

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Angiogenesis, a process which allows the formation of new vessels from pre-existing ones, is an essential phenomenon for tumor survival since it allows cancer cells to obtain nutrients and oxygen. This explains the increasing interest showed by many groups of research and pharmaceutical companies to find compounds with potential to disrupt at least one of the steps within the angiogenic process.

Hydroxytyrosol (3,4-dihydroxyphenyl ethanol) has been identified as the most important health-related phenolic compound of virgin olive oil because of its pleiotropic effects on multiple targets. In 2012, our group identified hydroxytyrosol as an anti-angiogenic compound able to inhibit several key steps in the angiogenic process. In the present study, the potential effects of six hydroxytyrosol derivatives are tested and compared with those exhibited by hydroxytyrosol by making use of several *in vitro* and *in vivo* assays. Results indicate that these are candidate new anti-angiogenic compounds with potential utility in anti-tumor and anti-angiogenic therapies.

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0145-R

Relevance of EGF receptor catalytic activity in liver regeneration associated with a progenitor response

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Adult progenitor liver cells, known as oval cells (OC), become activated and expand under chronic liver disease (CLD), being important players during liver regeneration. However, their role remains a matter of debate, being associated with pro-regenerative, pro-fibrogenic and pro-tumorigenic effects, depending on the specific context. Further investigation of the molecular mechanisms regulating OC fate is clearly needed. The EGFR pathway has a key role in liver regeneration and hepatocarcinogenesis, but its regulatory mechanisms on OC function remain elusive. We aim to evaluate the effect of inhibiting EGFR signaling in liver pathological contexts where a progenitor response is observed, using for that transgenic mice expressing in liver a truncated EGFR acting as a dominant negative mutant (ΔEGFR). WT and ΔEGFR mice were submitted to 0,1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-supplemented diet for 1-6 weeks, a model of liver cholestasis associated with ductular reaction and OC expansion. Results show that lack of EGFR catalytic activity leads to decreased liver damage, evidenced by lower serum levels of biochemical markers (AST, ALT, ALP and total bilirubin) and decreased fibrosis. This is accompanied by an amplified ductular reaction and higher induction of OC markers. Additional studies are being carried out in a model mimicking hepatocellular carcinoma development in chronically injured liver (Diethylnitrosamine + CCl4). Preliminary results also evidence differences in the liver response to damage. Collectively, our results support a key role for EGFR pathway in regulation of liver regeneration in CLD and in OC response while opening new perspectives to advance in our understanding on the mechanisms governing liver injury response.

0147-R

New cell genetic tracing and single-cell tools for the study of the molecular pathways involved in cancer metastasis

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Traditionally, metastasis has been seen as the final and often fatal step in the progression of solid malignancies. This vision of tumor progression has been recently challenged, as genetic analyses of circulating tumor cells and functional studies in animal models, have suggested that the dissemination of tumor cells can be a very early event, even at a premalignant stage. Whether this implies that the colonization of the distant tissue happens at such an early stage is still unclear. In accordance with an early metastatic implantation model, there is evidence in cancer of early parallel evolution of primary and metastatic tumors as well as tissue specific evolutionary branches among different metastases from the same tumor. Transcriptional analysis of cells with different metastatic potential in mouse tissues have been used to identify the genes and pathways involved in metastasis specificity. Moreover, intratumour heterogeneity has been observed in multiple cancers and has been postulated as a critical aspect for tumour metastasis and treatment resistance. In this context, the use of new molecular and sequencing strategies like cell lineage tracing systems and single-cell sequencing, to genetically modified mouse models, could provide new opportunities to unravel the molecular mechanisms behind metastatic potential. In order to construct a new fluorescent-based lineage tracing system, we have performed a systematic evaluation of the fluorescent characteristics of different proteins and have tried several orientation and locations of loxP sites in order to provide a random and proportional repertoire of fluorescent labelling after CRE recombination. Additionally, we have set up droplet-based microfluidic technology to perform single-cell sequencing on murine tumor primary samples. Here we have constructed a new allele able to produce up to 15 different colour combinations that can be uniquely identified by confocal microscopy and FACS. Additionally, we have set up the infrastructure and protocol to perform single-cell RNA-Seq and targetted sequencing on tumor primary samples. We have generated very promising new tools that could open new opportunities to study the molecular mechanisms behind metastatic potential in mouse and human tumors.

0150

HIV envelope protein-cholesterol interaction in the viral membrane: a promising requirement for cell entry

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The HIV-1 envelope protein (Env) contains an unusually large cytoplasmic tail (CT), in which three highly conserved amphipatic α-helical LLP (Lentiviral Lytic Peptide) domains are present. These LLPs have been described to play several key roles including the recruitment of Env to budding sites, its incorporation to the viral particle and its fusion capability, and to be responsible for the association of Env with lipid raft domains.

The aim of this work is to study the lipid environment of Env in the virus to determine if a specific interaction between the protein and cholesterol (chol) exists, a lipid commonly associated with lipid rafts. Also, the role of the LLPs in this interaction will be assessed. Additionally, the link between this lipid interaction and the viral entry capability, as well as the role that the LLPs play in this processes, have been studied. For this purpose, HEK293T cells were transfected with proviral plasmids expressing *wt* Env and two truncated Env proteins: ΔLLP1 and ΔLLPs. Virus producing cells were fed with radioactive photoactivatable chol, and viral particles containing this lipid were purified. This lipid allows the study of specific *in vivo* interactions as it covalently binds to any molecule closer than 3Å.

Our results clearly show that Env is associated to chol within the viral membrane, and that the truncation of the CT results in a significant loss of interaction of the protein with chol without any loss of chol amount in virus particle. This truncation induces a significant inhibition of viral entry capability, although the number of env proteins is three-fold increased. Together, these results may indicate that the loss of function derived from the truncation of CT could be related to the loss of interaction with cholesterol.

0151

The role of HIV-1 Env CT in cellular lipid interaction and the relationship with the recruitment of Env into virions

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The HIV-1 envelope protein (Env) plays a key role in the entry process, initiating viral infection. Despite some progress made in understanding the mechanism by which Env is incorporated into virions, it remains largely unknown. It has been proposed that the targeting of Env to budding regions requires lipid raft-like membrane domains, the Gag polyprotein, and the three LLP (Lentivirus Lytic Peptide) domains in the cytoplasmic tail (CT) of Env.

The aim of this work is to study the role that Gag polyprotein and LLP domains play in the interaction of Env with lipids and in its localization to the plasma membrane, and posterior incorporation of the protein into the virion.

To determine the effect of Gag, HEK 293T cells were transfected with either a plasmid that encodes only for *wt* Env or a proviral plasmid. The effect of the LLP domains was assessed by transfection with plasmids containing *wt* Env or two CT deletion mutants: ΔLLP1 and ΔCT. For lipid interaction experiments, radioactive photoactivatable lipids were used, which allow to research specific *in vivo* interactions due to the fact that they covalently bind to any molecule closer than 3Å.

Our results suggest that Env interacts with cholesterol and sphingolipids in the plasma membrane, independently of Gag. The CT of Env is shown to be a determinant of this interaction as the truncation of the three LLP domains induces a significant loss of lipid interaction. This truncation also alters Env's distribution, enriching the protein in the plasma membrane and inducing an increase in incorporation into virions. This suggests that the CT domain seems to be essential in the interaction of Env with lipids, taking part in the mechanism by which Env is recruited to the plasma membrane and incorporated into virions.

0173

Modelling genetic mechanisms of osteoarthritis using human induced pluripotent stem cells

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Osteoarthritis is an age-related degenerative joint disease that poses a substantial health, social and economic burden. Despite significant advances in recent years, the molecular basis of its pathogenesis is still poorly understood. The overall aim of our work is to model genetic mechanisms of osteoarthritis (OA) using chondrocytes derived from human induced pluripotent stem cells (hiPSCs). Using this human in vitro model, we aim at studying how individual genetic variation contributes, at cellular and molecular level, to the susceptibility to develop OA and the role that may play in disease progression. For the generation of the chondrocytes we are using standard open access hiPSC lines derived from healthy donors and fully characterised by the HIPSCI project (www.hIPSCI.org). The differentiation protocol recapitulates developmental stages observed during chondrogenesis in vivo and relies on chemically defined culture media. We are using the data from the molecular phenotyping of primary human samples and the hiPSC-derived chondrocytes, to select OA susceptibility loci that represent functional regions (promoter/enhancers), with particular emphasis on those involved in chondrocyte development and function. To understand their contribution to the disease phenotype, we will knockout the expression of the associated candidate genes in hiPSC using the CRISPR-Cas9 system and phenotype, molecularly and functionally, the derived chondrocytes. Overall, this study will contribute to understand the architecture of transcriptional networks characterising cartilage cells and the mechanisms by which changes in the genome affect OA disease susceptibility and progression. It will also help identify potential pathways and targets for OA diagnosis or disease-modifying drug development. Finally, this work will provide a proof of principle for the use of hiPSC-derived chondrocytes as an in vitro model to study OA and as a drug discovery platform.

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0194

Using Correlative SIM and cryo-SXT to explore Hsp90 inhibited myocardial fibroblasts by a new designed modular nanobiotechnological tool

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A chronic injured heart develops fibrosis and hypertrophy as defence mechanisms to restore function. These protective mechanisms can cause organ failure in the long term. This pathologic situation is becoming a socio-economic problem that cannot be treated with medical therapy implying ultimately aortic valve replacement. A possible pharmacological target for this problem is reducing the overexpression of collagen in the affected tissue. This could be accomplished by targeting the Hsp90 protein whose absence reduces collagen intracellular deposition. This project aims the Hsp90 inhibition by a new biosynthetic nanomolecule that combine a high Hsp90 affinity and a fluorescence nanocluster module (f-TPR) for intracellular localisation. Previously, it has been determined that f-TPR binds Hsp90 and reduces its activity leading to a reduction of collagen production and deposition. Our goal now is understanding the *in vivo* native interaction of the CTPR390-488 to dissect its efficiency in the reduction of the myocardial fibrosis. To do so we are adapting a high resolution correlative approach consisting of using super-resolution light microscopy, to detect the fluorescence signal of f-TPRs, and cryo soft X-ray nano-tomography (cryo-SXT) at the Mistralbeamline, to access to whole cell ultrastructure (Varsano et al., 2016). This correlative approach is key to provide the insights on the mechanism of Hsp90 inhibition by the f-TPR modulesand its relation with the final fibrotic/non-fibrotic phenotypes

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0198

Drug-Induced modulation of Clinical Response Genes to Antipsychotics (CRGAs) in Schizophrenia: transcriptome insights from human and CNS-derived cells.

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[1]IDIVAL Unidad de Psiquiatría, [2]IBBTEC/CIBERSAM Neurobiological basis of the mechanism of action of drugs acting at the CNS, [3]IBBTEC Neurobiological basis of the mechanism of action of drugs acting at the CNS

INTRODUCTION

The keystone of schizophrenia (SCZ) treatment is antipsychotic (AP) medication. APs can target G-protein coupled receptors (e.g. D2R, 5-HTR2A) in the cellular membrane, but their downstream effects controlling transcriptional mechanisms remain unknown. Recent results from our group in blood-based RNA-seq studies, revealed six CRGAs with altered mRNA expression (overexpressed) in naïve SCZ patients that can be reverted to normal levels after treatment using APs.

Our aim is to identify the main mechanisms that control CRGAs expression downstream of the receptors targeted by APs to improve our ability to diagnose and treat SCZ patients.

MATERIAL AND METHODS

- Schizophrenia patients were obtained from PAFIP program (HUMV) and SK-N-SH cell line was obtained from ATCC.

- Gene expression was measured by qRT-PCR.

- Protein phosphorylation was studied by Wester-Blot and transcriptional activity by Luciferase report assays.

RESULTS

Results in an independent cohort of clinically characterized patients validated the significant modulation of CRGAs in the onset of the disease and in response to APs. In SK-N-SH cells APs (Haloperidol, Clozapine, Risperidone and Aripiprazole) dynamically regulated CRGAs gene expression upon time. Also, in these cells, the use of selective agonists for the selected receptors positively regulated CRGAs gene expression whereas selective antagonists and antipsychotics, inhibited this activity. Finally, we show that downstream of these receptors several transcription factors can participate as mediators controlling the expression of the CRGAs.

CONCLUSIONS

Deregulated CRGAs can participate in the onset of the disease. Also, data suggest that APs can modulate CRGAs expression. GPCRs targeted by APs can trigger CRGAs expression by a mechanism possibly impinging the main transcription factors of these pathways

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0200-R

Deregulated mechanisms downstream of PLCG1 promoting Cutaneous T Cell Lymphoma progression

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IDIVAL Hematopatología traslacional

Introduction

Cutaneous T Cell Lymphoma (CTCL) is a heterogeneous group of non-Hodgkin lymphomas with unfavourable prognosis at advanced stages. The malignant network controling the development of CTCL is now starting to be characterized. NGS-derived studies have identified multiple molecular alterations in specific genes and signaling pathways including PLCG1 and JAK-STAT. It has been shown that up to 50% of CTCL cases develop deregulated PLCG1-downstream signaling as shown by activating *PLCG1* mutations and *PRKCQ* amplifications. Also, frequent mutations in *JAK1* and *JAK3* and in members of the STAT family have been identified, revealing a potentially important role of these signaling pathways in CTCL. We aimed at exploring the interactions between deregulated PLCG1 and JAK-STAT activity in CTCL using pre-clinical models and in samples from CTCL patients at different stages of the disease.

Materials & methods

HEK-BlueTM IL-6 and CTCL cell lines were used to study the signalling. Immunohistochemistry (IHC) of NFAT, pSTATs and NF-κB proteins was performed in 71 patient samples. The status of PRKCQ was analysed in 45 samples by fluorescent in situ hybridization.

Results

Deregulated PLCG1 activity elicited NFAT and STAT3 activation ex vivo. Also, pharmacological and genetic inhibition of PRKCQ negatively affected NFAT and STAT3 activation whereas a constitutively active PRKCQ mutant (A148E) was sufficient to trigger their transcriptional activity. Our data shows that downstream of PLCG1, PRKCQ can activate transcription mediated by both NFAT and STAT proteins. We also analysed nuclear accumulation of NFAT, STAT and NF-κB family proteins by IHC in patient samples. Interestingly positive P-STAT3 staining correlated with advanced diseases. Moreover, most CTCL-tumor stage cases studied were positive for *PRKCQ* amplification and/or 10q23 polysomy.

Conclusion

Our results support PLCG1 playing a mayor role at controlling CTCL progression to advanced stages. Downstream of PLCG1 or due to genetic amplification, PRKCQ can mediate the activation of NFAT and STAT3. Thus, mutations in *PLCG1*, *PRKCQ* amplification and/or nuclear NFAT and STAT3 accumulation can serve as diagnostic markers and provide rational to develop specific therapies targeting PRKCQ alone or in combination with CaN or JAK-STAT inhibitors for CTCL.

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0204

Effect of hypoxia on endothelial progenitor cells growth, gene expression, and exosome production.

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Background:

Endothelial Colony Forming Cells (ECFCs) are an Endothelial Progenitor Cell subpopulation with endothelium reparative as well as angiogenic and vasculogenic activities. Therefore, besides their potential therapeutic applications, they can be useful as a model for endothelium related pathologies such as cardiovascular diseases, as well as for pathophysiological angiogenic processes involved in tumour development. Hypoxia is one of the main

stimuli and drivers of angiogenic activity in the organism, so we are exploring at different levels the effect of low O2 tension in the behaviour of ECFCs in vitro.

Methodology:

ECFCs were isolated from healthy donor adult peripheral blood. Cell cultures were split at an early passage and maintained at 21% O2 (normoxia) or at 3% O2 (hypoxia). Cell growth was assessed throughout successive passages at the short and long time frame. Clonogenic assays were conducted, and cell cycle analysis and senescence assays complemented proliferation studies. mRNA levels of known specific genes regulated by oxygen availability (GLUT1, VEGFA, HIF1A, KDR) were determined. Finally, exosome and microvesicle isolation from cell culture supernatants obtained in normoxic and hypoxic conditions was accomplished by different protocols, and characterization assays were performed.

Results and conclusions:

No significant differences were observed in cell growth rates between normoxic and hypoxic conditions. Cell cycle progression did not differ between conditions and senescence in the cell population was slightly elevated in hypoxic conditions. However, an increased clonogenic capacity of ECFCs, considered as the appearance of larger proliferative colonies, was noted in hypoxic conditions, although it diminished throughout passages. GLUT1 and VEGFA gene expression was increased in hypoxic condition, while HIF1A and KDR were diminished, suggesting a metabolic adaptation to low oxygen concentration. Exosome and microvesicle isolation by different methods allowed to determine the best protocol in terms of efficiency and purity. No morphological differences were observed between exosomes derived from normoxic or hypoxic conditions.

0225

Making acute leukaemia initiating cells vulnerable by targeting the hypoxia signaling pathway

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CIC bioGUNE Metabolism and signaling in diseases

T-cell acute lymphoblastic leukaemia (T-ALL) is an aggressive malignancy produced by the uncontrolled proliferation of immature T lymphocytes. Despite the significant advance in the overall survival achieved in the past decades, overcoming resistance to chemotherapy is still the main therapeutic challenge in the treatment of T-ALL.

Seminal studies have shown that only discrete cell populations, known as leukaemia initiating cells (LICs), have the capacity to recreate the complete phenotypic heterogeneity of the primary malignancy and account for recurrence. Therefore, a better understanding of the key regulatory pathways that control LICs is vital for patients.

Low oxygen availability, or hypoxia, is a hallmark of the bone marrow stem cell niche and it is well known to affect the plasticity and properties of cancer stem-like/progenitor cells (CSCs) in solid tumours. Our results clearly stay a role for the hypoxia signalling pathway in the resistance of leukaemia cells against chemotherapy and the maintenance of LICs. Here, we will discuss our more recent results deciphering how hypoxia promotes LICs. Hence, targeting the hypoxia signalling pathway individually or in combination may provide new therapeutic approaches to eradicate residual malignancy and improve clinical outcomes for T-ALL patients.

0227-R/M

Implication of the Wnt/β-catenin pathway in the maintenance of cancer stem cells (CSCs) and tumor progression in bladder cancer

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Urinary bladder cancer is a major cause of morbidity and mortality worldwide, with over 400,000 new cases diagnosed per year. Most cases are urothelial carcinomas, which often have a good overall survival rate. However, about thirty percent of cases present as muscle-invasive carcinomas, which carry a higher risk of lethal metastatic disease. Muscle-invasive bladder carcinoma may need radical surgery and adjuvant chemotherapy with gemcitabine or platinum, and relapsed or refractory cases may require second-line therapies, which most often consist of chemotherapy with taxanes as paclitaxel or docetaxel. In several types of tumors, the presence of large populations of cancer stem cells (CSCs) and its relation to unfavorable prognosis of patients has been demonstrated. For this reason, the identification of CSCs markers, as well as other which plays a key role in the proliferation and maintenance of CSCs as Wnt/β-catenin pathway are necessary for the search of new therapeutic strategies against these CSCs. Because of that, the use of inhibitors of this signaling pathway is postulated as a possible therapeutic alternative. In this work we have shown that paclitaxel-resistant bladder cancer cells have higher levels of several markers of CSCs such as ALDH1A1, Oct-4, Sox-2 and SLUG, and higher levels of active Wnt/β-catenin pathway as demonstrated by the increase of active β-catenin and GSK3β phosphorylated (an inhibitor of this pathway). We have also observed that the pharmacological inhibition of β-catenin by XAV939 combined with paclitaxel could sensitize those paclitaxel-resistant cells, which present a more aggressive phenotype. In addition, ALDH1A1, Oct-4 and p-GSK3β may be new useful biomarkers for tumor aggressiveness and resistance to the therapy in patient biopsies of bladder cancer.

Founding: FIS:17/1240; SAF2017-87358-C2-(1R-2R)

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0233

Using CRISPR tools for the functional characterization of a novel mutation in EPHB4 that underlies a case of lymphangiomatosis.

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Lymphangiomatosis is a rare condition characterized by proliferation of lymphatic vessels. The specific presentation of the condition, and its severity, are determined by the location of the hyperproliferating lymphatic tissue. The molecular basis of lymphangiomatosis is largely not understood. We performed exome sequencing (WES) of DNA from a four-generation family of subjects with dominantly inherited lymphangiomatosis led to the identification of variant in *EPHB4* that is predicted to affect mRNA splicing. WES analysis revealed a heterozygous mutation in *EPHB4* (c.2334+1G>C) and RNA-Seq demonstrated that the *EPHB4* mutation creates a cryptic splice donor that results in retention of the intervening 12-bp intron sequence. We used CRISPR-Cas9 tools to generate a cellular model for *EPHB4* to confirm that the insertion causes the retention of the 12 bp of the intron and leads to an in-frame insertion of four-amino-acid in the highly conserved catalytic loop of protein kinase domain .The knock-in was confirmed by directly amplifying and sanger sequencing of the genomic region. Western blot analysis of the gene-edited cells with the *EPHB4* splice-altering mu-

tation displayed enhanced phosphorylation of p70S6K levels as compared with that of wild type cells suggesting that the Knockin of *EPHB4* mutation in HEK293T cells results in increased mTORC1 activity. In order to examine the functional consequences of this alteration of the EPHB4 protein, we have generated expression constructs representing the wild type and abnormally spliced variants of *EPHB4*, and expressed them exogenously in cell lines. The western blot analysis showed that under normal culture conditions, despite similar expression levels, the wild type protein is much more heavily tyrosine phosphorylated than the mutant. This indicates that the small insertion in the protein sequence caused by altered mRNA splicing has significant impact on protein functionality.

0237-R

The mutational and transcriptome landscape of infant B-cell acute lymphoblastic leukemia: The INTERFANT treatment protocol experience

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Infant B-cell precursor acute lymphoblastic leukemia (iBCP-ALL) has dismal prognosis, especially with *MLL*-gene rearrangements (*MLL*r) which are hallmark clonal leukemogenic drivers. Molecular pathogenesis of *MLL*r-iBCP-ALL remain somehow enigmatic and *in vivo* recreation of *MLL*r-iBCP-ALL is challenging.

Material and Methods

We performed whole-genome, exome, targetted and RNA-sequencing on an Interfant study discovery cohort of 50 iBCP-ALLs (27MLL-AF4+, including relapses, 5MLL-AF9+ and 10non-MLL). An independent validation cohort of 82iBCP-ALLs (43MLL-AF4+, 11MLL-AF9+, and 28non-MLL) was used for targeted DNA-sequencing/qRT-PCR.

Results and Discussions

iBCP-ALL shows an extremely low frequency of somatic mutations, irrespective of the presence/subtype of MLLr, with the predominant leukemic clone carrying a mean of 2.5 non-silent mutations. Recurrent mutations were exclusively found in *KRAS*and *NRAS*, which were more frequent in the MLL-AF4+ than in MLL-AF9+/non-MLL iBCP-ALL due to common *NRAS* mutations found in MLL-AF4+ infants (32%*vs*6%; p<0.01). These mutations were subclonal and frequently lost at relapse, despite a larger number of non-silent but non-recurrent mutations (19.5 mutations/patient). RNA-seq/qRT-PCR validation revealed that there are deregulated protein coding genes related to three importants pathways such as cell cycle regulation, DNA integrity check point and DSB DNA repair. Also, deregulated LncRNAs werefound that could provide further mechanisms of tumorigenesis. Furthermore, different isoforms of the reciprocal fusion *AF4-MLL* were expressed only in 55% of the t(4;11)+ patients, and HOXA cluster genes are uniquely expressed in *AF4-MLL*-expressing t(4;11)+ patients. AF4-MLL/HOXA-expressing patients displayed higher 2-year event-free survival than patients lacking AF4-MLL expression (65%vs34%, p=0.15). Opposite to pediatric/adult BCP-ALLs, BCR repertoire analysis revealed only minor, non-expanded B-cell clones in t(4;11)+iBCP-ALL.

Conclusion

iBCP-ALL shows a silent mutational landscape regardless the *MLL* status. The expression of *AF4-MLL* associates to a better prognosis and specific upregulation of HOXA cluster genes. A pre-BCR early progenitor/stem cell may represent the cell-of-origin for both the t(4;11) and RAS mutations.

0240

El hurón como modelo traslacional para estudiar la marronización del tejido adiposo periaórtico de aplicación en investigación sobre obesidad y enfermedad cardiovascular

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El tejido adiposo marrón es el responsable de la termogénesis facultativa que se induce en respuesta a determinados estímulos para producir calor e incrementar el gasto energético. La adquisición de características de tejido adiposo marrón (marronización) por parte del tejido adiposo blanco perivascular (TAPV) es un área de investigación en estudios de prevención de obesidad y enfermedades cardiovasculares. La mayoría de los estudios de marronización se han realizado en roedores, que no son el modelo más idóneo pensando en la extrapolación de datos a humanos. El hurón es un modelo animal con un tejido adiposo más similar al humano. Por ello, en este trabajo se ha analizado la marronización del TAPV aórtico (TAPVa) en respuesta al frío, el más potente estímulo de la termogénesis y de la marronización. Se ha realizado un análisis transcriptómico global del TAPVa en hurones de tres meses de edad aclimatados a 22ºC o a 4ºC, y se han comparado estos resultados con los obtenidos en el tejido adiposo inguinal (TAi). Para visualizar la marronización en ambos tejidos se ha llevado a cabo un análisis immunohistoquímico. Los datos del microarray revelan una fuerte respuesta a la exposición al frío en el TAPVa, observándose un aumento en la expresión de biomarcadores clave de la marronización en comparación con el TAi. Esto se traduce en una clara marronización del TAPVa, con la aparición de multitud de adipocitos positivos para la proteína termogénica clave, UCP1, en este tejido. La exposición al frío también disminuye la expresión de genes implicados en la respuesta inmune, siendo la respuesta inmunitaria la vía más afectada por la exposición al frío en el TAPVa. Por otro lado, en el TAi el frío incrementa la expresión de genes de la oxidación de ácidos grasos y del ciclo de ácidos tricarboxílicos, lo cual va asociado a una tendencia a reducir el tamaño de los adipocitos del TAi y a una disminución del peso de este depósito en los animales expuestos al frío. En resumen, la exposición al frío produce una marronización e inmunosupresión en el TAPVa de hurones. Debido a su semejanza con humanos, estos resultados presentan a los hurones como un modelo animal a tener en cuenta en investigación traslacional, pues muestran respuestas funcionales relevantes para estudios de obesidad y enfermedad cardiovascular.

0248

Identification of molecular targets of SWI/SNF alterations in cancer development

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SWI/SNF chromatin remodelling complex has been described to be altered in nearly 20% of all human tumour types, which places it as the most broadly mutated molecular system in human cancer, just after *TP53* [1]. However, the molecular mechanism underlying its involvement in tumour progression remains elusive. Among the different subunits of the complex, *ARID1A* has been identified as one of the most frequently mutated genes in several human malignancies, such as gynaecological and intestinal tumours [2, 3]. Therefore, one of the aims of our research is to get a further insight into the transcriptional alterations resulted from ARID1A-deficiency in different cellular contexts.

In order to achieve this set goal, we have generated stably-transduced cell lines for a doxycycline-inducible vector that directs the expression of different shRNAs targeting *ARID1A*in different human cancer cell lines. After the verification of an effective ARID1A-knock down, RNA-Seq experiments revealed both shared and tissue specific molecular pathways altered in the different cell lines. Among them, it should be highlighted an upregulation of genes belonging to proliferative pathways, as well as a downregulation of genes involved in apoptosis, which suggests an augmentation in their oncogenic capacities. What is more, there was also an upregulation of genes involved in cell migration, which might imply a potential increase in their metastatic capacities. Finally, gene-set enrichment analyses showed a significant upregulation of genes related to immune response. These results might help to clarify the molecular pathways underlying the role of *ARID1A* alteration in tumour progression and they could also suggest new therapeutic opportunities for SWI/SNF-deficient tumours.

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0258

The Protein Kinase PKR (EIF2AK2) is a Potential Predictive Biomarker in Metastatic Colon Cancer Patients Treated with 5-FU Based Chemotherapy

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The protein kinase R (PKR;EIF2AK2) is an interferon-inducible double-stranded RNA protein kinase with multiple effects on cells that contributes to the cellular response to numerous types of stress. PKR has been extensively studied for its relevance as an antiviral agent and a cell growth regulator. Recently, the role of PKR related with metabolism, inflammatory processes, cancer and neurodegenerative diseases has gained interest *(Marchal JA et al., 2014;Garcia MB et al., 2017).*The identification of PKR as a target of both conventional chemotherapeutic and novel drugs *(Garcia MA et al., 2011;Marchal JA et al., 2013; Cruz Lopez et al., 2017)* highlights the need to carry out translational studies with patients to validate its potential as a biomarker of important diseases like cancer. The objective of this study was to determine the expression levels of PKR as well as its cellular location in tumor tissue, respect to healthy tissue, and its relation with the clinical evolution of the patient. For this purpose, RNA extraction from paraffin tissue, immunohistochemistry of PKR and real time PCR of PKR expression were performed. Furthermore, the expression of the non-coding element regulating PKR called pre-mir-nc886 in the plasma of patients has been also analysed. To date, 80 metastatic colon cancer patients with unresectable liver metastases, treated in the first line with chemotherapy based on the use of 5-Fluorouracil (5-FU) drug have been evaluated. We have shown that both the expression and the location of PKR vary between the tumor and the healthy tissue of each patient, as well as between the different patients analysed. The data have been statistically related to the Objective Rate of Response of the patients to the chemotherapeutic treatment and have allowed to indicate that both the expression and the levels of PKR and nc886 tend to be statistically related to the evolution of the patient. Therefore, we must increase the "n" of patients, due to our results suggest that PKR may be a potential predictive biomarker of response to treatments based on the use of 5-FU in metastatic colon patients

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0259

DECIPHERING THE IMPACT OF RETROTRANSPOSITION IN THE EVOLUTION OF AN ANCESTRAL TRANSMISSIBLE TUMOUR

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Transposable elements represent around 40% of total content in mammalian DNA, keeping a main role in the evolution of the architecture of their genome. Mobilization of active copies can produce dramatic impact, ranging from inherent insertional mutagenesis till the eventual promotion of chromosomal rearrangements. This may alter the development of genetic diseases, both in germline and in somatic tissues.

Cancer encompass a set of genetic diseases where somatic retrotransposition may have an important role. The process of clonal evolution experimented by tumour lineages demand an input of genetic variation that retrotransposons can provide. However, the scope of this process over time and its relationship with the development of metastasis still greatly unknown.

For this reason, we have focused our research in the Canine Transmissible Venereal Tumour (CTVT), the oldest living cancer found in nature, and a great model for studying the evolution the low-frequency mutational processes within the cancer genome.

We have analyzed the genomic sequences from 15 CTVT tumours and their hosts, identifying a set of candidate somatic insertions. Our results depict high activity of retrotransposons from L1_cf and SINEC_cf families. We fully characterized 5336 retrotranspositions and estimated their relative age according to ancestral state phylogenetic reconstruction.

We detected 403 candidate somatic transductions and identified active LINE source elements in chromosomes 3, 25 and 32 which may be mastering retrotransposition activity in CTVT.

A total of 1473 candidate somatic insertions were found in the sequence of coding genes, including 86 loci included in COSMIC Cancer Gene Census. Remarkably, we found insertions on tumour suppressor genes (LRP1B, RSPO2, STAG1…) with an important role in the regulation of epithelial carcinogenesis.

We found 18 candidate somatic insertion disrupting the exonic sequence of coding genes. Among them stand out a 258 bp insertion affecting the exon 17 of gene CSMD3, which may arise as a driver event in the early evolution of this transmissible cancer.

These preliminary results suggest a long-term impact of somatic retrotransposition on tumour genomes which may have particular relevance for the development of metastatic cancers.

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0272

Effect of the silencing of the FUT8 gene on the composition of exosomes of SW480 and SW620 colorectal cancer lines

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Exosomes are characterized by having a nanoscale diameter (10-200 nm) and participating in the immune escape, neovascularization and metastatic spread of tumor cells. For the present study, exosomal fractions from 1) the isogenic lines of colorectal cancer (CRC) SW480 and SW620, derived from the same patient in two different malignant stages, and 2) their corresponding counterparts silenced with shRNA for the *FUT8* gene, have been obtained by acid precipitation. *FUT8* encodes the enzyme α(1,6)fucosyltransferase [α(1,6)FT], which catalyzes the a(1,6)fucosylation of the common pentasaccharide *core* of N-glycoproteins (core-fucosylation). Once the biochemical purity and morphological integrity of the exosome fractions were checked, the comparative study of the different wild-type (non-silenced) and *FUT8*-silenced clones of SW480/SW620 lines was carried out. The analysis performed consisted in the valoration by western blot of a panel of selected glycoproteins, with the aim of identifying alterations in the biochemical composition of exosomes linked to α(1,6)FT deficiency and malignization in CRC. The panel was composed of 1) the specific exosome markers heat shock protein 70 (HSP70) and ALG-2 interaction protein (ALIX), 2) some potential targets of α(1,6)FT-dependent fucosylation with reported evidences of involvement in CRC, such as the Hepatic Growth Factor Receptor (HGFR) and the Death Receptor 4 (DR4), and 3) the α(1,6)FT enzyme.

0278

Implications of CB2 cannabinoid receptors in the neurodegenerative process associated to tauopathies

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Background: Tauopathies are a group of neurodegenerative diseases characterized by the aggregation of TAU protein for which there is no effective treatment yet. This is the reason why new approaches are now being investigated, such as the endocannabinoid system. In Alzheimer's disease, it has been shown that the levels of type-2 cannabinoid receptors (CB2) are increased in the glial cells that surround amyloid plaques. Nevertheless, the effects that TAU accumulation has on CB2 expression and its implication in the neurodegenerative process are still unknown.

Results: We have seen an increase in CB2 expression in hippocampal neurons of 12 month-old transgenic mice overexpressing hTAUP301S protein. This alteration is an early event on the pathology, as CB2 levels are also increased in another animal model overexpressing hTAUP301L where there is not neuronal death. Moreover, CB2 expression in these mice can be modulated by dimethyl fumarate (DMF) treatment, an NRF2 inductor with neuroprotective effects. These results were reproduced *in vitro* when hTAUP301L was overexpressed in HT22 cells. Finally, we found an antioxidant response element on *CNR2* promoter and our analysis *in vitro* point out at the possibility that CB2 expression could be modulated by NRF2.

Conclusions: Overexpression of hTAU protein increases CB2 levels in neurons and can be modulated by DMF treatment.

0280

Búsqueda de Biomarcadores moleculares y celulares para el Síndrome Idic15: Estudio multidisciplinar sobre pacientes españoles

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El Síndrome Idic15 está causado por duplicaciones de la región 15q11-q13. Su baja prevalencia, la complejidad de la región génica (Prader-Willi/Angelman), la variabilidad en tamaño y disposición de las duplicaciones, y la débil correlación entre genotipo y clínica dificultan su diagnóstico y tratamiento tempranos, relegando éste último a medidas sintomáticas paliativas. La existencia en el Idic15 de alteraciones bioquímico-metabólicas detectables a nivel sistémico que puedan servir como marcadores moleculares para la enfermedad no ha sido evaluada. Estos marcadores serían una valiosa herramienta para el diagnóstico precoz, el pronóstico de la enfermedad o sus complicaciones, y la orientación y seguimiento de terapias farmacológicas y/o nutricionales. Esta posibilidad es la que aborda el presente Proyecto, un estudio observacional, caso-control, sobre pacientes españoles diagnosticados de Idic15 ((N=28) reclutados entre los adheridos a la Asociación Idic15 España http://www.idic15.es/) y controles (N=17) pareados en edad y sexo. Según el aCGH de los puntos de rotura (BP) descritos en la región 15q11-q13, la duplicación BP1-BP5 se observa en 16 pacientes, la BP1-BP3 en 10, y las BP1-BP2 y BP2-BP3 sólo en 1. Las principales manifestaciones clínicas incluyen hipotonía (61%), TEA (50%), infecciones recurrentes (50%), TGD y/o TDAH (50%) y epilepsia (43%). La relación entre estas observaciones y la búsqueda de biomarcadores asociados constituyen los objetivos del presente trabajo, en el que se hace uso de un abordaje multidisciplinar para evaluar exhaustivamente los aspectos bioquímico-metabólicos, (epi)

genéticos, transcriptómicos, proteómicos y citómicos del síndrome. Proyecto financiado mediante donaciones a la Iniciativa "Una casa una vida" promovida por Great Chance SLU.

0281-R/M

Calcitriol inhibits the protumoural properties of colorectal cancer-associated fibroblasts and the expression of its receptor in these cells predicts patient clinical outcome

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Vitamin D deficiency is associated with a higher risk of colorectal cancer (CRC). Accordingly, calcitriol, the most active vitamin D metabolite, inhibits the proliferation and promotes the epithelial differentiation of human colon carcinoma cell lines. Calcitriol action is mediated by the vitamin D receptor (VDR), a member of the superfamily of nuclear receptors that upon ligand binding regulates the transcription of target genes. Recent data indicate that fibroblasts are the principal cellular component of tumour stroma and contribute to tumourigenesis by numerous mechanisms.

Thus, we have investigated calcitriol effects on primary cultures of CRC patient-derived colon normal fibroblasts (NFs) and cancer-associated fibroblasts (CAFs). NFs and CAFs express VDR and respond to calcitriol. Remarkably, calcitriol inhibits two fibroblast protumoural properties: the ability to reorganize the extracellular matrix and the capacity to paracrinally promote the migration of CRC cells. Moreover, global transcriptomic analyses show that calcitriol regulates the gene expression profile of NFs and CAFs, and imposes in CAFs a gene signature that correlates with a better outcome of CRC patients.

We have also analysed the expression of VDR and of two calcitriol target genes (*CD82*, upregulated; and *S100A4*, downregulated) in 658 metastatic CRC patients. Importantly, high VDR expression in tumour stromal fibroblasts is associated with better prognosis. In addition, the expression of CD82 and S100A4 in these cells is associated directly and inversely, respectively, with that of VDR and with CRC patient clinical outcome.

In summary, our results indicate that the antitumoural action of calcitriol on CRC is mediated not only by its direct action on carcinoma cells, but also by inhibiting the protumoural properties of CAFs. We propose that treatment of CRC patients with VDR agonists could be explored in those patients that express VDR in tumour stromal fibroblasts even in the absence of VDR expression in carcinoma cells.

0284

Estudio citométrico de biomarcadores de diferenciación y activación en subpoblaciones leucocitarias de pacientes con Síndrome Idic15

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El Síndrome Idic15 está causado por duplicaciones de la región 15q11-q13, en la que se encuentran algunos genes relacionados con la respuesta inmunitaria. Un estudio observacional, caso-control, con 28 pacientes diagnosticados de Idic15, reclutados entre los adheridos a la Asociación Idic15 España y 17 controles pareados por edad, sexo y área geográfica, ha mostrado una elevada incidencia de infecciones recurrentes. Por ello, se ha abordado la posible relación entre duplicaciones 15q11-13 y cambios en la función inmunitaria a través de un estudio inmunofenotípico por citometría de flujo policromática en el que se ha cuantificado la expresión en la membrana de leucocitos de 19 proteínas (CD3, CD4, CD8, CD14, CD16, CD19, CD21, CD24, CD27, CD28, CD38, CD45, CD45RA, CD56, CD57, CD197, CD279, IgD, IgM), consideradas como biomarcadores de diferenciación y activación de subpoblaciones leucocitarias. Los datos inmunofenotípicos se han correlacionado con la manifestación de autismo y con el tipo de duplicación, según el aCGH de los puntos de rotura (BP) descritos en la región 15q11-q13 (BP1-BP5, BP1-BP3, BP1-BP2 y BP2-BP3). Nuestros resultados muestran que, en ausencia de diferencias cuantitativas en las principales poblaciones leucocitarias, los pacientes con idic15 muestran cambios en algunas subpoblaciones linfocitarias con respecto a los controles, así como variaciones relacionadas con la presencia o no de autismo y el tipo de rotura. Si bien estos resultados son consistentes con las observaciones descritas en la población general con autismo, representan el primer estudio específico en pacientes afectos de idic 15. Proyecto financiado mediante donaciones a la Iniciativa "Una casa una vida" promovida por Great Chance SLU.

0295

Applied diagnostics in liver cancer. Efficient combinations of sorafenib with targeted inhibitors blocking AKT/mTOR

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Hepatocellular carcinoma (HCC) is a very important health issue globally. It is the fifth most common cancer and the second one in mortality. The causes of HCC are HBV, HCV, NASH, obesity and diabetes, among others.

Around 30-40 % of newly diagnosed patients are in the very early/early stage (BCLC0/A), but their 5-year survival can be as low as 40%. Most patients end up having an advanced disease (BCLC-C), with only sorafenib as treatment option. Their median overall survival at this point is only 11 months, which is very poor and requires a huge effort from researchers and clinicians to improve this situation.

In this study, we have designed a sequencing platform to study somatic mutations in a selection of 112 genes (HepatoExome). Using this platform, we characterized 32 HCC cases and 6 HCC cell lines and we found mutations in 112 genes, many of them with inhibitors associated. This has allowed us to design specific targeted therapies that we have used alone and in combination with sorafenib.

Sorafenib alone, elicited heterogeneous responses to different signalling pathways, probably due to the different genetic background of the cells studied. This goes along with the effect that sorafenib has in patients with different etiologies and genetic contexts. Targeted therapy inhibited key signalling pathways such as Akt/mTOR and MAPK independently of sorafenib. The combination of both synergistically elicited anti proliferative effects.

We also found how, PRAS40, an important regulator of the mTOR pathway, was significantly inhibited in response to a targeted therapy. To further study the implication of PRAS40 in HCC, we have created mutants in 3 of its main phosphorylation sites. Then, we treated with activators thought

to have an important role on HCC (FGF, IGF). We saw how the activation of mTOR effectors is different in each case, which seems to place PRAS40 as a modulator of the AKT/mTOR pathway downstream of FGFR and IGFR activation.

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0297

Estudio Citométrico de Biomarcadores de Secreción Granular y Activación en Plaquetas de Pacientes con Síndrome Idic15

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El Síndrome Idic15 es una enfermedad rara con manifestaciones neurológicas, causado por duplicaciones de la región 15q11-q13. La función plaquetaria normal, al igual que la función neuronal, dependen de forma importante de la actividad secretora de gránulos. De hecho, estudios previos han mostrado una menor capacidad secretora y menor respuesta de activación en plaquetas de pacientes autistas. Por ello, se ha abordado la posible relación entre duplicaciones 15q11-13 y cambios en la función plaquetaria a través de un estudio por citometría de flujo en el que se ha cuantificado la expresión en la membrana de plaquetas de la molécula de adhesión P-selectina y de la forma activa del receptor de fibrinógeno en respuesta a la obtención de plaquetas de sangre periférica. El estudio ha sido observacional, caso-control, con 28 pacientes diagnosticados de Idic15, reclutados entre los adheridos a la Asociación Idic15 España y 17 controles pareados por edad, sexo y área geográfica. Los datos citométricos se han correlacionado con la manifestación de autismo y con el tipo de duplicación, según el aCGH de los puntos de rotura (BP) descritos en la región 15q11-q13 (BP1-BP5, BP1-BP3, BP1-BP2 y BP2-BP3). Nuestros resultados muestran que los pacientes con idic15 muestran una disminución en la expresión de la proteína intragranular P-selectina y de la forma activada del receptor de fibrinógeno, con respecto a los controles. No se han demostrado diferencias significativas entre los diferentes subgrupos de pacientes. Estos resultados apoyan observaciones previas en pacientes con autismo y representan el primer estudio específico en pacientes afectos de idic 15. Proyecto financiado mediante donaciones a la Iniciativa "Una casa una vida" promovida por Great Chance SLU.

0308-R/M

Determination of characteristic cancer stem cells miRNAs with prognostic and predictive value in patients with colorectal cancer

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Colorectal cancer (CRC) is the third most common cancer in the world [1]. Despite advances in treatment the mortality has not decreased enough. Cancer stem cells (CSC's), a subpopulation of cells within tumors that drive cancer initiation, growth and recurrence [2], are characterized by relative quiescence, slow cell cycle progression and, accordingly, high resistance to radiation and cytotoxic drugs, which finally accounts for metastasis formation and relapse after long periods of dormancy [3]. There is strong evidence that differentiated cells and CSC communicate with the host *via* miRNAs among others. Due to the difficulty of the direct detection of CSCs in tumor, the identification of miRNAS in liquid biopsies may be decisive for monitoring these subpopulations and their involvement in the resistance and relapse of the disease [4]. A correct diagnosis is essential for adequate and effective treatment. The objective of the study was to identify and select miRNAs characteristics of CSCs in patients with several CRC stages and evaluate biomarkers efficacy to predict response to treatment, resistance and disease recurrence. Firstly, we isolated CSC from established human CRC cell lines and collected samples (healthy tissue, tumor tissue and blood) from patients. After that, miRNAs isolation from both cells and samples was done. We select 8 relevant miRNAs in CSCs and quantified their expression in patients. Our results evaluated with the Kruskal-Wallis test show variability with non-casual distribution, with a statistically significant p value. The Hochberg comparison highlighted difference with statistical significance if compare the whole group of miRNAs in healthy tissue and blood and if compare these in tumor tissue and blood. A statistically significant major expression of two miRNAs in tumor tissue and healthy tissue was found. Dividing the patients in two groups, for presence or absence of metastasis, we observed that one miRNA in blood is significantly associated with metastasis. Our results indicate that circulating miRNAs related with CSCs detected in serum or plasma from patients could be used as biomarkers in CRC.

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0326-R/M

Aryl hydrocarbon receptor regulation by hsa-miR-Chr8:96 and its role in acute myocarditis

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Myocarditis is a Th17-mediated disease characterized by the presence of immune infiltrates that triggers cardiomyocyte necrosis and fibrosis of the heart and, therefore, progressive heart dysfunction. We have analyzed the role of the Aryl Hydrocarbon Receptor (AhR) during the development of myocardium inflammation and the progression to dilated cardiomyopathy. We found that lymphoid-compartment-specific AhR-depleted chimeric mice are protected from the heart dysfunction induced after Experimental Autoimmune Myocarditis (EAM) induction, according to echocardiography and magnetic resonance imaging analysis. The analysis of Th17 and Treg cells isolated from AhR-/- chimeric IL-17eGFP/Foxp3mRFP double reporter mice during EAM by small RNAseq, shows differential expression of miRNAs involved in the balance between Th17/Treg cells ratio. We have validated AhR as a target gene of one of the novel miRNAs expressed in Th17 cells and secreted into extracellular vesicles. As a result, we find that the levels of the novel miRNA are increased in mice with EAM in the absence of AhR in the lymphoid compartment, resulting in increased IL-22 and IL-10 and therefore, in exacerbated regulatory T cell responses. Finally, we have validated this novel miRNA as a Biomarker for myocarditis patients that can be easily detected in plasma samples.

0343

Methionine Metabolism and metabolic diseases: where is the link?

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The epidemic of obesity has led to increased prevalence of metabolic diseases. Although numerous drugs have been proposed there is still a need to provide new targets of treatment. Dietary methionine restriction leads to a reduction of adiposity and increased insulin sensitivity in mice. Besides, knockdown of nicotinamide N-methyltransferase or deficiency in phosphatidylethanolamine N.methyltransferase results in protection against diet-induced obesity (DIO) and insulin resistance. The first step in mammalian methionine metabolism is catalyzed by methionine adenosyltransferases (MAT) in which the isoenzymes MATI/MATIII are expressed primarily in the adult liver and are encoded by the gene MAT1A. Thus, here we investigated if MAT1A knockdown (kd) protected mice from DIO and the associated insulin resistance, fatty liver and dyslipidemia. We also treated genetically obese mice and analyze the reversion of the associated metabolic disorders. For this, antisense oligonucleotides were used. The results showed that the product of MAT1A gene was undetectable in brown or white adipose tissue under a chow or a high-fat diet. MAT1A kd avoided the DIO and the associated hypertriglyceridemia, fatty liver and insulin resistance. Increased energy expenditure and basal respiration was observed while food intake and locomotor activity maintained unchanged. MAT1A kd led to increased fatty acid oxidation (FAO) rate and mitochondrial oxygen consumption in brown (BAT) and white adipose tissue (WAT) while maintained unaltered in liver. MAT1A kd in DIO mice induced increased expression of adiponectin and lipolysis of WAT while avoided the liver lipid storage without promoting liver fibrosis or inflammation. MAT1A knockdown in obob improved the insulin resistance and when fed a high fat diet induced body weight loss, increased FAO in BAT and reduced the fatty liver. In all these models MAT1A kd increased FGF21 secretion. As a conclusion, knockdown of MAT1A channels consumption of lipids towards adipose tissues avoiding the obesity-related metabolic disorders.

0366

p38 MAPK, new avenues for a stress kinase

Antonia Tomas-Loba

The cell cycle is a tightly regulated process orchestrated in mammals by sequential activation of cyclin-dependent kinases (CDKs). Here, we

investigated the role of the stress-activated kinase p38γ in the control of the cell cycle. Upon stress stimulation, p38γ is activated and phosphorylates retinoblastoma protein (Rb), releasing hepatocytes from quiescence and initiating the cell cycle. p38γ compensated the loss of the cyclin-dependent kinases CDK1 and CDK2, inducing hepatocyte proliferation and liver regeneration after partial hepatectomy (PHx). p38γ thus plays a central role in the transition of hepatocytes from quiescence to proliferation by regulating cell cycle progression, similarly to CDKs. This finding has clinical implications since p38γ expression is increased in human liver fibrosis and human hepatocellular carcinoma (HCC) and is required for HCC cell proliferation. Genetic deletion or pharmacological inhibition of p38γ resulted in a lower tumour burden and extended the lifespan of mice with chemically-induced liver tumours. Our results establish the importance of p38γ in HCC initiation and development and strongly suggest that inhibition of p38γ could provide therapeutic benefit in HCC.

0392

THROMBOSPONDIN-1 ROLE IN LUNG PHYSIOPATHOLOGY:PULMONARY ARTERIAL HYPERTENSION AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Thrombospondin-1 (TSP1) is a trimeric matricellular protein that plays a significant role in both vascular homeostasis and disease through its binding to different cellular receptors. Beyond its widely accepted anti-angiogenic role, TSP1 has an emerging role in different physiopathological contexts, especially regarding the molecular origin, diagnosis and treatment of several pulmonary diseases. We have recently found that hypoxia, in a HIF-2α-dependent manner, elicits an increase on TSP1 levels in tissue and pulmonary artery cells mediating structural changes in the pulmonary vasculature. Our results from in vivo and in vitro models provide genetic evidence that TSP1 contributes to vascular remodelling during pulmonary arterial hypertension (PAH). As TSP1 has been found to be increased in several pulmonary diseases, our findings likely have implications beyond PAH. We are currently investigating the role of TSP1 in chronic obstructive lung disease (COPD), a disease characterized by progressive air-flow limitation and a decrease in oxygen tension in the blood and tissue. To date, TSP1 relation to this pathology has been suggested by almost exclusively genetic profiling of COPD patients. However our results from in vitro and in vivo models might unravel the potential of TSP1 as a prognosis marker or therapeutic target in the future of COPD clinical management.

0393

DYSFUNCTION OF mRNA METABOLISM IN MOTOR NEURONS OF SPINAL MUSCULAR ATROPHY: NUCLEAR RETENTION OF POLYADENYLATED mRNAs IN RNA GRANULES

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Childhood spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by the deletion or mutation of the Survival Motor Neuron 1 (SMN1) gene that causes degeneration of spinal cord motor neurons (MNs). Type I SMA is the most severe hereditary disease of infancy and early childhood. Degeneration and loss of MN caused by decreased levels

of SMN protein leads to myofiber denervation and, consequently, muscular atrophy and progressive muscular paralysis and death, usually within the first few months after birth. The main function of SMN is the biogenesis of spliceosomal snRNPs. Linked to this function, SMN deficiency in SMA produces alterations in this snRNP repertoire and causes widespread splicing defects resulting in a severe dysregulation of mRNA metabolism in MNs. Here, we aim to further understand the cellular and molecular mechanisms of RNA metabolism dysfunction involved in MN degeneration. We have used the SMNΔ7 mouse model, which reproduces the clinical and histopathological evolution of the human Type I SMA. In particular, we have analyzed the cellular reorganization of polyadenylated mRNAs associated with the splicing dysfunction. We demonstrate that SMN deficiency induces the abnormal nuclear accumulation of poly(A) RNAs in nuclear RNA granules (PARGs) enriched in the splicing regulator Sam68. However, these granules lack RNA-binding proteins essential for pre-mRNA processing and nuclear mRNA export such as PABPN1, TDP43, hnRNPA2/B1, hnRNPM3/M4, REF and Y14. Moreover, poly(A) RNA retention was accompanied by the cytoplasmic depletion of polyadenylated mRNAs for their translation. These effects were associated with the retention of the intron-containing pre-mRNAs of Chat, Chodl, Myh9 and Myh14, which are important for MN functions. Nuclear retention of polyadenylated mRNAs appears to be a stress-related neuronal response. This response might represent an essential component of RNA metabolism dysfunction in MNs and, therefore, an important contributor to MN degeneration and SMA pathogenesis. Our results also reveal that the loss of MNs causes neuromuscular denervation and, secondarily, cellular alterations in myofiber organization with segmental disruption of the sarcomere architecture, particularly, of the actin filaments. These alterations can compromise the physiology of muscle contraction.

R03 Biología del desarrollo y modificación genómica

0035

Angiogenesis in The Developing CNS: A Role for Membrane Type-4 Matrix Metalloproteinase (MT4-MMP)

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Angiogenesis during CNS development involves the migration of endothelial cells (EC) from the perineuronal vascular plexus (PNVP) on the brain surface into the neuroepithelium. Cues involved in this guidance process are poorly known and in this context, matrix metalloproteinases (MMPs) are good postulated candidates for regulating sprouting angiogenesis in the neural tube. We have focused on MT4-MMP (MMP17), a member of this endopeptidases family that is tethered to the cell membrane via a glycosylphosphatidylinositol moiety. Information about its function and substrates is very limited to date and little is known about its role during development. We have recently showed that Mt4-mmp is expressed in EC of the PNVP and it is also located in the first blood vessels that migrate and enter the neural tissue. In fact, the formation of the vascular plexus is impaired in the developing brain of mutant embryos. Whole-mount immunostaining of the embryonic hindbrain revealed vascular defects characterized by a reduced vessel density and number of branching points in a restricted time of development. In line with these data, number of macrophages is also impaired during angiogenesis, which may correlate with the vascular phenotype observed in the mutant embryos. Our results also demonstrate that the presence of MT4-MMP is necessary for VEGF-induced EC migration. Thus, mouse lung EC derived from Mt4-mmp-/- mice showed reduced migration compared to wild type animals in cell transmigration assays. In sum, Mt4-mmp activity is required during vascular development within the brain, possibly controlling VEGF-induced EC migration and macrophage proliferation and migration.

0189

La senescencia celular programada participa en la morfogénesis del oído interno durante el desarrollo temprano: papel de TGFbeta2

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El desarrollo embrionario requiere la regulación coordinada de los programas de apoptosis, supervivencia, autofagia, proliferación y diferenciación. La senescencia celular además de participar en envejecimiento, regeneración y cáncer es esencial para el desarrollo de varias estructuras de vertebrados. El objetivo de este estudio es evaluar el papel de la senescencia celular durante las primeras etapas del desarrollo del oído interno, así como los factores y las vías intracelulares que median su aparición. Mediante cultivos *ex vivo* de vesículas óticas de pollo tratadas con inductores de senescencia (Palbociblib), senolíticos (ABT-263), factores (TGFb2, IGF-1) e inhibidores de las rutas de señalización (SB431542, inhibidor de AKT VIII) se evaluó senescencia (SAβG, beta-galactosidasa asociada a la senescencia), apoptosis (TUNEL) y proliferación celular (incorporación de EdU). También se analizó la expresión (RT-qPCR) de los genes implicados en senescencia en las distintas condiciones.

Las células senescentes mostraron un patrón temporal altamente regulado en el primordio del saco endolinfático. La senescencia se asoció con áreas de arresto proliferativo y apoptosis. La eliminación de células senescentes con senolíticos bloqueó el desarrollo del primordio del saco endolinfático mientras que la inducción de la senescencia incrementó el tamaño del futuro saco endolinfático. TGFβ, sus receptores y su diana intracelular se expresan en explantes óticos durante los estadios estudiados. La modulación de la actividad de TGFβ potencia la respuesta senescente en la vesícula ótica e interactúa con las vías de señalización inducidas por IGF-1 para coordinar conjuntamente la dinámica celular durante la morfogénesis y la diferenciación del oído interno. En conjunto, estos resultados muestran que la senescencia es un proceso natural que es esencial para el desarrollo temprano del oído interno.

0241-R

NEURONAL DIFFERENTIATION CHANGES THE OLIGOMERIC STATE OF MECP2

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Rett syndrome (RTT; Rett, A. 1966) is a serious, incurable neurological disease that affects mostly girls with a frequency of 1 in 10,000 births and is considered the most frequent cause of mental retardation after Down syndrome. 95% of RTT cases are due to "de novo" mutations of the MeCP2 gene of parental germ cells. MeCP2 is a nuclear protein involved in neuronal ma-

turation. It is involved in the overall organization of chromatin by favoring transcriptional repression or activation by binding to methylated (5mC) or hydroxymethylated (5hmC) cytosines, respectively. But the molecular mechanisms by which protein dysfunction provokes RTT symptomatology are still unknown.

We think that this mechanism could be related with the oligomerization capacity of MeCP2. In fact, the expression of MeCP2 increases during differentiation and induces the clustering of heterochromatin *in vivo* (Brero et al, 2005). In addition, it is known that MeCP2 forms dimers (Ghosh et al, 2010) and associates with other proteins such as MBD2. These interactions could cross-link the chromatin fibers stabilizing their aggregation, and provided a novelty in the molecular mechanism of the global architecture of heterochromatin (Becker et al, 2013).

In this work, differences in the MeCP2 protein bands between proliferating cells and differentiated cells in neuron are being studied. For this purpose, human (SH-SY5Y) neuroblastoma cell line and human periodontal ligament-derived cells (stem cells from the neural crest) have been differentiated into neural-like cells. We found that neuronal differentiation changes the oligomeric state of MeCP2.

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0279-R/M

Analysis of the function of SP8 transcription factor in the limb ectoderm

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We recently showed that SP6 and SP8, two transcription factors members of the SP family, are expressed in the limb ectoderm where they work in a redundant and dose dependent manner downstream of Wnt/bcatenin to induce *Fgf8* and downstream of BMP signaling to activate *En1*. Thus, SP6 and SP8 constitute an important node in the gene regulatory networks operating in the limb ectoderm controlling proximo-distal (PD) and dorso-ventral (DV) patterning (Talamillo et al., 2010; Haro et al., 2014).

To understand the SP-dependent regulatory network, we have explored the direct transcriptional activity of SP8 by combining genome-wide ChIP-seq and RNA-seq analyses. The genome-wide binding pattern of SP8 was generated by ChIPmentation using E10.5 ectodermal hulls of mice homozygous for a Sp8-3xFLAG allele and identified 1,451 conserved and significantly enriched regions. De novo motif analysis identified a CG-rich motif, corresponding to the SP1 motif, as the most highly over-represented motif. Surprisingly, the second enriched motif was an AT-rich motif, described as the preferential motif of SP7 to indirectly bind the DNA through DLX5. The comparison between the global gene expression patterns (RNA-seq) in the limb ectoderm of WT and *Sp8* null mutant embryos identified 892 differentially expressed genes. Combining the ChIPmentation and RNA-seq datasets, 183 direct SP8 target genes were identified (30% repressed and 70% activated by SP8). Further analysis indicates that SP8 acts on these direct targets mainly from distally located binding sites either as an activator through AT-rich motifs (SP7/DLX5 motif) or as a repressor through CG-rich motifs (SP1 like motif). Currently we are evaluating for activity in mouse transgenesis the CRM associated with *Fgf8* and *En1*.

In addition, to understand the functional redundancy between SP6 and SP8 we have also investigated their possible protein-protein interactions by Co-Immunoprecipitation (CoIP) and by Bimolecular Fluorescence Complementation. Heterologous expression in HEK-293 cells showed that SP6/SP8 form homo and heterodimers through their zinc finger domain. CoIP also showed that SP8 formed heterodimers with DLX5.

Our study identifies a network of SP8 target genes that function in PD and DV patterning.

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0287

Insights into the function of Hoxc genes in limb ectodermic derivatives

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In mammals, Hox genes are organized in four genomic clusters and there is a correlation between the position of each gene in a cluster and its expression pattern along the main body axis: the more 3' a gene is positioned within the cluster, the earlier and more anterior it is expressed1,2. The collinear activation of Hox gene clusters results in a Hox code for various structures along the developing AP axis. In addition, the HoxA and HoxD clusters were coopted in the development of the tetrapod limb, where the temporal collinear activation of Hoxa and Hoxd genes is also observed3,4.

We have generated series of time-sequenced transcriptomes of the developing limb by separating mesodermal and ectodermal components and have uncovered a temporal collinear activation of the HoxC cluster in the limb ectoderm. This result prompted us to re-examine the limb phenotype of mice lacking the complete HoxC cluster (HoxCKO) focusing on ectodermal derivatives5. We noted the absence of the nail organ in HoxCKO newborns, an observation consistent with the expression of several Hoxc genes in the nail developing region and the malformed-nail phenotype described in Hoxc13 mutants6. The analysis of epithelial-layer markers revealed a failure in the differentiation of the nail organ in HoxCKO mutants and similar but milder alterations in Hoxc13 mutants.

We used the ATAC-seq technology to search for regulatory regions around the HoxC locus. We identified two putative regulatory regions 5' of the HoxC cluster, one conserved in mammals and the other in placentalia. Using mouse transgenesis, we showed that these regions can drive LacZ reporter activity in the limb ectoderm, including the nail developing region. We are currently analyzing the effect of the CRISPR-mediated deletion of these enhancer regions in mice, to reach a better understanding of the cis regulatory events driving Hoxc gene expression in the limb ectoderm.

*, $ These authors contributed equally to this work

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0340-R/M

Loss of enhancer-mediated Lmx1b autoregulation disrupts limb dorsalization and causes Nail Patella Syndrome

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Regulation of gene expression in time and space is essential during development. Amongst the different regulatory mechanisms, distal acting cis-regulatory modules (CRMs) or enhancers are under big survey due to their implication in development and disease. During mouse limb development the restricted expression pattern of the LIM homeodomain transcription factor *Lmx1b* to the dorsal limb mesoderm is required for dorsal patterning. In Lmx1b KO mice *Lmx1b* transcription is reduced 5.7-fold indicating a possible role for Lmx1b in its own expression. Lmx1b-targeted ChIP-seq in E12.5 limb tissue identified two conserved Lmx1b-bound non-coding regions 60 and 66 kb upstream of *Lmx1b*. Chromatin confirmation capture analyses identified the interaction between the *Lmx1b* locus and the upstream non-coding DNA region bound by Lmx1b that overlapped with multiple chromatin regulatory marks indicative of potential regulatory activity. Mouse reporter assays showed that each CRM exhibited limb activity patterns that overlapped *Lmx1b* expression, whereas chicken reporter assays showed that the activity was dependent on Lmx1b and we termed then Lmx1b associated regulatory modules (LARMs).

To investigate the role of LARMs in *Lmx1b* expression mice lacking the 6kb region encompassing both CRMs were generated. LARMs KO displayed a double ventral limb phenotype identical to that of *Lmx1b* KO with reduced expression of *Lmx1b* in the limb. The patella and the nails were absent whereas ventrally restricted footpads and sesamoid bones developed dorsally where lack of hair was evident. Besides the limb, Lmx1b knockout (KO) mice showed kidney, eye, and cerebellum anomalies not present in LARM KO mice, supporting a limb specific role for LARM in *Lmx1b* regulation.

In humans, haploinsufficiency of LMX1B leads to Nail-Patella Syndrome (NPS) characterize by nail dysplasia, absent or hypoplastic patellae, and progressive renal disease. Interestingly, a 4.5 Kb deletion in heterozygosis encompassing the LARM region was identified in patients suffering from NPS with no mutations in *LMX1B*. Collectively, our data demonstrates that the Lmx1b dependent LARM is required for *Lmx1b* expression during limb development, supporting the existence of an auto-regulatory mechanism for *Lmx1b* expression involved in limb development and disease.

0345

Regulación transcripcional de la diversidad neuronal

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El sistema nervioso está constituído por múltiples tipos neuronales que cumplen funciones muy especializadas. Para llevar a cabo dichas funciones cada clase de neurona ha de seleccionar, de entre todo el genoma, el complemento de genes que ha de transcribir. El genoma regulador es el que contiene la información necesaria para orquestar la expresión de los genes en los momentos y lugares aducuados . Mutaciones en el genoma regulador se asocian a multitud de enfermedades con componente genético. En nuestro laboratorio hemos utilizado el sistema modelo *C. elegans* para identificar las claves del genoma regulador que controlan la selección del transcriptoma de las neuronas serotonérgicas.

0359-R/M

Discovery of a new class of lipid mediators promoting cancer chemoresistance

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The introduction of receptor tyrosine kinase inhibitor (RTKis) targeted therapies has dramatically improved treatment options for many cancer types. Unfortunately, acquired drug resistance prevents patients from complete recovery and leads to relapse and emergence of more aggressive tumors. The mechanisms by which cancer cells acquire this resistant state are poorly understood. New evidence suggests the existence of a non-mutational, drug-tolerant persistent cell state that constitutes an intermediate step towards complete resistance. Specifically targeting these persister cells constitutes a significant therapeutic opportunity to prevent or delay the appearance of chemoresistance. Here we identified a novel group of lipoxygenase-derived lipid mediators specific to the persister cell state, persistins, that provide drug tolerance and stem cell-like properties to cancer cells. Using transcriptomics and untargeted metabolomics, we found lung cancer persister cells exist in an enhanced lipid peroxidation state sensitive to ferroptosis and lipoxygenase inhibition. This state supported the rapid and sustained production of persistins, which were quickly secreted to the extracellular environment. When transferred to sensitive cancer cells, persistins provided RTKi drug tolerance and facilitated the activation of stem cell-like gene programs involved in tumor progression. A systematic analysis for persistins in the NCI 60 tumor collection revealed their presence in numerous cancer types, including melanoma, leukemia and glioblastoma, and identified a strong positive correlation between persistins and therapy-resistance. Our results reveal an unrecognized fundamental role for high lipid peroxidation states and lipid signaling in aggressive cancer progression and chemoresistance. We anticipate these results will be a starting point to develop new therapies to treat highly aggressive RTK-dependent cancer types, and reveal persistins might hold promise as prognostic markers for detecting evolving tumor chemoresistance.

R04 Biología molecular computacional y bioinformática

0008-R/M

Modeling observed features in protein MECP2, the main target associated to Rett Syndrome

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It is estimated that 15-45% of the proteins encoded in the human genome are intrinsically disordered proteins (IDPs) or present intrinsically disordered regions (IDPRs).1 IDPs/IDPRs have been found to be commonly related to various human diseases, ranging from cancer to cardiovascular disease, to neurodegenerative diseases, to diabetes.2 We have recently focused our attention on the protein MeCP2 (methyl CpG binding protein 2), a multifunctional nuclear protein that participates in neuronal maturation and differentiation.3 Mutations in MeCP2 that result in a defective protein are associated with Rett syndrome, a rare disease affecting 1/10000 women and that is the main cause of mental retardation in girls.4 MeCP2 is organized into 6 domains (NTD, MBD, ID, TRD, CTDα y CTDβ) with abundant unstructured regions (~60%), which form the basis for multiple protein-protein and protein-DNA interactions of

moderate affinity.5 Here, we model -through atomistic molecular dynamics (MD) simulations- the stabilizing/destabilizing role of the flanking domains NTD and ID on the MBD (methyl binding domain) domain, an issue we have previously observed in experimental measurements. MBD domain along with the TRD (transcriptional repression domain) domain host all the mutations associated to Rett syndrome. Likewise, we apply a MD-based approach to find out the key mediating role of water molecules in the interaction of MeCP2 with methylated and non-methylated DNA.

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0078
Non-coding mutations in cancer

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The development of Next Generation Sequencing and bioinformatic tools has transformed our understanding of the genetic alterations involved in the tumorigenic process. Nonetheless, there are still tumors without mutations in driver genes, suggesting that other mechanisms might be involved in their development. In this context, non-coding mutations have emerged as novel players in the tumorigenic process, due to the fact that they can alter the function of a gene either at the structural or regulation level. In this regard, we have discovered a mutational hotspot in a small region of the 3'UTR of a gene involved in the NF-kB signaling pathway. This region is part of a translational silencing and destabilizing element and we have shown that the disruption of this structure by these mutations would lead to an increased production of this inhibitor, enhancing NF-kB-signaling.

Puente, X. S., Beà, S., Valdés-Mas, R., et al. (2015). Non-coding recurrent mutations in chronic lymphocytic leukaemia. Nature 526, 519-524.

0087
Inferencia de redes de regulación génica a partir de datos transcriptómicos y epigenéticos

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El desarrollo de tecnologías de alto rendimiento en biotecnología ha revolucionado los métodos de estudio en biología molecular, dando paso a un estudio de genomas completos. La genómica funcional se encarga de caracterizar funciones e interacciones de regulación génica a partir de datos de expresión génica y epigenética. Estos datos modelan redes de interacción cuyas dinámicas ayudan a entender la complejidad de sistemas biológicos.

Dada la cantidad de datasets publicados, se considera fundamental la implementación de nuevos métodos de integración y estudio a través de herramientas bioinformáticas como el meta-análisis.

En esta comunicación se presentan diversos métodos de integración de datasets a partir del análisis de datos de expresión génica (RNA-seq) y epigenética (ChIP-seq), que se fundamentan en la utilización de cambios de expresión y señal de marcas epigenéticas para inferir el grado de co-regulación de los distintos elementos genómicos y establecer relaciones de regulación génica.

Los métodos utilizados se realizaron a partir de lecturas de la secuencia de ADN alineadas frente al genoma humano para analizar la expresión génica diferencial. Además, se realizaron diversas estrategias para analizar datos tales como transformación, normalización y corrección de factores asociados a la variabilidad técnica. Para el análisis de los datos genómicos se utilizó el lenguaje estadístico R y el análisis de expresión diferencial se realizó mediante DESeq2. A partir de los resultados obtenidos se realizó un análisis de enriquecimiento funcional en listas de genes y así se valoró su contribución a rutas biológicas por medio de términos GO.

En el póster se muestra un ejemplo práctico de la integración de datos en genómica funcional, mostrándose de este modo la relevancia de la utilización de herramientas de meta-análisis para el estudio de los datasets.

0089
Systematic Characterization of Splice-Site-Creating Mutations by Integrating DNA and RNA Data

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Large-scale tumour sequencing studies have identified a wide variety of somatic genomic mutations which might contribute to tumour development. However, functional characterization of these alterations has been mainly focused on non-synonymous mutations that alter the protein sequence and mutations at splice sites(1). Recent studies from our group have shown that mutations outside of splice sites can have a major impact on RNA maturation, such as those described in NOTCH1 and CSF3R, resulting in the synthesis of oncogenic proteins(2,3). Despite the potential relevance of mutation-induced altered splicing, there has not been a comprehensive assessment in cancer.

For this aim, we have developed a bioinformatic tool called SpliceXeq that systematically evaluates whether mutations detected by whole genome or exome sequencing have an effect on splicing by using RNA-seq data. Applying SpliceXeq across >100,000 high-quality mutation calls from 272 chronic lymphocytic leukaemia samples has allowed us to identify >1,000 somatic mutations that had originally been mis-interpreted. In total, ~1,000 genes harbour at least one splice-site-creating mutation, including several driver genes of this pathology, such as ATM, BIRC3, CHD2, FBXW7, MAP4, and POT1. Moreover, we have discovered a recurrent silent mutation associated with changes in splicing in a gene not previously reported, reinforcing the hypothesis that silent mutations may frequently contribute to human cancer. Broadly speaking, integration of DNA and RNA data can provide a sound basis for discovering mutation-induced splicing events and for accurately understanding functional consequences of mutation in cancer and in other human diseases.

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0121
Pan-cancer analysis of whole genomes reveals over one hundred germline LINE-1 source loci with oncogenic potential

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Long interspersed nuclear elements (L1) are widespread repetitive sequences in the human genome. Most of the ~500,000 L1 copies in the reference genome are inactive elements not able to retrotranspose. However, a yet largely undetermined subset of them remains active in the human genome and epigenetic changes occurring in tumours usually promote their activation and mobilization. In order to characterize their role in oncogenesis, we studied the landscape of L1 somatic retrotransposition in 2,954 cancer genomes from 37 cancer types1. We identified 18,739 somatically acquired L1 integration events, affecting 35% of samples. We uncovered that some of this genomic variation is the consequence of the activation of 124 germline L1 source loci that are heritable structural variants in human populations2. Eight of them are particularly active (hot-L1s) and dominate the retrotransposition landscape. Hot-L1 elements display two extreme patterns of activity, which we have termed as Strombolian and Plinian, marked by their similarity to the patterns of volcano eruption types. Strombolian elements represent the calmest type, characterized by their frequent but modest activation in cancer genomes; while Plinian elements rarely become active, but their eruption is violent, mediating large number of retrotranspositions. Occasionally, these elements can promote large scale oncogenic rearrangements, such as deletions or tandem duplications. Thus, given the fact that each human being bears 50-65 of these elements in his genome, we believe this work underscores the role of L1 source elements as potential oncogenes and cancer risk genetic factors. This presentation summarizes the findings of the Retrotransposition Subgroup of the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium.

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0184-R/M

Looking for cancer-causing viruses in Pan-Cancer

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Viruses are the cause of about 10-15% of all cancers worldwide [1]. So far, seven human viruses have been found to cause human cancers. However, it seems that around 20% of all cancers could be promoted by viruses and other microorganisms. Thus, many other cancer-associated viruses remain undiscovered [2].

Due to the long time periods required for malignant transformation and diagnosis since primary infection occurs [3], a pathogenic virus would have left the infected cell before the tumour caused by its action is detected. However, some viruses can integrate their genetic material into the cancer cell genome [4-6], leaving hallmarks of their activity during infection that we believe could be used for the detection of their past activity along the evolution of a tumour.

Our main objective is to identify associations between virus and cancer in 2,700 whole-genomes, analysing next generation sequencing data generated within the framework of International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA) [7].

In order to do that, we have developed bioinformatic pipelines based on the analysis of paired-end sequencing, focusing on unmapped reads, discordant read pairs and on the depth of coverage. These pipelines have been validated using cell lines in which we were able to detect and identify episomal and integrated viruses.

Our preliminary analysis of a subset of tumoural and normal samples, showed viral integration in tumoural samples of different tissues: cervix, liver, bladder and head and neck. Occasionally, we observed that somatic insertion of foreign viral DNA triggers post-integration rearrangements in the cancer genome. Therefore, viral integrations not only allow us to detect viral hallmarks, but also represent another potential mechanism by which cancer clones acquire mutations that help them to survive and grow.

Given these results, the upcoming analyses of the whole dataset using these new bioinformatic tools seems promising.

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0288

Plasmid Cartography in the Postgenomic Era

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Plasmids are devices for gene propagation and, thus, fundamental agents of horizontal gene transfer (HGT) in bacteria. The myriad plasmids carry many differences in genetic structure, which are probably adaptive. Their genetic structure, together with environmental cues and barriers to plasmid dissemination, also limit the hosts they can infect. Determining the boundaries of plasmid host range is essential to understand the role of HGT in bacterial evolution and, more specifically, the dissemination of antibiotic resistance. The task is complicated by the difficulty in organizing plasmids into discrete, functionally equivalent categories. We analyzed the prokariotic plasmidome to determine whether plasmid genomes cluster together in genomically defined groups. Pairwise comparisons revealed that plasmids form discrete, coherent recombination groups, functionally equivalent to "molecular species". By analyzing the distribution of plasmid groups, we unveil a vast roadmap for HGT in enterobacteria. We also start to understand the functional roles of key plasmid genetic adaptations.

0309-R/M

Atomistic simulations of DNA bending and minicircle formation in the presence and absence of covalently bonded trabectedin (Yondelis®)

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Trabectedin (TRB, Yondelis®) is a potent antitumor drug composed of three fused tetrahydroisoquinoline subunits (A-C). The A and B subunits are responsible for DNA recognition through a direct readout mechanism involving specific donor-acceptor H-bonds. Covalent bonding of TRB to the exocyclic amino group of a guanine in the minor groove of selected double-stranded DNA (dsDNA) triplets is made possible by virtue of an essential reactive carbinolamine (hemiaminal) group present in subunit A. The C subunit, which projects out of the DNA minor groove both in the pre-covalent complexes and in the covalent adducts, is thought to interact with a variety of DNA-binding proteins.

An 18-bp dsDNA oligonucleotide containing (i) a single suitable site for adduct formation, namely an AGC triplet, and (ii) a 3-base 5'-overhang in each strand was used to determine possible alterations in DNA curvature by means of a standard DNA ligation assay followed by nondenaturing PAGE. Head-to-tail joining by T4 DNA ligase gave the expected linear (L) ligation products (L1x21, L2x21, …, L11x21) plus some closed circular (C) DNA species, mostly C6x21 and C7x21. Each of the linear TRB-DNA adducts showed retarded elec-

trophoretic mobility relative to its unmodified linear multimer counterpart and the proportion of minicircles was greater when the DNA contained the TRB adduct. Molecular modelling and molecular dynamics (MD) simulations of a dsDNA nonanucleotide containing the TRB adduct at a central AGC target site revealed that bending results from widening of the minor groove and introduction of positive roll at the ApG step.

To extend these early findings and assess the geometrical features and helical parameters of considerably longer free DNA molecules and drug-DNA adducts, we have now modelled and simulated the dynamic behaviour of several linear multimers L1x21, L3x21, and L6x21 plus covalently closed and nicked C6x21 and C7x21 minicircles making use of the AMBER suite of programs and the parmbsc1 set of parameters. Analysis with cpptraj and Curves+ of the snapshots collected from the MD trajectories have allowed us to gain structural insights into the persistence length of DNA oligonucleotides shorter than 150 bp, the influence of counterions and buffer components on DNA curvature, and the importance of torsional alignment for DNA ligation.

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0328

Bioinformatics approaches to prioritize therapeutic vulnerabilities in cancer

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The paradigm of personalized medicine is the identification of the appropriate drug for the right patient, using molecular profiles. In Oncology, it is well established that the anticancer drugs are effective in only a small subset of patients. Moreover, many of the new targeted therapies inhibit specific proteins, and they are only effective in tumors that are genetically altered. Consequently, the success of personalized treatment depends on each individual molecular profile, which a priori can be considered as very heterogeneous.

Here, we present new computational approaches based on the analysis and integration of genomic data (mutations, copy number variations or gene expression levels), with functional data (protein essentiality) and pharmacological data. These methods aim to identify those vulnerable molecular alterations that drive tumor progression and could be druggable based on the patient's molecular profile, and propose an individualized therapeutic strategy to guide clinical decision making for cancer patients.

R05 Biología química

0037-R

A FLEXIBLE THERANOSTICS PLATFORM FOR BREAST CANCER HER2+

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The idea of combining diagnose, drug transport and nucleic acid delivery capabilities in a unique nanoparticle is an attractive strategy. Further nanoparticle engineering to attain cell or tissue specificity it is also highly desirable. These particles are called teragnostics. However, their design, synthesis and targeting evaluation of these nanosystems is a time consuming process. In order to develop novel efficient protocols, we have designed a multidisciplinar approach by joining molecular biology, recombinant protein expression and organic chemistry for accessing a modular platform for the generation of teragnostic nanoparticles with cell targeting capabilities by the use of single chain antibodies that direct to specific receptors. These antibodies are attached to different complex in order to do different tasks: a) Polyethylenimine (for specific gene transfection), b) NIR (for diagnostic) and c) cyclodextrin (for drug delivery).

As a probe of concept, an anti-Her2 coding sequence has been cloned and assayed in breast cancer cells that over-express or not the Her2 receptor. The usefulness of the approach has been proven since the nanoparticle is able to selectively transfect Her-2 expressing cells and a plasmid coding for the green fluorescent protein. Moreover, the Her2 cell expression has been detected using confocal microscopy and NIR as ligand, and specific doxorubicin cell uptake using cyclodextrins has been achieved. The technique can be exploited to target other cell surface receptors by changing the coding sequence of the antibody used to direct the nanoparticles.

0058

Using Resurrected Ancestral Proviral Proteins to Study Virus Evolution

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We have recently reported that resurrected ancestral thioredoxins display substantial levels of functionality within Escherichia coli. Moreover, these ancient proteins are not efficiently recruited by the bacteriophage T7 in the infection process, thus preventing virus propagation (Delgado et al., 2017, Cell Reports 19, 1247–1256).

Bacteriophage T7 replisome is well known as a simple complex formed by four proteins. The replisome includes the following gene products: gp2.5, (single stranded binding protein), gp4 (DNA helicase-primase), gp5 (DNA polymerase), and thioredoxin, a processivity factor from E. coli that attaches to gp5.

Here, we describe preliminary experiments to explore virus evolution. Since gp5 is the only phage protein that interacts directly to thioredoxin, we are particularly interested in identifying the evolutionary relevant interactions between them. A detailed understanding of molecular interactions involved may have potential interest in engineering virus resistance.

Delgado et al., 2017, Cell Reports 19, 1247–1256

0162

Nano-production of biomimetic magnetoliposomes for their application as hyperthermia agent

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In the last few years, magnetic hyperthermia treatment has become an attractive alternative for cancer therapy. Therefore, many attempts have been done in the nanotechnology area with the goal of producing a nanomaterial that could behave as a good hyperthermia agent. In this context, biomimetic magnetic nanoparticles (BMNPs) mediated by MamC from Magnetococcus marinus MC-1 have recently been proposed as excellent potential nanocarriers for targeted drug delivery and for hyperthermia treatments. These particles, in comparison to other magnetic nanoparticles, show a larger magnetic moment per particle and, therefore, they are able to strongly respond to an external magnetic field, thus improving their guidance to the target site. In addition, the surface charge of those BMNPs allows their functionalization

with different molecules based on electrostatic interaction, thus making the adsorption-release processes pH-dependent. In the present study, in order to improve their cytocompatibility and to increase the chances of cell internalization, BMNPs were covered with liposomes, here referred as magnetoliposomes (BMNPs-ML), and their use as potential hyperthermia agents was evaluated. In particular, in the presence of an alternate magnetic field, BMNPs-ML raised the temperature of the suspension up to 44 °C in a few seconds and this temperature was maintained stable for, at least, 30 minutes. Moreover, transmission electronic microscopy of the BMNPs-ML after hyperthermia shows that the lipid cover disappeared upon hyperthermia treatment. These results indicate that BMNPs-ML display the suitable characteristics to become a potential agent for hyperthermia treatments and, thus, such a therapy might be used simultaneously in combination with targeted drug delivery by using the same nanocarrier.

0164

P1-nTBD mimetic peptide downregulates Cx43 and improves chondrocyte phenotype in osteoarthritis

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Osteoarthritis (OA) is the most extended rheumatic disease worldwide, but there is no cure or effective treatment to stop joint degeneration. During OA progression, chondrocytes in articular cartilage suffer phenotypic changes and senescence that leads to cartilage degradation. As in other wound healing disorders, chondrocytes from OA patients show a chronic increase in the gap junction protein connexin43 (Cx43), which also regulates signal transduction through recruitment or releasing of signalling factors. Hence, Cx43 has been identified as a new therapeutic target for OA and other wound healing disorders. In order to downregulate Cx43 activity in OA, we have designed new mimetic peptides with binding motifs and phosphorylation sites of the C-terminal domain of Cx43. Phenotypic characterization was studied by western blot, FACs and microscopy techniques, while localization of the peptides were studied by immunofluorescence and intercellular communication by exchange of lucifer yellow. Our results show that the peptide containing part of the tubulin-binding domain of Cx43 (P1-nTBD) decreases gap junction intercelular communication in OA chondrocytes. P1-nTBD did not colocalized with Cx43, however significantly reduced Cx43 protein levels and membrane localization in human primary chondrocytes in vitro. Downregulation of Cx43 by P1-nTBD led to redifferentiation of OA chondrocytes, increasing Col2A1 and proteoglycans synthesis, reducing mesenchymal stem cells markers and cell proliferation. These effects were positively associted with reduced levels of cellular senescence and restored regenerative capacity of cartilage. Our results indicate that P1-nTBD has potential use for the treatment of OA in order to halt cartilage degradation and foster regeneration by decreasing Cx43 activity to promote chondrocyte redifferentiation. Further studies will be performed in order to assess the potential use of this peptide as a new pharmacological approach in OA.

0203

Pattern Recognition Receptors: A Computational Exploration

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Toll-like receptor 4 (TLR4), as a pattern recognition receptor (PRR), perceives the presence of both damage-associated and pathogen-associated molecular patterns, e.g. bacterial lipopolysaccharides (LPSs), and its dimerization initiates the activation of innate immune system signaling pathways.[1] On the other hand, lectins (such as galectins, DC-SIGN and siglecs) specifically bind glycosides, and play a relevant role in cell adhesion, signal transduction and cell recognition.[2]

We have applied a combination of docking calculations, virtual screening techniques and drug reprofiling approaches, NMA and MD and CG simulations to characterize the architecture and membrane insertion mechanism, the binding mode and the agonist/antagonist mechanism of newly designed glycolipids,[3a] non LPS-like modulators,[3b] natural LPS, and glycomimetics as potential PRR ligands with interesting therapeutic properties.

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0261

Estudio del efecto de compuestos moduladores de gap junctions en líneas celulares de cáncer de mama triple negativo

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La conexina43 (Cx43) es una proteína transmembrana que forma canales de comunicación celular/gap junctions (GJs) y que puede presentar funciones independientes de la actividad del canal. Actúa como factor supresor tumoral en ciertos cánceres. Sin embargo, en carcinoma mamario se ha visto implicada en el proceso de metástasis cerebral, al participar en la comunicación celular entre astrocitos y células tumorales [1].

El objetivo del presente trabajo fue estudiar el efecto de compuestos moduladores de la Cx43 en líneas celulares de cáncer de mama triple negativo, subtipo tumoral que carece actualmente de terapias dirigidas. Los resultados obtenidos indicaron que el tratamiento con los compuestos moduladores de GJs tonabersat y PQ1 succinato reduce la proliferación celular y la capacidad de formación de colonias en las líneas tumorales triple negativo MDAMB231 (metástasis) y HCC1143 (tumor primario), que no presentan GJs funcionales ni contienen Cx43 en la membrana celular. Tonabersat 100 uM aumentó la isoforma de 20 kDa de la Cx43 en la línea de cáncer de mama triple negativo metastática, mientras que PQ1 succinato 4 uM redujo los niveles de la Cx43 en su forma nativa en la línea triple negativo de tumor primario.

El efecto del tonabersat, referenciado previamente como bloqueador de gap junctions de Cx43 entre astrocitos y células tumorales en metástasis cerebrales derivadas de carcinoma mamario triple negativo [1], sugiere que el empleo de este tipo de moléculas podría tener gran interés para la terapia de metástasis a nivel cerebral. Nuestros resultados demuestran que el tratamiento con ambos compuestos se traduce en modulación de la actividad independiente de GJs de Cx43, con una reducción de la capacidad proliferativa y formadora de colonias en HCC1143 y MDAMB231, revelando su potencialidad terapéutica en el contexto de crecimiento tumoral en carcinoma mamario triple negativo. La modulación de la actividad independiente de canal de Cx43 en MDAMB231 se podría vincular a la isoforma 20 kDa-Cx43, referenciada como factor implicado en un modelo de transición epitelio-mesénquima inducida

por TGF-β, donde su sobreexpresión es capaz de restaurar el tráfico de Cx43 a la membrana y la formación de gap junctions funcionales [2], así como en proliferación tumoral, ya que su upregulación en carcinomas primarios la reduce [3].

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0271

Biometalurgia: recuperación de cobre de residuos industriales mediante el uso de bacterias quimiolitotrofas hiperacidófilas

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Los procesos pirometalúrgicos de extracción de cobre a partir de sulfuros polimetálicos de cobre generan escorias fayalíticas con magnetita (Schlesinger et al., 2011) como parte de los productos de desecho del proceso extractivo (Aprox. 570-600 kg escoria / ton. concentrado). Las escorias muestreadas a la salida del horno flash están formadas por fayalita y magnetita. Además contienen pequeños gránulos esféricos de sulfuro de cobre y sulfuro de hierro (62-65 % Fe) con cantidades habitualmente inferiores a 1.5 % Cu. Las escorias constituyen un residuo del proceso y sus contenidos de cobre son considerados como pérdidas metalúrgicas. La producción diaria de cobre puede rondar aprox. 2400 ton /día/fundición. Los procesos biometalúrgicos pueden ser considerados como un tratamiento efectivo de este tipo de residuos.

La biolixiviación de sulfuros metálicos es un proceso donde confluyen mecanismos físico-químicos y procesos biocatalíticos derivados de la actividad de bacterias aerobias quimiolitoautótrofas habituales en los ríos afectados por el drenaje ácido de minas (DAM) (Watling, 2006). Dado que la consecuencia de estos mecanismos es la oxidación y solubilización de sulfuros metálicos, es por lo que decidimos abordar la recuperación del cobre contenido en escorias pirometalúrgicas (cedidas por Atlantic Copper, S.L.) mediante el cultivo controlado de bacterias aisladas de los ríos onubenses afectados por DAM (Grande et al., 1999; Nieto et al., 2007)

Las comunidades bacterianas aisladas, representadas esencialmente por Acidithiobacillus ferrooxidans (García-Moyano el al., 2007), se cultivaron en laboratorio con agitación continua en medio 9K de Silverman suplementado con hierro (Fe II) y/o azufre (So). Como fuente nutritiva se usaron las escorias, que contienen ca. 60% de hierro y un 1,3% de cobre. Tras un periodo de incubación de 21 días, se midió la presencia de cobre e hierro en el medio además de otros parámetros. La metodología empleada permitió recuperar casi un 20% del cobre encapsulado en las escorias, a la vez que se observó la fragmentación de las escorias mediante SEM y EPMA. El proceso de biolixiviación se acompañó de un descenso del pH, un aumento de la conductividad, un incremento del potencial redox y un aumento de la proporción Fe (III) / Fe (II).

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R06 Bioquímica de la nutrición

0075

Efectos antihiperlipidémicos de un extracto de hoja de aladierna (Rhamnus alaternus L.) en un modelo de hiperlipidemia aguda inducida con tritón en ratas y células humanas HepG2

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La aladierna, Rhamnus alaternus L., es un arbusto utilizado en medicina tradicional en países mediterráneos. El extracto metanólico de la hoja de este arbusto es rico en compuestos fenólicos, principalmente flavonoides; el contenido en compuestos fenólicos y flavonoides totales por g de extracto fueron 156 mg de equivalentes de ácido gálico y 75 mg de equivalentes de quercetina, respectivamente.

Nuestro objetivo fue explorar la posible actividad antihiperlipidémica de dicho extracto metanólico de la hoja de R. alaternus. Para ello, se evaluó la capacidad del extracto de contrarrestar la hiperlipidemia inducida por Triton WR-1339 en ratas, y sus efectos sobre la acumulación de lípidos intracelulares y la expresión de genes relacionados con el metabolismo de ácidos grasos en células humanas HepG2 de hepatoma, un modelo celular ampliamente utilizado en estudios del metabolismo hepático.

La administración oral del extracto redujo los niveles sanguíneos de colesterol y triacilgliceroles en ratas hiperlipidémicas (en un 60% y 70%, respectivamente, a la concentración de 200 mg/kg). En las células HepG2, la exposición al extracto disminuyó la acumulación intracelular de lípidos de forma dosis dependiente, que se acompañó con un aumento de los niveles de ARNm de la carnitina palmitoiltransferasa 1, enzima limitante de la oxidación mitocondrial de ácidos grasos.

En conclusión, este estudio aparentemente valida el uso medicinal popular de la aladierna y proporciona la primera evidencia de que puede funcionar como un agente hipolipemiante natural y frente al hígado graso. Es probable que estos efectos se deban a la acción de flavonoides y derivados de flavonoides cuya presencia en el extracto se demostró.

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0077-R/M

Protective effects of a commercial aged black garlic extract (ABG10+®) in obesity induced vascular alterations in rats

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Aged black garlic (ABG) not only exerts antioxidant and antiinflammatoy properties but also shows beneficial metabolic effects in an obesity context. However, its potential protective effects on the vascular alterations induced by a high fat/high sucrose diet (HFHS) have not been studied. The aim of this study was to analyze the effects of an ABG extract in the obesity induced alterations in vascular function. For this purpose, 3-month-old male Sprague Dawley rats were fed either with a standard diet (n=12; Controls) or with a HFHS diet (n=24) for 12 weeks. Between weeks 12 and 16 control rats and half of the rats fed with the HFHS diet were daily administered with vehicle (5ml/kg) by gavage and the other half were treated with and ABG extract (250µg/kg) supplied by Pharmactive Biotech Products S.L (ABG10+®). For the vascular reactivity experiments, after the sacrifice of the rats, the aorta was dissected and cut in 2mm segments. The perivascular adipose tissue (PVAT) was not removed from aorta segments in order to evaluate the possible role of this tissue in the vascular alterations induced by the HFHS diet. ABG10+® treatment significantly reduced body weight and insulin serum levels and improved the lipid profile in HFHS rats. In addition, ABG10+® treatment significantly reduced the obesity-induced-increase in the contractile response of aorta segments to KCl (100 Mm) and restored the obesity-induced-decrease in the vasodilating effect of aorta segments in response to acetylcholine 10-6 M. These beneficial effects in vascular function were associated with a significant downregulation in the gene expression of pro-inflammatory mediators such as IL-6, IL-1β, TNF-α and iNOS in PVAT of ABG10+® treated rats. In conclusion ABG administration reduces body weight, improves the lipid profile and prevents the obesity induced alterations in vascular function through changes in the genetic expression of PVAT to a less inflammatory profile. Due to its natural origin, this treatment could be used as an alternative to other anti-obesity drugs with fewer adverse effects.

0112-R

Alteraciones del metabolismo lipídico y la termogénesis en el Tejido Adiposo Marrón (TAM) en crías de madres alimentadas con dieta de cafetería durante la lactancia ante la exposición a una dieta obesogénica en la edad adulta

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Una disfunción en la termogénesis adaptativa ante la exposición a una dieta obesogénica puede contribuir al desarrollo de obesidad. El objetivo de este estudio ha sido analizar si el fenotipo de falsos delgados observado en las crías de madres alimentadas con dieta de cafetería durante la lactancia podría asociarse a alteraciones del metabolismo lipídico y la capacidad termogénica en el TAM a corto plazo y trascender hasta la edad adulta.

Tras el destete, las crías de las madres controles (O-C) y las crías de las madres alimentadas con una dieta de cafetería durante la lactancia (O-CAF) fueron alimentadas durante 4 meses con una dieta estándar (SD); después, la mitad de los animales de cada grupo fueron alimentados con una dieta Western (WD) hasta los 6 meses de edad. Se registró la ingesta, la evolución del peso corporal y la grasa corporal de estos animales, y se determinaron los niveles de expresión de genes implicados en el metabolismo en el TAM tras el destete y a los 6 meses de edad.

Tras el destete, las crías O-CAF en comparación con el grupo control presentaron menores niveles de expresión de los genes relacionados con la lipogénesis (Fasn), la lipólisis (Pnpla2), la captación de ácidos grasos (Cd36, Lpl) y la oxidación de ácidos grasos (Cpt1b). Además, los animales O-CAF presentaron mayores niveles de expresión de Adrb3, Ucp1 y Cidea. Por el contrario, en la edad adulta los animales O-CAF presentaron menores niveles de expresión de Ucp1 y Prdm16. En la edad adulta, mientras que los animales O-C incrementaron la expresión de Srebf1, Cpt1b, Adrb3, y Cidea en el TAM ante la exposición a la WD, los animales O-CAF presentaron una respuesta alterada a la exposición a la dieta obesogénica, y no se observaron cambios o incluso los niveles de expresión fueron menores que en condiciones de SD.

Estos resultados sugieren que la alimentación materna con una dieta obesogénica durante la lactancia trasciende a las crías resultando en una alteración de su respuesta termogénica en el TAM cuando son adultas y se exponen a una dieta obesogénica.

0136

Leptin as a breast milk component for the prevention of obesity La leptina como componente de la leche materna en la prevención de la obesidad

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La descripción de la presencia de leptina en la leche materna ha abierto un abanico de posibilidades en el ámbito científico, particularmente en el campo de la nutrición y la nutrigenómica en relación a la prevención de la obesidad y sus comorbilidades. Así, más allá de la primera función descrita para la leptina producida por el tejido adiposo en el control del peso corporal, esta hormona podría considerarse un requisito esencial durante la lactancia para garantizar que el sistema que controla la acumulación de grasa y la composición corporal esté bien organizado desde las primeras etapas del desarrollo. En este sentido, diversos estudios en animales y evidencias indirectas en humanos sugieren que una ingesta adecuada de leptina durante la lactancia tendría una función protectora en la futura salud metabólica del neonato, mientras que una ingesta inadecuada o la falta de ingesta, debido a obesidad materna, restricción calórica, o lactancia artificial, entre otros factores, podrían tener consecuencias negativas favoreciendo un mayor acúmulo de grasa corporal y otras alteraciones metabólicas relacionadas en la edad adulta. Estos hallazgos ponen de manifiesto la importancia de la lactancia materna, así como de la necesidad de establecer los valores óptimos o de referencia para la ingesta de leptina durante la lactancia, con el fin de diseñar unos patrones de nutrición personalizada desde la primera infancia.

0149

D-galactose intestinal transport is modulated by LPS and squalene

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The dynamic and complex interactions between enteric pathogens and the intestinal epithelium often lead to disturbances in the intestinal barrier, altered fluid, electrolyte and nutrient transport, and can produce an inflammatory response. Lipopolysaccharide (LPS) is a complex polymer forming part of the outer membrane of Gram negative bacteria. On the other hand, squalene is a triterpene present in high levels in the extra virgin olive oil that has beneficial effects against several diseases and it has also anti-oxidant and anti-inflam-

matory properties. The aim of this work was to study if the squalene could remove the LPS action on D-galactose intestinal transport in rabbit and Caco-2 cells. The results showed that squalene reduces the effects of LPS on sugar absorption. High LPS doses increased D-galactose uptake through paracelular via but also decreased the active sugar transport because the SGLT1 levels were disminished. However, the endotoxin effect on paracellular way seems to be more important than on the transcellular route. At the same time, an increased in RELM-β expression was observed. This even could be related to inflammation and cause a decrease of SGLT1 levels. In addition, the MLCK protein also is increased by LPS and this even could be related to the increase of sugar transport through tight junctions. At low doses, the LPS effect is related to intrinsic activity of SGLT1. Bioinformatic studies by docking confirm the interaction between LPS-squalene through the MLCK and NF-kB proteins

0217

Péptidos bioactivos con propiedades antihipertensivas

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Las enfermedades cardiovasculares tienen una gran incidencia en la sociedad actual, siendo la principal causa de mortalidad en Europa. Uno de los principales factores de riesgo del desarrollo de estas enfermedades es la hipertensión. En este contexto, aunque existen fármacos que son efectivos frente a la hipertensión éstos presentan efectos secundarios. Es por ello que se buscan compuestos de origen natural para su uso como tratamientos alternativos. Un ejemplo de estos compuestos son los péptidos procedentes de proteínas alimentarias, que han demostrado ser eficaces para reducir la presión arterial. Su efecto se debe principalmente a su capacidad para inhibir la enzima convertidora de angiotensina (ECA), clave para el control de la presión arterial.

En los últimos años, se ha investigado la posibilidad de obtener estos péptidos bioactivos a partir de las proteínas presentes en los subproductos de la industria alimentaria. Recientemente, nuestro grupo de investigación ha demostrado que las patas de pollo son una buena fuente para la obtención de hidrolizados proteicos antihipertensivos. Uno de estos hidrolizados fue muy eficaz disminuyendo la presión arterial en ratas espontáneamente hipertensas tras su administración aguda y tras su administración crónica en un modelo de hipertensión inducido por la dieta. Se demostró que su efecto estaba mediado principalmente por la inhibición de la ECA aunque también la mejora de la función endotelial y la reducción del estrés oxidativo parecen estar implicados en su efecto. Interesantemente, este hidrolizado no mostró un efecto hipotensor cuando se administró en ratas normotensas. Todas estas evidencias sugieren el gran potencial de este hidrolizado como ingrediente funcional o nutracéutico en el tratamiento o prevención de la hipertensión.

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0270

Maresin 1 regulates FGF21 in diet-induced obese mice and in cultured hepatocytes

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MaR1 is a DHA-derived specialized proresolving lipid mediator with anti-inflammatory and insulin sensitizing actions in obesity. The aim of the present study was to analyze MaR1 in vivo effects on FGF21 production as well as to characterize the tissue-specific regulation of Fgf21 and its receptor components β-Klotho and Fgfr1 in liver, skeletal muscle and white adipose tissue (WAT). For these purposes, diet-induced obese (DIO) mice were administered with MaR1 (50 µg/ kg, 10 days, oral gavage) and both serum FGF21 levels and liver, skeletal muscle and WAT Fgf21, β-Klotho and Fgfr1 mRNA expression were evaluated. Additionally, the effects of MaR1 were tested in mouse primary hepatocytes, C2C12 myotubes and 3T3-L1 adipocytes. In DIO mice MaR1 decreased circulating FGF21 levels in parallel with a reduction of HFD-induced hepatic Fgf21 mRNA expression. In addition, MaR1 increased hepatic β-Klotho and skeletal muscle Fgfr1 expression and counteracted the HFD-induced fall in the expression of both receptor components in WAT. In vitro experiments showed that MaR1 decreased Fgf21 expression in mouse primary hepatocytes in a concentration-dependent manner, in parallel with a down-regulation in Pparα mRNA levels. Preincubation with the AMPK inhibitor, Compound C, and the PI3K inhibitor, LY294002, did not modify the inhibitory effect of MaR1 on Fgf21, suggesting that neither AMPK nor PI3K/AKT pathways are involved in MaR1 effects on FGF21. Our results suggest that the ability of MaR1 to modulate FGF21 could be also mediating its previously reported beneficial metabolic effects.

0306-R

Dietary Advanced Glycation Endproducts (AGEs) are cytotoxic for keratinocytes

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Advanced Glycation Endproducts are formed during the Maillard reaction (glycation) initiated by a nucleophilic addition between the free amino group of a protein, aminophospholipid or nucleic acid and the carbonyl group of a saccharide. Protein glycation takes place both in vivo, in tissues and fluids under physiological conditions; and ex vivo, since this reaction occurs during food preparation such as baking, cooking or frying as well as during storage. Diet is the major source of AGEs in vivo. AGEs are discussed as to be involved in the pathogenesis of diseases such as diabetes, insulin resistance, cardiovascular diseases, kidney injury or age-related and neurodegenerative diseases. In the last 40 years, diets have changed in developed countries and nowadays, they are characterized by an elevated glucose or fructose intake mainly due to processed sweetened foods and drinks. Fructose consumption increases not only circulatory AGEs, but also their tissue accumulation.

The toxicity of AGEs has been previously proposed, however the nature of the compounds that are toxic is still unknown. In order to elucidate the possible cytotoxic effect of a particular AGE in the oral mucosa, we tested the toxicity of 7 different compounds, N-ε-[1-(1-carboxy)ethyl]lysine (CEL), N-ε-(carboxymethyl)lysine (CML), Methylglyoxal-derived hydroimidazolone 1 (MG-H1), 3-deoxyglucosone (3-DG), 3-deoxygalactosone (3-DGal), 3,4-dideoxyglucosone-3-ene (3,4-DGE) and Pyrraline (Pyr), synthetized and identified by our group. We incubated human keratinocytes (HaCaT) with several concentrations of each compound for 24, 48 and 72 hours. We found that 3-DGal and 3,4-DGE are cytotoxic compounds in a time and dose dependent manner. Thus, 3-DGal reduces viability to 31% and 3,4-DGE to 23% in the µM range. Here we first identify the toxicity, in a mucosa cell model, of 3-DGal, a dicarbonyl formed during Maillard reaction, which has been detected in milk and beer, and 3,4-DGE, an unsaturated intermediate in the degradation of glucose to 5-hydroxymethylfurfural, present in beer and honey.

0341

Evidencias sobre la biodisponibilidad y efectos en salud de los ácidos hidroxicinámicos y sus principales metabolitos.

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Los hidroxicinamatos son uno de los grupos de compuestos polifenólicos más ampliamente consumidos en la dieta. El café es una de las principales fuentes de estos compuestos, junto con la yerba mate en Sudamérica. Además, los ácidos hidroxicinámicos, también conocidos como ácidos clorogénicos, están ganando interés en los últimos años por sus diversos efectos beneficiosos en salud. Estos compuestos tienen una biodisponibilidad limitada, siendo ampliamente metabolizados, principalmente por la microflora colónica. No obstante, estudios in vitro y ex vivo han mostrado que los principales metabolitos biodisponibles mantienen una importante actividad biológica. Asimismo, estudios preclínicos en modelos animales y ensayos clínicos de intervención en humanos, tanto en sujetos sanos como en grupos de riesgo, han proporcionado resultados prometedores sobre el papel de estos compuestos mejorando distintos factores de riesgo cardiometabólico como dislipemia, hipertensión, inflamación u obesidad. Se presentarán evidencias recientes obtenidas por el grupo de investigación que apoyan el potencial en salud de estos compuestos fenólicos.

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R07 Bioquímica y biología molecular de plantas

0209

Desajustes redox en el cloroplasto por la falta de tiorredoxinas m en Arabidopsis provocan cambios en la estructura del mesófilo y en la fotosíntesis en condiciones de alta luz

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La luz, además de ser fuente de energía, actúa como señal ambiental en el proceso de desarrollo y crecimiento de las plantas. Sin embargo, un exceso de radiación puede comprometer la propia supervivencia de las plantas si no se activan los mecanismos adaptativos o de respuesta necesarios. Para adaptarse a los cambios ambientales de luz, las plantas cuentan con varios mecanismos de protección, entre ellos, el flujo cíclico de electrones (CEF), que ha sido descrito ser regulado por tiorredoxinas (TRXs). La regulacion redox a través de las TRXs plastidiales es uno de los mecanismos que utiliza la planta para mantener la homeostasis redox de las células no solo en condiciones normales de crecimiento si no también controlando los mecanismos de tolerancia/defensa en situaciones de estrés, tales como situaciones de alta luz.

Las TRXs han sido clasificadas en función de su localización subcelular: TRXs h (citosol y núcleo), TRXs o (mitocondria), y TRXs f, m, y, x y z (plastidio). Sin embargo, aún existe un gran desconocimiento de la clasificación funcional de las mismas. Las TRXs f y m tienen un papel reconocido en la regulación redox de las enzimas del ciclo de Calvin-Benson y los procesos fotosintéticos. En este trabajo se pretende conocer la importancia de tres de las cuatro isoformas de las TRXs m (m1, m2, m4) en la respuesta a los cambios lumínicos mediante la caracterización de líneas mutantes simples, dobles y triples de A. thaliana cultivadas en condiciones óptimas de luz o de alta irradiación. Los resultados preliminares muestran que la falta de las TRXs m1 y m4 activan mecanismos compensatorios modificando la expresión de las otras isoformas y provocan cambios importantes en la morfología, estructura celular y granos de almidón, y en los procesos fotosintéticos cuando están sometidos a alta irradiación.

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R08 Biotecnología molecular

0022-R

PRODUCTION AND CHARACTERIZATION OF A NEW IMMUNOTOXIN AGAINST COLON CANCER BASED ON A NON-IMMUNOGENIC VARIANT OF A-SARCIN

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Colon cancer is one of the most prevalent and mortal cancers in the world. Several drugs have been developed for its treatment, being the use of immunotherapy and monoclonal antibodies one of the most promising therapeutic tools. Regarding this, immunotoxins are chimeric proteins, composed by a target domain (an antibody or a derived from it) that directs the action of a toxic domain, mainly a bacterial or plant toxin, causing cell death. However, one of the main drawbacks of immunotoxins are those related to their immunogenicity, leading to a decrease in the half-life and antitumoral efficiency. In this work, it is shown the production, purification and characterization of a new immunotoxin (IMTXA33αSDI), based on the variable domains from mAbA33 (scFvA33) that recognize a specific tumoral marker from colorectal cancer (glycoprotein A33) and fused to a deimmunized variant of the fungal ribotoxin, α-sarcin (αSDI), that cleavages specifically a single phosphodiester bond, located in the ribosome. Recombinant IMTXA33αSDI has been produced in the yeast Pichia pastoris, and purified by Ni-NTA affinity chromatography. Functional characterization of both target and toxic domains was carried out, in order to evaluate the specific binding and toxic activity, respectively. IMTXA33αSDI showed similar structural and functional features as original IMTXA33αS, regarding their specificity and cytotoxic effects, leading to apoptosis in tumoral cells. Moreover, when immunogenicity assays were performed, it was shown that neither the non-immunogenic immunotoxin nor the non-immunogenic toxic fraction (αSDI) triggered any activation of immune cells, with IMTXA33αSDI being less immunogenic that IMTXA33αS leading to the conclusion that immunotoxins could be used without immunogenic drawbacks in patients as promising therapeutical tools against colon cancer.

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0031-R/M

Enzimas ancestrales para la producción de nanocelulosa

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La nanocelulosa es un nuevo biomaterial obtenido a partir de los materiales lignocelulosicos, la materia prima más abundante del mundo, que ha atraído la atención de la comunidad científica por sus propiedades como alta fuerza, baja densidad, pequeño tamaño, morfología, además es renovable, degradable y biocompatible. Existen numerosas aplicaciones en diferentes áreas de investigación e industrias formando parte de nanocomposites, films, dispositivos médicos y electrónicos. Se clasifica en diferentes tipos según su tamaño, origen y cristalinidad: las nanofibras de celulosa (CNFs) son largas, delgadas, con regiones cristalinas y amorfas; los nanocristales (CNCs) son pequeños y cristalinos. La nanocelulosa se puede obtener aplicando tratamientos mecánicos y químicos a las fibras de celulosa, ambos tienen un bajo rendimiento, alto gasto económico y gran impacto en el medio ambiente. La alternativa más ecológica es la hidrolisis enzimática aunque también tiene bajo rendimiento y alto coste, por ello necesitamos optimizar el proceso y desarrollar enzimas con mayor actividad específica capaces de degradar sustratos lignocelulosicos. En nuestro grupo hemos reconstruido una endocelulasa ancestral de 3000 millones de años usando técnicas de reconstrucción ancestral. Estas enzimas han demostrado tener una mayor actividad y promiscuidad por diferentes sustratos lignocelulosicos en comparación a las enzimas actuales. En este trabajo hemos desarrollado un nuevo método para la producción de nanocelulosa utilizando las propiedades de estas enzimas pudiendo controlar el tamaño de las partículas obtenidas, tanto nanofibras como nanocristales, y caracterizarlas fisicoquímicamente con técnicas como el microscopio de fuerzas atómicas, infrarrojos por transformada de Fourier, difracción de rayos X, resonancia magnética nuclear en estado sólido C13 y análisis termogavimétrico. Además con el fin de optimizar el proceso hemos transformado la bacteria Bacillus subtilis para secretar nuestras enzimas ancestrales al medio de cultivo, simplificando y aumentando su producción.

Perez-Jimenez, Raul, et al. "Single-molecule paleoenzymology probes the chemistry of resurrected enzymes." Nature Structural and Molecular Biology 18.5 (2011): 592.

0032-R

Reconstruction of ancestral enzymes for lignocellulosic biomass degradation

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Improving the breakdown of the recalcitrant materials forming lignocellulose by enzymes has become a main goal in biochemistry. Recently, Lytic polysaccharide monooxygenases (LPMO) have been discovered as powerful oxidative enzymes. Together with other lignocellulosic enzymes can cleave lignocellulose to very valuable products such as bioethanol, lignin or nanocellulose. Nevertheless, performing enzymatic assays in industrial conditions is still a challenge. In our work, we use Ancestral Sequence Reconstruction (ASR) as an effective method to improve some features of lignocellulosic enzymes for their biotechnological applications. ARS allows us to track the evolutionary history of genes and proteins to obtain information about extinct species. This way, we are able to deduce the sequence of proteins that lived in the harsh environments of ancient Earth. We reconstruct bacterial laccase that show outstanding temperature resistance and a wide pH range. Moreover, we have reconstruct LPMO, endo-1, 4-beta-xylanase and an endoglucanase with improved features and synergy with lignocellulosic enzymes. We also improve their activity by immobilizing them as Nano flowers and in industrial beads. Our results show that ARS is a promising method for enzyme design.

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0036

Engineering de novo enzymatic functions in ancestral protein scaffolds

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Generation of de novo and non-natural enzymatic activities is one of the main challenges in protein engineering and biotechnology in general. Resurrected ancestral proteins have been proved to display high stability and enhanced promiscuity likely linked to conformational flexibility/diversity. These biophysical properties are advantageous features for protein engineering and design, as they contribute to protein evolvability[1]. In a previous work, we designed a new active site in an ancestral β-lactamase through a single hydrophobic-to-ionizable amino acid replacement that generated substantial Kemp elimination activity[2]. Preliminary results suggest that directed evolution of this engineered ancestral protein increases the levels of Kemp elimination activity while maintaining high thermostability. Thus, we highlight the potential of directed evolution using ancestral proteins as a feasible tool to engineer de novo enzymatic functionalities.

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0047-R

Expresión de prolactina humana bioactiva mediante el uso de un sistema de expresión lentiviral

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La prolactina (PRL) es una hormona peptídica sintetizada principalmente por la pituitaria anterior y por diferentes tejidos periféricos, considerándose una hormona pleiotrópica con más de 300 funciones biológicas. A pesar de que se han descrito los mecanismos moleculares desencadenados por PRL durante la lactancia, aún se desconoce el papel de esta hormona en la fisiología del resto de órganos diana y sus implicaciones patológicas1. Por esta razón, se necesitan nuevas herramientas biotecnológicas que permitan estudiar la acción de PRL tanto a nivel de fisiológico como patológico.

Los lentivectores (LVs) son vehículos ideales para la manipulación genética y el estudio de las funciones de los genes, gracias a su eficacia para transducir una gran variedad de células, tanto en división como quiescentes, y a su capacidad para integrar su información genética en el genoma de la célula diana2. En este trabajo hemos generado un sistema lentiviral de segunda generación derivado del VIH que permite la expresión de una prolactina humana (hPRL) recombinante. Para ello, hemos usado el péptido 2A para expresar hPRL junto a la proteína verde fluorescente (GFP) como un único péptido auto-catalítico. Este transgén generado, hPRL-P2A-GFP, se ha clonado en un sistema lentiviral bajo el control de un promotor constitutivo con el objetivo de transducir diferentes tipos celulares. Los resultados muestran que los LVs generados son capaces de transducir diferentes tipos celulares, tanto líneas establecidas como cultivos primarios. Además, las células transducidas secretan hPRL bioactiva al medio extracelular y acumulan en el citosol la proteína GFP. Por lo tanto, nuestro sistema de expresión, que permite tanto la producción de hPRL bioactiva como la detección de las células diana transducidas, podría ser útil para ayudar a comprender la acción fisiológica de la hPRL y su impacto en las patologías asociadas.

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0052-R

Development of a nanodevice for exosomes detection based on an antibody recognition approach

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Exosomes are vesicles ranging in size from 30 to 100 nm with an important role in tumor microenvironment maturation and cancer progression. Due to a common biogenesis path, most of exosomes contain proteins involved in the endosomal network such as the tetraspanins family's proteins CD63 and CD81, with potential use as malicious exosomes markers. Metastasis is the main cause of death due to melanoma cancer and needs to be treated by standard therapies. In metastasized melanoma, molecular signatures of the exosomes derived from melanoma cells must be identified for its recognition, which is difficult to evaluate. As a proof of concept, a novel nanodevice was developed, conjugating exosome specific antibodies (anti-CD63 and anti-CD81) to polystyrene nanoparticles (PS-NPs) in order to recognize these exosomes by flow cytometry. The simultaneous conjugation of a fluorophore allows to track PS-NPs by flow cytometry and confocal microscopy. The nanotool's functionality was evaluated using cancer stem cell (CSC) exosomes isolated from MEL1 primary melanoma cells. The resulting nanodevice was innocuous to MEL1 cells, displaying a high cellular uptake, being successfully localized inside the cells. Furthermore, the recognition of the exosomes by the nanodevice was analysed by TEM images, confocal microscopy and flow cytometry.

0059-R

Desarrollo de herramientas genéticas para aplicaciones biotecnológicas bacterianas

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Actualmente, las bacterias suponen una enorme fuente de recursos para el desarrollo de procesos biotecnológicos, ya sea para la obtención de cepas mejoradas de uso en alimentación como para la producción bacteriana de metabolitos o de enzimas de interés industrial entre otras aplicaciones. En este trabajo se han seleccionado dos tipos diferentes de bacterias para el estudio de su potencial biotecnológico: dentro de las bacterias gram-negativas, se ha escogido a la cianobacteria Arthrospira platensis por su alto valor nutricional, su capacidad de formación compuestos bioactivos de interés industrial y por tener escasos requerimientos nutricionales para su crecimiento; en cuanto a las bacterias gram-positivas, se ha seleccionado al género Rhodococcus por su interés en la industria farmacéutica debido a su capacidad para degradar esteroides, lo que permite obtener una amplia variedad de sintonas o esteroides de gran valor.

Mediante el uso de la ingeniería metabólica hemos diseñado herramientas para la modificación genética de Rhodococcus, lo que nos ha permitido manipular su metabolismo y generar compuestos esteroideos de interés. Por otro lado, estamos desarrollando protocolos de modificación genética de Arthrospira platensis con el fin de mejorar sus aplicaciones biotecnológicas.

0085-R

Efecto de la expresión en trans de sulA sobre la morfología y sobre el acúmulo de polihidroxialcanoatos en *P. putida U.*

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La principal limitación para el uso de los polihidroxialcanoatos (PHAs) como sustitutos de los termoplásticos de origen petroquímico es su alto coste de producción. Se han propuesto varias estrategias para reducir estos costes. En nuestro laboratorio hemos obtenido mutantes superproductores de PHAs derivados de la cepa Pseudomonas putida U. La producción de PHA en estos mutantes parece haber alcanzado el límite fisiológico de contención en el interior celular. Aquí proponemos una estategia basada en la modificación del volumen celular mediante la alteración de proteínas representativas del elongasoma y del divisoma bacteriano, con el fin de incrementar el volumen citoplasmático y, así, intentar que las cepas superproductoras sean capaces de acumular un mayor contenido de PHAs. En este sentido, se ha expresado en trans la proteína SulA de P. putida U, de manera constitutiva. Esta proteína participa en la respuesta SOS frente a estrés celular (tal como deterioro del DNA e inhibición de la replicación del cromosoma). En Escherichia coli y en Salmonella enterica se ha descrito que SulA interacciona con FtsZ durante el proceso de división celular asegurando la correcta división del cromosoma bacteriano. La represión del proceso de división se manifiesta como un fenotipo de filamentación celular. La expresión constitutiva de esta proteína en P. putida U y en sus mutantes superproductores también da como resultado un fenotipo filamentoso, observándose la formación de gránulos de PHAs a lo largo de las fibras cuando estas cepas son cultivadas en un medio mínimo suplementado con precursores de PHAs. Sin embargo, pese al fenotipo mostrado y al evidente aumento del volumen celular, no se ha observado un incremento de la capacidad para acumular PHA en estas células. A pesar de ello, el fenotipo filamentoso de las cepas posee un gran potencial biotecnológico, ya que podría facilitar el procesamiento downstream de las cepas superproductoras, concretamente, la recolección de las células con el fin de extraer y procesar los PHAs acumulados.

0090

Characterization of ABCG2-targeted nanoparticles and evaluation of citotoxicity of doxorubicin-conjugated anti-ABCG2 nanoparticles to selectively eliminate cancer stem cells in a in vitro model of small cell lung cancer

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Lung cancer is the first cause of cancer deaths in the world, being the small cell lung cancer (SCLC) the most aggressive and malignant subtype. At first, the SCLC patients have a good response to current treatments, but most of them usually generate metastasis and have relapses rapidly due to the development of resistance to treatments.

According to previous studies, the existence of a small subpopulation of undifferentiated cells, known as cancer stem cells (CSCs), that exhibit the ability to self-renewal and differentiate into an heterogeneous progeny, could be a possible explanation for the behaviour of SCLC. These cells are responsible for tumor initiation, tumor progression, metastasis, recurrence and resistance to chemotherapy and radiotherapy. A possible and innovative strategy to overcome this issue would be the development of a targeted therapy based on targeting drugs to specific surface markers of CSCs to eradicate this subpopulation and consequently improve the prognosis of patients with SCLC. Nowadays, nanomedicine plays an important role in drug targeting systems since the use of nanocarriers (NCs) improves the bioavailability of the drug and its therapeutic efficacy.

In this study, we characterized the ABCG2 transporter as a surface marker in two human cell lines, a bronchial cell line (BEAS-2B), as a control, and a SCLC cell line (H69V). Subsequently, we studied the internalization of ABCG2-targeted nanoparticles (NPs) and the citotoxicity of these nanoparticles conjugated with doxorrubicin, an antitumoral drug, in the same cell lines. Our results reveal higher ABCG2 surface expression in the SCLC cell line compare to that of control cells. In addition, anti-ABCG2 NPs specifically bound to ABCG2 and induced an endocytic process in our cells. The results obtained from the in vitro cell viability assay seem to indicate that this ABCG2-targeted therapy could be effective in SCLC.

Therefore, the ABCG2 transporter could be a good target for the development of a targeted drug system using NPs for the treatment of SCLC.

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0103

Proteínas fotosintéticas con funciones alternativas en la diatomea *Phaeodactylum tricornutum*

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Las algas diatomeas presentan un mayor requerimiento de hierro en su cadena redox fotosintética, tanto porque tienen citocromo c550 asociado al fotosistema II (PSII), como porque la mayoría de las diatomeas usan citocromo c6 soluble como único transportador de electrones entre el complejo citocromo b6f y el fotosistema I (PSI). Este requerimiento extra de hierro limita la adaptabilidad de las diatomeas a los bajos niveles de este elemento existentes en grandes áreas de los océanos. Por el contrario, en el linaje verde, que comprende algas verdes y plantas, la proteína de cobre plastocianina reemplaza al citocromo c6, mientras que el citocromo c550 es sustituido por la proteína PsbP, que no contiene hierro.

El análisis mediante técnicas de coinmunoprecipitación e inmunomicroscopía electrónica indican que aunque el citocromo c550 de la diatomea modelo Phaeodactylum tricornutum se localiza principalmente en el dominio tilacoidal del cloroplasto, esta proteína también se puede encontrar en el pirenoide, un microcompartimento subcelular relacionado con el mecanismo de concentración y asimilación de CO2, lo que sugiere nuevas funciones alternativas para este citocromo.

Por otra parte, hemos construido una cepa de P. tricornutum capaz de expresar en el cloroplasto, bajo condiciones limitantes de hierro, una plastocianina mutada del alga verde Chlamydomonas reinhardtii, que ha demostrado previamente una eficiente interacción con el PSI de la diatomea in vitro. El análisis de esta diatomea modificada reveló una mayor tolerancia a la limitación de hierro, con un incremento de crecimiento de entre el 30-50% en condiciones limitantes de hierro, en relación con la estirpe silvestre.

0111

The genome of *Labrenzia sp.* PHM005 reveals a complete pederin family gene cluster

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Polyketide synthases (PKS) are wide spread multi-enzymatic modular complexes that currently produce a large number of biomedically important natural products. Pederin is a cytotoxic compound that belongs to a family of polyketides synthetized by trans-AT PKS. Few pederin biosynthetic gene clusters have been identified so far in symbiotic bacteria of beetles and marine sponges 1. However, a free-living marine Alphaproteobacterium Labrenzia sp. PHM005 has been recently reported as a producer of a pederin analogue 2. Here we show the in silico characterization of a putative complete pederin-like gene cluster responsible for the production of this pederin analogue. By comparative analysis with its phylogenetically related species such as Labrenzia alexandrii DFL-11, a non-producer of pederin, using Mauve alignment and Rapid Annotation using Subsystems technology (RAST) we have identified the precise genome integration site of the cluster. The domains of PKS and non-ribosomal peptide synthases (NRPS) as well as its specific substrates were analyzed using AntiSMASH. Based on these analyses we have proposed a putative biosynthetic pathway for the production of a pederin family compound. In addition, we have used qPCR to determine the expression of the genes in a liquid culture. The presence of this cluster in a cultivable bacterium opens the possibility for its functional genetic analysis and encourages further studies on the role of pederin-like molecule in this bacterium.

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0114

Synthetic mating aggregate assemblers switch broad host range plasmids into efficient liquid conjugators

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Horizontal gene transfer governs bacterial genome evolution, allowing adaptation and survival of microorganisms in specific environments. Plasmids have also driven the development of a myriad of genetic engineering applications and play nowadays an important role in systems and synthetic biology as backbones to store, modify and transfer genetic information. However, scaling-up conjugation by e.g. robotic platforms, has been hampered by the fact that most plasmids show low mating efficiency in liquid, compared with conjugation rates obtained on solid surfaces1.

Here we report on the use of a synthetic bacterial cell adhesion system2 to dramatically increase conjugation frequencies in E. coli. Specific mating assemblers consist on nanobodies and their cognate antigen peptides, genetically encoded in either plasmid donor or recipient cells. Outer membrane translocation of nanobody and peptide is achieved by fusion to the anchoring domains of Intimin and EhaA autotransporter proteins from enterohemorrhagic E. coli (EHEC O157:H7), respectively3. Synthetic cell anchoring increases IncP, W and N broad-host-range plasmids conjugation frequencies in liquid media, to practically reach the same yields obtained on solid surfaces. Furthermore, in the presence of the assemblers, mating efficiency either of the liquid-conjugating IncF plasmids or in solid media remained invariable, con-

firming the absence of interference of our system with the cells conjugative machinery. This synthetic cellular adhesion system enables liquid conjugation automation while sheds light on the mechanisms limiting plasmid spread among microorganisms.

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0140

Structural and thermodynamic determinants of HIV-1 inhibition by small proteins targeting gp41

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During the HIV-1 infection, the envelope subunit gp41 folds into a trimer of helical hairpins formed by the interaction between three N-terminal (NHR) and three C-terminal (CHR) heptad-repeat regions. This folding process brings the cell and viral membranes into close proximity and facilitates membrane fusion and infection. Therefore, interfering with the NHR-CHR interaction of gp41 is a promising therapeutic approach against HIV-1.

In our previous work (1), we rationally designed single-chain protein constructs that mimic the NHR trimeric coiled-coil surface. The proteins were built by connecting with short loops two parallel NHR helices and an antiparallel one, followed by engineering stabilizing interactions. These proteins (named covNHR) show high structural stability and solubility, bind the CHR region of gp41 with high affinity and possess a strong HIV-1 inhibitory activity in vitro.

In this work, we compared the biophysical properties of two covNHR variants differing in two point mutations on the CHR-binding surface. We performed a detailed isothermal titration calorimetry study to dissect the thermodynamics of binding of the covNHR proteins to their target in gp41. The high-resolution crystallographic structure of the complex between one of the variants and a CHR peptide allows a structural rationalization of the binding. The activity of the covNHR variants to neutralize cell infection by diverse HIV-1 strains in vitro showed no correlation with the binding affinity to the CHR target, suggesting kinetic limits to the inhibition process. This study helps in the understanding of the physicochemical and structural basis of the HIV-1 inhibition and could guide the development of future therapeutics against HIV-1.

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0146-R/M

Generation of hybrid extracellular vesicles as a novel nanocarrier for molecular therapies and a gene therapy system against cardiac fibrosis

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Cardiovascular diseases are the most common cause of death in developed countries. They usually lead to secondary pathologies, like cardiac fibrosis, whose reduction promotes a better prognosis of the patients. There are not pharmacological solution to reduce pathological fibrosis for this reason, we are designing new molecular and pharmacological approaches for the treatment of cardiac fibrosis. We have generated through the method of isothermal assembly a plasmid construction that will try to recover the myocardial tissue from the fibrotic state by the breaking down of extracellular matrix, the cause of fibrosis. This vector is composed by the gene encoding metalloproteinase II (MMP-2), whose role is the depletion of collagen (main component of extracellular matrix). The plasmid used presents a secretion signal for MMP-2 protein to be secreted to the extracellular space. All this construction is controlled by the inducible promoter of type β myosin heavy chain protein (βMHC), only activated when cardiac tissue is damaged (1, 2). On the other hand, we are developing a novel nanocarrier system based on the fusion of molecular engineered liposomes and extracellular vesicles (EVs) from cells (3, 4). Liposomes provide the encapsulation vehicle and the control over lipid membrane composition to facilitate fusion. Extracellular vesicles provide the molecular mechanisms of specificity and effectiveness that liposomes lack. We achieved to purify EVs from HeLa cells and we generated liposomes of phosphatidylcholine. We fused them by thermo shocks and we encapsulated a novel engineered fluorescent protein CTPR390 with antifibrotic properties as a probe of concept and the plasmid construction of our invention to check its efficiency. In this project, we are giving the first steps forward to opening up a new field of tissue specific treatments. We are in our way to develop two novel strategies as alternatives to classic surgical treatments to cardiac fibrosis.

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0156-R

Heterologous synthesis of docosahexaenoic acid in *Escherichia coli*

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Some marine bacteria, such as Moritella marina, are able to produce certain amounts of the nutraceutical docosahexaenoic acid (DHA) thanks to a specific enzymatic complex called Pfa synthase. Moreover, E.coli heterologously expressing this pfa gene cluster from M. marina is also able to produce DHA [1]. The aim of this study was to find genetic or metabolic conditions to improve DHA production in E. coli or any other microorganism. Firstly, we studied the expression of Pfa promoters in E. coli and replaced these promoters by inducible pBAD promoters in order to increase the production of the final product. Secondly, we altered the canonical carbon flux in E. coli to improve the availability of substrates for Pfa complex by two strategies: using the exogenous compound cerulenin, and deleting a initiation enzyme of a competing pathway, FabH [2]. Both strategies exploit a substrate competition mechanism between the native fatty acid synthase from E. coli and the heterologous Pfa complex from M. marina. Finally, we improved E. coli growth at low temperature by introducing two psychrophilic chaperonins, Cpn10 and Cpn60 from Oleispira antarctica [3], in order to improve protein folding. These approaches have increased the overall efficiency of DHA synthesis in E. coli, and could be used for biotechnological optimization in different organisms to synthesize DHA or other polyunsaturated fatty acids.

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0159-R/M

Label-free, multiplexed, single-molecule characterization of protein-DNA complexes with biological nanopores

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Protein-DNA interactions are key for the regulation of gene expression and replication. Thus, they are ideal targets in cancer treatments and against antibiotic resistance. Nonetheless, the study of the interactions of these two macromolecules brings several challenges. Nanopore technology is based on measurements of the ionic currents passing through a single nanopore embedded in a lipid bilayer. It detects single molecules passing through the pore by the decrease in the ionic current. These measurements have high temporal resolution and sensitivity. Indeed, it is used for next-generation DNA sequencing, as each nucleotide of a single-stranded DNA causes a different ionic current drop when interacts with the nanopore. Here we present an experimental strategy that allows to measure single molecules of protein-DNA complexes and to obtain the kinetic and affinity constants of the interaction. It works with an oligonucleotide reporter that self-hybridizes in the 3 'end forming a hairpin where is located the binding site of a target protein, followed by a single-stranded DNA that pulls the reporter through the nanopore detector. We take advantage of the sequencing ability of the pore to distinguish different DNA sequence-patterns and using different reporters we multiplex the measurements. We use two oligonucleotides with distinct ssDNA tails and protein binding sites that allow us to identify which protein-DNA complex molecule is passing through the nanopore. We also demonstrate that it is possible to analyze the inhibitory effect of small compounds on each protein-DNA complex. Our current aim is to expand the multiplexing capability of our approach by generating a single-molecule barcoding system. This would allow simultaneous measurements of hundreds of different protein-DNA interactions within the same sample using a single nanopore detector.

1. Celaya et al. ACS Nano 2017, 11, 5815–5825

0163-R/M

Correlative cryo SXT and XRF to localize an iridium metallodrug in breast cancer cells

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In recent years organometallic compounds with potent (nanomolar) cytotoxic activity have begun to emerge. Metallodrugs offer unprecedented versatility in medicinal chemistry because of the different building blocks from which they can be constructed, the variety of available interactions (H-bond, p-stacking, coordinative bond, spatial recognition), and their redox properties. The latter makes them extremely attractive as potential biocatalysts in cancer research. Understanding the intracellular fate of this class of drugs is crucial to further their development towards their clinical use. Previous work has confirmed intracellular accumulation and distribution inside the cells through cell fractionation and elemental quantification using Inductively Coupled Plasma Mass Spectrometry (Romero-Calderón et al., 2012). However, cross-contamination during cell manipulation and fraction separation, metal efflux during sample handling, and sensitivity too close to the detection limit depending on the specific cell fraction, are major drawbacks of this type of experiments. Therefore, to elucidate the intracellular trafficking and the overall cellular accumulation of Ir compounds with potent cytotoxic activity we have chosen the following correlative approach. Cryo Soft X-ray tomography (Chichón et al., 2012) is used to obtain the 3D ultrastructural information of whole cells treated with an Ir metallodrug, at resolutions better than 50 nm. This 3D structural information is then correlated with elemental specific information obtained by cryo X-ray fluorescence. This novel strategy allow us to shed light on the cellular accumulation trafficking and localizing unambiguously which organelles are involved in the intracellular Ir metallodrug accumulation. Our data strikingly show a clear preference for the mitochondria, which can help design the next new generation of highly potent anticancer metallodrugs.

Chichón FJ, Rodríguez MJ, Pereiro E, Chiappi M, Perdiguero B, Guttmann P, Werner S, Rehbein S, Schneider G, Esteban M, Carrascosa JL. Cryo X-ray nano-tomography of vaccinia virus infected cells. J Struct Biol. 2012 Feb;177(2):202-11. Romero-Calderón I, Pizarro AM, Habtemariam A and Sadler PJ. Contrasting cellular uptake pathways for chlorido and iodido iminopyridine ruthenium arene anticancer complexes. Metallomics, 2012, 4, 1271–1279.

0166-R/M

DNA transfer and genomic integration by conjugative relaxases. Using Type IV Secretion System as a genomic modification tool.

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We study the possibility of using bacterial Type IV Secretion System (T4SS) as a genomic modification tool in mammalian cells. T4SS are involved in bacterial conjugation. In this process, the relaxase cleaves one strand of the transferred plasmid at the oriT sequence, binds covalently to it and transfers the complex to the recipient cell. We have previously shown that foreign DNA could be introduced into human cells by the T4SS of Bartonella henselae (which is involved in the translocation of effector proteins during the infection process) 1. Also, we have seen that the relaxase of the conjugative plasmid R388, TrwC, is able to promote non-specific integration of the incoming DNA in to the human genome 2. We wanted to explore if this activity is only for TrwC and this T4SS or if this happens with other relaxases and secretion systems. Here, we report that MobA (the relaxase from the plasmid RSF1010) can deliver too DNA into target cells with higher efficiency than TrwC and it promotes DNA integration although with lower rates than TrwC. In addition, we found that DNA can be translocated through others T4SS, the ones of two intracellular human pathogens, Legionella pneumophila and Coxiella burnetii 3. Taking these data together, we propose that this DNA transfer activity is extended to the T4SS familiy. Having this T4SS-based DNA transfer and integration system, we want to improve it by combining the system with site-specific nucleases.

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0186-R/M

Reconstitution of a Type IV secretion system from the human pathogen *Bartonella henselae* in *Escherichia coli* for DNA delivery into human cells

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Type IV secretion systems (T4SSs) are bacterial multiprotein complexes used for a variety of biological functions, including the injection of effectors into host cells by pathogens, or the exchange of genetic material with other bacteria (1). Bartonella henselae, an intracellular human pathogen, uses a T4SS to translocate effector proteins to the host cells. In addition, we have previously shown that B. henselae can also transfer DNA to human cells through its T4SS, conferring T4SS a high biomedical and biotechnological potential for the development of useful tools for in vivo genetic modification (2). The

direct application of these T4SSs for DNA transfer into human cells is hindered by the pathogenicity of the host bacterium. In this work, we have

rationally designed the heterologous expression of the T4SS VirB/D4 of Bartonella henselae in the nonpathogenic strain E. coli K-12, following the strategy previously reported to reconstitute a Type III secretion system in a non-pathogenic environment (3). To this end, the required genes for the assembly of the T4SS were amplified in three operons and integrated in different chromosomal loci of E. coli under the control of a tac promoter. We have confirmed the expression and assembly of the synthetic T4SS by western blot. Now, we are testing the functionality of the reconstituted T4SS for protein and DNA delivery into human cells.

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0208

IDENTIFICACIÓN Y CARACTERIZACIÓN BIOQUÍMICA DE HIDROLASAS DE FUENTES TERMALES

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La metagenómica es una herramienta especialmente útil en el descubrimiento y estudio de los productos génicos de microorganismos no cultivables en laboratorio. En concreto, el estudio de los metagenomas de ambientes termófilos puede dar lugar al descubrimiento de productos con propiedades de termoestabilidad y actividad a elevadas temperaturas que los hacen interesantes desde el punto de vista biotecnológico. En este trabajo se han construido metagenotecas a partir de muestras de aguas termales de la provincia de Ourense (Galicia) y se ha realizado una búsqueda funcional de diversas actividades hidrolíticas con potencial aplicación industrial con sustratos específicos. Tras la identificación, se ha procedido a determinar los parámetros bioquímicos de las mismas para verificar que sus propiedades las hacen interesantes en el contexto de sus posibles aplicaciones.

0238-R/M

Specific targeting to CD44 receptor by novel transfection reagents based on polyethyleneimine and hyaluronic acid

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In recent years, gene therapy is receiving increased interest for the treatment of several diseases, such as cancer, but there still exist many limitations. One of the most important steps in gene therapy, is the delivery of exogenous genetic material into the cells by transfection method. The transfection techniques based on viral vectors are very effective, but they have associated problems of toxicity, immunogenicity and in vivo carcinogenesis. On the other hand, chemical reagents, such as cationic polymers, are suitable for transfection of cell cultures as well as for in vivo studies. However, it is still needed to develop new efficient transfection reagents that could specifically direct genetic material to molecular targets involved in different pathologies. In this sense, we have developed novel nanoparticles based on polyethyleneimine (PEI) decorated with hyaluronic acid as a director molecule to CD44 receptor, overexpressed in many types of cancer cells. These synthesized reagents were evaluated in MDA-MB-231, Hela and HEK cells concluding that they have a high DNA binding and protection capacities during transfection, high transfection efficiency and low toxicity in different cell types. At the same time, by ligating PEI to fluorescent probes, for example near infrared fluorophore (NIR), it has been possible to monitor and determine specific localization of the transfection reagents in mouse experimental models by IVIS. In the next future we would like to extend the use of these reagents to transport anticancer drugs to their target cells.

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Mattheolabakis G et al., Hyaluronic acid targeting of CD44 for cancer therapy: from receptor biology to nanomedicine, 2015, J Drug Target 23(7-8): 605-618. He YY et al., Polyethyleneimine/DNA polyplexes with reduction-sensitive hyaluronic acid derivatives shielding for targeted gene delivery, 2013, Biomaterials 34: 1235-1245. Ito T et al., DNA/polyethyleneimine/hyaluronic acid small complex particles and tumor suppression in mice, 2010, Biomaterials 31: 2912-2918.

0283-R

Silenciamiento del gen Smurf1 mediante un sistema combinado de ASOs y nanopartículas lipídicas para promover la regeneración ósea: Prueba de concepto.

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El proceso de reparación del hueso utilizando Células Madre Mesenquimales (CMMs) requiere la administración de Proteínas Morfogenéticas Óseas (BMPs) para promover la diferenciación de dichas células una vez implantadas en el tejido receptor. Sin embargo, múltiples estudios sugieren la existencia de importantes efectos secundarios adversos asociados al uso de BMPs en la clínica1, debidos fundamentalmente a su administración en dosis suprafisiológicas, algo necesario dada la baja estabilidad de las BMPs recombinantes.

A fin de reducir la dosis de BMPs necesaria para activar la formación de hueso, hemos recurrido al silenciamiento del gen Smurf1, una ubiquitin ligasa que inhibe la cascada de señalización iniciada por la unión de las BMPs a su receptor celular, mediante la degradación de las proteínas que actúan en la transducción de dicha señal2.

Nuestro grupo ha puesto a punto un sistema de regeneración ósea en el que, mediante el silenciamiento del gen Smurf1 en CMMs se acelera el proceso de formación de hueso tanto in vitro como in vivo sin necesidad de utilizar dosis

suprafisiológicas de BMPs. Las CMMs son implantadas en un scaffold biocompatible y transfectadas in situ con LNA-ASOs3 (Locked Nucleic Acid-Antisense Oligonucleotides) que bloquean la expresión de Smurf1. El uso de nanopartículas lipídicas inocuas para facilitar la entrada de los ASOs en la célula, en contraste con el modelo de gimnosis usado en este tipo de terapias4 permite reducir las dosis de estas moléculas del orden de 10.000 veces, haciendo que este procedimiento sea también coste-efectivo. La utilización de scaffolds de alginato que liberan pequeñas dosis de BMPs (3µM) de forma controlada, induce en este sistema una diferenciación osteogénica eficiente de las CMMs en las que se ha silenciado Smurf1 y la formación de matriz ósea madura.

El silenciamiento de genes mediante esta técnica es ideal para procesos que requieran un tratamiento local y transitorio. La reparación de lesiones óseas presenta estas dos características y por tanto podría beneficiarse en gran manera de la aplicación de esta tecnología.

1 Mroz TE et al. Complications related to osteobiologics use in spine surgery: a systematic review. Spine. 2010 2 Rodríguez-Évora M et al.Smurf1 knocked-down mesenchymal stem cells and BMP-2 in an electrospun system for bone regeneration. Biomacromolecules. 2014 3 Wahlestedt, C., et al. Potent and nontoxic antisense oligonucleotides containing locked nucleic acids. P.N.A.S. USA. 2000. 4 Li, Z. & Rana, T.M. Therapeutic targeting of microRNAs: current status. Nat Rev Drug Discov. 2014

0291-R/M

Plasmid conjugation for synthetic biology: tuning up the machinery.

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IBBTEC Genética

Biological computation is a fundamental issue in synthetic biology. Synthetic computation devices are typically based on gene regulation networks able to modulate reporter genes in response to chemical or physical inputs.

This approach poses some problems, specially related to scaling the computing system:

- Diffusible chemical signals are non-directional

- There are few Input-regulator pairs available

- Gene regulation is a stochastic process with important fluctuations associated

- Output concentrations produced in a first step of computation may not match with the range of input concentrations that can be sensed by the next element in the circuit.

To address these issues we propose bacterial conjugation as the mechanism for transmission of information. It is a directional, modular, and binary system of communication, the computing modules may be used as much as they are needed in a network and there are plenty of different orthogonal channels described (~50 relaxase-oriT pairs).

Bacterial conjugation consists on the cut and transport of a bacterial plasmid from a bacterium to another in contact using a Type IV secretion system. Conjugative plasmids contain all the necessary genes. Plasmids lacking the secretion system, but containing an origin-of-transfer sequence (oriT) are called mobilizable.

Every gene involved in the process is essential, they can be complemented in trans (allowing conditional operations and building AND gates).

These essential components are exclusive for almost each conjugative plasmid. This allows us to build OR gates using equivalent components from different conjugative systems in the same plasmid.

In this work we characterized conjugation of model plasmid R388 (40kb, IncW) and some synthetic constructs in E.coli as the keystone in the bacterial computer

0298

Búsqueda de nuevas β-galactosidasas en las aguas termales de As Burgas

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Las β-galactosidasas se emplean en la industria alimentaria para reducir el contenido en lactosa de leche y productos lácteos, lo que tiene una doble aplicación. En primer lugar, la de hacer accesibles estos alimentos a los individuos con intolerancia a la lactosa. En segundo lugar, la revalorización de los lactosueros; subproductos de la fabricación de quesos cuyo vertido ocasionaría un grave problema medioambiental. La utilización de β-galactosidasas termorresistentes presenta la ventaja de hacer compatible la temperatura de actividad de la enzima con algunas fases del procedimiento industrial. Además, permite trabajar en condiciones menos restrictivas dado que, las enzimas termoestables, a menudo son resistentes a diversos agentes desnaturalizantes además de la temperatura, como el pH, detergentes o solventes orgánicos. Esta característica, convierte a las enzimas procedentes de ambientes geotermales en potenciales biocatalizadores de elevado interés para la industria. Sin embargo, el cultivo tradicional en el laboratorio de microorganismos extremófilos para la obtención de sus enzimas es difícil y costoso, por lo que, en los últimos años, se han desarrollado nuevas metodologías, entre ellas la metagenómica. Esta aproximación, basada en el estudio de todo el material genético de un ambiente determinado, permite el análisis de toda la comunidad microbiana de un ecosistema y, con ello, encontrar nuevas enzimas presentes en dicho ambiente. En el presente estudio, la construcción de una metagenoteca a partir de las aguas termales del manantial de As Burgas (Ourense), ha permitido la detección, aislamiento y caracterización de una nueva β-galactosidasa termoestable, no descrita hasta el momento, mediante metagenómica functional.

0302-R

La bacteria que funde el medio de cultivo: bioprospección de enzimas degradadoras de polímeros

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El interés creciente en la búsqueda de actividades degradadoras de polímeros ha llevado al descubrimiento de muchas enzimas microbianas que pueden ser de gran utilidad. La bioprospección de ambientes con ciertas características que ejerzan una presión selectiva sobre la microbiota allí existente puede ser un buen motor de búsqueda dirigida hacia estas actividades. En este estudio, se han muestreado sedimentos marinos del Mediterráneo, con el objetivo de encontrar estas actividades hidrolíticas. El cultivo en medios gelificados con el polisacárido agar-agar ha llevado a la obtención de microorganismos con capacidad degradadora de éste y que, debido al crecimiento colonial, producen agujeros en las placas de cultivo. Este tipo de actividad degradadora de polisacáridos derivados de algas ya se han descrito anteriormente [1]. El interés en uno de los microorganismos que hemos aislado recae en la gran capacidad hidrolítica del polisacárido agar-agar que presenta, puesto que, con pocos días de incubación, deshace por completo una placa de cultivo. Para su caracterización, se ha comprobado su crecimiento en diversos medios de cultivo para determinar la dependencia de otros nutrientes y la capacidad de crecer en una única fuente de carbono. Además, debido a la dificultad de su obtención en cultivo puro cabe la posibilidad de que se trate de un consorcio microbiano, capaz de conseguir esta potente hidrólisis polisacarídica por complementación de actividades enzimáticas. Se han utilizado técnicas de biología molecular para su identificación y clasificación. Más adelante, nos planteamos la caracterización molecular de la(s) enzima(s) responsables de

esta degradación. Estas actividades tienen un gran interés en el campo de la producción de biocombustibles a partir de macroalgas, ya que, producen la hidrólisis completa del agar en azúcares monoméricos que pueden ser fermentados para la obtención de etanol o butanol. En esta comunicación se mostrarán los resultados obtenidos en cuanto a su identificación, así como diversas pruebas de la gran capacidad hidrolítica de agar que presenta.

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0319-R

A chimeric protein engineering strategy as a method to produce a recombinant version of human pulmonary surfactant protein SP-B

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Respiratory dynamics subjects pulmonary alveoli to drastic changes in pressure, so it is not surprising that they produce and secrete a surface-active substance that modulates the stability of the air-liquid interface, the location where gas exchange takes place, along compression-expansion breathing mechanics. Such substance is a lipid-protein complex called pulmonary surfactant. One of the most relevant components for its biophysical function is surfactant-associated protein B (SP-B), a small and very hydrophobic protein with the ability to interact with phospholipids and mediate fusion, distortion and lysis of surfactant lamellar structures to facilitate their adsorption into the interface (Olmeda, Garcia-Alvarez et al. 2013). The particular properties of SP-B limit its purification from natural sources and make difficult its handling in many structural and analytical techniques. A method for production and purification of human SP-B in recombinant biofactories could allow overcoming these obstacles and facilitate the detailed characterization of SP-B three-dimensional structure. This work summarizes a new attempt in the production of a recombinant version of SP-B. A cloning strategy consisting on a Staphyloccocal nuclease-SP-B derived construction similar to that for the production of recombinant SP-C (Lukovic, Plasencia et al. 2006) has been engineered, and protein expression was optimized to produce SP-B as a soluble chimeric protein in E. coli. The His-tagged protein could be purified by affinity chromatography. Preliminary results in the digestion of the chimeric protein to release mature recombinant SP-B suggest that this strategy could be suitable as a reproducible and affordable way to produce human SP-B, although optimal conditions must be further optimized.

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0342

Standard Genetic Tools for engineering complex phenotypes in bacteria

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Centro Nacional de Biotecnología Systems Biology

Synthetic biology pursues to reprogram microorganisms to execute new-to-nature functions. New technologies have brought about the possibility not only to efficiently manipulate bacterial genomes but to insert genetic devices to program specific bacterial phenotypes in a consistent way. However, deep genetic engineering assignments need to optimize two factors to maximize reprogramming efficiency; (i) it is fundamental to possess a suitable standard genetic toolbox repertoire and (ii) it is important to select a manageable bacterial chassis that offers good biotechnological properties in diverse environments.

To this end, we developed the Standard European Vector Architecture (SEVA) repository that includes a collection of plasmids composed of standardized functional parts assembled in a modular fashion. In that way, users can exchange functional parts of different plasmids at their will to obtain the specific tool for their desired application [1, 2]. Among these, we have tools to stably implant genetic networks into bacterial genomes and also to eliminate/exchange undesired genes/operons using homologous recombination techniques [3, 4, 5]. Then, since classic genome engineering is still a time-consuming process, we recently adopted ssDNA recombineering, the use of oligonucleotides combined with the protection delivered by a phage recombinase, together with the use of the CRISPR/Cas9 system to quickly and efficiently perform bacterial genome editing [6, 7].

The combination of both technologies worked with a high efficiency for entering a suite of mutations in the chromosome of Pseudomonas putida KT2440. These results pave the way for automated and multiplexed editing of this bacterium. This non-pathogenic and soil bacterium is endowed with a broad metabolic versatility and a considerable tolerance to multiple organic compounds that make it an optimal choice for different biotechnological purposes [8].

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0350

Got Enzymes?. A la Carte Design through Molecular Modeling

VICTOR GUALLAR

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We are clearly witnessing a rise of computational predictions in biotechnology applications; top pharmaceutical companies, for example, are signing large contracts with modeling software companies. This, has been made possible by the improvement in the techniques (algorithms) but also by the rise in computational power. At BSC, we are developing the next generation molecular modeling software, capable of a la carte engineering of improved biomolecules, such as industrial enzymes and antibodies (as well as small drugs). In this line we are combining PELE, our Monte Carlo sampling technique highlighted as an outstanding achievement in the latest CSAR blind test, with machine learning algorithms, high performance computing and improved 2D/3D visualization techniques. In this talk we will center on our recent results in enzyme and antibodies engineering, illustrating how to activate an enzyme for additional substrate activities or our recent success in adding a second active site.

Dr Guallar completed his PhD between UAB (Spain) and UCBerkeley (USA), with a postdoctoral position at Columbia University. After a tenured assistant professor at Washington University School of Medicine (USA), he was awarded an ICREA professor position at the Barcelona Supercomputing Center. He is also co-founder of Nostrum Biodiscovery. He has published more than 100 papers in reputed journals and has been the recipient of presitioug grants like an Advanced ERC from the European Union.

0394

Directed evolution of the synthetic ligninolytic secretome: the case story of unspecific peroxygenase

MIGUEL ALCALDE GALEOTE

(IPC, MADRID)

Among the broad repertory of the ligninolytic enzyme consortium, the unspecific peroxygenase (UPO) is shining as a versatile biocatalyst with potential applications in organic chemistry. UPO is a heme-thiolate peroxidase with mono(per)oxygenase activity for the selective oxyfunctionalization of C-H bonds. Fueled by catalytic concentrations of H2O2, which acts as both oxygen donor and as final electron acceptor, this stable, soluble and extracellular enzyme performs dozens of transformations that are of considerable interest in organic synthesis. In this talk I will describe the main attributes of this versatile enzyme, while reflecting on the directed evolution campaigns recently followed in our laboratory that set out to enhance the functional expression of UPO in yeast and improve the activity, as well as approximating its properties to the required industrial standards.

R09 Enseñanza de la bioquímica

0007-R

Seminarios de Bioquímica: diseño de preguntas eficaces para favorecer el aprendizaje colaborativo

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La incorporación de las TICs en la sociedad ha favorecido el acceso directo a todo tipo de información. Sin embargo, la adquisición inmediata de tanta información a menudo conlleva la falta de una reflexión, necesaria para comprender conceptos, integrar los conocimientos adquiridos y poder aplicarlos en situaciones complejas. Por otra parte, resulta difícil formular preguntas que puedan ser resueltas mediante el análisis y la discusión en grupo utilizando la información de la red.

El objetivo de la experiencia que presento es dar a conocer un ejemplo práctico de una propuesta mixta de preguntas cerradas y de respuesta breve que facilite el aprendizaje colaborativo y la reflexión en Bioquímica. El ejemplo que propongo se enmarca en la competencia del Metabolismo, que corresponde a la asignatura *Bioquímica Médica* del Grado de Medicina en la Universidad del País Vasco. En particular, se centra en la *Fosforilación oxidativa,* tema especialmente adecuado para realizar este tipo de seminarios.

Agradecemos al profesorado de los grupos docentes de Bioquímica Básica y Médica por sus contribuciones en la práctica docente de estas asignaturas.

0011

CÓMO ENSEÑAR (CIENCIA) EN EL SIGLO XXI

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En nuestro actual sistema socioeconómico, basado en el conocimiento y la innovación, globalizado y en permanente actualización tecnológica necesitamos formar personas capaces de comprender conceptos complejos, de trabajar creativamente, de generar nuevas ideas, teorías y productos; capaces de evaluar críticamente lo que lee, expresarse con claridad y comprender el pensamiento científico y matemático. Su formación debe capacitarlas además para asumir la responsabilidad de su propio aprendizaje, continuo y permanente.

Sin embargo el análisis del panorama, en lo que a los modelos de enseñanza que se vienen aplicando en nuestro sistema de educación superior se refiere, muestra que si se quieren alcanzar eficazmente estos objetivos, se requieren profundos cambios curriculares, organizativos y de enfoque de la enseñanza. Cambios que, en lo que a la enseñanza/aprendizaje de la ciencia se refiere, permitan presentar la ciencia y el pensamiento científico como una forma de conocer; que propicien la implantación de metodologías informadas por los resultados que aportan la investigación en ciencias de la educación y las neurociencias.

En esta comunicación se revisarán algunos aspectos que, a la luz de estas consideraciones, deben informar el nuevo modelo de aprendizaje, sin perder de vista tampoco referencias al qué enseñar y para qué enseñar.

0016

LA FLAVODOXINA DE *ANABAENA* COMO HERRAMIENTA DOCENTE EN PRÁCTICAS DE BIOLOGÍA MOLECULAR

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Las flavodoxinas son proteínas monoméricas solubles de pequeño tamaño ~20 kDa que contienen FMN no covalente como centro redox. En cianobacterias, la flavodoxina (Fld) se induce en condiciones de deficiencia de hierro, sustituyendo a la proteína sulfoférrica ferredoxina (Fd) en la transferencia de electrones del fotosistema I a la enzima ferredoxina-NADP+ reductasa (FNR), encargada de la síntesis de poder reductor (NADPH) [1]. La Fld de la cianobacteria *Anabaena* es fácilmente sobreexpresada por vía recombinante en *Escherichia coli* y aislada con un alto grado de pureza mediante métodos relativamente sencillos [2]. Su gran estabilidad la hace muy adecuada para trabajar en el laboratorio con los estudiantes. Por otra parte, su color amarillo-anaranjado permite identificarla fácilmente a lo largo del proceso de purificación, tal y como ocurre con otras proteínas que contienen grupos prostéticos coloreados [3]. En este trabajo se presenta una práctica que integra diferentes conceptos y actividades relacionadas con la Biología Molecular y la Ingeniería Genética y que tiene como base la expresión de Fld recombinante y la mutagénesis dirigida de uno de sus residuos implicados en la interacción con FNR. La mutación escogida, D150K, se verifica fácilmente comparando mapas de restricción de las proteínas silvestre y FldD150K. Esta práctica consta de 5 sesiones de 4 horas de duración y está dirigida a estudiantes de último curso de los grados en Bioquímica, Biología o Biotecnología, y se presenta a los alumnos como la ejecución de un pequeño proyecto de investigación.

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0030

A student congress as an example of integration of both biochemistry and genetics curricula

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Among the best practices for teaching biochemistry in health science degrees we want to remark the use of integrated learning. Here, we present a student congress competition successfully conducted at the *Universidad Europea de Madrid*. For this purpose, both Medicine and Biotechnology students worked to share their knowledge and to acquire research skills in the resolution of 23 clinical cases/problems. These presentations were about rare inheritable metabolic diseases with an emphasis in the genetic and biochemistry fundaments. The 350 students were working in groups along the whole academic year to conduct the specific research in order to solve the case of presentation. As an interdisciplinar activity, Medical students faced the challenge looking at diagnoses, prognosis and molecular explanation and Biotechnology students were more worried about methodological approaches used as diagnostic methods or treatments for those diseases. Through this activity both undergraduate students, with different backgrounds, are trained in real situations similar to professional scenarios. At the end of the activity, they were able to improve their apprenticeship and they have to defense their work in the format of a scientific poster session (72 poster) open to all the University collective.

The assessment of the poster included both peer and professor assessment. Academic results from last years will be analyzed in order to show the suitability of this non-traditional teaching methodologies in undergraduate students.

0033
MOOC SOBRE BIOLOGÍA MOLECULAR: BASES Y APLICACIONES

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La manera de enseñar evoluciona, pasando del en la universidad a desde la universidad. Desde ese punto de vista, se plantea el presente *Massive Open Online Course* (MOOC). Nuestro objetivo principal consiste en facilitar al alumnado interesado en la Biología Molecular una vía de inicio en su estudio, partiendo de un conocimiento mínimo de la biología de los seres vivos. Si bien ese alumnado puede haber cursado algunas asignaturas de grado asociadas a la biología, el módulo 1 (Bases) resume de manera básica el tipo de conocimientos necesarios para poder seguir el curso de manera satisfactoria.

El curso se estructura en diferentes módulos:

Módulo 0. Presentación del curso. Introducción y distribución de temas

Módulo 1. Bases principales que permitan enfocar y aprovechar el curso.

Módulo 2. Técnicas en biología molecular

Módulo 3. Organismos modelo (no pluricelulares)

Módulo 4. Organismos modelo (pluricelulares)

Módulo 5. Aplicaciones en biomedicina

Módulo 6. Aplicaciones en alimentación

Módulo 7. Aplicaciones en agricultura

Módulo 8. Otras aplicaciones de la biología molecular

Se pretende que el alumnado asimile conceptos básicos en biología molecular durante las primeras cuatro semanas del MOOC. Las semanas 5-8 requieren de una preparación más específica, si bien el objetivo didáctico-divulgativo del curso debería permitir introducirle a las diferentes aplicaciones en las que se centra el curso. Cada una de los lecciones se ha impartido/grabado teniendo como base una presentación de diapositivas con fotos/diseño de acceso libre. Cada tema se acompaña de documentos (pdf) de ayuda y en algunos casos se enlaza con otros recursos seleccionados por el profesorado. El curso incluye además *tests* autoevaluables para ir comprobando el nivel de aprendizaje tras seguir las lecciones.

Al finalizar el curso, el alumnado debe haber adquirido un conocimiento amplio sobre el tema, que podrá ampliar cursando grados o másters (presenciales), como los ofrecidos por la Universitat de València en el que participan las personas implicadas en este MOOC. El profesorado, fundamentalmente de la UV, está perfectamente complementado por científicos del resto de centros de investigación de la ciudad de Valencia.

El curso puede seguirse a través de la plataforma MiríadaX, *partner* educativo de la Universitat de València.

0076
Implementación y evaluación de una «game-based learning activity» para promover la participación y el aprendizaje del alumnado en el aula

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El juego es una herramienta de aprendizaje que en el aula incentiva el aprendizaje formativo de los alumnos. Hemos implementado una «game-based learning activity» (GBL) basada en plataformas de aprendizaje virtuales disponibles de forma gratuita, para promover la dinamización de las tutorias en grupo en asignaturas de laboratorio, y evaluado el grado de satisfacción así como, objetivamente, el impacto en el nivel de conocimiento asimilado por el alumnado.

Se desarrolló un banco de preguntas utilizando la plataforma Kahoot sobre los contenidos de la asignatura. Durante dos cursos académicos, justo antes de la prueba de evaluación, se implementó en dos sesiones opcionales de tutorías en grupo la actividad GBL (20 preguntas por sesión). La mayoría del alumnado asistió al menos a una sesión (82 de 92 alumnos). La prueba de evaluación consistió en dos partes equivalentes: una reforzada con la actividad GBL y la otra no. Se realizó también una encuesta de satisfacción al alumnado. Los resultados de la evaluación se compararon con los obtenidos en cursos anteriores y con las opiniones del alumnado sobre la actividad docente del profesorado recogidas por el Servicio de Estadística y Calidad de la UIB (SEQUA).

La valoración global del alumnado sobre la actividad GBL fue muy buena, les motivó y ayudó en su proceso de aprendizaje, y aumentó el dinamismo de las sesiones en comparación con una tutoría grupal convencional. Los resultados de la prueba de evaluación mostraron que la participación en al menos una sesión aumenta de manera significativa la puntuación obtenida, siendo particularmente significativo en el alumnado que obtuvo una puntuación inferior a 7,5. Por otro lado, también mejoraron las opiniones del alumnado sobre la actividad docente del profesorado recogidas por el SEQUA.

Estos resultados reflejan la buena aceptación entre los estudiantes y nos alientan a introducir las actividades GBL como herramientas para promover la dinamización y el aprendizaje en el aula en asignaturas de laboratorio tanto en Grado como en Máster.

Agradecimiento: Proyecto de innovación y mejora de la calidad docente del IRIE (Ayuda 171830)

0099

Presentan las áreas de ciencias experimentales, humanidades y ciencias sociales la misma eficacia con las nuevas metodologías docentes?

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Uno de los principales objetivos tras la declaración de Bolonia es el aprendizaje centrado en el estudiante. El profesor, por tanto, trata de implantar nuevas metodologías docentes que fomenten el aprendizaje activo. La innovación implica la utilización de metodologías sin un previo conocimiento de lo adecuadas que pueden llegar a ser en las diferentes áreas de conocimiento. De hecho, en la mayoría de los casos, se utilizan las mismas metodologías para la enseñanza de la Bioquímica y áreas afines, así como en áreas de Humanidades y Ciencias Sociales. **Objetivo**: de este trabajo es evaluar la eficacia del método *design thinking* –técnica de saturación-agrupación o *clustering*– consistente en: Fase 1, cribar la información recopilada aleatoriamente; fase 2, identificar y agrupar la información que contribuye a consolidar o completar los conocimientos. Para ello, se recurre a la elaboración de notas donde los alumnos registran ideas de un tópico propuesto al inicio de la sesión. Terminada la fase de saturación, se lleva a cabo la de agrupación de ideas en bloques temáticos para construir una historia completa y ordenada, generada a partir de dicho tópico. Los grupos de trabajo (control y experimental) fueron alumnos de diferentes asignaturas (Bioquímica, Química Física en representación de Ciencias Experimentales (CE) y Literatura y Derecho Constitucional en representación de Humanidades y Ciencias Sociales (HCS). Se encontraron claras diferencias en la adquisición y consolidación del conocimiento entre las materias de CE y HCS utilizando la misma metodología docente; observándose que, aunque todos los alumnos implicados valoraron la experiencia de forma muy positiva, los resultados académicos de los alumnos de HCS fueron notablemente mejores al compararlos con los de su grupo control (metodología clásica) que en el caso de los alumnos de CE. **Conclusión:** La adaptación de la metodología docente empleada en cada área de estudio es importante. Se ha comprobado que la interacción entre profesores de diferentes áreas es siempre enriquecedora a la hora de la innovación.

An Introduction to Design Thinking. Process Guide. Hasso Plattner, Institute of Design at Stanford. Design Thinking. Tim Brown. Harvard Businnes Review. 84-95. 2008.

0102

La investigación basada en el diseño como una metodología útil para la preparación de recursos educativos en la enseñanza de la Bioquímica a nivel universitario.

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Es un deseo ampliamente compartido entre la mayoría de los docentes de Ciencias, y en particular del profesorado de las asignaturas del área de la Bioquímica, lograr una transferencia relevante de los conocimientos pertinentes a su alumnado, mediante un efectivo proceso de enseñanza-aprendizaje. Por otra parte, y de una manera voluntariosa y entusiasta, algunos de estos profesores, avalados por una dilatada y amplia experiencia en el aula, diseñan y desarrollan recursos didácticos de diferente tipología y perfil en la creencia de que estos facilitarán una mejor consecución de los objetivos didácticos de una manera más amable y atractiva para los estudiantes.

Desafortunadamente, muchas veces, el desarrollo de estos recursos educativos carece de una aproximación metodológica rigurosa que vele por una coherente correlación entre los objetivos didácticos, los contenidos a enseñar, las actividades de enseñanza-aprendizaje, el desarrollo de competencias científicas y el proceso de evaluación del desempeño del alumnado.

La aplicación de metodologías de investigación provenientes del ámbito de la Educación puede ser de gran ayuda para obtener conclusiones válidas sobre la adecuación de un determinado recurso didáctico a las finalidades deseadas. En este trabajo se presenta la *investigación basada en el diseño*1-2 como una aproximación metodológica, ampliamente conocida y usada en el área de las Ciencias de la Educación, como una alternativa útil para la generación de nuevos recursos educativos válidos en el área de las Ciencias. A modo de ejemplo se presenta su aplicación al diseño de un *proyecto práctico* sobre análisis enzimático empleado desde hace varios años en asignaturas del área de la Bioquímica Aplicada en la Universidad de Málaga. También, y más recientemente, esta metodología educativa se está utilizando en el desarrollo de nuevos *casos* para la enseñanza del metabolismo, mediante la metodología del *aprendizaje basada en problemas (ABP).*

[Estos recursos educativos se están desarrollando bajo la cobertura de dos proyectos de innovación educativa - PIE17-065 y PIE17-145 -, financiados por la Universidad de Málaga]

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0113-R

Diseño de un juego de preguntas y respuestas mediante la plataforma gratuita Kahoot! para "gamificar" las tutorías grupales en el aula

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Las tutorías grupales en el aula presentan importantes ventajas sobre las tutorías individuales que clásicamente se han desarrollado en el ámbito universitario al fomentar algunas competencias relacionadas con la expresión oral y el trabajo en grupo. No obstante, existen algunos inconvenientes como la reducción del esfuerzo individual y la difusión de responsabilidades en la toma de decisiones asociada a la escasa creación del discurso verbal y discusión de ideas por parte de los alumnos que llevan a su aburrimiento. Así, nos planteamos elaborar un juego de preguntas y respuestas o Quiz mediante la plataforma gratuita Kahoot! para "gamificar" las tutorías grupales en el aula. Lo que es una actividad lineal y aburrida para los alumnos queremos convertirla en algo divertido, y si se divierten, aprenden más.

En concreto hemos creado un banco de preguntas de opción múltiple en la asignatura Laboratorio Integrado I de segundo curso del Grado de Bioquímica definiendo para cada pregunta el tiempo límite de respuesta, las respuestas, información adicional, o una imagen/vídeo (muy interesante, especialmente en asignaturas de contenido práctico, para contextualizar la pregunta o para plantear preguntas alrededor de la imagen/vídeo insertado). En el aula, las preguntas aparecían en una pantalla compartida y los alumnos/jugadores respondían en sus dispositivos y sus respuestas eran almacenadas en la aplicación que a su vez les asigna una puntuación. Dado el temario a reforzar y su magnitud, así como el tipo de alumnado, se vio que 13 preguntas en 1h era una cantidad apropiada, al abrir un espacio de comunicación, conversación y orientación suficiente, donde los alumnos tenían la posibilidad de trabajar, revisar y discutir, junto con el profesor, también entre ellos, los contenidos a reforzar.

Hemos constatado que esta estrategia didáctica permite una mayor in-

teracción profesor-alumno en ambas direcciones (permitiendo un mejor seguimiento de los progresos del alumno y sirviendo como refuerzo educativo), además de potenciar algunas habilidades/actitudes que permanecían inadvertidas en el sistema de en enseñanza-aprendizaje anterior, proporcionando una formación más completa y mejorando a su vez la calidad educativa.

Agradecimiento: Proyecto de innovación y mejora de la calidad docente del IRIE (Ayuda 171830)

0126

Escuela de iniciación a la investigación en pregrado de la Universidad CEU San Pablo.

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Dado el creciente número de alumnos internos y becarios pregrado que se incorporan a los laboratorios de investigación de los diferentes Departamentos de la Universidad CEU San Pablo de Madrid (39 en la convocatoria 2017/2018 sólo en la Facultad de Farmacia), se hace interesante organizar una serie de actividades generales y transversales para cubrir las necesidades formativas más básicas de estos alumnos. Los objetivos son: 1) Centralizar la formación básica que cada tutor/investigador da de manera individual a estos alumnos internos y becarios pregrado. 2) Ofrecer una visión amplia del mundo científico y de los diferentes campos de investigación que se desarrollan en la Universidad, para que el alumno disponga de toda la información posible a la hora de decantar su futuro investigador. Se realizó un proyecto piloto durante el curso 17/18 con los alumnos del área de Bioquímica y Biología Molecular (11 alumnos). Durante el curso desarrollan 2 tipos de actividades formativas: asistencia a cursos (formación en método científico y orientación hacia el trabajo de fin de grado, búsqueda de información y estadística aplicada) y asistencia a seminarios de investigación semanales de los diferentes grupos de la Facultad y profesores invitados a la Escuela de Doctorado. El resultado de las encuestas de satisfacción de los alumnos sobre los cursos fue de 9.15. La nota media de las calificaciones en el TFG de los alumnos de último curso pertenecientes a la Escuela de Iniciación a la Investigación ha sido de 9.3. Durante el curso 2018/2019 el programa se ampliará al resto de áreas de la Facultad, por lo que se espera multiplicar el número de alumnos miembros de este programa. Además, se añadirá una nueva actividad consistente en una sesión específica dentro del Congreso de Bioquímica y Biología Molecular y áreas afines que se realiza en la Facultad de Farmacia, donde los alumnos puedan presentar su TFG.

0131

Bioquímica ecológica: proyecto de mejora de la coordinación intradepartamental

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En la actualidad la multidisciplinariedad es uno de los pilares de la educación a todos los niveles que permite desarrollar diversas competencias y la coordinación entre el profesorado. En este sentido, el alumnado de grado no es una exención; pero para lograr alcanzar una educación de calidad, los grupos docentes deben estar comprometidos con la innovación y la mejora de la docencia. La coordinación entre grupos docentes será básica para conseguir una enseñanza sin solapamientos de conceptos y actividades incrementando la eficiencia de la utilización del tiempo que el alumnado invierte en su aprendizaje. Sin embargo, la coordinación entre departamentos diferentes suele ser deficiente y puede conducir al solapamiento de contenidos a algunas asignaturas derivado de esta falta de coordinación. La asignatura de bioquímica ecológica es una asignatura optativa del Grado de Biología, pero impartida por profesores del Departamento de Biología Fundamental y Ciencias de la Salud. Por ello, el presente proyecto pretende conseguir la colaboración del profesorado de ambas áreas de conocimiento: bioquímica y ecología y que, además, forman parte de departamentos diferentes para conseguir adecuar los contenidos y las formas de trabajar de la asignatura de forma integrada. Este proyecto quiere evitar posibles solapamientos de contenidos con el resto de asignaturas que imparte el área de ecología, optimizar recursos haciendo coincidir salidas de campo de cara a mejorar la calidad de la docencia impartida. Una vez finalizado el curso los alumnos han contestado una encuesta donde han manifestado no encontrar solapamientos con otras asignaturas y valorando de forma muy favorable las prácticas y los seminarios multidisciplinares.

Agradecimientos: el proyecto a sido financiado por la Universitat de les Illes Balears – Institut de Recerca i Innovació Educativa (PID 171805).

0143

LEARNING CONTRACT, CO-OPERATIVE AND FLIPPED LEARNING AS USEFUL TOOLS FOR STUDYING METABOLISM

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Undergraduate students in Biology identify *Metabolic Biochemistry* as a particularly difficult subject. This is due to the fact that students need to interconnect properly all the contents of its syllabus throughout their study of the subject in order to get a global insight of the complex regulatory features controlling metabolic pathways within the metabolic network under different physiologic and pathologic conditions, as well as metabolism as a whole. Due to these objective difficulties, a high percentage of our students face the study of this subject as a very hard task beyond their forces and capacities. This perception leads to high rates of premature dropout. In previous years, less than 40% of all the registered students attended the examinations of *Metabolic Biochemistry* (a subject in the second year of the Degree of Biology at our University). Even worse, less than 25% of our students passed the exams.

From the academic year 2015/16 on, we are developing innovative teaching projects (PIE15-163 and PIE17-145, funded by University of Malaga) aimed to increase our student loyalty to the subject (and hence to increase their attendance to exams) and to help them to learn more effectively metabolism and its regulation. These innovative teaching projects are based on the use of several powerful tools: a learning contract and problem-based learning within the framework of group tasks promoting an actual collaborative learning in a flipped classroom.

0161

Inclusión de contenidos y competencias de igualdad de género en primer curso del Grado de Bioquímica

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La desigualdad de género, a nivel académico, se traduce en una ocupación mayoritariamente masculina de los cargos de rango superior, a pesar del predominio de mujeres graduadas en numerosas titulaciones. Este proyecto pretendía incorporar formación específica relacionada con la igualdad de género en el Grado de Bioquímica. En la asignatura de Biología (primero de Bioquímica), se trabajó en la igualdad de género a través de diferentes actividades, cuya realización permitía al alumnado ir sumando puntos que podían llegar a suponer el 5% de la nota final. Adicionalmente, el alumnado contestó, al inicio y al final del curso, una encuesta anónima. Al inicio del curso, el 92,1% del alumnado era capaz de dar una definición satisfactoria del término "feminismo", mientras que dicho porcentaje se incrementó al 96,6% al final del cuatrimestre. Por su parte, sólo el 65,1% del alumnado conocía el lenguaje inclusivo al inicio, porcentaje que se incrementó hasta el 89,7% al final de la asignatura. Este alumnado concibe el machismo como un problema social, es consciente de la desigualdad laboral entre hombres y mujeres y ve adecuado que se trate la igualdad de género o feminismo en una carrera universitaria de ciencias. Al término del curso, el alumnado conocía un mayor número de mujeres científicas que al inicio de éste. La nota media de este apartado de la asignatura fue de 0,29 (sobre 0,5 puntos). Si bien no se observaron diferencias en los resultados entre alumnas y alumnos, al inicio del curso los alumnos tienen una percepción ligeramente más alta que las alumnas de la discriminación laboral por razón de género. En conclusión, el alumnado de nuevo ingreso al grado de Bioquímica de la UIB se percibe a sí mismo como mayoritariamente feminista. La inclusión de contenidos de género en una asignatura de primer curso de dicho grado conciencia al alumnado sobre la necesidad de trabajar por la igualdad y la visibilización de las mujeres científicas.

0236

Elaboración de vídeos y utilización de la autoevaluación en trabajos grupales para la promoción de competencias educativas en estudios de ciencias

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Como parte de un proyecto de innovación docente de la Universidad de las Islas Baleares (UIB) se ha analizado el impacto formativo de la elaboración de trabajos grupales en formato de vídeo y de la autoevaluación entre el alumnado. Con la implementación de ambas estrategias se ha pretendido crear un entorno de aprendizaje innovador y mejorar la efectividad educativa. La experiencia se ha realizado en dos asignaturas: "Nutrición y Metabolismo" del grado de Biología, y "Fuentes y Financiamiento de I+D+i Alimentaria", del máster en Nutrigenómica y Nutrición Personalizada de la UIB. Han participado entre 20-25 alumnos por asignatura durante el curso académico 2017-18. Los vídeo-trabajos se realizaron con aplicaciones informáticas gratuitas, y la consecución de los objetivos se valoró mediante encuestas. Los resultados evidencian que los alumnos consideran que el formato de vídeo-trabajo ha sido eficaz y ha ayudado a mejorar la compresión de los temas preparados, con una tendencia (p=0.07) a una percepción ligeramente más positiva para los alumnos de postgrado. En la asignatura de grado se realizaron dos actividades utilizando vídeos, una al inicio y otra al final de la asignatura; la percepción positiva se mantuvo en el tiempo. Las encuestas también muestran que los alumnos consideran que la realización de los vídeo-trabajos tiene un grado de dificultad adecuado. En cuanto a la autoevaluación, en la asignatura de grado no hubo diferencias entre la nota del profesorado y las notas de los propios alumnos a los trabajos de los otros grupos y a su propio trabajo grupal. Esto contrasta con los resultados del curso académico anterior, donde se realizaron presentaciones grupales tradicionales en aula. En el curso anterior se observó una diferencia significativa entre la evaluación del profesorado y la del alumnado sobre sus compañeros, siendo la nota de estos últimos más estricta, del orden de un punto inferior. La mayor accesibilidad del recurso a evaluar podría ayudar a explicar la coincidencia en la evaluación entre profesores y alumnos. En resumen, los vídeo-trabajos son percibidos positivamente por parte del alumnado, y del profesorado. Además, la evaluación entre semejantes se ha uniformizado con la del profesorado al utilizar vídeo-trabajos respecto a cuándo se realiza evaluación de presentaciones entre semejantes en aula.

Agradecimiento: Este trabajo se ha desarrollado dentro del proyecto PID 171854, financiado por una ayuda de la "convocatoria de ayudas para proyectos de innovación y mejora de la calidad docente" de la Universidad de las Islas Baleares en colaboración con el Instituto de Investigación e Innovación Educativa (IRIE). Año académico 2017-18.

0246

Mini Congreso: La importancia de la Bioquímica y Biología Molecular en Medicina

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Los alumnos del grado de Medicina tienden a pensar que la asignatura de Bioquímica y Biología Molecular no contribuye de manera significativa a su formación. Normalmente es recordada como una asignatura difícil e inútil, ya que no les aporta ningún valor clínico en su formación. En la UIB se ha implantado recientemente el grado de Medicina, donde en el primer curso los alumnos tienen dos asignaturas de Bioquímica y Biología Molecular (I y II). Después del primer año los alumnos criticaron la Bioquímica y Biología Molecular II, en la encuesta de satisfacción por no desarrollar aplicaciones clínicas, olvidándose de todos los ejemplos clínicos que se presentaron en el desarrollo de la asignatura.

Así nos plateamos subsanar este aspecto, introduciendo una nueva actividad: mini congreso aplicaciones clínicas de la Bioquímica y Biología Molecular. Los alumnos se dividieron en grupos de 4-7 personas para diseñar y presentar la comunicación, donde debían elaborar una comunicación sobre una patología (definición, base molecular, diagnóstico, tratamiento y perspectivas de futuro).

En la clase final del curso se realizó un *poster party*, cada grupo defendió durante 15 minutos su poster y contesto a las preguntas de sus compañeros y profesorado. La jornada terminó con un *chocolate break* y se procedió a entregar los premios a los mejores grupos según contenido, diseño y defensa.

Los alumnos participaron activamente y consideraron esta actividad muy enriquecedora en su formación, dando valor a los conceptos que habían estado trabajando durante todo el cuatrimestre en que se desarrolló la asignatura.

Agradecimiento a los alumnos de primero de la 2ª promoción del Grado de Medicina de la UIB.

0247

Un Lab Meeting como herramienta de evaluación.

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La evaluación del grado de aprovechamiento e implicación del alumnado en la parte práctica de una asignatura sigue siendo hoy en día tema de controversia, ya que se debe evaluar desde distintos puntos de vista el alumnado.

Así pues, el profesorado de la asignatura de Métodos y Técnicas en Biología Molecular, que se imparte en tercer curso del grado de Bioquímica de la *UIB* y dedica 45 horas a prácticas de laboratorio y su evaluación, decidimos introducir un *Lab Meeting* como herramienta de evaluación.

Las prácticas comienzan con sesión de diseño, donde el alumnado tiene un papel relevante, y se estable un detallado cronograma; así mismo se explica al alumnado que la evaluación de esta asignatura se llevará a cabo mediante una exposición pública (restringida al grupo de prácticas) de los resultados obtenidos y una pequeña discusión de los problemas que puedan haber surgido durante las prácticas. El profesorado procura fomentar una dinámica de intercambio de ideas y discusión de los resultados entre los grupos de trabajo. Para ello cuentan, únicamente, con el apoyo gráfico de una presentación que tienen que realizar ellos mismos con los resultados obtenidos en las prácticas.

El *Lab Meeting*, que lleva implantado 3 cursos académicos, permite al profesorado ser más justo en la evaluación, ya que se pone de manifiesto el aprendizaje significativo del alumnado, así como su capacidad de discusión de los resultados obtenidos, y está muy bien valorado entre los alumnos, dejándolo muchas veces por escrito en las encuestas de satisfacción de la asignatura. Además, consideramos que dota al alumnado de competencias que le serán muy útiles en su futuro como bioquímicos.

0253

Biomodel: 20 años construyendo y compartiendo bioquímica interactiva

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La sede web "Biomodel.UAH.es" está diseñada para la elaboración de recursos interactivos de apoyo al aprendizaje en bioquímica y biología molecular, útiles como ayuda docente pero también para el trabajo autónomo del estudiante. Comenzó en el otoño de 1998 con una instalación local en sala de ordenadores, pero ya desde abril de 1999 ha estado disponible en acceso abierto desde servidores web de la Universidad de Alcalá. Durante este prolongado periodo, los contenidos han ido creciendo en número y aplicaciones, alimentados por las necesidades para impartir la clase, por ideas surgidas durante la actividad en el aula o por inspiración en el trabajo de otras personas. Se han dado ocasiones fructíferas de confluencia y colaboración con colegas que también desarrollaban sus recursos docentes; entre ellas cabe destacar BioROM, una experiencia extraordinaria cuyos contenidos se actualizaron anualmente entre 2001 y 2010 llegando a involucrar a 31 autores de 19 universidades en 5 países. Superada la necesidad de trabajar sin conexión y al aumentar la disponibilidad de recursos en español, BioROM dejó de renovarse pero Biomodel ha seguido su proceso de ampliación y actualización constantes. El objetivo se ha mantenido firme: la elaboración y oferta de complementos para facilitar y enriquecer el aprendizaje, aportando aquello que no se puede hacer en el papel. Ahí encontramos modelos tridimensionales, animaciones, estructuras y esquemas interactivos, simulaciones de procesos y laboratorios virtuales. El impacto de Biomodel ha sido relevante, con abundantes reseñas y enlaces en guías docentes y páginas web universitarias, también desde profesores de secundaria y asimismo desde el otro lado del océano. Algunos materiales están disponibles también en inglés, destacando la guía de la doble hélice del DNA, obra original de Eric Martz que trasladada a Biomodel se ha modernizado y se ofrece en 13 idiomas.

0254

Use of an oral health customized case based on a Work Station Learning Activity (WSLA) as a tool to improve Biochemistry teaching

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In Health Sciences, teaching basic subjects relating to the professional practice is a milestone in the university curricular organization. Focusing on dental education, 1st year students consider Biochemistry not very relevant for their future profession. This reality leads us to design a practical laboratory activity entitled "Saliva and Oral Health in a case of Eating Disorder". In this practice, basic biochemistry concepts are used to analyze and understand a real clinical scenario. Eating disorders (ED) have a direct impact on oral health. In fact, it is often the dentist the first Health Care Practitioner who detects alterations directly related to some of these pathologies. The case presented here is a real scenario for dentists, since ED are becoming prevalent pathologies among youth and child populations. Therefore, a good biochemical background is essential to understand the molecular processes of these pathologies. The WSLA (Work Station Learning Activity) is a flexible tool conceived to teach basic subjects into a clinical context. It is customized to handle big groups of students with a minimum number of instructors. Following this model, three workstations were designed to address three learning objectives: the composition and function of saliva, its use as a diagnostic fluid, and the effect of pH on dental tissues in ED. The activity was performed during the first midterm of the academic course 2017-2018, involving a total of 426 dentistry students. All the members of the Biochemistry team noticed an increase in student's motivation since students integrated basic biochemistry concepts to solve a real dentistry case.

0267

APRENDIZAJE COLABORATIVO Y EL MODELO DE CLASE INVERTIDA COMO AYUDA PARA EL APRENDIZAJE DEL METABOLISMO

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Los siguientes párrafos resumen la presentación oral que será expuesta en este XXI Congreso de la SEBBM, y que se dedicará a describir los fundamentos de las metodologías de aprendizaje colaborativo (AC) y de inversión de la clase (CI) (*flipped learning)* y su utilidad en la mejora de la enseñanza/aprendizaje de la Bioquímica y el metabolismo para estudiantes de Biología. Materia, que muchos alumnos consideran difícil por ser muy amplia, no tener tiempo para estudiarla e integrar sus contenidos, y donde echan de menos el que no se resuelvan más ejercicios en clase.

La transición del modelo educativo tradicional centrado en la enseñanza hacia el emanado del EEES focalizado en el aprendizaje y la adquisición de competencias por el estudiante supone un cambio del paradigma educativo que obliga a complementar las clases magistrales con metodologías activas que realcen el papel central del alumno en su proceso de aprendizaje.

El AC tiene su base en el constructivismo social, que considera el aprendizaje un proceso social que se construye no sólo con el profesor, sino también con los compañeros, el contexto y el significado de lo que se aprende. El AC no sólo favorece el rendimiento académico de los estudiantes sino que les permite adquirir importantes competencias transversales de gran utilidad en su desarrollo profesional. No obstante, su aplicación exige al profesor nuevas competencias y más esfuerzo que impartir clases magistrales; por ello, y por la idea de que se verán obligados a renunciar a partes del temario no son muchos los profesores que utilizan el AC en la docencia de la Bioquímica. Gran parte de estos problemas podrían paliarse asociando el AC con metodologías que como las de CI permiten "ganar tiempo" sin sacrificar temario. Los diferentes papeles de profesores y alumnos en este nuevo escenario, sus ventajas e inconvenientes, serán analizados durante la charla.

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R10 Estructura y función de proteínas

0002-R/M

STRUCTURAL BASIS AND ENERGY LANDSCAPE FOR THE CA2+-GATING AND CALMODULATION OF THE KV7.2 K+ CHANNEL

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Kv7.2 (KCNQ2) channel is the principal molecular component of the slow voltage-gated non-inactivating K+ M-current, a key controller of the neuronal excitability. To investigate the calmodulin-mediated Ca2+ gating of the channel, we used NMR spectroscopy to structurally and dynamically describe the association of helices *h*A and *h*B of Kv7.2 with calmodulin (CaM), as a function of Ca2+ concentration. The structures of the CaM/Kv7.2-hAB complex at three different calcification states are here reported. In the presence of a basal cytosolic Ca2+ concentration (10-100 nM) only the N-lobe of calmodulin is Ca2+-loaded and the complex (representative for the open channel) exhibits collective dynamics in the millisecond timescale towards a lowly populated excited state (1.5%) that corresponds to the inactive state of the channel. In response to a chemical or electrical signal, intracellular Ca2+ levels rise up to 1-10 μM, triggering Ca2+ association to the C-lobe. The associated conformational rearrangement is the key biological signal that shifts populations to the closed/inactive channel. This reorientation affects C-lobe of CaM and both helices in Kv7.2, allosterically transducing the information from the Ca2+ binding site to the trans-membrane region of the channel.

Bernardo-Seisdedos, G. et al (2018). Structural basis and energy landscape for the calcium-gating and calmodulation of the Kv7.2 potassium channel. PNAS. Doi: 0.1073/pnas.1800235115

0003

Exploring the role of nucleophosmin and its interaction with APE1 in DNA repair mechanisms

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Nucleophosmin (NPM1), an abundant protein in nucleoli, performs multiple functions affecting cell growth and homeostasis, such as assistance in ribosome assembly and export. NPM1 is also involved in the cell response to stress and DNA damage repair, e. g. in the double strand breaks repair pathway. However, whether NPM1 takes part in other repair pathways and which is its role within these mechanisms remains to be deciphered. Recently, NPM1, considered a hub of the nucleolar interactome, has been reported to interact with apurynic apyrimidinic endonuclease 1 (APE1), a key enzyme in the Base Excision Repair (BER) pathway, which corrects lesions of DNA bases, suggesting that NPM1 might participate in this route. We have focused on the interaction between NPM1 and APE1 to understand how it could be related to BER regulation. We have demonstrated, based on native electrophoresis, the formation of stable complexes between the two recombinant proteins, and presently we are characterizing the thermodynamic parameters of the interaction by isothermal titration calorimetry (ITC). We have also analyzed the binding of APE1 to an abasic oligonucleotide, finding, by circular dichroism (CD) spectroscopy, that the enzyme is conformationally stabilized upon complex formation with the substrate. On the other hand, we have found, in agreement with other studies, that NPM1 slightly stimulates the APE1 incision activity on abasic DNA. Furthermore, anisotropy experiments with a fluorescently-labelled abasic oligonucleotide suggest that NPM1 can compete with DNA for binding to APE1. Based on the available information on NPM1/APE1 interaction, we propose that NPM1 might cooperate in the BER process favouring the withdrawal of APE1 from the abasic, incised, DNA.

383PX-FLAG_OF_SCOTLAND.JPG.PNG

0014

NON-ANTIBIOTIC FDA-APPROVED DRUGS INHIBIT THE IN VITRO ACTIVITIES OF ESSENTIAL TRANSCRIPTIONAL REGULATORS FROM *PSEUDOMONAS AE*

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In February 2017, the World Health Organization published its first ever list of antibiotic-resistant "priority pathogens" for which new antibiotics are urgently needed. The WHO list comprises several multi-drug resistant bacterial pathogens that pose at present the greatest threat to human health, including *Pseudomonas aeruginosa* and *Helicobacter pylori* [1]. A major challenge in the current antibiotic crisis is the identification of novel microbial targets, essential for *in vivo* growth or pathogenicity, whose inhibitors can overcome the currently circulating resistome of human pathogens. Transcriptional regulators (TRs) modulate gene expression in bacteria, being in some cases essential proteins for cell viability and/or microbial pathogenesis. Hence, TRs are currently considered promising targets in the development of new antimicrobial drugs, while some TR inhibitors discovered to date have shown strong antibacterial activity in both *in vitro* and *in vivo* experiments [2]. In the present study, we screened the Prestwick Chemical Library®, a collection of 1120 small size FDA-approved drugs, for compounds that specifically bind to essential TRs of *P. aeruginosa* and *H. pylori* and potentially inhibit their functions. High throughput screening of binding capabilities to target proteins was conducted by a fluorescence-based thermal shift assay [3]. *In vitro* inhibition of TR biological activities were determined by electrophoretic mobility shift assays. Four non-antibiotic FDA-approved drugs sensibly inhibited the biological function of the *H. pylori* essential regulator ArsR, while two drugs appeared effective as inhibitors of the *P. aeruginosa* target protein Fur.

[1]. Tacconelli et al. Lancet Infect Dis 2018; 18: 318-327. [2]. González et al. Future Med Chem 2018; 10: 541-560. [3]. Cremades et al. ACS Chem Biol 2009; 20: 928-938.

0020-R/M

Un represor poliamoroso: descifrando la estrategia evolutiva de las islas de patogenicidad bacterianas para su diseminación en la naturaleza.

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Las islas de patogenicidad de *Staphylococcus aureus* (SaPIs) son elementos genéticos móviles que diseminan súper-antígenos y genes de virulencia. Residen pasivamente en el cromosoma bacteriano reprimidas por el regulador StI. Tras la infección o inducción de un fago residente en la bacteria, las SaPIs son desreprimidas mediante interacción de una proteína específica del fago con StI, dando lugar a su escisión, replicación y encapsidación en partículas del fago. Las SaPIs han desarrollado un fascinante mecanismo para asegurar la promiscuidad de su transferencia mediante la interacción de StI con diferentes proteínas de fagos que, aunque estructuralmente no están relacionadas, tienen una idéntica función. Combinado una aproximación de biología estructural con la caracterización funcional *in vitro* e *in vivo* desciframos el mecanismo molecular de esta elegante estrategia por la que la isla "piratea" un proceso del fago para sensar su entrada en ciclo lítico.

Nuestros estudios estructurales muestran que el represor StI de la isla SaPIbov1 auna un dominio canónico HTH en posición N-terminal para su función de unión a DNA, al que va adicionando dominios que funcionan como de módulos reconocimiento específico de diferentes proteínas de fago (anti-represores). Nuestros datos *in vitro* e *in vivo* descifran el mecanismo molecular subyacente de interacción entre el represor StI y los diferentes anti-represores de fagos, mostrando cómo cada módulo mimetiza el sustrato para un tipo de anti-represor. La interacción de StI con los diferentes tipos de anti-represores siempre supone la ruptura del dímero StI, implicando la disociación del DNA y la de-represión de la isla. Nuestros resultados establecen el mecanismo molecular del evento de interacción detonante para la transferencia intra- e inter-género de SaPIs de relevancia clínica en la naturaleza.

Elife 2017. doi: 10.7554/eLife.26487. PlosPath 2017. doi: 10.1371/journal.ppat.1006581.

0041

Production of thermostable proteins involved in ribosome maturation using cDNA from *Chaetomium thermophilum*

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Thermophilic proteins are known to be more stable than their mesophilic counterparts and therefore thermophilic genes from bacteria and archaea have been widely used in Structural Biology. Despite homologs of medically relevant proteins might not be found in thermophilic eubacterial and archeal genomes, this approach has been traditionally almost unused in eukaryotic organisms. Recent developments are overcoming the limited use of rare and often difficult to culture thermophilic eukaryotes. Since the sequencing of the fungus *Chaetomium thermophilum* in 2011 (1), more than 360 entries in the PDB correspond to this source organism, including large particles with many proteins. This approach has been particularly successful in the study of the eukaryotic ribosome biogenesis process (2). The frequent presence of introns in the Chaetomium thermophilum genome often discards the possibility of gene amplification from genomic DNA and therefore cDNA templates are more suitable.

We have grown C. thermophilum on oatmeal agar plates at 50°C and extract total RNA from mycelium. cDNA was obtained by reverse transcription with oligo-dTs and used directly as template for amplification of selected genes. Using several cloning approaches and vectors we have cloned, overexpressed and purified different proteins involved in the synthesis of the eukaryotic Large Ribosomal Subunit, like NOC2 and the RNA helicases MAK5 and HAS1. Resulting constructs have been overexpressed in *Escherichia coli* and purified using FPLC techniques.

1. Amlacher, S et al. Cell, 146 (2011), pp. 277-289. 2. Baβler, J et al. Protein Science, 26 (2017), pp. 327-342.

0049-R

Mechanism of autoinhibition of the guanine nucleotide exchange factor C3G

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C3G is a guanine nucleotide exchange factor (GEF) for Rap1/2 and R-Ras GTPases. The structure of C3G consists of three parts with different structural and functional characteristics: (I) the N-terminal region is rich in α-helical content and harbors a binding site for E-cadherin. (II) The central region contains five proline-rich sequences that bind to SH3 domains of adapter proteins such as Crk, p130CAS and Cbl. (III) The C-Terminal region is formed by two domains: a Ras Exchange Motif (REM) and a Cdc25 homology domain (Cdc25h). The latter is responsible for the catalytic activity (GEF activity). Here we show that C3G is autoinhibited by an intramolecular interaction. A sequence of 110 residues out of the catalytic region has been found to bind and inhibit the Cdc25h domain. Additionally, mutagenesis analysis showed that three single substitutions within the inhibitory segment compromised the inhibitory interaction. As a result, these mutations produce a constitutive activation of C3G in the context of the full length protein. Inte-

restingly, similar mutations were found in patient sample databases. Overall, this study deepens our understanding of C3G regulation and suggests a possible role of C3G misregulation in the development of human diseases.

0060

An evolutionary study of protein folding landscapes

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We have recently characterized the chevron plots of a resurrected Precambrian thioredoxin and its modern counterpart from *E. coli*. A significant rate enhancement of the folding reaction for the ancestral protein suggests an adaptation to inefficient Precambrian folding chaperones.

Here, we use an experimental mutational analysis to explore the evolution of protein folding landscapes over billions of years. Our working hypothesis relies on the fact that the evolutionary history of glycine residues may potentially affect high-energy regions of folding landscapes and alter the folding trajectories of polypeptide chains, thus, providing clues about evolution of protein landscapes. A detailed kinetic analysis of a number of variants reveals the relevant residues for efficient folding in primordial thioredoxins. We believe these results might have potential interest in biotechnological applications of proteins.

Candel AM, Romero-Romero ML, Gamiz-Arco G, Ibarra-Molero B, Sanchez-Ruiz JM. Fast folding and slow unfolding of a resurrected Precambrian protein.

0063

A Hub for 3'-end Processing: Structural Insights into mRNA Polyadenylation

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Almost all eukaryotic pre-mRNAs must undergo 5' capping, splicing and 3'-end processing before they can be transported to the cytoplasm for their translation into proteins. 3'-end processing involves over 20 different protein factors that also co-ordinate transcription termination. The cleavage and polyadenylation factor (CPF) is an essential component of the 3'-end machinery that cleaves pre-mRNA transcripts and adds the 3' polyA tails. Despite its fundamental importance, we are still far from understanding the molecular mechanisms of CPF. Here, we identify a sub-complex of the yeast CPF, the polyadenylation module (pAm), which acts as a hub for protein-protein interactions. Using cryo-EM we determine a 3.5 Å structure of the Cft1-Pfs2-Yth1 subunits of pAm. This consists of 4 beta propellers in Cft1 and Pfs2 that are strikingly similar to other interaction hubs involved in DNA and RNA processing. The zinc finger Yth1 protein extends from the side, providing an RNA binding surface. Biochemical studies confirm the structural observations and indicate the important role of pAm as the scaffold element of CPF to assemble other CPF subunits, including the poly(A) polymerase, and accessory factors of the 3'end processing machinery on RNA. We now aim to understand how the enzymatic activities are regulated. Our most recent results will be presented.

Casañal A., Kumar A., Hill C. H., Easter A.D., Emsley P., Degliesposti G., Gordiyenko Y., Santhanam B., Wolf J., Wiederhold K., Dornan G. L., Skehel M., Robinson C. V., Passmore L. A. Science (2017).

0071-R/M

Structural analysis of the adaptor protein Nck1

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The family of Nck (non-catalytic region tyrosine kinase) proteins, Nck1 and Nck2 in humans, are adapter proteins that participate in signalling events that involve tyrosine phosphorylation. Nck1 and Nck2 have a very similar size (47 kDa) and domain structure. They contain three Src homology 3 domains (SH3-1, SH3-2, SH3-3) and a C-terminal SH2 domain that mediate the interaction with a large repertoire of proteins. The SH3 domains bind to Pro-rich motifs and each SH3 domain exhibit very distinct ligand specificity. The SH2 domain binds to phospho-Tyr. These globular domains are connected by linkers between 29 and 49 residues long. An intramolecular interaction has been described between the SH3-2 domain and a basic sequence in the linker between the SH3-1 and SH3-2 domains, which blocks the Pro-rich motif binding site [1]. Other than that, the domain architecture of Nck1 remained largely uncharacterized. We have combined x-ray crystallography and small angle x-ray scattering (SAXS) to analyze the structure of human Nck1. We have solved the crystal structure of the SH3-2 domain to 1.2 Å resolution. SAXS analysis of full length Nck1 indicates that it is a monomeric protein in solution with a radius of gyration of ~34 Å and a maximum dimension of ~124 Å. Analysis of the scattering data using the Ensemble Optimization Method [2] indicates the presence of conformational heterogeneity. This suggests flexibility in the relative orientation of at least some domains, possible due to the relatively long inter-domain linkers and the lack of stable contacts between the domains.

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0084-R/M

Cryo-EM structures of KdpFABC reveal K+ transport mechanism via two inter-subunit half-channels.

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P-type ATPases ubiquitously pump cations across biological membranes to maintain ion gradients. Among those, the heterotetrameric high affinity potassium ion uptake system KdpFABC from prokaryotes is unique. While ATP hydrolysis via a phosphoenzyme intermediate is accomplished by P-type ATPase subunit KdpB, potassium ion transport is supposed to be mediated by channel-like subunit KdpA. The chimeric nature of KdpFABC promises unperceived insights into transporter and channel mechanisms. A first crystal structure uncovered the overall topology of the complex and led to the suggestion of a two-way coupling mechanism between KdpA and KdpB via a subunits-connecting tunnel initiating KdpB phosphorylation and a coupling helix controlling the cytoplasmic gate in KdpA. Here we report two cryo-EM structures of KdpFABC with 3.94 Å and 4.00 Å resolution representing a nucleotide-bound E1 and an E2-P state, respectively. Unexpectedly, the structures revealed two half-channels at the interface of KdpA and KdpB suggesting a new translocation pathway via KdpA and KdpB, while KdpA's pore remained closed in both states. Based on these findings we hypothesize that KdpFABC has evolved to unite the alternating-access mechanism of actively pumping P-type ATPases with the high affinity and selectivity of a potassium ion channel.

0093

Fosfohidrolasas extracitoplasmáticas implicadas en la interacción patógeno-hospedador: una ectoenzima de la pared celular de Streptococcus suis, relacionada con la CpdB periplásmica de *Escherichia coli*

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Las fosfohidrolasas bacterianas extracitoplasmáticas han adquirido un renovado interés por su potencial actividad sobre dinucleótidos cíclicos (cDN) producidos y secretados por el patógeno o producidos por el hospedador infectado, con efectos sobre el sistema inmunitario innato. La CpdB de *E. coli* es una enzima periplásmica que hidroliza, entre otros sustratos, 3′-nucleótidos (3N), 2′,3′-mononucleótidos cíclicos (23cN) y cDN, pero no 5′-nucleótidos [1]. Se sintetiza como un precursor con un péptido señal que es eliminado durante su secreción al periplasma. La CpdB madura tiene un dominio llamado 5_nucleotid_C (5C), con dos tirosinas que pueden formar un *sandwich* con la base nitrogenada del sustrato, y un dominio Metallophos (MP) que contiene el centro catalítico con un residuo de histidina y un centro dimetálico. 5C y MP están unidos por un espaciador que, por analogía con la 5′-nucleotidasa UshA [2], podría permitir un giro de 5C que llevaría el sustrato al centro catalítico en MP. El gen *cpdB* codificador de CpdB es un factor que aumenta la colonización a largo plazo de órganos internos de aves infectadas [3]. En el género *Streptococcus* se han identificado proteínas homólogas a CpdB, pero están ancladas a la pared celular como ectoenzimas, y han sido denominadas SntA en *S. suis* [4] o CdnP en *S. agalactiae* [5]. Además de los dominios 5C y MP unidos por un espaciador, tienen un dominio C-terminal de anclaje a la pared y actúan como factores de virulencia. SntA limita la acción del complemento [4] y CdnP disminuye la producción de IFN-β por el hospedador gracias a la hidrólisis del cDN diadenilato cíclico [5]. Nosotros hemos clonado molecularmente el homólogo de CpdB de *S. suis*, hemos expresado como proteína recombinante su parte central, que contiene 5C y MP, y hemos preparado mutantes puntuales de los dos residuos de tirosina que pueden formar el *sandwich* con el sustrato en 5C, para caracterizar sus actividades fosfohidrolasa sobre 3N, 23cN, cDN y otros sustratos conocidos de la CpdB de *E. coli*. (Grupo de Enzimología del SECTI, Ayudas GR15143 e IB16066 de la Junta de Extremadura, cofinanciadas por FEDER)

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0106

Biophysical characterization of Erb1 interactors

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The mammalian PeBoW complex, composed of Pes1, Bop1 and WDR12 (or Nop-7 subcomplex: Nop7, Erb1 and Ytm1 in yeast) is critical for the processing of the 32S pre-ribosomal RNA in ribosome biogenesis. However, the exact role of PeBoW in biogenesis is not well understood and the interactions within the complex have been poorly characterized. Erb1 is the core of the trimer and interacts with Nop7 through a region within its N-terminal domain. The exact site within Nop7 involved in binding to Erb1 in the complex is not yet known. Moreover, we have previously described the interaction of Ytm1 and Erb1 through their β-propeller domains. The most recent data show Erb1 is functionally correlated with other factors involved in 5.8Ss formation and was found part of pre-ribosomal complexes that included Drs1 and Has1 helicases, Noc2 and Nog1 by using large scale yeast two-hybrid analysis and tandem affinity purification. In this work, we focus on the association between Nop7 and Erb1 N-terminal domains and Erb1 with other interactors: Noc2, Nog1, Has1 and Drs1.

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0110-R/M

Mechanical architecture and folding of *E coli* type 1 pilus domains

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CIC nanoGUNE Nanobiomechanics

Uropathogenic *Escherichia coli* attach to tissues using the pilus type 1. This organelle is composed by thousands of coiled FimA domains followed by the domains of the tip fibrillum, FimF-FimG-FimH. All the domains are linked by β-strands that must resist mechanical forces during bacterial attachment. Here, we use single-molecule force spectroscopy to measure the mechanical contribution of each domain to the stability of the pilus and monitor the oxidative folding mechanism of a single Fim domain assisted by the chaperone-like FimC and the oxidoreductase DsbA. We discovered that the pilus domains bear high mechanical stability following a hierarchy by which domains close to the tip are weaker than those close to or at the pilus rod. We also show that during folding, the remarkable stability is achieved by the intervention of DsbA that not only forms strategic disulfide bonds but also serves as a true chaperone assisting the folding of the domains that are stabilized and transported by FimC.

Alonso-Caballero A, Schönfelder J, Poly S, Corsetti F, De Sancho D, Artacho E & Perez-Jimenez R. Mechanical architecture and folding of E coli type-1 pilus domains (2018). Nature Comm, in press.

0117-R

Co-translocational unfolding of a conjugative protein-DNA complex monitored by nanopore technology

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The transfer of antibiotic resistance genes by bacterial conjugation requires a sophisticated machinery, named Type IV secretion system (T4SS). In our model system, plasmid R388, DNA is transferred through the secretion channel in complex with TrwC, a protein that cleaves one of the DNA strands at the origin of transfer and remains bound to the 5´ end ADDIN EN.CITE *Guasch20031 < ![endif]-->Here we report DNA methylation and hydroxymethylation dynamics at nucleotide resolution during highly efficient transcription factor-induced somatic cell to iPS cell reprogramming. We found that gene regulatory elements of key pluripotency factors become demethylated within*

one day after induction of the Yamanaka factors. Throughout reprogramming we observed successive waves of hydroxymethylation at enhancers, concomitant with a decrease in methylation. This suggests active demethylation, consistent with the finding that ablating the DNA demethylase Tet2 almost completely abolished reprogramming. Three distinct transcription factors, namely C/EBPa, Klf4 and Tfcp2l1, were shown to interact with Tet2 and recruit the enzyme to the DNA. Some of these sites maintain high levels of 5hmC, suggesting that here hydroxymethylated cytosines act as an epigenetic mark. Surprisingly, we also discovered regions in which methylation changes preceded chromatin opening. These included sites where Klf4 was bound without leaving a detectable footprint, suggesting a novel type of pioneer factor activity.

0120

Nucleophosmin as a pharmacochaperon target: a study in acute myeloid leukemia cells

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Nucleophosmin (NPM1), a nucleo-cytoplasmic shuttling protein enriched in nucleoli, is essential in cell growth regulation, being involved in ribosome biogenesis and export, control of centrosome duplication and stabilization of some tumor suppressors. Alterations of structure, function, or cellular localization of NPM1 are related with both solid tumors and hematological malignancies. In particular, mutated nucleophosmin is characteristic and probably a driver event of one third of acute myeloid leukaemia (AML) patients. Mutations prevent correct folding of NPM1 C-terminal domain, and confer the protein a novel nuclear export signal, which jointly determine the aberrant localization of mutant NPM1 in the cytoplasm of AML blasts. Therefore NPM1 has become a target in AML therapy and different strategies have been proposed to circumvent NPM1 mutations. We have envisaged NPM1 C-terminal domain refolding mediated by pharmacological chaperones as a strategy to relocalize NPM1 into the nucleolus.

Based on our previous studies showing that certain compounds increase the thermal stability of the wild-type C-terminal domain, we have analyzed the effect of these compounds on the compactness and stability of the AML-like C-terminal domain. Furthermore, we have proceeded to investigate the effect of the best hits on NPM1 subcellular localization in human blasts corresponding to AML cell lines. We provide evidence that the selected compounds induce a partial enrichment of NPM1 in the nucleolus although not completely avoiding the presence of NPM1 in the cytoplasm of AML cells. Thus we show that these compounds may serve as structural scaffolds that promote proper folding and localization of the NPM1 mutant and are therefore endowed with interest as therapeutic leads for treatment of AML.

Urbaneja MA, Skjærven L, Aubi O, Underhaug J, López DJ, Arregi I, Alonso-Mariño M, Cuevas A, Rodríguez JA, Martinez A, Bañuelos S. (2017) Conformational stabilization as a strategy to prevent nucleophosmin mislocalization in leukemia. Sci Reports 7:13959

0175-R/M

Architecture and force activation in membrane associated adhesion complexes

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(alternatively for R12 or R19)

Focal Adhesion Kinase (FAK) is a key signalling component in focal adhesion (FA) complexes. These are large protein complexes arranged in layers that couple the actin cytoskeleton across the membrane to the extracellular matrix and play important roles in cell migration and cancer invasion. They initially assemble in absence of force and are then force activated by contractile actomyosin fibers to trigger crucial FA signals. FAK forms the first layer closest to the membrane in FAs and is activated once force builds up in the FA complex. Both, the molecular architecture and mechanism of force activation in FAs is currently unknown.

Using a combination of cryo-electron crystallography and single particle cryo-electron microscopy we recently obtained structural information to 6.8 Å of the first layer in FAs comprised by FAK bound to the membrane. This reveals large conformational changes of FAK upon membrane binding that trigger the assembly of FAK oligomers. This oligomeric membrane bound state of FAK represents a primed, but not yet activated state of FAK and we propose that forces in FAs will trigger full catalytic activity of FAK by opening the structure and releasing the kinase domain from autoinhibitory intramolecular and membrane interactions. We employed atomic force microscopy based force spectroscopy to test this hypothesis and probe the mechanical properties of FAK under force. We were able to optimize our experimental setup to detect a force event in the low pico newton force range, which we show to be associated to conformational activation. This event occurs prior to FAK unfolding in the force range known to occur natively in FAs.

In conclusion, our study for the first time provides details on the structural arrangement of the first layer in FAs formed by FAK oligomers and how mechanical force in FAs might be translated into biochemical signals to trigger FA activation.

Goñi GM, Epifano C, Boskovic J, Camacho-Artacho M, Zhou J, Bronowska AK, Martín MT, Eck MJ, Kremer L, Gräter F, Gervasio FL, Perez-Moreno M, and Lietha D (2014) Phosphatidylinositol 4,5-bisphosphate triggers activation of focal adhesion kinase by inducing clustering and conformational changes. Proc Natl Acad Sci USA, 111(31), 3177-86 Lietha D, Cai X, Ceccarelli DF, Li Y, Schaller MD and Eck MJ (2007) Structural basis for the autoinhibition of Focal Adhesion Kinase. Cell, 129, 1177-1187

0178

Deciphering the mechanism of action of toxins delivered by the Type-VI Secretion System (T6SS) in *Pseudomonas aeruginosa*

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Almost a million people worldwide die each year as a result of bacterial infections resistant to antibiotic treatment, a number which is predicted to rise tenfold by 2050. To fight the rapid rise of antimicrobial resistance, the scientific community is making a constant effort to understand the molecular mechanisms underlying microbial resistance.

Along these lines of research, we investigate Pseudomonas aeruginosa, a multidrug resistant pathogen, which has acquired sophisticated antibiotic resistance mechanisms. Importantly, this microorganism is the third most commonly isolated nosocomial pathogen and is lethal to patients with Cystic Fibrosis. In a setting of polymicrobial infections, this pathogen outcompetes other bacteria by using a Type-VI Secretion System (T6SS) to deliver toxic effectors into neighbouring bacteria or host cells. Thus, the T6SS provides a fitness advantage that allows the bacteria to thrive and facilitates host colonization [1,2].

Very recently, we have identified the full set of toxins delivered by the T6SS in P. aeruginosa by a Transposon Directed Insertion Sequencing (TraDIS) approach (unpublished results). Based on these results, we have selected for further studies one of these toxins, which we have named Tse8 (type six

exported 8). We have deciphered the 3D molecular structure of Tse8 at 1.9 Å resolution, showing it belongs to the Amidase Signature (AS) superfamily; enzymes in the family catalyse the hydrolysis of amide bonds (CO–NH2), although the family has diverged widely with regard to substrate specificity and function. Tse8 shares the canonical AS sequence motif found in the family members that consist of ca. 130 residues containing a region rich in serine and glycine residues and a unique, highly conserved, Lys84 – Ser162 – Ser186 catalytic triad used for amide hydrolysis.

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0188

THE METASTASIS SUPPRESSOR KISS1 IS AN INTRINSICALLY DISORDERED PROTEIN SLIGHTLY MORE EXTENDED THAN A RANDOM COIL

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CIC bioGUNE Structural Biology

The metastasis suppressor KISS1 is involved in the progression of several solid neoplasias, making it a promising molecular target for controlling their metastasis1. We present the first structural characterization of KISS1 by CD, MALS, SAXS and NMR2. Backbone NMR chemical shifts do not reveal a deviation from a random coil ensemble. The backbone 15N transverse relaxation times indicate a mildly reduced mobility for two regions that are rich in bulky residues. The SAXS data of KISS1 KISS1 is likewise consistent with a predominantly random coil ensemble, although an ensemble optimization analysis indicates some preference for extended conformations, possibly due to positive charge repulsion between the abundant basic residues. Our results support the hypothesis that KISS1 mostly samples a random coil conformational space, which is consistent with its high susceptibility to proteolysis and the generation of Kisspeptin fragments.

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0218

Protein expression and biochemical characterization of two new putative carboxypeptidase inhibitors: RARRES1 and Ovocalyxin-32

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Metallocarboxypeptidases (MCPs) are proteolytic enzymes that catalyse the cleavage of C-terminal amino acids in proteins and peptides. These enzymes have been associated with different biological roles, which range from food digestion to the precise control of neuropeptides activity. Due to its biological relevance, these proteins can be used as biotechnological and biomedical targets.

The activity of MCPs can be regulated by Carboxypeptidase Inhibitors (CPIs), that could mostly be chemical molecules, although a small number are of proteinaceous nature. This latter group are composed typically by small proteins (4 to 7 kDa) with a large number of disulphide bonds that confers to these inhibitors high thermal resistance. These molecules have a C-terminal tail which interact with the catalytic site of the target enzyme blocking the access of substrates.

During the last years, novel endogenous carboxypeptidase inhibitors has been discovered in humans and other vertebrates. These inhibitors display much larger sequences compared to those previously described and share a different and complex secondary structure which confers them a different inhibitory mechanism. The first member identified was latexin, which was discovered in 1995 in rat brain tissue and its structure was resolved in 2005 in complex with human carboxypeptidase A4 (hCPA4). In 1996, a second human protein with a 31% sequence homology to latexin was discovered. This protein was named as Retinoic Acid Responder 1 (or RARRES1), since responds to retinoic acid.

In the present work, we present a comprehensive biochemical characterization of both RARRES1 and its chicken homolog Ovocalyxin-32 (OCX32). OCX32 is an eggshell matrix protein that, displays homology to human RARRES1 and structural similarity with to human latexin. Despite the function of RARRES1 and OCX32 remains unknown, it is likely that such proteins can act as functional carboxypeptidase inhibitors.

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0219-R

Exploring the relevance of USP29 and PCNA interaction

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CIC bioGUNE RMN y HypoxiPATH

The family of DeUBiquitinating enzymes (DUBs) removes mono- and poly-ubiquitin from the target proteins. DUB's activity impacts on multiple biological processes, including DNA replication. Because of their direct or indirect implication and because of their potential druggability, DUBs have become of increasing interest in recent years. USP29 is a poorly characterized member of the large family of DUBs. USP29 has been suggested as a potential oncogene as it is involved in the regulation of p53, claspin and more recently HIF-α. Furthermore, USP29 expression levels correlate with the Gleason score in prostate cancer patients. Our data show that USP29 interacts with the DNA sliding clamp Proliferating Cell Nuclear Antigen (PCNA), which mono- or poly-ubiquitination (mUb or polyUb) plays a key role to deal with DNA lesions encountered by replication forks.

We hypothesized a role for PCNA/USP29 in the cellular response to replication stress through the deubiquitination of polyUb-PCNA and/or PCNA-interacting proteins by USP29. Here, we will discuss the results of the characterisation of the interaction between human USP29 and PCNA.

0224-R

Effect of a mutation in MamC on the formation of magnetic nanoparticles

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Magnetotactics bacterias (MTB) consist of a group of microorganisms capable of aligning and swimming along the magnetic field of the Earth thanks to the formation, through a process of controlled biomineralization, of magnetosomes. These are composed of magnetite or greigite crystals surrounded by a lipid bilayer membrane, which makes them a useful tool in different biomedical applications (being carriers of drugs, etc). However, increasing its production at an industrial level is complicated due to the difficulty of MTB growth. Therefore, at present, different alternatives are being studied to achieve this objective. One of them is based on the production of biomimetic magnetite nanoparticles using certain magnetosome-associated proteins that seem to control the biomineralization process.

Our studies are focused on MamC, an integral protein of the magnetosome membrane, whose secondary structure is unclear, although it is predicted to have two helical transmembrane domains connected by an a-helical loop oriented towards the magnetosome lumen, which contains two negatively charged amino acids (Glu66 and Asp70) with an important role in the protein-mineral interaction.

According with the latter, in this study we introduce a double mutation in the sequence of the protein E66A and D70A, place in the loop involved in the interaction between the protein and the crystal. We will study the effect of such mutations on the formation of magnetite, as well as on the stability and/or the conformation of the protein by physical chemistry techniques: circular dichroism, fluorescence and FTIR spectroscopy.

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0235-R

ArdC Antirectriction Protein, Crystallographic Structure and Function

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Plasmids, when transferred from one cell to another by conjugation, have to overpass the restriction-modification (RM system) control of the recipient cell, which is responsible for distinguishing between foreign or own DNA. Some conjugative plasmids encode their own antirestriction proteins, called Ard (alleviation of restriction of DNA), allowing the plasmid to overcome the recipient restriction barriers (Belogurov et al., 2000).

In this communication, we describe the crystal structure of ArdC protein coded by the broad host range plasmid R388. ArdC is composed of a non-specific ssDNA binding N-terminal domain and a C-terminal metalloprotease domain of type gluzincin. The metalloprotease active site contains a divalent metal binding motif HEXXH (Cerdà-Costa & Gomis-Rüth, 2014). We solved the structure in the absence of metal as well as in the presence of Zn2+ and Mn2+.

ArdC is encoded by a gene located in the establishment module of R388. We constructed the mutant R388ΔArdC and showed that, although ArdC is non-essential for conjugation between Escherichia coli strains, it is required for conjugation to Pseudomonas putida. Thus, ArdC helps the plasmid to conjugate to hosts with different "immigration controls".

We also found that the closest structural homologous protein is the metalloprotease PprI/IrrE from Deinococcus radiodurans. By the cleavage of the transcriptional regulator DdrO, PprI/Irre plays a central regulatory role in the DNA protection or repair pathways in response to radiation (Wang et al., 2015). We propose that ArdC plays a similar regulatory role, activating the expression of genes needed for the establishment of the plasmid after conjugation. We are presently addressing the molecular mechanism by which ArdC exerts its function.

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0269

Structural characterization of a previously unrecognized group of *Legionella pneumophila* E3 ubiquitin ligases

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The eukaryotic ubiquitylation machinery catalyzes the covalent attachment of the small protein modifier ubiquitin to cellular target proteins in order to alter their fate. Microbial pathogens exploit this post-translational modification process by encoding molecular mimics of E3 ubiquitin ligases, eukaryotic enzymes that catalyze the final step in the ubiquitylation cascade. Here, we show that the Legionella pneumophila effector protein RavN belongs to a growing class of bacterial proteins that mimic host cell E3 ligases to exploit the ubiquitylation pathway. The E3 ligase activity of RavN was located within its N-terminal region and was dependent upon interaction with a defined subset of E2 ubiquitin-conjugating enzymes. The crystal structure of the N-terminal region of RavN revealed a U-box-like motif that lacks the central alpha helix commonly found in other U-box domains of eukaryotic E3s. These structural characteristics indicate that RavN is an E3 ligase relic that has undergone significant evolutionary alteration. Substitution of residues within the predicted E2 binding interface rendered RavN inactive, indicating that, despite significant structural changes, the mode of E2 recognition has remained conserved. Using hidden Markov model-based secondary structure analyses, we identified and experimentally validated four additional L. pneumophila effectors that were not previously recognized to possess E3 ligase activity, including Lpg2452/SdcB, a new paralog of SidC. Our study provides strong evidence that L. pneumophila is dedicating a considerable fraction of its effector arsenal to the manipulation of the host ubiquitylation pathway.

Lin YH, Lucas M, Evans TR, Abascal-Palacios G, Doms AG, Beauchene NA, Rojas AL, Hierro A, Machner MP. RavN is a member of a previously unrecognized group of Legionella pneumophila E3 ubiquitin ligases. PLoS Pathog. 2018 Feb 7;14(2):e1006897

0273

Impact of redox status on biological activity of Fur from the strictly anaerobic pathogen Clostridium difficile

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Clostridium difficile is a strict, spore-forming anaerobic bacterium, being the main cause of nosocomial diarrhoea [1]. Different C. difficile antibiotic-resistant strains with altered expression of both redox proteins and iron metabolism have emerged in recent years [2]. To survive and cause infection, C. difficile needs iron as an essential nutrient, whose acquisition is controlled by the ferric uptake regulator Fur [3]. Although it is considered a strictly anaerobic pathogen, C. difficile induces a large number of electron transporters when is exposed to atmospheric O2, suggesting that mechanisms for tolerating oxidizing conditions occur in this bacterium [4]. In order to investigate the impact of redox status on the C. difficile Fur (CdFur) activity, we overexpressed in Escherichia coli and purified by immobilized metal ion affinity chromatography (IMAC) the CdFur repressor and all its seven single cysteine mutants (C51A, C81A, C93A, C101A, C104A, C141A, and C144A). Biological activities of native recombinant CdFur and its cysteine mutants were analyzed in vitro by electrophoretic mobility shift assays (EMSA). Results demonstrated that recombinant CdFur was biologically active under reducing conditions, while residues C101, C104, C141, and C144 appeared essential for DNA binding activity. In vitro DNA binding affinity was strongly affected by oxidizing conditions, but this effect appeared reversible. Residue C81 seemed to be involved in redox modulation of CdFur DNA binding activity, since mutant C81A remained active under non-reducing conditions. The structural Zn2+ ion of CdFur was removed from the native protein under oxidizing conditions. Residue C104 appeared essential for coordination of structural Zn2+.

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0335

When Vulcan met a titan: redox structural biology of titin

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The giant protein titin is a major determinant of the mechanical activity of myocytes, as evidenced by the fact that mutations in the titin gene are a leading cause of cardiomyopathies and musculoskeletal disease. In my talk, I will review recent findings that link redox modification of cysteines in titin domains with modulation of the mechanical activity of the protein. So far, we have described three different levels of regulation: S-thiolation of buried cysteines, disulfide formation, and disulfide isomerization. Most of the immunoglobulin domains of titin contain conserved cysteines that can engage in these modes of regulation. Our laboratory is focused on understanding their physiological impact in the function of the heart in health and disease.

Alegre-Cebollada J, Kosuri P, Giganti D, Eckels E, Rivas-Pardo JA, Hamdani N, Warren CM, Solaro RJ, Linke WA, Fernandez JM (2014) S-glutathionylation of cryptic cysteines enhances titin elasticity by blocking protein folding. Cell 156: 1235-1246 Giganti D, Yan K, Badilla CL, Fernandez JM, Alegre-Cebollada J (2018) Disulfide isomerization reactions in titin immunoglobulin domains enable a mode of protein elasticity. Nat Commun 9: 185

0307

Estudios estructurales del complejo ARN Polimerasa I en estado activo

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Un factor esencial para el funcionamiento normal de la célula es el mantenimiento de la integridad del ADN (1). Cambios químicos o estructurales en este provocan inestabilidad en el genoma y pueden obstaculizar procesos fundamentales en la célula, como la transcripción (2). Dentro del nucléolo, la enzima ARN Polimerasa I (Pol I) transcribe el ADN ribosomal (ADNr) para producir el ARN precursor, el cual tras su maduración formará el esqueleto de los ribosomas. La transcripción de los genes ADN ribosomales es un paso limitante para el crecimiento y supervivencia de las células, y la desregulación de la síntesis del ARN ribosomal está ligado a la proliferación de células cancerosas (3,4). En este trabajo, se muestra la estructura obtenida por criomicroscopía de Pol I con una modificación química en el ADN a una resolución de 3.6 A. La estructura revela que dicha modificación induce un intermedio de translocación con características únicas. Este resultado, junto con los resultados bioquímicos abren el camino para desentrañar los mecanismos moleculares subyacentes para el reconocimiento de anomalías en el ADN ribosomal.

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0357

New artificial peptides for endosomal escape and cytoskeleton mimics.

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Supramolecular Chemistry allows us to study and to better understand the fundamental interactions that govern the biological processes.1,2

These lessons that we learned from Supramolecular chemistry can be applied to the transport of macromolecules across the membrane of the cells.2 This "controlled delivery" of exogenous molecules is of fundamental important in chemical biology, medicine and beyond. The non-covalent conjugation of cationic artificial carriers and anionic biomacromolecules (nucleic acids, proteins) is an excellent non-viral strategy with potential applications in gene therapy and cancer treatments.2 We apply dynamic covalent bonds (i.e. hydrazone or oxime) to modify the control the molecular structure of polymers and peptides and to trigger the membrane transport and delivery of macromolecules with biological relevance.3,4 We have recently achieved the efficient delivery of different nucleotides (siRNA and DNA) as well as functional proteins including Cas9 for gene edition by the CRISPR/Cas9 system.3,4

We are also interested in applying synthetic and supramolecular chemistry to the fabrication of artificial tubular networks and cytoskeleton mimics. The controlled self-assembly of simple peptide structures in confined spaces constitute and excellent synthetic tool for the development of bottom up approaches for minimal cell-like entities in synthetic biology.

Conceptual drawing of amphiphilic vehicles and nucleotide cargos for

membrane translocation and cell delivery.

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R11 Genómica y proteómica

0009-R

VALIDACIÓN E IDENTIFICACIÓN DE BIOMARCADORES EPIGENÓMICOS CLÍNICOS ÚTILES PARA EL DIAGNÓSTICO PRECOZ Y LA RESPUESTA A FÁRMA

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El cáncer de pulmón (CP) es la principal causa de fallecimiento por cáncer en todo el mundo, habiendo sido responsable de la muerte de 1,7 millones de personas en 2015. A pesar de que se ha logrado un progreso importante en la comprensión de la biología y del tratamiento del CP, las tasas de supervivencia solo han aumentado ligeramente en los últimos años pero siguen siendo muy bajas, debido a la carencia de herramientas de predicción temprana y de terapias personalizadas. Nuestro laboratorio llevó a cabo un cribado de alto rendimiento para la identificación de biomarcadores epigenéticos en cáncer de pulmón no microcítico (CPNM), mediante microarrays de metilación del ADN, llegando a la conclusión de que los niveles de metilación en las regiones promotoras de los genes LAD1 y GRHL2, estaban asociados con la respuesta a los inhibidores de tirosina quinasa (ITQs) de EGFR.

Este proyecto de máster ha validado in vitro el papel funcional de estos genes y su posible relación con la resistencia a los ITQs en líneas celulares de CPNM dirigidas por mutaciones activadoras de EGFR. Además, dada la relación de ambos genes con el fenotipo epitelial, nos propusimos estudiar si su represión podía inducir la TEM (Transición Epitelio-Mesénquimal). Nuestros resultados indican que el silenciamiento de GRHL2 aumenta la expresión de marcadores mesenquimales y disminuye la de los epiteliales, confirmando que el fenotipo de la TEM se asocia a una mayor resistencia a los ITQs y que GRHL2 constituye no solo un biomarcador de respuesta, sino que aparece como un modulador importante de este proceso. Serán necesarios más estudios in vitro (p.e., la sobreexpresión ectópica de GRHL2 en líneas celulares con resistencia adquirida a los ITQs) e in vivo (como el análisis de metilación en pacientes de CPNM que reciben tratamiento con ITQs) para establecer de manera precisa el impacto funcional y traslacional de estos hallazgos.

0215

Evaluación de la integridad del transcriptoma extraído de aceituna

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La calidad organoléptica y nutricional del aceite de oliva está relacionada con su perfil fenólico. El estudio de la expresión génica que regula la biosíntesis de los compuestos fenólicos mejoraría el control de la calidad del aceite. El análisis de expresión génica mediante PCR reversa cuantitativa requiere ARN de calidad. Por ello, el objetivo de este trabajo fue determinar la integridad del ARNm obtenido a partir de aceituna, mediante el ensayo 3':5' (Nolan et al., 2006), basado en la ratio entre valores Cq de tres amplicones (región 3', C (central) y 5') a lo largo del ARNm de un gen de referencia.

Se diseñaron cuatro sistemas para olivo, tres basados en secuencias disponibles en el NCBI, NADH DH subunidad F (AF130163: NADH), poliubiquitina OUB2 (AF429430: OUB2) y proteína intrínseca de tonoplasto (DQ202710: TIP), y un cuarto sistema diseñado en base a la secuenciación del gen codificante de la gliceraldehído-3-fosfato deshidrogenasa B (GAPDH). Tras optimizar las condiciones y comprobar la amplificabilidad, los sistemas seleccionados fueron OUB2 y GAPDH. Las curvas de melting para el sistema OUB2-5' mostraban dos picos, por lo que fue descartado. El sistema seleccionado como sistema de referencia fue el GAPDH. Sus límites de detección fueron 3,125 ng para C y 3' y 0,195 ng para 5'. Finalmente, el sistema GADP se aplicó en cuatro variedades de aceituna (Arbequina, Cornicabra, Hojiblanca y Picual) en cinco estados de maduración tras floración (90, 102, 116, 132 y 144 d). Los resultados obtenidos (ratios < 3) sugieren que, para todas las variedades y tiempos de maduración, el ARNm extraído de aceitunas presentaba una integridad adecuada para su utilización en estudios de expresión génica.

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0245-R

Identification of tumor associated antigens in colorectal cancer patients by immunoprecipitation coupled to mass spectrometry

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Ninety percent of patients suffering from the 8 most common cancer types can be successfully treated if early diagnosed. The humoral immune response has been demonstrated useful for cancer diagnosis, predating clinical symptoms up to 3 years. Therefore, we have here aimed at developing a methodological approach based on the specific immunoprecipitation of seroreactive proteins to IgGs of colorectal cancer (CRC) patients and their identification by mass spectrometry to complete the panel of CRC tumor associated antigens that could help establishing a diagnostic signature of the disease.

Serum samples from CRC patients at stages III and IV were used to immunoprecipitate specific seroreactive proteins from protein extracts from CRC cell lines with different metastatic properties. Protein extracts were previously clarified with IgGs from healthy individuals to eliminate non-specific proteins. Eluted proteins with selected reactivity to cancer patients' autoantibodies were identified by mass spectrometry using a LTQ-Orbitrap Velos.

A total of 1743 peptides corresponding to 442 proteins seroreactive to CRC patients' sera and controls were identified, with 79 proteins showing a unique and specific seroreactivity to CRC IgGs. To avoid false positive targets and to select those proteins with a higher potential as CRC diagnostic markers for validation, the dataset was analysed using the CRAPome database and different bioinformatics tools. With the CRAPome, we selected 31 proteins more prone to be actual targets of CRC autoantibodies. Among them, 29 protein showed genetic variation in CRC and 7 showed a statistically significant expression deregulation in CRC. Lastly, one of the identified

proteins has already been described as colorectal and renal cancer prognostic marker.

Overall, we have developed a methodological approach to identify specific proteins target of autoantibodies of colorectal cancer. We have selected a total of 13 proteins for their validation and evaluation of their inclusion in diagnostic panels.

0260-M

Non-SUMOylated Cx43 changes the recruitment of cellular components into exosomes switching the role of these vesicles in metastatic melanoma

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Connexin43 (Cx43) a transmembrane protein involved in cell-cell communication and signalling, has been described as a tumor suppressor factor in melanoma, however its role in disease progression remains under debate. Exosomes are circulating nanovesicular carrier of molecules, which provide signals and "educate" neighboring or distant cells. The presence of Cx43 in exosomes provides these particles with an additional capacity to exchange small molecules such as RNAs, metabolites or ions with target cells via gap junction channels (GJs). In this study we have investigated the role of Cx43 and exosomal Cx43 in metastatic melanoma. Low Cx43 levels and SUMOylated Cx43 in BRAF-mutant human melanoma cell lines was associated with cytoplasmic distribution and low incidence of dye coupling (GJIC). Ectopic Cx43 gene expression using vectors incremented total Cx43 levels, restored Cx43 native band detected by western-blot, plasma membrane localization and raised GJIC. Interestingly, exosomes isolated from BRAF-mutant melanoma cells overexpressing Cx43 only contains the non-SUMOylated Cx43. When different melanoma cell lines were exposed to exosomes containing Cx43, these extracellular vesicles (EVs) significantly decreased cell proliferation and blocked colonies growth. The effect of exosomal Cx43 was compared to the overexpression of the protein (using a vector) suggesting that EVs might now act as a potent tumor suppressor during melanoma progression. Besides, the presence of Cx43 in exosomes significantly increased the sensitivity of BRAF-mutant metastatic melanoma to drugs such as Dabrafenib and Trametinib. The RNA and proteomic component identified by RNA-Seq and mass spectrometry revealed that exosomal Cx43 through its scaffolding function could be involved in the recruitment of proteins and compounds such as small RNAs to the exosomes switching the messages and therefore the role of these EVs in melanoma. Our results indicate that exosomal particles containing non-SUMOylated Cx43 are potent vehicles to combat metastatic melanoma. Further understanding of the role of Cx43 in the exosomes will have implications for the development of new therapeutic strategies. For instance, we demonstrated their ability as drug carriers to combat metastasic melanoma when these vesicles contain Cx43.

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0299

Comparative analysis of MOBQ4 plasmids demonstrates that MOBQ is a cis-acting-enriched relaxase protein family

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A group of small putatively-mobilizable plasmids is increasingly being reported in epidemiology surveys of enterobacteria. Some of them encode colicins, while others are cryptic. All of them encode a relaxase belonging to a previously non-described MOB subfamily, MOBQ4. While highly similar in their mobilization module, two subfamilies with unrelated replicons can be distinguished, MOBQ41 and MOBQ42. The members of two subfamilies were compatible and stably maintained in E. coli. MOBQ4 plasmids were mobilized by conjugation. They contained two transfer genes, mobA coding for the MOBQ4 relaxase and mobC, which was non-essential but enhanced the plasmid mobilization frequency. The origin of transfer was located between these two divergently transcribed mob genes. MPFI conjugative plasmids were the most efficient helpers for MOBQ4 conjugative transmission. No interference in mobilization was observed when both MOBQ41 and MOBQ42 were present in the same donor cell. Remarkably, MOBQ4 relaxases exhibited a cis-acting preference for their oriTs, a feature already observed for other MOBQ plasmids. These findings indicate that MOBQ4 plasmids can spread among enterobacteria aided by coresident IncI1, IncK and IncL/M plasmids, while ensuring their self-dissemination over highly-related elements.

0300-R

Metagenomic study of microbial α-diversity during the recovery of iron deficiency anaemia with fermented goat's and cow's milks

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It is widely held that gut microbial α- diversity might be a key factor in the development of several diseases, since impairment of this diversity is normally associated with disorders such as hepatitis C(1) or inflammatory bowel disease(2). Moreover, it has been suggested that greater bacterial richness may also be beneficial, as individuals categorized as having a low stool bacterial gene count had more overall adiposity, insulin resistance, and dyslipidemia as well as a more pronounced inflammatory phenotype(3). However, the specific role of α- diversity in the genesis and resolution of ferropenic anaemia has not been characterised to date. In this study, we aim to analyze microbial α-diversity in healthy and experimentally-induced anaemic rats, fed with either a diet of fermented cow's milk or fermented goat's milk. During the pre-experimental period, forty rats were divided into two groups, one receiving AIN-93G diet (48 mg/kg Fe) (Control group) and the other a low-Fe diet (6 mg/kg Fe) (Anaemic group), inducing an iron-deficiency anaemia over a period of 40 days. Then the animals were subjected to an experimental period in which both groups were fed for 30 days with fermented milk-based diets prepared with cow's or goat's milk powder. Stool samples were collected at the end of the experimental period and analyzed by high- throughput 16S rRNA gene sequencing. All the data were processed by bioinformatic analysis using specific software. Both diets

showed beneficial effects on the recovery of the α-diversity in anaemic animals, but fermented goat's milk-based diet produced the greatest microbial diversity. Thus, goat's daily products might promote intestinal health through the modulation of the gut microbiome.

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0304

ESTUDIO METAGENÓMICO DE DOS ECOSISTEMAS SEMIÁRIDOS CON DIFERENTES ESTADOS DE DEGRADACIÓN.

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Las zonas áridas y semiáridas (~41% de la superficie del planeta) se encuentran en un grave riesgo de degradación. En tales ecosistemas, los suelos aparentemente desnudos entre plantas, desarrollan unas complejas comunidades de líquenes(biocostras), las cuales enriquecen el suelo en C y N y conducen a un aumento del tamaño de las comunidades microbianas de los suelos subyacentes. Sin embargo, son comunidades altamente sensibles a la degradación, lo que podría influir de forma indirecta en la estructura de las comunidades microbianas asociadas. El objetivo del presente trabajo fue estudiar la biodiversidad, riqueza y estructura de las comunidades microbianas asociadas a biocostras y suelos subyacentes en dos ecosistemas semiáridos con diferentes estados de degradación. Se estudiaron dos zonas, Balsa Blanca (BB) y Amoladeras (AMO) localizadas en el SE de España (Almería), siendo AMO el ecosistema más degradado respecto a BB. Se muestrearon en ambos sitios tres réplicas espaciales de: (i) biocostras dominadas por el liquen Diploschistes diacapsis (C1), (ii) el primer 1.5 cm de suelo justo bajo la biocostra (S2) y (iii) el suelo a partir de 1.5 cm de profundidad hasta el regolito a 10-15 cm de profundidad (S3). Las muestras se secuenciaron usando la plataforma Miseq (Illumina) con lecturas pareadas y química de los kits V.3 (300+300 ciclos). El análisis bioinformático y eliminación quimeras se realizaron utilizando Mothur y CHIMERA-UCHIME. La riqueza de especies fue superior en BB, aunque los índices de biodiversidad fue similar en ambas zonas; si bien existieron diferencias significativas en biodiversidad entre C1, S2 y S3 en ambas zonas. BB presentó una mayor abundancia relativa de los Phylum Acidobacteria, Actinobacteria y Cyanobacteria mientras que en AMO fueron más abundantes Proteobacteria y Bacteroidetes.

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0305

METAGENÓMICA DE BIOCOSTRAS DE LIQUENES Y SUELOS SUBYACENTES A DIFERENTES PROFUNDIDADES EN ZONAS SEMIÁRIDAS

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Las biocostras son comunidades representativas de las zonas áridas y semiáridas llegando a ocupar hasta un 70% de su superficie, desempeñando funciones clave para el funcionamiento de esos ecosistemas. La presencia de consorcios microbianos selectivos en biocostras liquénicas y la lixiviación de ciertos metabolitos secundarios con propiedades antibacterianas desde las biocostras al suelo inmediatamente subyacente, junto con CO-Lábil, carbohidratos, polifenoles y otros nutrientes, podrían influir y seleccionar la composición de las comunidades microbianas del suelo. En este trabajo se llevó a cabo un estudio metagenómico de los principales géneros de bacterias asociados a biocostras liquénicas dominadas por Diploschistes dicapsis, suelos subyacentes bajo las biocostras y suelos desnudos (sin biocostras) en dos ecosistemas semiáridos del Parque Natural del Cabo de Gata (Almería). El objetivo fue encontrar indicadores biológicos y determinar el grado de influencia de la biocostra en las comunidades bacterianas de suelos subyacentes justo bajo la biocostra, hasta 1.5 cm de profundidad (S2), y a mayor profundidad, desde 1.5 cm a 10-15 cm (S3). Suelos desnudos próximos a las biocostras fueron usados como control. En todas las muestras se extrajo el ADN usando un kit comercial y se secuenciaron usando la plataforma Miseq (Illumina) con lecturas pareadas y química de los kits V.3 (300+300 ciclos). El análisis bioinformático y tratamientos de datos se llevó a cabo utilizando Mothur y CHIMERA-UCHIME. Se hallaron géneros exclusivos de las biocostras (Bryocella, Ohxyphotobacteria, Methylobacterium, Acidipila) en ambos ecosistemas. Los suelos S2 presentaron un mayor número de bacterias comunes a las biocostras (Bryobacter, Rhodocytophaga, Nostocales). Por el contrario, los suelos S3 y SD tuvieron el mayor número de bacterias comunes entre ellos (Gaiellales, Rodhoplanes, Rokubacteria sp) sugiriendo una menor influencia de las biocostras en las bacterias de S3.

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0312

METAGENOMIC INSIGHTS INTO MICROBIAL COMMUNITY STRUCTURE IN RANGELANDS FROM SW SPAIN

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Rangelands are one of the major ecosystems of the Earth. It provides multiple goods and services including the maintenance of biodiversity. Rotational grazing has been the main land use during the 20th century, but in the last decades, demographic pressures on rangelands were increasing worldwide leading to progressive land degradation. Soil bacteria play an essential role in the maintenance of ecosystem services and are extremely sensitive to human or natural perturbations. The objective of this study is to investigate the impact of grazing intensification on soil microbial community structure in rangelands. Four representative sites were selected having similar environmental characteristics and only differing in grazing intensity: grazing exclusion, low, moderate and high grazing intensities. Soils were sampled at two depths 0-5 and 5-10 cm. Soil DNA was sequenced using Miseq (Illumina) platform and the taxonomy estimated from 16S rRNA gene data. Two-way PERMANOVA analysis at the genus level using grazing intensity and soil depth as fixed effects showed statistical signification in both factors and its interaction. Pair-wise tests detected significant differences in the bacterial community among the four levels of grazing and two soil depths. Univariate PERANOVA showed 64 taxa significantly influenced by grazing, 37 by soil depth and 33 by its interaction. Aridibacter, Ferruginibacter, Ohtaekwangia, Nocardioides, Dongia, Romboutsia, Turicibacter, Nitrospira and Gemmatimonas progressively increased while Acidobacteria Gp3, Gp1, Bradyrhizobium, Conexibacter, Chthonomonas/Armatimonadetes gp3, Burkholderia, Armatimonas/Armatimonadetes gp1, WPS 2 genera incertae sedis and Beijerinckia decreased with grazing intensification. These taxa are related with soil quality/development indicating a deterioration of soil functioning with grazing intensification, even at low grazing intensities,

thus enabling these taxa to be used as sensitive bioindicators of grazing intensification.

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0333

Respuesta inmune humoral en patologías crónicas: Identificación de biomarcadores mediante proteómica para su diagnóstico temprano

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Las enfermedades crónicas en España son culpables del 85% de las muertes y el 80% del gasto sanitario se debe a su diagnóstico y tratamiento. El refuerzo de la detección en etapas tempranas como método preventivo supone la mejor estrategia para su control ya que permitiría su tratamiento antes de que evolucionen, mejorando la calidad de vida y supervivencia de los pacientes, además de disminuir su gasto sanitario asociado, pues es mucho más económico tratar a los pacientes en estadíos tempranos, cuando la enfermedad es curable. Para ello es necesario, por un lado, identificar y validar biomarcadores útiles para el diagnóstico temprano y, por otro lado, desarrollar nuevas herramientas diagnósticas que permitan la detección múltiple y simultánea de dichos biomarcadores.

Nuestro trabajo se centra en ambos enfoques identificando biomarcadores de respuesta humoral, principalmente, en pacientes de cáncer colorrectal (CCR), y, más recientemente de la enfermedad de Alzheimer (EA), pues los autoanticuerpos frente a proteínas alteradas durante su formación y progresión se pueden detectar entre 1 y 3 años antes de que los pacientes desarrollen síntomas clínicos, lo que demuestra su gran utilidad como marcadores de diagnóstico temprano.

Mediante el empleo de técnicas de proteómica basadas en microarrays de proteínas de alta densidad y en espectrometría de masas, hemos encontrado un panel de 40 proteínas diana de autoanticuerpos en CCR que permiten una detección temprana de la patología. Adicionalmente, en la enfermedad de Alzheimer hemos encontrado un panel de 6 proteínas diana de autoanticuerpos que permiten discriminar entre sueros de pacientes y controles. Por otra parte, estamos integrando dichos autoantígenos en plataformas diagnósticas autoensambladas -ELISA, Luminex y biosensores- basadas en el uso de proteínas fusionadas a HaloTag expresadas in vitro y que permitan la detección temprana de CCR, y su implementación para el diagnóstico de la EA.

0348

Aplicaciones de la proteómica en biomedicina. Mecanismos y biomarcadores en la enfermedad hepática

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La proteómica es una disciplina cuyo fin es el estudio del conjunto de las proteínas de un organismo (proteoma) así como su regulación en un entorno biológico. El desarrollo tecnológico experimentado en los últimos 15 años permite hoy día el estudio preciso y sistemático de las alteraciones que se producen en miles de especies proteicas en respuesta a un estímulo fisiológico o patológico. En el seminario se revisarán los principales recursos para el estudio masivo de proteínas, accesibles a la comunidad científica desde la Plataforma en Red del ISCIII, ProteoRed (http://www.proteored.org) y se ilustrará su aplicación en la investigación de los mecanismos moleculares subyacentes a la progresión de enfermedades hepáticas.

En el marco de la iniciativa cuyo objetivo es el estudio del hígado en el Human Proteome Project, nuestro laboratorio se ha centrado en el análisis de los mecanismos asociados a la progresión de enfermedades hepáticas. La caracterización del proteoma de hígado y suero nos ha permitido identificar a la ruta de grupos monocarbonados (OCM) como un proceso esencial en el mantenimiento del fenotipo diferenciado y quiescente de los hepatocitos. Además de las alteraciones en la abundancia de proteínas clave para la regulación de la supervivencia y proliferación celulares, se discutirán las modificaciones observadas a nivel de R-metilación de proteínas, especialmente de proteínas de unión a RNA, como la quaking protein 1, cuya metilación induce un incremento de p27kip1. Finalmente, asumiendo la relevancia del OCM, se describirá el desarrollo de un método para la cuantificación de las enzimas implicadas mediante espectrometría de masas dirigida (Selected Reaction Monitoring, SRM) que podría considerarse como un ejemplo de biomarcador funcional.

12 Mecanismos moleculares en membranas celulares: Fisiología, patología, biotecnología

0017-R

Desregulación epigenética del transportador OCT1 en el cáncer hepático: Utilidad como diana de terapia génica quimiosensibilizante

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Introducción: Una de las causas de la falta de respuesta a la quimioterapia con sorafenib es la incapacidad de este fármaco de acceder a su diana molecular, situada en el interior de la célula. Previamente hemos demostrado que el transportador de cationes orgánicos OCT1 (SLC22A1) es una vía determinante en la captación del sorafenib y que su expresión está disminuida en tumores hepáticos, como el carcinoma hepatocelular (HCC) y el colangiocarcinoma (CCA).

Objetivo: Caracterizar los mecanismos que determinan la pérdida de expresión de OCT1 en estos tipos de cáncer, así como evaluar la utilidad terapéutica de restaurar la función de OCT1.

Métodos y resultados: El análisis in silico de información obtenida de la base de datos TCGA reveló una caída de la expresión de OCT1 y una hipermetilación de su promotor. El tratamiento de líneas celulares de HCC y CCA con agentes desmetilantes e inhibidores de histona desacetilasas restauró la expresión de OCT1 y aumentó la captación de sorafenib (medida por HPLC-MS/MS). En la disminución de los niveles de OCT1 también intervienen una

elevada proporción de formas aberrantes de splicing alternativo (determinadas por RT-PCR), y la sobreexpresión de hsa-miR-330 y hsa-mir-141. La sobreexpresión de OCT1 mediante vectores lentivirales aumentó la captación de sorafenib y su actividad citostática in vitro. En modelos animales de inducción de HCC o CCA también se observó una disminución de la expresión de los ortólogos de OCT1 y de la captación de sorafenib en el tejido tumoral. Como una prueba de concepto, se realizó la implantación ortotópica de tumores de CCA en ratones nu/nu, que se trataron con sorafenib y vectores adenovirales que contenían la ORF de OCT1 bajo el control de un promotor específico de células tumorales, lo que aumentó significativamente el efecto antitumoral del fármaco.

Conclusión: Existen diversos mecanismos epigenéticos involucrados en la disminución de la expresión de OCT1 en los tumores hepáticos. La terapia génica dirigida a restaurar la expresión de OCT1 en el tejido tumoral es una estrategia prometedora para mejorar su respuesta al sorafenib.

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0043

Intra-helical salt-bridges in transmembrane segments

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Helical membrane proteins are mainly composed by stretches of apolar amino acids spanning the membrane core. However, many transmembrane (TM) segments have charged amino acid residues in their sequence that may conflict with the hydrophobic environment. The free energy cost ΔG of partitioning TM segments harbouring charged residues is unfavourable and strongly affects current membrane protein prediction methods. However, in some cases, the predicted values do not correlate with the experimental ones obtained for this type of TM segments. On the other hand, the experimental data suggest that with the proper orientation (in the same helix face), amino acids with opposite charges could form intra-helical salt-bridges, lowering the cost of partitioning into the hydrophobic environment of the membrane core. By a computational analysis of the composition and distribution of charged residues in TM segments, we have observed that most of the charged amino acids are at a distance of +1, +3 or +4 residues, thus being aligned on one face of the helix and allowing the formation of salt-bridge interactions between them. The aim of our work is to identify and characterize this type of non-covalent interactions both on model (alanine/leucine based) and native TM sequences by using an in vitro translation system in the presence of microsomal membranes, and to precisely estimate the energy contribution of the salt-bridges in the membrane insertion process. Beyond the conceptual issues related to the mechanism of membrane insertion, we note that the availability of quantitative experimental data on the contribution of salt-bridge formation to ΔG will make it possible to refine current prediction methods.

0081

Hypoxia and AQP4 contribute to the origin of age related hydrocephalus

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AQP1 expressed in choroid plexus epithelial cells and AQP4 present in ependymal cells, glia limiting membranes and pericapillary astrocytes foot processes; play an important role in the cerebrospinal fluid (CSF) production and may participate in the pathophysiology of age-dependent hydrocephalus. The finding that brain AQPs expression is regulated by low oxygen tension and ageing led us to analyze how hypoxia and elevated levels of cerebral AQPs may increase the CSF production that could be associated with the hydrocephalus onset. In this study we explored in young and aged WT mice exposed to hypoxia whether expression levels of AQP4 and AQP1 were affected. Choroid plexus, striatum, cortex and ependymal tissue were analyzed separately both for mRNA and protein levels. Furthermore, parameters such as intraventricular pressure (IVP), outflow rate of CSF and ventricular compliance measured by intra ventricular recordings in live animals, as well as total ventricular volume, measured by resonance magnetic images (RMI), were evaluated in WT, AQP4-/- and AQP1-/- mice. Our data prove that hypoxia participates in the origin of hydrocephalus by a process that depends on AQP4 as a main route for CSF movement. Significant increases in AQP4 expression that occur along animal´s aging contribute to produce a considerably worse hydrocephalus situation related with hypoxic events, with impairment of the cognitive function. We hypothesized that physiological events and/or pathological situations coursing with brain hypoxia/ischemia, along live span would contribute to development of chronic adult hydrocephalus.

0135

Papel de las bombas ABCC4 y ABCC5 en la quimiorresistencia del cáncer gástrico

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Antecedentes: La respuesta del adenocarcinoma gástrico (ACG) al tratamiento farmacológico de primera línea con 5-fluorouracilo (5-FU) y cisplatino es menor del 5%. Uno de los mecanismos responsables es la eficaz expulsión de los fármacos por las células tumorales. Objetivo:Dilucidar el papel de las bombas ATP-binding cassette (ABC) en la quimiorresistencia del ACG. Métodos: La expresión de bombas ABC en muestras de ACG procedentes de los Biobancos de Salamanca y San Sebastián se determinará mediante Taqman Low Density Arrays (TLDA) seguido de RT-QPCR convencional de confirmación. Las secuencias codificantes de las bombas seleccionadas se han clonado en vectores lentivirales a partir del cDNA obtenido por RT de alta fidelidad del mRNA de una línea celular de ACG humano. Las proteínas ABC con mayor nivel de expresión en ACG fueron ABCC4 y ABCC5. Debido a su gran tamaño se clonaron por fragmentos que se reconstruyeron en un vector pWPI. En ambos casos se secuenció la construcción y se obtuvieron lentivirus completos para la transducción de células HEK-293. Se comprobó la capacidad de sobre-expresar ambas bombas por determinación de los niveles de mRNA y por western blot. Su localización en la membrana se analizó por inmunofluorescencia. Se llevaron a cabo pruebas funcionales por citometría de flujo para comprobar si la expresión de ABCC4 y ABCC5 confería a las células la capacidad de expulsar sustratos fluorescentes Fluo-3 y 6-carboxifluoresceína, respectivamente, y si este fenómeno se reducía en presencia de diclofenaco, un inhibidor general de estas bombas. Por último, los estudios de viabilidad celular mediante ensayos de MTT mostraron que ABCC4 confería resistencia a 5-FU y a algunos fármacos de segunda línea como SN38, sorafenib, regorafenib y panobinostat, pero no a cisplatino. Por otra parte ABCC5 confirió resistencia a 5-FU y a 6-tioguanina. Conclusión: Las bombas ABCC4 y ABCC5 juegan un papel importante en la falta de respuesta a la quimioterapia del ACG por lo que constituyen interesantes dianas farmacológicas para el desarrollo de nuevas estrategias quimiosensibilizantes.

0168-R/M

Myocardial fibrosis protection. Unraveling the mechanism of action of new therapeutic fluorescence engineered-proteins

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Cardiac fibrosis is defined as a disproportionate accumulation of mainly collagen type I fiber in the myocardial interstitium constituting a serious health problem widely distributed among the world population. Although some conventional therapies reduce fibrosis in humans, myocardial fibrosis persists in patients with cardiac diseases. Myofibroblasts are the cell type responsible for the overproduction of collagen in the extracellular matrix. This study is focused on the inhibition of Heat Shock Protein 90 (Hsp90), as possible therapy of this disease. Hsp90 is a chaperone that is associated with the signaling cascade of TGF beta (main cytokine activating the cellular collagen synthesis) and acts as a positive regulator. For this purpose, two different biotechnological particles (CTPR390-488, 22kDa) (CTPR390-Au, 44kDa), specially designed to inhibit the regulatory function of Hsp90 will be used.

The studies were carried out in NIH 3T3 cell line, and in primary fibroblasts extracted from the heart of mice. Fluorescence microscopy and western blot methods were used for the visualization and localization of the biotechnological particles inside the cell. We demonstrated the Inhibition of collagen production through q-PCR assays.

In order to elucidate the mechanism of action of the inhibitors proposed we defined a cell system with a constitutively active TGFbeta signaling cascade.

We also perfomed in silico simulations of the inhibition, and the first steps through the crystallization of the proposed interaction between Hsp90-TGFbeta type I receptor and Hsp90 with and without the biotechnological particles.

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0191

DSPE-PEG coating improves Solid Lipid Nanoparticles as efficient drug carriers

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One of the challenges of medicinal chemistry is to design new therapeutic compounds that have good solubility and permeability through the biological barriers. Otherwise, even the most promising therapeutic molecules would never reach its target. In order to improve bioavailability of drugs that present bad solubility, poor absorption or low cell membrane permeability different drug delivery strategies are being developed. Apart from improving drug stability and solubility in aqueous dispersions, the incorporation of drugs into delivery systems can increase their efficiency and reduce solvent-related degradation and high dose-related side effects.

In this regard, Solid Lipid Nanoparticles (SLN) are one of the most promising nanocarriers for controlled drug delivery. They are able to incorporate both hydrophilic and lipophilic drugs, present no biotoxicity and drug release kinetic is controlled thanks to their solid core. Moreover, their most important characteristics are their ability to pass through some biological barriers, such as the blood-brain barrier and their tendency to accumulate around solid tumor areas. Many SLN compositions have been developed and tested and it has been demonstrated that interactions of these nanoparticles and cells depend on the SLN composition and characteristics. Therefore, systematic determination of SLN application is not possible and each preparation should be tailored to the purpose.

It has been demonstrated that modification of nanoparticle surface by adding polyethilenglycol stabilizes different SLN suspensions and it can improve the nanoparticle biocompatibility. To analyze the effect of the surface modification, different amounts of phosphatidilethanolamine-polyethilenglycol (DSPE-PEG) were added to SLN composed of stearic acid, Epikuron 200 and sodium taurodeoxycholate. Obtained nanoparticle suspensions were characterized by the analysis of the following parameters: particle size, polidispersity index, ζ-potential, cell toxicity and cell incorporation. We have observed that DSPE-PEG improves cell incorporation of the nanoparticles without affecting their physicochemical characteristics.

0192

Gating mechanisms of Kv11.1 (hERG) without covalent connection between voltage-sensing and pore domains

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Voltage-dependent gating of the K+ channel Kv11.1 (hERG) can be reconstructed from non-covalently associated voltage-sensing domain (VSD) and pore domain (PD) modules (Split channels). This finding challenges the longstanding idea that VSD/PD coupling depends upon their covalent connexion through the S4-S5 linker, that acting as a rigid mechanical lever couples the movement of the voltage-sensor to pore opening (1).

We show here that when the split point is systematically shifted from the end to the beginning of the S4-S5 linker, a gradual negative shift in activation voltage dependence, an almost complete abolition of the Kv11.1 sigmoid shape activation time course and a significant slowing of voltage-dependent deactivation kinetics are induced. This indicates that a strong destabilization of the channels closed state(s) is taking place in channels disconnected near the S4 helix. We also found that the isochronal ion current mode-shift magnitude is clearly reduced in the different splits. However, the accessibility rates of the cysteine-modifying reagent MTSET to an engineered cysteine in the upper S4 helix remained essentially unaltered, indicating no gross alterations of the conformational changes of the voltage-sensing domain in the different splits.

Finally, using a site-directed cysteine mutagenesis and disulfide chemistry approach to analyze the interactions between VSD and PD, we demonstrate that a physical proximity exists between the N-tail of the VSD and the helix S6 bottom in the PD. Our results support the hypothesis that functional integrity of Kv11.1 split channels is conferred by an allosteric coupling of the S4 helix base with the channel gate region near the bundle crossing of S6, and that the disordered and flexible N-tail acting as part of a dynamic network of interactions constitutes an important component of the Kv11.1 gating machinery (2).

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0195

Lipid changes with age in the autophagosomal and mitochondrial membranes after parkin deletion.

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Mitophagy is the selective degradation of mitochondria by autophagy and is a key process in order to establish an appropiate number of mitochondria in the cells. Parkin dependent mitophagy is one of the best characterized ways of selective degradation of mitochondria.

Lipids have a central role in autophagy acting like molecular signals, binding factors or alterating the curvature state of subcellular membranes. Lipid changes make possible the fussion/fission of membranes that are necessary for the progression of the autophagic processes.

In our work we have characterized the lipidomic composition of autophagosomes and mitochondrial membranes isolated from livers of Wild Type and Parkin Knockout mice and we have seen changes in lipid levels due to aging and Parkin deletion

0201

A RIPPLED LAMELLAR PHASE IN SPHINGOMYELIN BILAYERS

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Recent interest in SMs arises from their interaction with cholesterol in generating cholesterol-rich lateral membrane domains, their specific binding to and regulation of particular membrane proteins, and their involvement as precursors to simpler sphingolipids in cell signaling events. Egg SM (ESM) stands out as the most homogeneous of the natural SMs, therefore from the viewpoint of composition, ESM is the closest natural extract to the pure N-palmitoyl sphingomyelin (PSM). Even the nature of the generic SM main transition has been unclear, by analogy with saturated PC lipids; the transition is often inferred to be from ripple phase to fluid (liquid-crystalline or liquid-disordered) phase if a pretransition is observed and gel-to-fluid phase if it is not.

This work studies both PSM and ESM in the temperature regime from 3oC to 55oC using X-ray diffraction and X-ray diffuse scattering on hydrated, oriented thick bilayer stacks. The aim of this work is to characterize the structure of the phases, particularly for T < TM, using oriented hydrated samples. We observe clear evidence for a ripple phase for ESM in a large temperature range from 3oC to the main phase transition temperature (TM) of ~38oC. This unusual stability of the ripple phase was not observed for PSM, which was in a gel phase at 3oC, with a gel-to-ripple transition at ~24oC and a ripple-to-fluid transition at ~41oC. Our study demonstrates that oriented lipid films are particularly well-suited to characterize ripple phases since the scattering pattern is much better resolved than in unoriented samples.

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0206-R

Allosteric connection between the third sodium site and the chloride site in the neuronal glycine transporter 2

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Neurotransmitter removal from glycine-mediated synapses relies on two sodium driven high-affinity plasma membrane glycine transporters (GlyTs) that control neurotransmitter availability. Mostly glial GlyT1 is the main regulator of glycine synaptic levels, whereas neuronal GlyT2 promotes the recycling of synaptic glycine and supplies neurotransmitter for presynaptic vesicle refilling. The GlyTs differ in sodium:glycine symport stoichiometry, showing GlyT1 a 2:1 and GlyT2 a 3:1 sodium:glycine coupling. Sodium binds to the GlyTs at two conserved Na+ sites: Na1 and Na2. The location of GlyT2 Na3 site remains unknown, although Glu650 has been involved in the coordination. Here we have used comparative molecular dynamics simulations of a GlyT2 model constructed by homology to the crystalized dopamine transporter from Drosophila melanogaster by placing the Na3 ion at two different locations. By combination of in silico and experimental data obtained by biochemical and electrophysiological analysis of GlyTs mutants, we provide evidences suggesting the GlyT2 third sodium ion is held by Glu250 and Glu650, within a region with robust allosteric properties involved in cation-specific sensitivity. Substitution of Glu650 in GlyT2 by the corresponding methionine in GlyT1 reduced the charge-to-flux ratio to the level of GlyT1 without producing transport uncoupling. Chloride dependence of glycine transport was almost abolished in this GlyT2 mutant but simultaneous substitution of Glu250 and Glu650 by neutral amino acids rescued chloride sensitivity, suggesting that protonation/deprotonation of Glu250 substitutes chloride function. The differential behavior of equivalent GlyT1 mutations sustains a GlyT2-specific allosteric coupling between the Na3 site and the chloride site.

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0226-R/M

Trafficking properties of the neuronal glycine transporter GlyT2 and mutant variants associated to human hyperekplexia

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Hyperekplexia is a neuromotor syndrome characterized by hypertonia and exaggerated startle response to trivial stimuli that can provoke death in neonates due to cardiorespiratory problems. Hyperekplexia is caused by defective inhibitory glycinergic neurotransmission. The neuronal glycine transporter GlyT2 reuptakes glycine from the synaptic cleft and supplies neurotransmitter to the presynaptic terminal for the maintenance of the quantal glycine content inside synaptic vesicles. Some mutations in the human GlyT2 gene (SLC6A5) cause presynaptic hyperekplexia. In the present work, we study the intracellular trafficking and regulation properties

of four missense GlyT2 mutants which retain partial activity: A277T, L308V, Y707C and G769R. By measuring [3H]glycine transport, surface biotynilation and immunocytochemistry on heterologous cells expressing the mutant variants we have accurately quantified their functional and trafficking properties. As compared to the wild type, mutants A277T and Y707C show lower activity, reduced surface expression and diminished proportion of mature fully glycosylated transporter probably due to an altered maturation along the secretory pathway. In agreement with these results, these defective variants show increased interaction with the chaperone calnexin. A277T also presents reduced interaction with the COP II complex protein Sec24D, what demonstrates a partial arrest in the endoplasmic reticulum. Besides, ubiquitination assays show that most of the variants except Y707C are degraded by the proteasome to a greater extent than the wild type transporter. To improve folding, maturation and membrane arrival of the A277T and Y707C mutants we assayed different compounds with potential to act as pharmacochaperones: glycine derivatives, GlyT2 specific inhibitors and ligands able to stabilize the inward facing conformation of the SLC6 transporters. Our data may help the design of more specific compounds of therapeutic potential.

0242

Posttranslational cysteine modification of the glycine neurotransporter GlyT2

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Glycine transporter GlyT2 is a plasma membrane protein located in presynaptic neurons of caudal areas of the central nervous system. The transporter introduces glycine from the synaptic cleft into the neurons trough cotransport with three sodium ions and one chloride ion. GlyT2 is involved in the termination of the inhibitory glycinergic transmission and its activity represents the main source of neurotransmitter for synaptic vesicle refilling. Mutations in the human GlyT2 gen (SLC6A5) have been detected in hyperekplexia patients. Hyperekplexia (OMIM 149400) is a rare neurological disorder caused by disruption of glycinergic neurotransmission characterized by a pathological startle reflex. The disease may cause severe respiratory problems or even sudden death due to neonatal hypertonia. Dissecting the causes of inactivity of GlyT2 mutants may allow therapeutic approaches. The traffic or activity of GlyT2 can be modulated by posttranslational modifications such as palmitoylation and nitrosylation, which regulate several aspects of many presynaptic proteins. In this report, we demonstrate GlyT2 is acylated. We also characterize different GlyT2 cysteine mutants, which have been generated based on bioinformatics predictions of palmitoylation in order to identify the palmitoylated residues of GlyT2. The effects of 2-bromopalmitate (2-BP), an inhibitor of palmitoyl transferases, have been evaluated. Our data suggest that at least one of the candidate cysteines is palmitoylated.

0256

Omega-3 polyunsaturated fatty acids do not fluidify bilayers in the liquid-crystalline state.

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Long-chain polyunsaturated, omega-3 fatty acids have been repeatedly described as "unique" in their membrane fluidifying properties. This work reports on the comparative effects of docosahexaenoic (C22:64,7,10,13,16,19) acid (DHA), and eicosapentaenoic (C20:55,8,11,14,17) acid (EPA), with oleic (C18:19) acid (OA) as a control, on the gel-liquid crystalline phase transition of dipalmitoyl phosphatidylcholine (DPPC). Mainly differential scanning calorimetry has been used, together with Laurdan fluorescence, and confocal fluorescence microscopy. All three fatty acids DHA, EPA and OA exhibited fluidifying properties when added to the DPPC bilayers, decreasing the main transition temperature. DHA and EPA were somewhat more effective than OA in this respect, but the effects of all three were of the same order of magnitude, thus the long-chain omega-3 fatty acids failed to exhibit any peculiar fluidifying potency. The same was true when the omega-3 fatty acids were esterified in the sn-2 position of a phosphatidylcholine. In agreement with previous studies, the omega-3 fatty acids also failed to have any effect on the fluidity of egg phosphatidylcholine bilayers in the liquid-crystalline state. It can be concluded that no physiological effects of long-chain omega-3 fatty acids could be related to their special fluidifying properties.

0257

Macroautophagy: Different properties of LC3/GABARAP family proteins

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Macroautophagy mediates the degradation of long-lived proteins and organelles through the de novo formation of double-membrane autophagosomes (APs) that sequester cytoplasmic material and deliver it to the lysosome for its elimination. This process requires the concerted actions of a distinctive set of proteins named Atg which include two ubiquitin-like systems, Atg12 and Atg8. The final objective of these systems is the covalent attachment of the Atg8 protein to the lipid phosphatidylethanolamine (PE) in the autophagosomal membrane. Their membrane association has been related to both membrane expansion and membrane fusion, but the underlying molecular mechanisms are poorly understood. The LC3/GABARAP family includes six mammalian orthologs of yeast Atg8 namely LC3A, LC3B, LC3C, GABARAP, GABARAPL1, GABARAPL2. This existence of this variety of proteins raises the possibility that each of them plays a different role in autophagy, either in the various steps during autophagosome biogenesis or in cargo recognition during selective autophagy. To clarify the possible roles of these proteins during macroautophagy we have explored their differential properties. First, we have reconstituted in vitro the enzymatic lipidation reaction of the LC3/GABARAP family members and compared their membrane tethering and fusion activities in order to know which proteins might be involved in the elongation of the autophagosome. Second, to explore which of the orthologs could be involved in mitophagy, we have performed a quantitative biophysical analysis of CL interaction with these proteins using model membranes and we have analysed their colocalization with mitochondria in cells upon induction of mitophagy by rotenone treatment. The GABARAPs but not LC3A or LC3B promote membrane tethering and fusion. However, LC3s interact preferentially with CL-containing vesicles and colocalize with mitochondria upon rotenone treatment in human glioblastoma cells. Therefore, our results support the notion that GABARAPs might be involved in fusion events during autophagosomal growth while LC3s proteins might be in charge of cargo recognition as in the case of rotenone-induced mitophagy.

0266

Folding of the transmembrane helices in the ribosome exit tunnel

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Membrane-spanning domains of integral membrane proteins must achieve the final folded structure in a very different environment compared to that experienced by soluble proteins. This integral membrane proteins are predominantly formed by alfa-helix bundles that are assembled into the ER membrane via a continuous ribosome-translocon channel. Previous measurements suggested that a transmembrane (TM) segment adopt a compact, presumably alfa-helical structure while the nascent polypeptide is still in the exit tunnel near the peptidyl transferase center and moves through the tunnel in this conformation (1-4). We used an in vitro translation of truncated nascent chains of different length and different helical sequences (TM or soluble helices) trapped within the ribosome tunnel to estimate the compactness of test peptide segments. Our results confirm that hydrophobic TM segments in the ribosome exit tunnel assume a compact, likely helical conformation within the tunnel and potential helices from soluble proteins do not. Furthermore, modulation of the hydrophobicity and helix-forming propensity by introducing mutations into a particular TM segment supports the conclusion that it is alfa-helix formation that causes the observed effects.

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0276-R

Unveiling the critical role of nanoscale lipid organizations in IFN-γ Receptor signaling

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Receptors are a class of proteins that can receive and/or respond to signals from either the internal or the external cellular environment upon binding of signal molecules, thus promoting their molecular recognition and signal transduction. A large number of receptors are membrane proteins that are embedded within biomembranes.

Interferon-gamma (IFN-γ) is a cytokine that orchestrates many critical cell functions and signaling processes through transcriptional activation and regulation of a vast number of genes. Among others, INF-γ plays crucial roles in controlling host defense, immunopathological processes, and fighting tumors. IFN-γ mediates its pleiotropic effects on cells binding to IFN-γ receptor (IFN-γR), a pre-assembled heterodimeric transmembrane protein, expressed on the membrane surface of a large variety of cells.

Understanding how membrane nanoscale organization controls transmembrane receptors signaling activity remains a challenge mainly due to a lack of accurate methodology. The work aims to investigate the role that specific lipids have on IFN-γR partitioning into lipid nanodomains, and therefore to act as a docking site for receptor phosphorylation and JAK-STAT signaling cascade activation.

We show that IFN-γR localizes, in vivo, in lipid nanodomains and that IFN-γ addition induces a conformational change in IFN-γR that leads to specific IFN-γR-sphingolipid and -cholesterol interactions with receptor chains 1 and 2, respectively. Furthermore, we described the impact of these proteo-lipid assemblies in receptor oligomerization, and in modulating the JAK-STAT signaling pathway.

This work established the critical importance of dynamic interactions with lipid nanodomains in IFN-γR signaling, and shed new light on the role of membrane protein-lipid interaction in the partitioning of transmembrane receptors into lipid nanodomains and receptor signaling, in vivo.

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0290-R

The yeast exomer complex: a TGN platform involved in the distribution of amino acid permeases

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Receptors, channels, transporters and enzymes use the secretory and endocytic pathways to reach their final destinations. Along these highly conserved routes, the main sorting station is the trans-Golgi-Network (TGN). Here, proteins can be sent to multiples destinations: endosomes, vacuole/lysosomes, plasma membrane (PM) or earlier Golgi compartments. To address these purposes, cells use the lipid language, the cytoskeleton, several small GTPases, adaptors and coats. Although some general mechanisms have been well described, in last decades the number of routes and players described has been continuously growing. In this context, the yeast exomer complex was described as a model of protein platform for cargo sorting from the TGN to the PM.

Exomer complex was originally described in Saccharomyces cerevisiae as an effector of Arf1 necessary for the transport of a narrow pool of proteins to PM (Chs3, Fus1 and Pin2). It is formed by two molecules of Chs5 bound to a dimer of any of the four ChAPs: Chs6, Bud7, Bch1 and Bch2. The ChAPs proteins will act not only as adaptors for the cargoes but they will play additional roles in exomer functions (1).

Recently, we have described exomer implications on correct transport of other transmembrane proteins: the cation extrusion pump Ena1 and the RIM101 pathway sensor (2). However, contrary with what can be observed for the previous exomer cargoes, the absence of exomer complex does not generate an absolute blockade of these proteins at TGN. These phenotypes point out a more general function of exomer at TGN instead of the already described cargo adaptor function. In that sense, evolutionary studies have revealed that exomer complex subunits have suffered duplications and neofunctionalization which have allowed functional diversification in Saccharomyces genus. These results strongly support the idea that cargo adaptor could not be an exomer conserved function (3).

Here we will show that exomer is involved in nitrogen metabolism by managing the localization of amino acid permeases. And in the same line, results seem indicate that exomer is involved in transmembrane protein distribution by a general mechanism instead of working as protein adaptor complex at TGN. A function which could be more conserved along fungi exomer complexes.

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0293-R/M

Effect of conjugation inhibitors in natural environments

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Bacterial conjugation is one of the main mechanisms of antibiotic resistances (AbR) spread among bacterial species1. The constant exposition of environmental bacteria to antibiotics, either added in food industry or derived from wastewater, can trigger the appearance of AbR. AbR are usually encoded in mobile genetic elements such as plasmids, which could get to animal or human pathogens, thus causing serious health problems2. Conjugation Inhibitors (COINs) are compounds that inhibit bacterial conjugation by targeting the conjugative machinery in a specific manner. 2-Hexadecynoic acid (2-HDA) is a synthetic unsaturated fatty acid (UFA) that has shown strong inhibitory activity against a number of conjugative plasmids3. Here, we show the effect of 2-HDA on conjugation in a natural microcosms.

We set up an aquatic microcosm as model environment for AbR emergence and transfer, and populated it with zebrafish. We used the conjugative plasmid pOX38, a derrepressed derivative from F plasmid, contained in Escherichia coli, as subject for the experiments. Aquarium water (AqW) was not suitable for conjugation, but when amended with LB broth to simulate a polluted environment, conjugation occurred at high frequencies. In non-amended AqW, Zebrafish gut resulted as a concentrator for bacterial cells, facilitating mating, and conjugation frequency arose in comparison with results in AqW. When 2-HDA was added either to AqW or fed to zebrafish, conjugation frequencies were significantly diminished. COINs effect applied in mammals, tests carried out in mice gut gave similar results. The effect of 2-HDA on conjugation in an aquatic microcosm and in mammalian's gut shows the potential use of COINs as a mean to control the spread of AbR in natural systems, or as adjuvants of antibiotics.

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0368

Transmembrane substrate docking and helical unwinding in intramembrane proteolysis

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Presenilin, the intramembrane-cleaving protease (I-CLiP) that constitutes the catalytic subunit of the γ-secretase complex, cleaves the transmembrane helix of the amyloid precursor protein (APPTM) to produce the amyloid-β peptides (Aβs) involved in Alzheimer's disease (AD). The mechanisms underlying the ability of presenilin and other I-CLiPs to recognize and hydrolyze transmembrane helices is not known. Using deep-UV Resonance Raman (dUVRR) and NMR spectroscopies, complemented by molecular dynamics simulations, we show that while predominantly in a-helical conformation the transmembrane helix of APPTM and the established rhomboid I-CLiP substrate, Gurken (GurkenTM), display deviations in canonical helical geometry. Binding experiments indicate these helical substrates bind to presenilin homologues and rhomboid with high-affinity at a docking site distinct from the active site. Our spectroscopic analysis indicates that docking of the substrate is coupled to a conformational change that facilitates local transmembrane helix unwinding for intramembrane proteolysis, thus providing insight into how dormant pools of helical membrane protein signaling precursors that play crucial roles in cell physiology and human disease are liberated. Analysis of APPTM variants involved in familial AD also provides insights valuable for AD drug discovery.

0378

Single-molecule insight into membrane protein translocation

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Proteins are known to fold at the exit of the ribosome and to unfold at the entrance of the proteasome. The other main scenario of folding and unfolding in the living cell is during membrane protein translocation. While this process is universal and affects a large number of proteins (> 30%) our knowledge is still scarce. Here I will present a methodology that allows to measure the unfolding and refolding of a protein substrate translocating a membrane pore. Remarkably, our measurements are made on single substrate molecules, which allow us to obtain detailed information on the kinetics of these processes. We observe that unfolding occurs in several steps and that refolding is slowed down by the appearance of kinetic traps. Interestingly, these results are similar to those obtained on substrate unfolding at the proteasome and in folding at the ribosome exit, suggesting that all these processes have a common vectorial component (the folding/unfolding reaction starts through one end) that makes their folding landscape different to that observed in bulk.

R13 Metabolismo del nitrógeno

0118

Estudio filogenético de las cianobacterias fijadoras de nitrógeno atmosférico de los campos de cultivo del Bajo Guadalquivir

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El sistema de cultivo basado en los fertilizantes de síntesis tiene un importante efecto negativo sobre las características ecológicas del entorno de las zonas cultivables. Por otro lado, la fijación bacteriana de nitrógeno

atmosférico tiene una gran relevancia en el enriquecimiento del suelo con nitrógeno combinado. Debido a esto, y como alternativa a la fertilización de síntesis, en los últimos años se viene extendiendo el uso de biofertilizantes, más conocidos como inoculantes microbianos. Los más comunes incluyen bacterias no fotosintéticas (Rhizobium, Azotobacter…), cianobacterias y hongos micorrícicos.

Las cianobacterias son un grupo de organismos fotosintéticos que pueden proliferar fácilmente con requisitos mínimos de luz, dióxido de carbono (CO2), agua y algunas sales minerales. Son fototróficos y viven de forma natural en prácticamente todos los ecosistemas, desde la Antártida a los polos árticos, incluyendo muchos ecosistemas agrícolas. Algunos géneros de cianobacterias cumplen con sus propias necesidades de nitrógeno mediante su capacidad de fijar nitrógeno atmosférico (N2) hasta compuestos nitrogenados, que son fácilmente asimilables por las plantas, como amonio o aminoácidos. Además producen compuestos bioactivos que promueven el crecimiento de los cultivos, los protegen de patógenos y mejoran el estado nutricional del suelo. Estas capacidades, y en particular la de fijación de nitrógeno, son muy beneficiosas para las plantas y algunas de ellas establecen relaciones de simbiosis con algunos de estos microorganismos fotosintéticos.

En este trabajo se ha realizado un estudio de la presencia de cianobacterias fijadoras de nitrógeno atmosférico en los cultivares del Bajo Guadalquivir. Se ha realizado un amplio muestreo de la región y se han estudiado filogenéticamente los microorganismos aislados. Para esto, se ha puesto a punto un nuevo método de análisis basado en una región polimórfica, exclusiva de cianobacterias filamentosas, que permite una discriminación entre especies.

R14 Neurobiología molecular

0027

Maternal hydroxytyrosol supplementation modifies the brain neurotransmitter profile in a pig model at different ages

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Hydroxytyrosol (HTy) is a polyphenol with antioxidant, anti-inflammatory and immuno-modulatory properties. The present study aimed to determine whether supplementing the maternal diet with HTy during pregnancy can modify pre- and early post-natal developmental of the offspring.

Experiment was performed in Iberian sows to study the effects of HTy on monoaminergic neurotransmitters (NTs) in several brain areas. A group of sows were treated daily with 1.5 mg HTy per kg of feed between Day 35 of pregnancy until delivery (30% of total gestational period), whilst another group was left untreated.

Three groups of animals were obtained: foetuses at 100 days of gestation (n=100), 1 month old (n=52) and 6 months old (n=50). After sacrifice, brains were rapidly obtained and the amygdala, prefrontal cortex and hippocampus were dissected.

The concentration of catecholamines (noradrenaline (NA), dopamine (DA), DOPAC, HVA) and indoleamines (serotonin (5-HT), 5-HIAA) in brain extracts was quantified by HPLC-ED. The results indicated that the concentration of almost all NTs was modified by age in the three brain areas, as well as the ratios DOPAC/DA, HVA/DA and 5-HIAA/5-HT, that are considered indicators of the pathways turnover.

Treatment with HTy lead to a clear change in monoaminergic NTs in 100 days-old foetuses, in the amygdala and prefrontal cortex (dopaminergic and serotoninergic) and in the hippocampus (dopaminergic pathway). In contrast, very few changes were observed between HTy-treated and untreated animals at 1- or 6-month-old.

These results indicate that maternal antioxidant treatment affects neurochemical pathways during prenatal development, while they are not affected at postnatal ages, if animals are fed with a standard diet without supplementation.

0044

Carnitine palmitoyltransferase 1 deletion in AgRP neurons increases energy expenditure by enhancing brown adipose tissue activity

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Food intake and whole-body energy balance are regulated by the brain through a sophisticated neuronal network located mostly in the hypothalamus. In particular, the hypothalamic arcuate nucleus (ARC) is a fundamental sensor for the hormones and nutrients that inform about the energy state of the organism. The ARC contains two populations of neurons with opposite functions: anorexigenic proopiomelanocortin (POMC)-expressing neurons and orexigenic Agouti-related protein (AgRP)-expressing neurons. Activation of AgRP neurons leads to an increase in food intake and a decrease in energy expenditure . It has been suggested that lipid metabolism in the ARC plays an important role in the central control of whole-body energy balance. Yet it is unclear whether lipid metabolism regulates the activity of AgRP neurons specifically. To answer this question, we studied mutant mice lacking carnitine palmitoyltransferase 1A (CPT1A) specifically in AgRP neurons. CPT1A regulates the rate-limiting step in the mitochondrial oxidation of fatty acids (FAs) and therefore plays a central role in the metabolism of lipids. The results of our research demonstrate that the deletion of Cpt1a in AgRP neurons: 1) reduces body weight without affecting food consumption. 2) increases energy expenditure and the activity of brown adipose tissue (BAT). 3) decreases white and brown adiposity and increases the expression of genes involved in lipolysis, such as Atgl, in BAT. Altogether, our results suggest that CPT1A and FAs oxidation in AgRP neurons impact peripheral energy balance without affecting food intake and highlight this pathway as a possible target for therapeutic strategies to decrease body weight.

0051-R/M

Cdk5 modulates neuronal ischemic tolerance through the control of the MDM2/p53 pathway

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Brain preconditioning (PC) is based on a stressful, but non-damaging, cellular stimulus to induce an endogenous adaptive response to tolerate a subsequent severe stress, including ischemia. The serine/threonine kinase cyclin-dependent kinase-5 (Cdk5) plays physiological and pathophysiological roles in the brain. In fact, aberrant Cdk5 overactivation contributes to stabilization and activation of p53 stress sensor, promoting ischemia-associated neuronal apoptotic death.

Our previous results demonstrate that PC prevents ischemia-induced p53 stabilization due to post-translational modifications. Here, we aimed to study the possible role of Cdk5 in PC-promoted ischemic tolerance.

To do that, primary cortical neurons were exposed to a validated in vitro model of PC prior to oxygen and glucose deprivation (ischemia in vitro). Levels of Cdk5 were modulated by specific designed siRNA and neuro-

nal apoptosis (Annexin-V-staining) was analysed by flow cytometry, after 4 hours of reoxygenation. mRNA and protein levels were determined by RT-qPCR and western blotting, respectively.

Our results show that PC decreased Cdk5 mRNA and protein levels after ischemia. Indeed, PC reduces calpain-mediated cleavage of p35 into p25, avoiding the generation of the Cdk5/p25 complex, which is linked to ischemia-induced apoptotic death. Moreover, PC prevents the phosphorylation of p53, as well as the accumulation of active caspase-3 and its transcriptional target, PUMA, induced by ischemia/reoxygenation. Thereby, pharmacological inhibition of Cdk5 activity with Roscovitine and Cdk5 knockdown with siRNA confered neuroprotection against the ischemic insult by inducing level expression of the p53-specific E3 ligase MDM2, which promoted MDM2-p53 interaction and p53 destabilization. These results suggest that Cdk5 is involved in PC-induced ischemic tolerance through the control of the MDM2/p53 pathway.

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0056-R

Relevancia de la E3 ubiquitina ligasa APC/C-Cdh1 en el desarrollo de la red neuronal y la estabilidad dendrítica

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La formación de la red dendrítica es un proceso complejo y estrechamente regulado, que se lleva a cabo en el cerebro durante el desarrollo. La estabilidad de las dendritas confiere a la población neuronal la capacidad de mantener su integridad dentro de la red, lo que es esencial para el correcto funcionamiento de la actividad neuronal. Recientemente, nuestro grupo ha demostrado que la E3 ubiquitina ligasa APC/C-Cdh1 (Anaphase promoting complex/cyclosome-Cdh1) regula la estabilidad dendrítica en el cerebro adulto. Así, la deficiencia de Cdh1 provoca la desestructuración dendrítica y, en consecuencia, la pérdida de sinapsis y deterioro cognitivo.

Para estudiar la función de APC/C-Cdh1 en el establecimiento y estabilidad de la red dendrítica en el cerebro en desarrollo, hemos utilizado ratones knockout (Cdh1 cKO) de Cdh1, de manera condicional a la expresión de Nestin-Cre, así Cdh1 está ausente en los precursores neurales, ya desde la etapa embrionaria (E9). A su vez, estos ratones se cruzaron con ratones que expresan GFP en las neuronas piramidales, con objeto de visualizar la formación de las redes neuronales durante el desarrollo.

Mediante inmunohistoquímica, observamos que la deficiencia de Cdh1 provocó la desestructuración de las dendritas, ya desde los 7 días de vida (P7). Este efecto fue más evidente a los 21 días (P21), momento en el que observamos una clara reducción del peso del encéfalo, así como una dilatación de los ventrículos cerebrales, respecto a los animales control. La desestructuración dendrítica fue muy evidente en corteza e hipocampo, que son áreas cerebrales con una función relevante en los procesos de aprendizaje y memoria.

Con todos estos resultados podemos concluir que APC/C-Cdh1 desempeña una función esencial en el establecimiento y estabilidad de la red dendrítica durante el desarrollo, lo que posiciona a Cdh1 como un factor a tener en cuenta en el desarrollo de patologías causadas por alteraciones en el neurodesarrollo, como es el autismo.

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0057-R

Función neuroprotectora de la terapia antioxidante en la isquemia cerebral

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El ictus representa la tercera causa de muerte y la primera de discapacidad en adultos. La disminución del flujo sanguíneo cerebral provoca la muerte neuronal, principalmente por excitotoxicidad y estrés oxidativo. Asimismo, activa mecanismos endógenos de protección y reparación responsables de la mejora funcional. El restablecimiento del flujo sanguíneo es actualmente la única terapia eficaz en el ictus isquémico. Sin embargo, todavía el 40% de los pacientes recanalizados presenta mal pronóstico. Ello puede ser debido al incremento en la generación de radicales libres de oxígeno (ROS) durante la reperfusión del tejido dañado, por lo que una terapia antioxidante coadyuvante a la recanalización podría mejorar el pronóstico de los pacientes de ictus.

La utilización de un modelo animal que expresa catalasa en la mitocondria, de forma constitutiva (mCat), nos ha permitido estudiar la función neuroprotectora de los mecanismos antioxidantes en la isquemia cerebral. En primer lugar, comprobamos que la diferenciación neuronal no resultó afectada por la presencia de catalasa en la mitocondria. Además, observamos similares concentraciones de $O2^{\cdot-}$ y $H2O2$ en neuronas procedentes de ambos genotipos en condiciones basales. Sin embargo, la presencia de catalasa previno la generación de ambos oxidantes en neuronas sometidas a un daño excitotóxico (100 µM de glutamato) o tras un protocolo de isquemia in vitro (privación de glucosa y oxígeno). Estos resultados confirman la importancia del estrés oxidativo en el daño isquémico. Es más, la expresión de mCat en la mitocondria previno la despolarización mitocondrial y la subsecuente apoptosis neuronal causada por el insulto excitotóxico.

En conjunto, nuestros resultados confirman, mediante un modelo endógeno, la función neuroprotectora de los mecanismos antioxidantes en la isquemia cerebral y sugieren la importancia de una terapia antioxidante coadyuvante a la recanalización tras un infarto cerebral.

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0169

Identificación de biomarcadores lipídicos séricos de enfermedad para la esclerosis lateral amiotrófica

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La esclerosis lateral amiotrófica (ELA) es la enfermedad neurodegenerativa de motoneurona más frecuente, no existiendo hasta la fecha un tratamien-

to eficaz. Actualmente existe la necesidad de encontrar biomarcadores que permitan el diagnóstico y el pronóstico de la enfermedad. Parte del proceso patogénico de la ELA lo conforman defectos energéticos que provocan un estado de hipermetabolismo sistémico. Esta alteración hipermetabólica podría generar en la sangre cambios en las concentraciones de metabolitos que pudieran relacionarse con la evolución y la supervivencia de la ELA. Inicialmente, en análisis metabolómico no dirigido del suero de una cohorte de 71 sujetos en ayunas (32 controles y 39 pacientes de ELA) mediante cromatografía líquida de ultra alta resolución (UHPLC) se detectaron 446 metabolitos, de los cuales 30 estaban alterados en pacientes de ELA. Se observó un aumento general de los triglicéridos con ácidos grasos de cadena larga/muy larga y un aumento del ácido nervónico (24:1 n-9). Además, destacó la reducción del cociente 16:1/16:0, indicativo de una actividad delta-9-desaturasa deficiente. En un segundo estudio lipidómico, mediante cromatografía de gases con detección por ionización de llama, se corroboraron estas alteraciones y se observaron cambios adicionales en varios ácidos grasos poliinsaturados de las familias n-6 y n-3, destacando el ácido eicosapentaenoico (20:5 n-3). La reducción de la actividad delta-9-desaturasa se asoció con tiempos más cortos de ventilación mecánica no invasiva (VMNI, HR 5,03 p<0,01), gastrostomía (HR 23,7 p<0,001) y fallecimiento (HR 4,68 p<0,012, 3 años de seguimiento). Se puede concluir que, aunque no se ha encontrado en suero una huella metabólica que discrimine pacientes con ELA, se han detectado cambios en ciertos parámetros lipídicos individuales (cociente 16:1/16:0) que podrían ser posibles biomarcadores predictores de la enfermedad.

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0179

Señalización mGluR1/5-mTOR en un modelo murino de síndrome de Down

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El síndrome de Down (SD) es la principal causa genética de discapacidad intelectual. Nuestro laboratorio ha demostrado previamente que la vía de señalización mTOR está hiperactiva en el hipocampo del ratón Ts1Cje (un modelo de SD) debido a un incremento de los niveles de BDNF/pro-BDNF (Troca-Marín et al., 2011). Puesto que la vía mTOR también se activa por glutamato, recientemente hemos abordado el estudio de la señalización mGluR1/5-mTOR en este modelo.

Nuestros resultados preliminares muestran una alteración de la mGluR-LTD en el hipocampo de los ratones Ts1Cje. La mGluR-LTD es un tipo de plasticidad sináptica que depende de la vía mTOR y de la traducción local dendrítica de ciertas proteínas bajo el control de FMRP (Fragile X Mental Retardation Protein). Entre las proteínas cuya traducción local depende de FMRP se encuentra APP. Nuestros resultados preliminares también apuntan a una alteración de los niveles dendríticos de APP en neuronas de hipocampo de los ratones Ts1Cje. Los avances en estas líneas de investigación serán presentados.

Troca-Marín, Alves-Sampaio, Montesinos (2011) An increase in basal BDNF provokes hyperactivation of the Akt-mammalian target of rapamycin pathway and deregulation of local dendritic translation in a mouse model of Down's syndrome. J Neurosci 31:9445- 9455.

0264-R

IMPACT OF FATTY ACID ß-OXIDATION ON ASTROCYTIC BIOENERGETICS

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Fatty acids metabolism in the brain in vivo at the cellular level is a yet unexplored issue. Previous evidence, based in primary culture, suggests that astrocytes utilize fatty acids. However, whether the metabolic fate of acetyl-CoA is entirely the tricarboxylic acid (TCA) cycle to obtain energy, entirely the biosynthesis of ketone bodies, or a mixture of both metabolic pathways has not been deciphered (1,2). During normoglycemia, astrocytes contribute to neuronal energy metabolism by shuttling to neurons metabolic glucose-derived precursors, such as lactate (3). During fasting, fatty acids contribute to maintain energy homeostasis of tissues by either serving as direct energy precursors, or by being previously converted in ketone bodies in the liver, which then serve as energy substrates for other tissues, such as the brain. However, it is unknown whether neurons utilize liver- or astrocytic-generated ketone bodies as energy precursors, during normoglycemia or fasting, to maintain neurotransmission (4,5). The lack of this information is most likely due to the absence of an appropriate experimental model specifically designed to respond to this question. Here, we aimed to address this issue by generating a mouse genetic model unable to perform ß-oxidation specifically in astrocytes. This was achieved by confining carnitine palmitoyltransferase-1A (CPT1A) gene ablation in astrocytes using the LoxP system combined with glial-fibrillary acidic protein (GFAP) promoter driven Cre recombinase expression (6). Here, we present the initial characterization of this genetic model and preliminary results indicating the efficacy of this approach, both in astrocytes in primary culture and in vivo. We believe that this novel genetic tool will be very useful to address fundamental biologically relevant questions such as whether there is a astrocyte-neuron ketone body shuttle in mouse in vivo.

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0265

PERSISTENT STIMULATION OF GLYCOLYSIS IN MOUSE NEURONS IN VIVO IMPAIRS COGNITION AND MOTOR COORDINATION BY REDOX STRESS

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In contrast to the strong glycolytic phenotype of astrocytes, neurons very scarcely consume glucose through glycolysis (1,2). Amongst the possible factors dictating this metabolic difference, the protein stability of the pro-glycolytic enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), plays an essential role (3). Thus, PFKFB3 protein is constantly degraded by the action of the E3 ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C)-Cdh1 (4). By degrading PFKFB3, APC/C-Cdh1 coordinates the balance between glucose consumption through glycolysis and through the pentose-phosphate pathway (PPP) in neurons. In astrocytes, APC/C-Cdh1 activity is low and accounts for the high PFKFB3 stability and glycolytic phenotype (4). However, the biological significance of the low glycolytic activity in neurons in behaving mice is uncertain. To address this issue, we generated a genetically-engineered mouse able to constitutively express PFKFB3 in neurons in vivo since the first week of life. We found that neuronal PFKFB3 protein stabilization yielded mice exhibiting cognitive and motor coordination impairment as from 3-months of age, leading to an obese and glucose intolerance picture, along with neuronal dendrite disruption at the 8 months of age. Interestingly, this phenotype was rescued by preventing, in a neuron-specific manner, redox stress. These results demonstrate, in behaving mice, that the glycolytic activity in neurons must be kept low to support survival and function, and suggest that neuron-specific glycolytic inhibitors, likely PFKFB3 inhibitors, might be potentially useful drugs to prevent the cognitive impairment associated with several neurological disorders.

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0286-R/M

Desing of nanosystems conjugated with antibody fragments for treating brain infections

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Sleeping sickness, or African trypanosomiasis, is a serious health problem with an added socio-economic impact in sub-Saharan Africa, due to direct infection in both humans and their domestic livestock. There is no vaccine available against African trypanosomes and the main reason is the ability of the parasite to change the major surface glycoprotein (VSG) avoiding antibody-mediated responses. The current drugs used to treat African trypanosomiasis are effective, but all of them have limitations, ranging from problems with poor efficacy and acute toxicity to drug resistance. Clearly, novel, safe, and affordable treatments are needed.

Searching for new strategies to treat African trypanosomiasis we developed a drug delivery system to treat the acute phase of the disease: a drug transporter consisting in polimeric nanoparticles conjugated with a single-domain antibody derived from camel heavy-chain antibodies (termed nanobody NbAn33)1-2, that specifically recognizes conserved cryptic epitopes on the parasite surface. In vitro and in vivo studies demonstrated that the nanodevice reduced the curative dose of pentamidine about 100 fold, and most significantly, overcome drug resistance as a result of mutations in the surface transporter that mediate drug uptake.

However, the effectiveness of this nanocarrier in the encephalitic stage of the disease has not been evaluated. Transcytosis is the process by which the epithelial cells of the blood-brain barrier transport macromolecules across the interior of a cell and expel them on the other side. Thus, transcytosis provides the means for brain delivery of drugs across the blood-brain barrier. Receptors of transferring and LDL represent the majority of the receptors present in endothelial cell of the blood-brain barrier. In this sense, we have modified NbAn33 fusing a peptide corresponding to the ligand of LDL receptor to the amino terminal end of the nanobody. Our results showed an increase in the penetrance through the BHE of the modified Nb33_LDL in comparison with the Nb33 alone.

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0310

Exploring the regulation of frataxin expression by neurotrophic factors in the mouse cerebellum after physical exercise

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Friedreich's ataxia is a predominantly neurodegenerative disease caused by recessive mutations that ultimately lead to a deficiency of frataxin (FXN) protein. It mainly affects the spinocerebellar system, thus leading to lack of motor coordination and loss of balance. However, little is known about the regulation of frataxin gene expression under different physiopathological situations. Our group has recently shown the prominent role of neurotrophic factors (specifically the Brain-derived Neurotrophic Factor; BDNF) to elicit neuroprotection against frataxin deficiency both in vitro and in vivo. Compelling evidence has pointed out a link between physical activity and the expression of neurotrophic factors in the nervous system through several molecular mechanisms In this work, we aim to explore the link between physical exercise, neurotrophic factors and frataxin gene expression in the mouse cerebellum. To achieve this, we subjected our mice to a spontaneous exercise protocol lasting 8 weeks, allowing us to identify, between the "runners" a sub group of "high runners" in order to check for "activity-amount" specific effects. We tested, through qPCRs, the levels of mRNAs of the factors we have previously demonstrated are involved in frataxin regulation such as BDNF, Neurotrophin 3 (NT3) and Sonic Hedgehog (SHH) as well as FXN itself. Our results show an increment in the levels of mRNAs for NT3 and SHH, while we were not able to detect significant changes in FXN or BDNF levels. Then we checked FXN protein levels by performing an ELISA assay, showing a significant increment in FXN protein in an amount directly related to the physical activity performed by the mouse. Similar, but not significant, results for BDNF protein levels were found. To search for more possible mediators of the effect of physical exercise on FXN protein level, we analyzed the levels of multiple cytokines using a protein array, which have led to the identification of several potential candidates for FXN upregulation. In view of these data, we suggest that physical exercise up-regulated FXN protein possibly through a posttranscriptional mechanism. A more thorough knowledge of the mediators and molecular mechanisms underlying FXN up-regulation may provide some clues for new therapeutic approaches to curb neurodegeneration in Friedreich's ataxia.

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0313-M

Fibroblast Growth Factor-21 promotes ketone body utilization in neurons through activation of AMPK

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Energy supply to the brain is essential to ensure correct neuronal function, and glucose is the main fuel utilized by neurons (1). In metabolically challenging situations when glucose availability is restricted, brain cells may switch to alternative carbon substrates. This ensures energy supply to preserve the functions of the central nervous system. Moreover, metabolic switching in the brain has profound effects on neuronal physiology and survival (2, 3). In this regard, ketone bodies, a by-product of fat metabolism, play a key role. They can replace glucose as the main source of ATP in the brain when glucose availability is very low, such as during fasting, extenuating exercise, or pathological situations such as diabetes (4). However, the mechanisms through which brain cells reprogram their metabolism are not fully understood.

Fibroblast growth factor-21 (FGF21) is an endocrine hormone that contributes to modulate systemic adaptation to fasting, and it is known to regulate ketone body metabolism in peripheral tissues. However, its role in the

brain, except for neuroendocrine regions, has not been studied in depth (5).

In this work, we show that FGF21 increases ketone body utilization in cortical neurons through a mechanism partly dependent on AMP-dependent kinase (AMPK). We propose that this mechanism contributes to prepare the brain for fasting, thus preventing metabolic decline.

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0327

Mechanisms of plasticity loss during development in the hippocampus

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During development, critical periods of synaptic plasticity facilitate the reordering and refining of neural connections, allowing the definitive synaptic circuits responsible for correct adult physiology to be established. In the hippocampus, presynaptic spike timing-dependent long-term depression (t-LTD) exists, which depends on the activation of NMDARs and that probably fulfills a role in synaptic refinement. This t-LTD is present until the 3rd postnatal week in mice, although it disappears in the 4th week of postnatal development. We were interested in the mechanisms underlying this maturation related loss of t-LTD. We found that at CA3-CA1 synapses, presynaptic NMDA receptors (preNMDARs) are tonically active between P13 and P21, mediating an increase in glutamate release during this critical period of plasticity. Conversely, at the end of this critical period (P22-P30) and coinciding with the loss of t-LTD, these preNMDARs are no longer tonically active. Using immunogold electron microscopy, we demonstrated the existence of preNMDARs at Schaffer collateral synaptic boutons, where a decrease in the number of preNMDARs during development coincides with the loss of both tonic preNMDAR activation and t-LTD. Interestingly, this t-LTD can be completely recovered by antagonizing adenosine type 1 receptors (A1R), which also recovers the tonic activation of preNMDARs at P22-P30. By contrast, the induction of t-LTD was prevented at P13-P21 by an agonist of A1R, as was tonic preNMDAR activation. Furthermore, we found that the adenosine that mediated the loss of t-LTD during the fourth week of development is supplied by astrocytes. These results provide direct evidence for the mechanism that closes the window of plasticity associated with t-LTD, revealing novel mechanisms probably involved in synaptic remodeling during development.

0346-R/M

Class IIa HDACs in Schwann cell differentiation and myelin development

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Schwann cells respond to cyclic adenosine monophosphate (cAMP) halting proliferation and expressing myelin proteins.

We show that cAMP signaling induces the nuclear shuttling of the class IIa histone deacetylase (HDAC)–4 in these

cells, where it binds to the promoter and blocks the expression of c-Jun, a negative regulator of myelination. To do it, HDAC4

does not interfere with the transcriptional activity of MEF2. Instead, by interacting with NCoR1, it recruits HDAC3 and

deacetylates histone 3 in the promoter of c-Jun, blocking gene expression. Importantly, this is enough to up-regulate Krox20

and start Schwann cell differentiation program–inducing myelin gene expression. Using conditional knockout mice, we also

show that HDAC4 together with HDAC5 redundantly contribute to activate the myelin transcriptional program and the

development of myelin sheath in vivo. We propose a model in which cAMP signaling shuttles class IIa HDACs into the nucleus

of Schwann cells to regulate the initial steps of myelination in the peripheral nervous system.

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0351

Peptides based on Connexin43 to target glioma cells without toxic effects on neurons and astrocytes

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Connexin43 (Cx43), a protein that forms gap junction channels and hemichannels in astrocytes, is down-regulated in high-grade gliomas. These are the most common primary brain tumor that unfortunately have a very poor prognosis. In our laboratory we have designed a cell-penetrating peptide based on Cx43 (TAT-Cx43266-283) that mimics the inhibition of the oncogenic activity of c-Src promoted by Cx43. The inhibition of c-Src requires phosphorylation at tyrosine 527 mediated by C-terminal Src kinase (Csk) and dephosphorylation at tyrosine 416 mediated by phosphatases, such as phosphatase and tensin homolog (PTEN). Pull-down assays showed that TAT-Cx43266-283 recruits c-Src together with CSK and PTEN and as a consequence inhibits the oncogenic activity of c-Src in glioma stem cells (GSCs), including those derived from patients. Indeed, TAT-Cx43266-283 reduced GSC proliferation, motility, as analyzed by time-lapse microscopy, and strongly reduced their invasive ability. Interestingly, we investigated the effects of TAT-Cx43266-283 on freshly removed surgical specimens as undissociated glioblastoma blocks, which revealed a dramatic reduction in the growth, migration and survival of these cells. Finally, we have studied the effect of that TAT-Cx43266-283 on neurons and astrocytes and we have not found any significant effect on their morphology and viability. In conclusion, TAT-Cx43266-283 impairs glioma stem cells without affecting neurons and astrocytes, suggesting that this is a promising sequence for the development of new therapies against malignant gliomas.

R15 Parasitología molecular

0025

Identificación de antígenos inmunogénicos de *Plasmodium falciparum* asociados a IgM en una población africana hiperendémica de malaria.

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La malaria es una de las enfermedades infecciosas más amenazantes, en términos de salud pública e impacto socioeconómico, de países tropicales y subtropicales en vías de desarrollo. En la lucha por su control, es manifiesta la carencia de diagnósticos eficaces para la detección de personas infectadas con bajas parasitemias, la mayoría de casos en áreas endémicas debido a la inmunidad parcial que se desarrolla. Por ello, la búsqueda de marcadores que proporcionen información acerca del estado de enfermedad y de protección individual se considera imprescindible.

El hito principal de este trabajo es la detección de nuevos marcadores proteicos que determinen el perfil de respuesta humoral frente a malaria de habitantes de regiones hiperendémicas, incluyendo a aquellos con malaria subclínica que actúan de reservorio de la enfermedad. En la mayoría de la bibliografía, el nivel de exposición e inmunidad frente al parásito se analiza a través de la inmunoglobulina G (IgG), sin embargo, la IgM y la IgA resultan ser potenciales marcadores serológicos poco estudiados hasta el momento que aportan una información complementaria dada su biosíntesis diferencial. Por ello, en este estudio se analizó la producción de las tres clases de anticuerpos específicos de P. falciparum en plasma, aportando una visión global de su dinámica de expresión a nivel poblacional de acuerdo a parámetros demográficos y epidemiológicos, lo que nos permitió clasificar a las personas según su estado inmunológico y de enfermedad. Finalmente, hemos identificado nuevos antígenos reconocidos por IgM a través de técnicas de inmunoprecipitación e inmunómica (LC-MS/MS MALDI-TOF/TOF) en los distintos grupos de inmunidad que abren perspectivas para su uso como marcadores en el control de la malaria.

0042

Caracterización estructural y funcional de la enzima Endonucleasa G de *Leishmania infantum*, factores que regulan su actividad nucleasa.

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La enzima mitocondrial endonucleasa G del parásito Leishmania infantum (LiEndoG) posee una serie de características estructurales y funcionales altamente conservadas en las endonucleasas G de organismos eucariotas superiores. Sin embargo, LiEndoG se caracteriza por la presencia de cuatro dominios, no presentes en otras EndoGs, que hacen de esta proteína una enzima única. Esta singularidad se ve reflejada en el papel dual que lleva a cabo LiEndoG dentro del parásito: por un lado, se sabe que participa activamente en el proceso de muerte celular programada, migrando al núcleo celular donde degrada el ADN genómico; y por otro, se ha demostrado que LiEndoG es una proteína esencial para el parásito, puesto que el desarrollo normal del organismo está condicionado por la correcta expresión de la misma. Ante la falta de una estructura cristalográfica que nos permitiera caracterizar estos dominios y estudiar el papel dual de la enzima, fue necesario realizar un modelo molecular de la proteína. El modelo nos muestra una enzima homodimérica completa, cuyos sitios activos se encuentran en lugares opuestos a la interfaz de dimerización. Un análisis detallado del modelo nos permitió (i) asignar una función a cada uno de los singulares dominios, que fue validada experimentalmente en dos de los cuatro casos, (ii) entender el motivo por el cual LiEndoG contiene una secuencia SRGH en su lugar de corte en vez del canónico DRGH que presentan la mayoría de los miembros de la familia a la que pertenece, (iii) argumentar la clara preferencia de corte de esta enzima por el ADN de cadena sencilla y (iv) abrir un nuevo camino al estudio de LiEndoG como parte de un complejo proteico que estaría implicado en replicación, recombinación y/o reparación del ADN.

0153-R/M

La infección con la cepa atenuada de *Leishmania infantum* ΔHSP70-II genera protección a largo plazo frente a un reto infeccioso con *L. amazonensis* en ratones BALB/c.

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Las leishmaniosis son un conjunto de enfermedades causadas por la infección de parásitos del género Leishmania en diferentes hospedadores vertebrados. Dentro de las cepas cutaneotrópicas, destaca la especie L. amazonensis por la gravedad de la leishmaniosis que genera en pacientes humanos donde las lesiones cutáneas pueden complicarse con la dispersión del parásito a órganos internos. No existe vacuna contra esta forma de leishmaniosis y las estrategias basadas en la leishmanización (generación de inmunidad tras la recuperación de las lesiones patológicas localizadas por infección experimental con parásitos infectivos de L. major) son inviables por la severidad de la patología que causa L. amazonensis.

Este trabajo muestra el potencial profiláctico de una vacuna basada en una cepa atenuada de L. infantum, deficiente en los genes hsp70-II codificantes para una isoforma de la HSP70 implicada en la viabilidad del parásito en el hospedador vertebrado (1). Como antecedente, esta vacuna generó protección en modelos murinos frente a la infección con L. major (2).

Doce semanas después de la vacunación con LiΔHSP70-II, ratones BALB/c fueron retados con un inóculo infectivo de L. amazonensis. Como control se infectó un grupo de animales que habían recibido una dosis del tampón fosfato salino (vehículo vacunal). Por un lado, se analizó la evolución clínica en ambos grupos, monitorizándose semanalmente el tamaño de las lesiones en la almohadilla plantar infectada. Por otro, se determinó la carga parasitaria en diferentes localizaciones al final del periodo de estudio. Los resultados de dos experimentos independientes pusieron de manifiesto un elevado grado de protección, caracterizado por la ausencia de lesiones y un control de la carga parasitaria en el grupo vacunado. Se concluye que la administración de la vacuna atenuada es capaz de generar inmunidad frente a leishmaniosis cutáneas causadas por especie del Viejo y del Nuevo mundo en modelos de ratón.

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0158

Iron overload in mice modifies early antioxidant and immune responses in malaria infection

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UCM Bioquímica y Biología Molecular

The co-endemicity of malnutrition, erythrocytopathies, transmissible diseases and iron-deficiency contribute to the prevalence of chronic anaemia in many populations of the developing world. Although iron dietary supplementation is applied or recommended in at risk populations, its use is controversial due to undesirable outcomes, particularly regarding the response to infections, including highly prevalent malaria. We hypothesized that a boosted oxidative stress due to iron supplementation have a similar impact on malaria to that of hereditary anaemias, enhancing innate response and conditioning tissues to prevent damage during infection. Thus, we have analysed antioxidant and innate responses against lethal Plasmodium yoelii during the first five days of infection in an iron-supplemented mouse. This murine model showed high iron concentration in plasma with upregulated expression of hemoxygenase-1. The sustained homeostasis after this extrinsic iron conditioning, delayed parasitemia growth that, once installed, developed without anaemia. This protection was not conferred by the intrinsic iron overload of hereditary hemochromatosis. Upon iron-supplementation, a large increase of the macrophages/dendritic cells ratio and the antigen presenting cells was observed in the mouse spleen, independently of malaria infection. Complementary, malaria promoted the splenic B and T CD4 cells activation. Our results show that the iron supplementation in mice prepares host tissues for oxidative-stress and induces unspecific cellular immune responses, which could be seen as an advantage to promote early defences against malaria infection.

0174

Heme-binding or defense molecules? Deciphering the role of the MF6p/HDM family of proteins from trematode parasites

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Food-borne trematodes are responsible for a variety of diseases that have a great impact on human and animal health. A rational strategy to develop new drugs or vaccines against these parasites is to identify those molecules involved in metabolic pathways that are exclusive to trematodes and crucial for their survival. In this sense, our group has previously identified a 7.8 kDa heme-binding protein (FhMF6p) which is abundantly present in parenchymal cells and secreted antigens of Fasciola hepatica. This protein belongs to a family of proteins which so far have only been described in trematodes. The particularities of its binding to heme and ability to abrogate oxidation and peroxidase-like activity of heme suggest a possible role for this family of proteins as heme scavengers and/or as source of heme for heme-protein synthesis1,2. Although the transport mechanism of FhMF6p through the parasite tissues is unknown, we were able to demonstrate the ability of this protein to interact with cell membranes in different biological systems (erythrocytes, Trypanosoma and Leishmania). In parallel with our investigations, FhMF6p and orthologs were also described as helminth defense molecules (HDMs) due to their structural homology to cathelicidin-like antimicrobial peptides3. Specifically, the F. hepatica FhMF6p/FhHDM-1 was reported to have immunomodulatory functions that could contribute to inhibit the establishment of a Th1 type immune response during infection in favor of parasite survival4. These data do not contradict the ideas above, as many proteins with a primary function in the physiology of parasites are known to play a substantial role in host-parasite interactions. Thus, in this presentation we will discuss the most recent findings on both research lines.

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0232

In house libraries of human protein kinase inhibitors as a source for new leishmanicidal agents

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Nowadays, the current treatment against leishmaniasis relies exclusively on chemotherapy. The scarce number of drugs available are threatened by rising resistance and severe side effects.

The quest for new drugs to solve this compelling need includes combination therapies, and screening of available libraries composed by natural products or by small organic molecules developed for other clinical purposes. The kinome of parasites are an appealing druggable target with some kinases already validated for this aim.

We have selected a representative set of 60 compounds from our in-house library of small size organic compounds with a wide variety of scaffolds. This library was formerly developed for the search of new inhibitors with different modes of action against human protein kinases, including one compound under clinical trial for neurodegenerative diseases.

These compounds were tested both for inhibition of parasite proliferation and purified L. donovani recombinant GSK3. The range of inhibitory concentrations reached low or submicromolar for the two activities. However, the correlation between them was quite modest. The molecular bases for specific LdGSK3 recognition by compounds with the highest LdGSK3 inhibition were disclosed by molecular docking.

The feasible use of drug libraries available for human pathologies as a source for new antiparasitic compounds under a drug repurposing approach and strategies to improve their selectivity on the parasite will be discussed.

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0239

Mapeado de epítopos de antígenos inmunodominantes de *P. falciparum* por Inmunoglobulina G de una población endémica de malaria

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UCM Bioquímica y Biología Molecular

La malaria promueve una gran heterogeneidad de respuestas humorales que dificultan tanto el desarrollo de una inmunidad natural esterilizante, como el de una vacuna o incluso un diagnóstico fiable dado que la mayoría de infecciones de bajas parasitemias no son fácilmente detectables por microscopía o tests de diagnóstico rápido.

Con el objetivo de determinar las regiones inmunogénicas de IgGs en cinco nuevos antígenos inmunodominantes de Plasmodium falciparum (Pf) de una población de un área endémica de malaria en Ghana, hemos mapeado sus secuencias completas mediante microarrays de péptidos de 15 aminoácidos solapantes.

Del mapeado exhaustivo (2.327 péptidos) se identificaron regiones de alta y baja antigenicidad y se observó que la reactividad de estas regiones no

se modifica entre sueros de personas con alta o baja concentración de IgGs Pf-específicas, demostrando que un aumento en la producción de anticuerpos no modifica los patrones de reconocimiento de epítopos.

Entre todos los péptidos analizados se seleccionaron las 8 secuencias con mayor señal de reconocimiento por IgG para ser sintetizados y utilizados como diana en un ELISA diagnóstico. Mediante este análisis de reactividad de péptidos de forma individual es posible definir epítopos que diferencian individuos infectados y sanos (p<0,0001). Por otra parte, se ha observado un aumento, aunque no significativo, en el reconocimiento por IgG circulante de la mayoría de péptidos por aquellos individuos con malaria submicroscópica respecto a los que presentan parasitemias clínicas.

Las dos antígenos de Pf con mejores resultados respecto a la diferenciación entre infectados y sanos fueron StAR-RLTP y PDI.

En resumen, el mapeo de epítopos antigénicos puede permitir diagnósticos precisos para la identificación de personas infectadas con altas o bajas parasitemias en regiones hiperendémicas de cara a ofrecer tratamientos específicos a gran escala, dependientes de la infectividad e inmunidad poblacional, para la eliminación de la malaria.

0263-R/M

Expression of immune-related genes in human neutrophils after infection with the pathogen *Acinetobacter baumannii*

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IDIVAL Microbiología clínica y molecular

Acinetobacter baumannii is a common cause of health care associated infections worldwide. The molecular and genetic basis of Acinetobacter baumannii virulence remains poorly understood, and there is still lack of knowledge in host cell response to these bacteria.

To gain knowledge about the immune response elicited by A. baumannii in contact with human cells, we used naïve inactivated primary human neutrophils as a model for Acinetobacter infection.

Using confocal laser and scanning electron microscopy, immunofluorescence, and live-cell imaging, we shown that immediately after bacteria-cell contact, neutrophils rapidly and continuously engulf and kill bacteria during at least 4 hours of infection in vitro. Interestingly, using scanning electron microscopy and immunofluorescence, we observed that human neutrophils used very large filopodia (more than 50 µm) to sense the environment, but also to detect and retain bacteria. Furthermore, we used this ex vivo model to assess the expression profiles of 84 immune relevant genes up to 2 h post infection by means of q-PCR-arrays, finding that a number of inflammation-promoting genes downstream of the TLR signalling pathways, including those that code for IL1B, CXCL8, IRAK2, NFKB1, PTGS2 and TNF were upregulated.

This model system could be usefull in the design of specific immunomodulatory therapies for Acinetobacter and other important opportunistic infections.

0329

A novel approach to inhibit trypanothione reductase in Trypanosomatids: Dimerization inhibitors

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Leishmaniasis is a neglected tropical disease caused by several protozoan species of the genus Leishmania. Current treatment of leishmaniasis is mainly based on chemotherapy, which includes systemic agents such as antimonials, amphotericin B, miltefosine, paromomycin and pentamidine. Even though these drugs are relatively effective, most of them are expensive and the treatments are long and accompanied by severe side effects. The discovery and development of new drugs against Leishmania parasites as well as the identification of new drug targets is crucial to overcome these problems. For this purpose, it is always preferable to focus on parasite-specific processes that may provide selective drug targets. Leishmania parasites lack the glutathione/glutathione reductase (GSH/GluR) system that is used in nearly all eukaryotes and prokaryotes to maintain the intracellular thiol redox homeostasis. Instead, they possess the rather unique trypanothione/trypanothione reductase (T[SH]2/TryR) system, the blockage of which has been used over the last decades to design and develop new drugs for the treatment of leishmaniasis (1,2).

TryR is a homodimeric NADPH-dependant oxidoreductase whose dimeric state is essential for its catalytic activity. At the core of the dimer interface there is a pair of parallel helices that are regarded as the dimerization and folding nucleus of TryR. Based on these helices, our group designed a 13-mer α-helix inhibitory peptide (TRL35) with the ability to disrupt the dimer (3). Our results show that TRL35 is a time-dependent inhibitor of Li-TryR that, upon dimer disruption, leads to enzyme precipitation. Detailed analysis of the residues present in this α-helix allowed us to identify the three essential ones responsible for the inhibitory activity and then to design a pharmacophoric model which constitutes the bases for the design of new small peptide-mimetics.

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0336

Metabolomic analysis of Trypanosoma cruzi infection: the road to clinical management of Chagas' disease

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Chagas disease (CD) is a complex disease, presenting various clinical outcomes such as spontaneous healing, asymptomatic infection, cardiac, visceral, or cardio-visceral forms. Furthermore, the etiologic agent of CD, the parasite Trypanosoma cruzi, exhibits high genetic diversity, proposed to be associated to the diverse clinical outcomes of CD. Understanding the pathogenic mechanism may have deep implications in the clinical management of this disease. Despite the discovery of this disease more than 200 years ago, still there are many controversies about its pathogenesis.

To address this issue, we have employed a model system by using multiple T. cruzi strains, of the different main genetic lineages (DTUs) to infect mice. We performed global metabolomic analysis in the serum and in the heart of those infected mice in the acute and chronic phase.

We have found many metabolic alterations in experimental acute and chonic T. cruzi infection. Some metabolites can discriminate acute from chronic phase, infecting T. cruzi strain, autoantibody response or T cruzi replication and damage in the heart. Many of these suggest a stressful condition in the heart such as: increased glycolysis, respiratory chain impairment, lipid accumulation, ROS production and uric acid formation and altered Arginine/ADMA ratio were observed in the heart of infected mice. They likely reflect the heart hyperthrophic alterations, although we found elevated levels in serum metabolites not found in other clinically related cardiac alterations, (i.e. idiopathic dilated cardiomiopathy).

Those serum metabolites not found in other cardiac alterations, may be specific biomarkers in this disease. Moreover, our analysis also suggested pathogenic mechanisms amenable of therapeutic intervention. Studies in animal modesl have provided evidence that this therapeutic approach is feasible.

R16 Radicales libres y estrés oxidativo

0024-M

Modificaciones oxidativas de proteínas de membrana eritrocitaria en pacientes con hemocromatosis hereditaria

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La hemocromatosis hereditaria (HH) es una enfermedad en la que se altera el metabolismo del hierro, favoreciendo una elevada concentración de este metal en suero. Una de sus principales consecuencias es el aumento de la producción de especies reactivas de oxígeno que provocan una mayor oxidación a nivel molecular y alteran, en último término, la homeostasis celular y la adaptación de la respuesta metabólica.

Con el objeto de estudiar la oxidación en células circulantes (susceptibles de promover el daño tisular) en pacientes de HH, se analizaron proteínas de membrana eritrocitaria de pacientes con HH de tipo 1 y 4. Mediante electroforesis bidimensional e inmunotransferencia se detectaron proteínas con dos tipos de modificaciones oxidativas: carbonilaciones generales y aductos formados por unión con 4-hidroxi-2-nonenal. En general, se pudo observar que los pacientes con HH poseen un número mayor de proteínas modificadas oxidativamente en comparación con controles.

Mediante huella peptídica (MS-MALDI-TOF/TOF) fue identificada una selección de tales proteínas oxidadas, tanto comunes entre pacientes y controles como diferenciales entre ambos grupos. Así, en pacientes con HH se detecto una marcada oxidación de proteínas del citoesqueleto (susceptibles de modificar la deformabilidad de la membrana), de proteínas encargadas de la adhesión celular y de la migración, e incluso de proteínas relacionadas con la inhibición del sistema de complemento.

Esta oxidación proteica diferencial en HH pone de manifiesto el daño que la exposición persistente al hierro causa en los tejidos de los pacientes, lo que a su vez se espera que facilite el diseño de sistemas diagnósticos asociados a fenotipos patológicos basados en la identificación específica de tales lesiones oxidativas.

0092

The natural stilbene vitisin A triggers cytotoxicity in the HepG2 human hepatoma cell line

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Resveratrol represents the most investigated stilbene. This family of phytochemicals is found in numerous plants, and is remarkably enriched in the Vitis vinifera species. However, numerous other stilbenes are also found in this plant at concentrations at least as high as resveratrol. In the present study we have explored some biological properties of vitisin A, a tetramer derived from resveratrol, in HepG2 as the cellular model. Vitisin A triggered cytotoxicity on HepG2 dose- and time dependently (IC50 lower than 10 mM at 72 h of treatment). The stilbene had no significant effect on HH4 cells, a non-tumoral human fetal hepatocyte-derived cell line. Treatment of HepG2 with vitisin A increased the intracellular steady-state levels of total ROS as measured by flow-cytometry. However, it did not modify superoxide anion levels as detected with the MitoSOX probe. Cell cycle-analysis and caspase activities detection showed an apoptotic mechanism of cell death, without casapase-2 or -9 intervention. These results indicate that vitisin A is a promising natural compound that is worth researching in depth on its biological properties.

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0133

Características funcionales de PON1 en la diabetes mellitus tipo 2

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Las HDL constituyen uno de los factores protectores independientes más importantes frente al riesgo aterosclerótico al que se encuentran sometidos los pacientes con diabetes mellitus tipo II (DM2). Sin embargo, se ha observado que un aumento de HDLc no reduce la tasa de enfermedades cardiovasculares (ECV), lo que sugiere que los aspectos funcionales de las HDL atribuibles a su composición proteica son más importantes que la cantidad circulante de las mismas en el desarrollo de ECV. Las investigaciones se han dirigido hacia el estudio de otras proteínas que se encuentran asociadas a las HDL, tales como PON1. En este trabajo se ha llevado a cabo un estudio para comprobar la hipótesis de que el enzima PON1 presenta características distintas en pacientes con DM2, analizando la concentración y la actividad arilesterasa del enzima y su interacción con las HDL, teniendo en cuenta también el control glucémico y el polimorfismo Q192R del gen. No se pudo confirmar que la concentración y la actividad del enzima estuvieran relacionadas con la enfermedad. Sin embargo, existen características funcionales diferentes de este enzima que, en parte, están mediadas por la distinta composición acílica de sus lipoproteínas transportadoras, y cuya trascendencia fisiológica será tema de futuras investigaciones.

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0170

Capacidad antiinflamatoria del ácido nitro-oleico (NO2-OA) presente en el Aceite de Oliva Virgen Extra (AOVE)

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Los ácidos grasos nitrados (NO2-FAs), resultado de la interacción del óxido nítrico (NO·) con ácidos grasos insaturados, han sido detectados y caracterizados ampliamente en sistemas animales. Entre ellos, el ácido nitro-oleico (NO2-OA) está relacionado con la modulación de la activación de los macrófagos, la mejora de la salud cardiovascular promoviendo la vasorelajación y, debido a su carácter antiinflamatorio, actualmente se está utilizando como fármaco en el tratamiento de enfermedades que cursan con inflamación (1, 2, 3). Hasta el momento, no se había descrito la presencia de NO2-OA en ningún alimento. En este trabajo se presenta su detección y caracterización por primera vez en el AOVE, mediante cromatografía líquida de alta resolución acoplada a espectrometría de masas de triple cuadrupolo (HPLC-MS/MS) (4).

En base a estos antecedentes y con el objeto de caracterizar la capacidad antiinflamatoria del NO2-OA presente en el AOVE, se analizaron biomarcadores de inflamación en cultivos de sangre humana completa utilizando el sistema de inmunoensayo "Bio-Plex ProTM Human Inflammation, Biorad". El análisis de los resultados obtenidos mostró la modulación negativa de marcadores proinflamatorios en los cultivos de sangre tratados con NO2-OA, lo que evidencia que el AOVE constituye una fuente directa de ácidos grasos nitrados que pueden regular el metabolismo y la resolución de procesos inflamatorios.

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0205

Neutrophils exposure to low levels of hydrogen peroxide induce the expression of pro-inflammatory and antioxidant genes

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Disruption of redox control and imbalance in favour of pro-oxidant species leads to the exposure of cells to high and uncontrolled levels of radical and non-radical oxidant by-products of oxidative metabolism. Hydrogen peroxide (H2O2), due to its stability under physiological conditions, exhibits a longer lifetime that allows it to participate either as a secondary messenger molecule or a damaging oxidant. Understanding the molecular mechanisms of the H2O2 biology that make one of these responses more likely than another has far-reaching practical significance since several pathological states are characterized by altered redox biology. Neutrophils of peripheral blood from patients with Metabolic Syndrome were purified and exposed to two H2O2 levels: high rate of H2O2 production overwhelmed cellular antioxidant defenses and low rate of H2O2 production were equilibrated by cellular antioxidant defenses. We determine the genetic expression of key antioxidant and inflammatory proteins in these physiological situations. We eclectically selected proteins implicated in the immune response (COX2, TLR2, TLR4, NFkB), pro-inflammatory markers (IL6, Il8, TNFα), anti-inflammatory mediators (IL1rα, IL1β), antioxidant enzymes (catalase, SOD Mn, SOD Cu/Zn) and mitochondrial dynamics related proteins (Mtf1, Mtf2, Tfam and NRF2). The molecular mechanisms of the H2O2 to provoke variations in mRNA levels remains to be a conundrum but we have established a methodology that allows tracking 'ex vivo' differential cellular responses to external stimuli as it is H2O2. Exposure of neutrophils to low levels of H2O2 is enough to induce a pro-inflammatory response, but also to activate the expression of antioxidant enzymes.

0216-R/M

Total or endothelium specific PGC-1α deficiency is associated with alterations in vascular function in mice

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Endothelial dysfunction is a common alteration in cardiovascular diseases that is produced by increased oxidative stress and a vascular pro-inflammatory state. This condition is associated with an imbalance between endothelium-derived relaxing factors such as nitric oxide (NO) and contracting factors such as endothelin-1 (ET-1). PGC-1α is a transcriptional coactivator that regulates oxidative metabolism and controls oxidative stress. Previous in vitro studies suggested that, PGC-1a activity could prevent endothelial dysfunction induced by hyperglicaemia. The aim of this work was to analyze in vivo, using a preclinical model, if PGC-1α deficiency results in alterations in vascular function. For that purpose, knockout mice for PGC-1α (KO) and mice with a specific deletion of PGC-1α in the vascular endothelium (Tg-TIE2-Cre/PGC-1af/-) or their controls were used. Aorta segments were set in an organ bath to perform vascular reactivity experiments in response to both vasodilators such as acetylcholine (Ach) and vasoconstrictors such as noradrenaline (NA), angiotensin II (Ang II) or ET-1 in presence/absence of lipopolysaccharide (LPS). Aorta segments from PGC-1α KO mice showed increased vasoconstriction in response to NA, Ang II and ET-1 in basal conditions and decreased relaxation in response to Ach only after pre-incubation with LPS. Similarly, aorta segments from TIE mice showed increased vascular contraction in response to ET-1 and Ang II and decreased relaxation in response to Ach only when LPS was present. The increased response to vasoconstrictors seemed to be mediated by COX-2 activation as it was blocked in the presence of the COX-2 inhibitor meloxicam (10-7 M). The decreased relaxation in response to Ach in LPS-preincubated segments appears to be mediated by increased oxidative stress, and the subsequent inactivation of NO, since it was attenuated in the presence of ROS scavengers, and in the presence of a NO donor the relaxation response was fully restored. In conclusion, total or endothelium specific PGC-1α deficiency results in alterations in the vascular response of arterial segments to both vasoconstrictors and vasodilators, especially under an inflammatory challenge. Thus, PGC-1α might be a promising target of study against vascular diseases.

0255-R

Mitochondrial pore opening and loss of Ca2+ exchanger NCLX levels in a cardiac model of Friedreich Ataxia

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Friedreich ataxia (FA) is a rare disease caused by decreased expression of Frataxin, a nuclear encoded protein with mitochondrial localization [1]. Frataxin is highly expressed in the heart and the nervous system, and consequently these tissues are among the most affected in FA. The first symptoms of the disease are usually neurologic while the primary cause of death in FA patients is related to cardiomyopathy [2]. In order to investigate the cellular mechanisms involved in cardiomyopathy, we have been using Frataxin-deficient neonatal rat cardiomyocytes as cell models of this disease [3]. We have observed that frataxin depletion results in mitochondrial swelling, lipid droplet accumulation, oxidative stress and altered calcium homeostasis. In order to investigate the relationship between these different phenotypes, we have analyzed the effect of several compounds on the phenotype of frataxin-deficient cardiomyocytes. We have observed that mitochondrial swelling can be reverted by cyclosporine A, an inhibitor of the mitochondrial permeability transition pore (mPTP) opening, suggesting that frataxin-deficient mitochondria are more sensitized to mPTP opening. Lipid droplet accumulation may be a consequence of mPTP opening, as it is also reverted by cyclosporine A [4]. Indeed, carnitine treatment reverts lipid droplet accumulation, but not mitochondrial swelling. As mPTP opening can be caused by oxidative stress, we have also analyzed the effect of several antioxidants on mitochondrial swelling. However, we have only observed a partial reversion of this phenotype, suggesting that mPTP opening is not caused by oxidative stress. Regarding calcium homeostasis, we show that frataxin-deficient cardiomyocytes display reduced levels of the mitochondrial calcium transporter NCLX that can be restored by calcium-chelating agents These results highlight the importance of calcium homeostasis and that targeting mitochondrial pore by repurposing cyclosporin A, could be envisaged as a new strategy to treat the disease.

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0277-R

Estudio del estado Redox intracelular de diferentes cepas mutantes de *Rhodococcus equi* afectadas en sus micorredoxinas

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Rhodococcus equi es un micoorganismo perteneciente al grupo de las actinobacterias que se encuentra en hábitats muy variados y que es el agente causal de bronconeumonía en ciertos animales (principalmente potros) y ocasionalmente en humanos inmunosuprimidos. R. equi es patógeno intracelular de macrófagos alveolares donde se ve sometido a altas concentraciones de agentes oxidantes (Radicales Libres de Oxígeno y Nitrógeno, RLONs). Actualmente no se conocen los mecanismos de defensa empleados por R. equi para hacer frente a estas condiciones, pero se han identificado genes con alta homología a genes para micorredoxinas de otras actinobacterias (mrx1, mrx2 y mrx3), con potencial implicación en procesos reductivos para el mantenimiento del estado Redox celular que promueva la supervivencia de R. equi en los macrófagos.

Para el estudio de las micorredoxinas de R. equi, se han desarrollado mutantes que presentan deleciones en los genes Re-mrx y se ha estudiado la viabilidad celular de los mutantes bajo diferentes condiciones oxidantes y fisiológicas (temperaturas y pH). Además se han empleado sensores de estrés oxidativo, como son (i) roGFP2 y (ii) Hyper3, para valorar los cambios en el estado Redox intracelular de los mutantes así como la actividad de las micorredoxinas. En el caso de roGFP2, se ha fusionado dicho sensor a ReMrx3 y se ha usado la construcción para complementar la actividad Mrx3 de la cepa delecionada de la micorredoxina-3. Las cepas recombinantes se sometieron a distintos ensayos in vitro de crecimiento frente a diferentes agentes oxidantes y se analizaron los "ratios" de fluorescencia del sensor frente al tiempo en función de la presencia de diferentes concentraciones de agentes oxidantes.

0349-R/M

Coenzyme Q10 deficiency: therapeutic approaches in preclinical models

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Coenzyme Q10 (CoQ10) deficiency causes a mitochondrial syndrome with variability in the clinical presentations. Patients with CoQ10 deficiency show inconsistent responses to oral ubiquinone-10 supplementation, with the highest percentage of unsuccessful results in patients with neurological symptoms (encephalopathy, cerebellar ataxia or multisystemic disease). Failure in the ubiquinone-10 treatment may be the result of its poor absorption and bioavailability. Using a mouse model of fatal mitochondrial encephalopathy due to CoQ deficiency, we have tested the therapeutic efficacy of different CoQ10 formulations as well as alternative molecules related and unrelated to CoQ metabolism. The results show variable responses to the different treatments in terms like CoQ levels, mitochondrial bioenergetics, morphological changes and animals phenotype and survival. Overall, our data provide the options to improve the treatments of patients with CoQ10 deficiency.

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0352

Efecto de la concentración de oxígeno en el cultivo de células madre. Aplicaciones prácticas

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Las células madre se dividen asimétricamente para producir una copia de sí mismas y una segunda célula que seguirá su camino hasta convertirse en una célula diferenciada y especializada. En particular, las células madre de pulpa dental humana (CMPD) han demostrado ser una buena fuente de células para terapias celulares. Sin embargo, requieren de un elevado número de células, para lo cual es necesaria una etapa de expansión in vitro previa a la implantación. Este proceso lleva implícito la senescencia o envejecimiento celular. Las células senescentes no tienen la capacidad de entrar en alguna vía de muerte celular por sí mismas, para lo cual el fenotipo secretor es el encargado de avisar al sistema inmunitario para que proceda a la eliminación de dichas células. De hecho, con el envejecimiento del individuo, el sistema inmune decae, y las células senescentes se acumulan.

El cultivo in vitro de células madre se sigue llevando a cabo bajo tensión de oxígeno ambiental (21% pO 2), la cual difiere de la tensión de oxígeno fisiológica dentro del organismo. Ello, conlleva un estrés oxidativo, reflejado por una mayor producción de especies reactivas del oxígeno (ROS), una mayor oxidación lipídica y carbonilación proteica, así como un menor potencial de membrana mitocondrial. Todo ello, acompañado de cambios morfológicos, una menor tasa de proliferación, una mayor actividad de la enzima β-galactosidasa asociada a la senescencia y un incremento considerable de los niveles de p16 INK4a, uno de los principales reguladores del ciclo celular. Estos resultados en conjunto demostraron que las CMPD cultivadas al 21% pO2 estaban sometidas a un envejecimiento prematuro en comparación con sus homólogas cultivadas al 3% pO2 (tensión de oxígeno fisiológica).

Paralelamente, el análisis de la expresión de los genes de pluripotencia (SOX2, OCT3/4, KLF4 y c-MYC) revela una correlación inversa con dicho envejecimiento. Es decir, el envejecimiento prematuro inducido por estrés oxidativo está asociado con una pérdida de pluripotencia.

Por tanto, la obtención y el cultivo de células madre debe realizarse bajo condiciones de oxígeno fisiológicas que producen una menor alteración de sus características propias de células madre.

0363

REDOX SIGNALING IN ACUTE PANCREATITIS

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Acute pancreatitis is an inflammatory process of the pancreatic gland that eventually may lead to a severe systemic inflammatory response. A key early event in pancreatic damage is glutathione depletion, which is not associated with glutathione oxidation or protein glutathionylation. Importantly, pair cystine/cysteine as well as protein cysteinylation and gamma-glutamyl cysteinylation markedly increase in pancreas during acute pancreatitis. Indeed, cystine levels rise in pancreas after induction of pancreatitis. Two types of targets of disulfide stress were identified: redox buffers, such as ribonuclease inhibitor or albumin; and redox-signaling thiols that include thioredoxin 1, APE1/Ref1, Keap1, tyrosine and serine/threonine phosphatases, protein disulfide isomerase. These targets exhibit great relevance in DNA repair, cell proliferation, apoptosis, endoplasmic reticulum stress, and inflammatory response. Redox-sensitive PP2A and tyrosine protein phosphatase activities decreased in pancreatitis and this loss was abrogated by N-acetyl cysteine. Disulfide stress would be a specific mechanism of redox signaling independent of glutathione redox status involved in inflammation. In addition, PGC-1a serves as a link between redox signaling and the inflammatory cascade as it binds phospho-p65 in pancreas during pancreatitis greatly restraining the systemic inflammatory response.

R17 Regulación de la expresión génica y dinámica del genoma

0012

CONSEQUENCES OF THE REVERSAL OF THE ASYMMETRIC PATTERN OF DISTRIBUTION OF MICROTUBULE-ORGANIZING CENTERS IN BUDDING YEAST

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Cellular division during mitosis is usually envisioned as a process involving an equal distribution of the genome and the cellular components between the mother and the daughter cell. However, there are many examples of cells that display an asymmetric division, among which the stem cells from animals are a stereotypical example. The asymmetry during the division of stem cells allows them to self-renew while at the same time producing another cell that, based on the inheritance of specific cell-fate determinants, enters a particular differentiation program. Defects during the asymmetric division of stem cells can lead to tissue degeneration or aging if stem cell number is reduced or, alternatively, to tumorigenesis or tissue hyperplasia if their number is increased. Therefore, the analysis of the regulation of asymmetric cell divisions is of pivotal importance. An extremely interesting phenomenon associated to some asymmetric cell divisions is the differential inheritance of the microtubule-organizing centers (MTOCs) from which the microtubules that form the mitotic spindle emanate. This phenomenon has been described during the division of budding yeast, neuroblasts and male germline stem cells from Drosophila, radial glia cell progenitors from mice, or human neuroblastoma cell lines. However, little is known about how pre-established MTOC inheritance patterns are generated and, more importantly, about what could be their biological relevance. Using budding yeast as a model and taking advantage of the powerful molecular tools developed for this organism, we have generated a genetically-designed system to evaluate the functional consequences of this phenomenon.

0039-R

Methylation in the promoter region of SRD5A genes in patients with different grade of prostate cancer

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Background: Prostate Cancer (PCa) is a cause of public concern in the Western World. Within the prostate, the enzyme steroid 5α-Reductase (5α-R) converts circulating testosterone (T) into dihydrotestosterone (DHT), the primary androgen responsible for the development, maturation and function of the prostate gland and also implicated in the pathogenesis of prostatic diseases. 5α-R occurs as three isozymes, with a controversial role for 5α-R3 in the androgen metabolism and prostate growth. Currently, there are no prognosis biomarkers for PCa, but the need for personalized treatment for each patient illustrates that the discovery of new markers are becoming increasingly urgent. DNA methylation is an important epigenetic mechanism for gene expression regulation, and plays an essential role in the initiation and progression of tumours, where hypermethylation of critical genes are associated with gene silencing.Thus, this epigenetic mark could be potential biomarker and a target for treating PCa. Epigenetic research uses powerful techniques for the study of DNA methylation, such as sodium bisulfite modification of DNA associated with polymerase-chain-reaction procedures. One of these approaches is the Methylation-Sensitive High Resolution Melting (MS-HRM), a new sensitive and specific method for the detection of methylation. It allows analyzing the methylation status of an unknown sample by comparing its dissociation or melting profile with the melting profiles of methylated and unmethylated DNA controls.

We hypothesized that the methylation status of CpG islands in the promoter region of SRD5A genes may play a role in the development and progression of PCa. The objective of this study is to stablish an optimal workflow using MS-HRM in order to detect changes in the methylation levels of SRD5A1 and SRD5A2 promoters, in normal and malignant prostate tissues

from patients with different grade of PCa.

Patients and Methods: We used formalin-fixed paraffin-embedded (FFPE) tissue samples from 18 patients with a different grade of PCa (n=9 low-grade PCa; n=9 high-grade PCa). Due to the challenging use of this type of starting material, we stablished an optimal methodology to isolate the DNA with the highest concentration and quality and we optimized the design of primers for a MS-HRM downstream analysis.

0080-R

The MYC antagonist MNT as a possible new regulator of the NF-κB signaling pathway

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MNT is a transcription factor of the MXD family known to be an important antagonist of the oncoprotein MYC. Both MYC and MXD proteins are basic helix-loop-helix leucine zipper (bHLHLZ) transcription factors that heterodimerize with MAX, bind to E-boxes within regulatory regions of target genes, and generally activate (MYC) or repress (MXD) their transcription. However, little is known about MYC and MXD functions beyond MAX interaction. Our preliminary results in UR61 cells (derived from rat pheochromocytoma and deficient in MAX) based on proteomic analysis suggest a possible interaction between MNT and c-REL (REL) that is MAX-independent. REL is a member of the REL/NF-κB family which takes part in several biological processes (immunity, inflammation, differentiation, cell growth, apoptosis) and it is found altered in a variety of cancer types, mainly in B and T malignancies. Here we confirm this interaction in other murine (C6, Neuro-2a) and human (CEM and LoVo) cell lines by co-immunoprecipitation and proximity ligation assays. Next, we analyze the possible role of MNT in NF-κB pathway by its knockdown through a short-hairpin construct in LoVo cells. Reduced levels of MNT caused an increase in the activity of luciferase reporters carrying either several NF-κB binding sites or the promoter of NFKBIA, a bona fide target of the pathway that codes for IκBα. In the complementary approach, MNT overexpression led to a decrease in the luciferase activity of these constructs. These results suggested a possible repressor role of MNT. Moreover, the knockdown of MNT caused the translocation of REL into the nucleus, as assessed by immunofluorescence studies. The localization of p65 was not affected, pointing out a possible functional implication of the MNT-REL interaction. Then, in order to clarify whether the effect of silencing MNT on NFKBIA promoter was direct or not, we scanned by ChIP the proximal promoter and the first intron of NFKBIA, and we identified a peak of MNT binding that mapped at +171 to +343 bp of the human gene. These findings raise the possibility of a new MAX-independent function of MNT as a negative regulator of NF-κB signaling and the functional importance of the novel interaction found between MNT and REL. They also establish a relationship between the MYC and NF-κB oncogenic pathways.

0094

Insights into SND1 oncogene regulation

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The staphylococcal nuclease and Tudor domain containing 1 gene (SND1, also known as Tudor-SN, TSN or p100) encodes an evolutionarily conserved protein with invariant domain composition. It contains four repeated staphylococcal nuclease domains and a single Tudor domain that confers it nuclease activity and extraordinary capacity for interacting with nucleic acids, individual proteins and protein complexes. Originally described as a transcriptional coactivator, SND1 not only plays fundamental roles in the regulation of gene expression, including RNA splicing, stability and editing, but also in the regulation of protein and lipid homeostasis. Moreover, SND1 has gained attention as a potential disease biomarker due to its positive correlation with cancer progression and metastatic spread. This functional diversity of SND1 marks this gene as interesting for further analysis in relation with the multiple levels of regulation of SND1 protein production. In this work, we summarize the SND1 genomic context and promoter architecture, the set of transcription factors that can bind the proximal promoter, the miRNAs targeting the transcript 3'UTR, and the evidence supporting the regulation of SND1 gene transcription by a number of signal transduction pathways operating in different cell types and conditions. Elucidation of such regulatory mechanisms is of fundamental importance to understand how SND1 contribute to both health and disease.

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0104

Effects of the transcriptional regulator CTCF in the control of erythroid cell differentiation

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The transcriptional regulator CTCF (CCCTC-binding factor) is a highly conserved zinc finger protein that was identified as a repressor of the MYC gene. The functions described for CTCF included the regulation of transcription, insulator binding protein, global organization of chromatin and epigenetic regulation of many cancer related genes. Erythropoiesis is the process of red blood cell formation originating from hematopoietic stem cells. It is highly regulated by growth factors, transcription factors and miRNAs. Previous results of our group indicate that overexpression of CTCF induces differentiation towards the erythroid lineage in leukemia cells, accompanied with the differential expression of genes involved in erythropoiesis. In this study, we investigate the effects of CTCF during erythroid cell differentiation and the regulation of erythroid genes. CTCF was silenced using lentiviral transduction of short hairpin constructs in K562, a multipotent human leukemia cell line, and CD34+ hematopoietic stem cells purified from cord blood. Erythroid differentiation was induced by cytosine arabinoside (Ara-C) or Imatinib in K562 cells and by erythropoietin (EPO) in CD34+ cells. Downregulation of CTCF in K562 and CD34+ cells reduced cell proliferation as well as the percentage of benzidine positive cells and the expression of erythroid marker genes (γ-Globin and GATA-1) analyzed by Western-Blot and RT-qPCR. The binding of CTCF to the regulatory regions of genes involved in erythropoiesis was studied using Chromatin Immunoprecipitation (ChIP). CTCF occupancy in the regulatory regions of erythroid specific genes was found. Moreover, CTCF binding to these genes changed upon induction of erythroid differentiation. Our results indicate that CTCF downregulation inhibits erythropoiesis through the regulation of erythroid specific genes. We suggest a pivotal role of CTCF in the erythroid differentiation of hematopoietic cells.

0129

PP4 phosphatase enhances resection in response to DNA damage

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Eukaryotic cells are constantly threatened by innumerable sources of ge-

notoxic stresses that cause DNA damage. In order to maintain genome integrity, cells have developed a coordinated signalling network known as the DNA Damage Response (DDR). While numerous kinases have been thoroughly studied during the activation of the DDR, the role of protein phosphatases remains elusive. In the recent years, protein phosphatase 4 (PP4) has been implicated in several steps of the DDR, most of them related to its capacity to deactivate the DNA damage checkpoint and promote cell cycle re-entry. This function is mainly attained by counteracting the effect imposed by the checkpoint kinase Mec1 over Rad53 once the DNA lesion has been repaired. Interestingly, elimination of PP4 activity also renders cells to a hyper-phosphorylated state of Rad53 under continuous DNA damage, suggesting a role of the phosphatase in the control of Rad53 phosphorylation during the repair process itself. Corroborating this observation, Rad53 was identified in a screening looking for PP4 substrates operating during the repair of a DNA lesion. Surprisingly, PP4 was involved in generating single-stranded DNA around the break, a vital mechanism called resection that is necessary to execute recombinational DNA repair. Inactivation of the phosphatase restrains resection mainly in a Sgs1/Dna2-dependent manner. Importantly, the defects in DNA resection observed in the absence of PP4 can be reverted by just decreasing the levels of Rad53 phosphorylation, suggesting that the phosphatase controls resection by attenuating Rad53 activity. Accordingly, Rad53 hyper-phosphorylation drastically affects the capacity to restore induced DNA breaks when long-range resection is needed, a condition that can be alleviated by reducing Rad53 phosphorylation. This implies that excessive checkpoint activation is not compatible with DNA repair pathways that relies on long-range resection for its achievement and envision that both DDR-dependent kinases and phosphatases cooperate along the damage response to couple checkpoint activation and repair efficiency. This balance in checkpoint activity ensures a precise DNA damage response, strong enough to activate an accurate G2/M cell cycle arrest, but not too robust as to negative influence in the repair of the DNA lesion.

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0130

The Epstein Barr virus receptor CD21/ CR2 as a potential MYC target gene

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MYC is a transcription factor that belongs to the basic-helix-loop-helix leucine zipper protein family. MYC is deregulated in at least half of human tumor cases. In Burkitt´s lymphoma (BL) MYC is translocated in almost all cases. The endemic variant of BL, typical in equatorial Africa, is usually related with Epstein Barr virus (EBV) infection and the sporadic one, typical from developed countries, is related with EBV infection around 30% of the cases. EBV enters B-cells through its receptor, the membrane protein CD21 or CR2. Although MYC translocation and EBV infection contribute to the development of BL, it is still unknown the relation between this two events. The current hypothesis suggests that the expression of EBV latent genes provoke genomic instability and cell proliferation that cooperates with MYC translocation and eventually leads to B cells proliferation.

Previous results in our lab indicated that CR2 could be regulated by MYC and we demonstrated that this regulation is independent of protein synthesis and cell cycle. Altogether, the data indicate that CR2 up-regulation is a direct MYC transcriptional effect. The ENCODE project data showed that MYC could directly bind to CR2 promoter. We address this issue performing promoter activity assays with luciferase reporter of the human CR2 promoter and chromatin immunoprecipitation of MYC on CR2 promoter. We have also shown that silencing of CR2 in Raji cells (Burkitt´s lymphoma cell line, EBV positive) impairs cell proliferation and decrease the activity of the ERK2 signaling pathway. Thus, CR2 expression could be one mechanism by which MYC stimulates or maintain Raji cell proliferation. We finally asked whether MYC-induced CR2 expression had an impact on EBV infection. We found that MYC downregulation, mediated by short-hairpin constructs in B cells, is related with poor EBV infection.

This open the hypothesis that the concomitancy of MYC translocation and EBV infection in African BL may occur by a "reverse" mechanism by which cells with MYC translocation express more CR2and make B-cells more susceptible to EBV infection.

0144

Transcription factors drive Tet2-mediated enhancer demethylation to reprogram cell fate

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Here we report DNA methylation and hydroxymethylation dynamics at nucleotide resolution during highly efficient transcription factor-induced somatic cell to iPS cell reprogramming. We found that gene regulatory elements of key pluripotency factors become demethylated within one day after induction of the Yamanaka factors. Throughout reprogramming we observed successive waves of hydroxymethylation at enhancers, concomitant with a decrease in methylation. This suggests active demethylation, consistent with the finding that ablating the DNA demethylase Tet2 almost completely abolished reprogramming. Three distinct transcription factors, namely C/EBPa, Klf4 and Tfcp2l1, were shown to interact with Tet2 and recruit the enzyme to the DNA. Some of these sites maintain high levels of 5hmC, suggesting that here hydroxymethylated cytosines act as an epigenetic mark. Surprisingly, we also discovered regions in which methylation changes preceded chromatin opening. These included sites where Klf4 was bound without leaving a detectable footprint, suggesting a novel type of pioneer factor activity.

0167-M

Physical and functional interaction between MNT and CCDC6

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CCDC6 gene was first isolated fused to RET, forming the PTC1 oncogene in papillary thyroid carcinomas. The gene product, termed H4 (D10S170) or CCDC6, is a 65 kDa phosphoprotein located in both cytoplasm and nucleus that contains a coiled coil domain for protein-protein interactions. Our preliminary results from proteomic screenings showed that CCDC6 interacts with MNT, a protein from the MXD family that contains a basic-he-

lix-loop-helix-leucine zipper (bHLH-LZ) domain. MNT is known as an important antagonist of the oncoproteinMYC in several models. In this project, we have confirmed the CCDC6-MNT interaction in the HEK293T cell line. We also aimed to delimitate the interaction domain transfecting different CCDC6 and MNT mutants and performing co-immunoprecipitation assays. The results suggest that the C-terminal domain of CCDC6 and that both the LZ and the N-terminal domains of MNT are necessary for the interaction. We have also studied the nucleus or cytoplasmic localization of the MNT-CCDC6 complex in HeLa cells, finding out that they interact mainly in the cytoplasm. In order to evaluate the effects of CCDC6-MNTon cell proliferation, we transfected PTC1 and HeLa shCCDC6 stable cell lines, which expression of CCDC6 is very low, with a CCDC6 overexpressing vector and a short-hairpin construct against MNT. The data suggest that these two events together impair proliferation, as assessed by clonogenic assays.In order to test a complementary approach, we transfected HeLa and HeLa shCCDC6 cells with a MNT overexpressing vector.. Furthermore, to study the role of MNT-CCDC6 in DNA damage, a viability analysis was performed in HAP1 (from Chronic Myelogenous Leukemia) and HAP1 MNT KO cells treated with cisplatin and etoposide. Data suggest that MNT confers partial resistance against etoposide. Finally, we studied the effects of CCDC6 silencing on apoptosis and cell cycle in HAP1 MNT KO versus HAP1 control. For this purpose, we silenced CCDC6 and observedno effect on cell proliferation but a possibleantiapoptotic role, mostly in the absence of MNT. In summary, our work has shed light into different biochemical and biological functions of these two important oncoproteins. This opens the possibility of a role of MNT on DNA damage control through CCDC6.

0196

Plk1 overexpression suppresses tumor development by inducing chromosomal instability

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Polo-like kinase 1 (Plk1) is overexpressed in a wide spectrum of human tumors, being frequently considered as an oncogene and an attractive cancer target. However, its contribution to tumor development is unclear. Using a new inducible knock-in mouse model we report here that Plk1 overexpression results in abnormal chromosome segregation and cytokinesis, leading to the formation of polyploid cells with reduced proliferative potential. Mechanistically, these cytokinesis defects correlate with defective loading of Cep55 and ESCRT complexes to the abscission bridge, in a kinase-dependent manner. In vivo, Plk1 overexpression prevents the development of Kras-and Her2-induced mammary gland tumors, in the presence of increased rates of chromosome instability. In patients, Plk1 overexpression correlates with improved survival in specific breast cancer subtypes. Therefore, despite the therapeutic benefits of inhibiting Plk1 due to its essential role in tumor cell cycles, Plk1 overexpression has tumor suppressive properties by perturbing mitotic progression and cytokinesis.

0197

Establishment of High Content Imaging based cell panel for drug repurposing

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Breast cancer (BC) is the second most common cancer worldwide after lung and the leading cause of cancer death in women [1]. Developing a brand new drug is a process that requires a lot of time and effort as well as an important monetary inversion. One alternative strategy is drug repurposing (finding new indications that can be treated with a drug that is already approved). However, reports of successful repurposing of drugs as anticancer agents have been limited [2].

High Content Imaging (HCI) is a powerful technique that allows testing of a high number of compounds to assess their specific activity in tumor cells by monitoring multiple readouts simultaneously. Consequently, it is a helpful tool to guide drug repurposing.

Malignant cells present an increased proliferation rate, therefore investigating mitotic specific biomarkers might be a viable option to improve drug efficacy. Interestingly, in BC some mitotic gene overexpression (BIRC5, CCNB1, CCNB2, ECT2, MAD2L1, NDC80, PRC1, PTTG1 and TPX2) might lead to poor or good prognosis depending on the tumoral subtype. This led us to think that these mitotic regulators might be a bona-fide biomarker to improve drug sensitivity and/or efficacy, and for which the correlation to drug sensitivity is yet to be demonstrated.

Firstly, to study whether these mitotic regulators might help drug repurposing, we optimized a BC cell line panel to measure cell proliferation inhibition by HCI. A subset of kinases inhibitors were tested in this panel and helped us validate the correct performance of the assays by finding sensitivity patterns already described in the literature.

Secondly, we have identified BC cell lines with over and under-expression for the mitotic genes of interest and confirmed the protein levels by western blot. Preliminary data showed sensitivity profiles associated to level of expression for some of the genes, thus target cell lines have been transformed with an inducible expression system. We are performing a drug sensitivity screening to gain insights on the relationship between mitotic gene expression profile and drug treatments.

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0220-R

HIF1A mRNA stability: a new target to control the hypoxia signalling pathway

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CIC bioGUNE Physiopathology of the hypoxia signalling pathway (HypoxiPATH)

Adaptation to hypoxia is a puzzling and tightly regulated challenge. This adaptability involves a severe gene expression rewiring, which is mainly triggered by the Hypoxia Inducible transcription Factor (HIF). HIF acts as a heterodimer composed by a ubiquitously expressed β subunit (HIF-β) that binds to the O2-sensitive α subunit (HIF-α). Canonicaly, the regulation of the hypoxia signalling pathway mostly relies on HIF-α protein stability, which has been described to be exquisitely regulated through the Ubiquitin Proteasome System (UPS). However, we argue that HIF1A post-transcriptional modifications might also contribute to the adaptative programme. Indeed, transcripts processing and turnover, and the rate of translation provide additional control points, which determine the amount of protein delivered.

Here, we will describe a novel and robust mechanism of HIF-α regulation through the control of HIF1A mRNA stability that could shed light on new targets to tune the hypoxia signalling pathway.

0275-R/M

Exploring the role of the mRNA Export factor ALY in genome instability in human cells

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CABIMER/Universidad de Sevilla Genética

THO is a eukaryotic conserved complex that acts at the interface between transcription and mRNA export. It has been shown that THO depletion in human cells impairs transcription elongation, affects mRNA export and increases genome instability in an R loop-dependent manner. This complex interacts physically and functionally with the mRNA binding export factors Sub2/UAP56 and Yra1/ALY. The cellular levels of Yra1 in yeast are tightly regulated and its overexpression leads to transcription-dependent genome instability, replication impairment and telomere shortening. Interestingly, human ALY is overexpressed in tumor cells and in proliferative normal cells. To gain insight into the role of ALY in genome instability in human cells, we have analyzed wether downregulation of ALY in siRNA transfected cells and its overexpression have any effect on DNA damage and genome instability. The results will be presented and discussed.

Domínguez-Sánchez et al., 2011. PLoS Genet 7(12): e1002386. Genome Instability and Transcription Elongation Impairment in Human Cells Depleted of THO/TREX. Gavaldá et al., 2016. PLoS Genet 12(4): e1005966. Excess of Yra1 RNA-Binding Factor Causes Transcription-Dependent Genome Instability, Replication Impairment and Telomere Shortening. Aguilera and García Muse, 2013. Annu Rev Genet 47:1–32. Causes of Genome Instability.

0325

The helicase PIF1 facilitates resection over G-quadruplex structures in human cells

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CABIMER Biología del Genoma

DNA breaks are complex lesions that can be repaired either by non-homologous end-joining (NHEJ) or by homologous recombination (HR). The decision between these two routes of DNA repair is a key point of the DNA Damage Response (DDR) that is controlled by DNA resection. The core machinery catalysing the resection process is well established. However, little is known about the additional requirements of DNA resection over DNA structures with high complexity. Here, we found evidences that the human helicase PIF1 has a role in DNA resection, specifically for defined DNA regions such as G-quadruplexes. Indeed, PIF1 is recruited to the site of DNA damage, physically interacts with proteins involved in DNA resection and its depletion causes DNA damage sensitivity and a reduction of HR efficiency. Moreover, G4 stabilization by itself hampers DNA resection, a phenomenon suppressed by PIF1 overexpression.

0344-R

A role for Mog1 in H2Bub1 and H3K4me3 regulation affecting RNAPII transcription and mRNA export

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Monoubiquitination of histone H2B (to H2Bub1) is required for downstream events including histone H3 methylation, transcription and mRNA export. The mechanisms and players regulating these events have not yet been completely delineated. In this talk I will show that the conserved Ran-binding protein Mog1 was required to sustain normal levels of H2Bub1 and H3K4me3 in Saccharomyces cerevisiae. Mog1 was needed for the correct recruitment to gene bodies of factors such as Rad6, Bre1 and Rtf1 that are involved in H2B ubiquitination, showing genetic interactions with them. We provide evidence that the absence of MOG1 impacted on cellular processes such as transcription, DNA replication and mRNA export that are linked to H2Bub1. Importantly, the mRNA export defect observed in mog1D strains, was exacerbated by the absence of factors that decrease H2Bub1 levels. Consistent with a role in sustaining H2Bub and H3K4me3 levels, Mog1 co-precipitated with components that participate in these modifications such as Bre1, Rtf1 and the COMPASS associated factors Shg1 and Sdc1. These results reveal a novel role for Mog1 in H2B ubiquitination, transcription and mRNA biogenesis.

R18 Regulación metabólica

0023-R

The molecular mechanisms of NMN-dependent activation of mitochondrial metabolism in yeast requires Hst4 deacetylation activity.

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The pyridine nucleotide NAD+ has emerged like a new strategy of therapy to mitochondrial diseases. It is possible to raise NAD+ levels by supplementation with its precursor, the nicotinamide mono-nucleotide (NMN). In PubMed is possible to find numerous studies showing potential benefits of NMN in different organs and tissues. It has proposed NAD+ as regulator of mitochondrial metabolism by a double action; increasing mitochondrial biogenesis by PGC1α and increasing mtDNA expression by TFAM 1. However, it is not possible to discard other pathways based on mitochondrial sirtuins such as SIRT3 2 can be involved in the activation of mitochondrial metabolism.

We have studied the effect of NMN on mitochondrial metabolism using as model Saccharomyces cerevisiae. NMN supplementation increases NAD+ in whole cells and mitochondria, and increases the growth rate of respiring cells in non-fermentable carbon sources. That increase is dependent on Hst4, a deacetylase orthologous to human SIRT3. NMN increases the growth rate in respiratory conditions producing an early and specific expression of proteins encoded by mtDNA such as Cox2 and Atp6.

Our hypothesis is that mtDNA expression can be regulated by the acetylated state of proteins associated to nucleoids 3. During respiration, the population of proteins bound to nucleoids changes from Abf2 (a basic protein with a protective function) to mitochondrial enzymes such as aconitase or α-KDGH, being this location dependent of its acetylated state that is regulated by the Hst4 activity and NAD+ level.

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0040-R/M

Influence of G protein-coupled receptor kinase 2 (GRK2) levels on β-cell functionality.

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Introduction: G protein-coupled receptor kinase 2 (GRK2), classically involved in the desensitization of G protein-coupled receptors (GPCR), has been recently described to impair insulin signaling and glucose homeostasis in different mice models of obesity and insulin resistance. However, a possible role for GRK2 on insulin secretion has not been studied so far. In this regard, insulin secretion is a tightly regulated process controlled by different mechanisms, including GPCR activation.

Objectives: In this work we address the physiological implications of reducing GRK2 levels on pancreatic functionality focusing on insulin secretion.

Methods: Wild-type (WT) and global GRK2 hemizygous (GRK2+/-) mice were subjected to different physiological conditions to stimulate insulin secretion. Further characterization was performed using functional analyses, ELISA, immunohistochemistry and Western blotting.

Results: GRK2+/- mice present differences in the pancreatic insulin secretion profile compared to their WT littermates in the absence of changes in blood glucose levels. No differences were found in total islet area of the pancreas between WT and GRK2+/- mice. We are currently characterizing the secretory capacity of GRK2+/- islets and studying the molecular mechanisms underlying the differences observed.

Conclusions: Our results demonstrate that GRK2 levels may modulate the physiological response of the β-cells to feeding stimuli.

0045-R

Carnitine palmitoyltransferase 1A in AgRP neurons play a key role in the metabolic flexibility in response to fasting

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Whole-body energy balance and food intake are regulated by the hypothalamus. Specifically, the arcuate nucleus (ARC) acts as a sensor for nutrient and hormonal signals informing about the energy status of the organism. The ARC contains two populations of neurons with antagonistic effects: appetite-suppressing neurons, which express proopiomelanocortin (POMC) and appetite-stimulating neurons, which express agouti-related protein (AgRP). Some studies have pointed out that fatty acids (FAs) in the ARC might play a role in the control of appetite and energy homeostasis. However, the paper of FAs in specific hypothalamic neurons is unknown. Here, we have generated a mouse model lacking carnitine palmitoyltransferase 1A (CPT1A) specifically in AgRP neurons (*Cpt1aAgRP-/-*). CPT1A is a key enzyme in FAs metabolism, because it regulates the entry of these metabolites into the mitochondria for their oxidation. Male *Cpt1aAgRP-/-* mice exhibit decreased cumulative food intake without changes in body weight. Moreover, *Cpt1aAgRP-/-* mice show reduced food intake and energy expenditure in response to fasting when compared to control littermates. These observations can be explained at least in part because *Cpt1aAgRP-/-* mice display a decreased response to ghrelin, a hormone secreted by the stomach that binds to AgRP neurons to promote food intake. Altogether, our results indicate that deletion of *Cpt1a* in AgRP neurons promotes metabolic inflexibility in fasting conditions and identify a new potential pathway for therapies targeting a reduction in food intake.

0046-R

Validation of a method to generate a transplantable brown adipose tissue to treat obesity

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Despite titanic social and scientific efforts, the obesity epidemic and its associated comorbidities are increasing worldwide. In the last decade, active brown adipose tissue (BAT) has been rediscovered in adult humans. Importantly, BAT activity is decreased in obese patients. BAT utilizes fuel such as fatty acids (FAs) to regulate energy expenditure by thermogenesis, therefore increasing BAT's thermogenic fat-burning power is a potential therapeutic approach to treat obesity and associated complications. The oxidation of FAs is regulated by the enzyme carnitine palmitoyltransferase 1A (CPT1A), which catalyzes the first step in the entry of FAs into the mitochondria. Previous results from our group show that expression of a constitutively-active form of CPT1A (CPT1AM) in brown adipocytes in culture increases their thermogenesis and mitochondrial activity (1). The aim of this project is to validate a method to generate functional CPT1AM-expressing BAT able to improve the obese phenotype of mice after its transplantation. To pursue this approach we propose to isolate adipose tissue-derived mesenchymal stem cells (Ad-MSCs), transduce them with CPT1AM, differentiate them into adipocytes and later transplant them back to donor mice. Our results demonstrate that Ad-MSCs isolated from either white adipose tissue or BAT have multipotent characteristics and can be differentiated *in vitro* to 3 lineages: 1) adipocytes; 2) osteoblasts and 3) chondrocytes. Moreover, adipocytes derived from Ad-MSCs express specific markers such as *Zic1*, *Tmem26* and *Ucp1*. Using a lentivirus vector we are able to express CPT1AM in Ad-MSCs and we show that this genetic modification does not impair the ability of these cells to differentiate into adipocytes. Altogether, our results validate a method to generate functional CPT1AM-expressing adipocytes from Ad-MSCs. Next steps will include the transplantation of these modified adipocytes into an obese recipient mice to increase FAs oxidation and energy expenditure and thus reverse obesity.

0064

Modulación del metabolismo de la placenta en partos gemelares.

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La placenta humana es un órgano complejo, de rápido desarrollo y altamente especializado situado en la interfaz materno-fetal, que cumple funciones esenciales para el correcto crecimiento y desarrollo del feto. Se trata de un tejido metabólicamente activo, capaz de adaptarse a las diferentes necesidades fetales y maternas durante toda la gestación. Los requerimientos durante la gestación varían a lo largo de ésta, tanto en gestaciones simples o múltiples, lo que conlleva cambios adaptativos a nivel del metabolismo de la placenta.

El objetivo de este trabajo fue determinar las diferencias que existen entre las placentas de partos gemelares y partos simples, tanto en su cara fetal como en la materna, estudiando la expresión de genes relacionados, entre otros, con el transporte de ácidos grasos (FATP2, ANGPTL4 y CD36) y de glucosa (GLUT1 y GLUT3), así como de enzimas del metabolismo lipídico (ACACA, ACSS1, LPL, LPIN). En el estudio se incluyeron 28 mujeres con embarazos normales, no patológicos (Servicio de Ginecología y Obstetricia del Hospital Universitario de la Paz).

Los resultados obtenidos muestran que la proporción de TG acumulados en la cara fetal respecto de la cara materna es significativamente mayor en los partos gemelares que en los sencillos. En el mismo sentido la expresión de GLUT 1 y AQP 11 en la cara fetal respecto de la materna fue significativamente mayor en las placentas de partos gemelares que en las de parto sencillo. Estos cambios sugieren una modulación de la captación y del metabolismo de las placentas de partos gemelares con el fin de mantener un correcto aporte de nutrientes hacia el feto.

0066-R/M

Papel de la pleiotrofina en el metabolismo lipídico

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La pleiotrofina, es un factor de crecimiento de unión a heparina implicado en la proliferación celular, la angiogénesis y en el proceso de la adipogénesis. Sin embargo, su papel en el metabolismo lipídico en el hígado es completamente desconocido.

El objetivo del presente estudio fue caracterizar el papel de la pleiotrofina en el metabolismo lipídico hepático en un modelo de ratón en el que se ha delecionado el gen de la pleiotrofina (Ptn-/-) y en sus correspondientes controles (Ptn+/+) a los 3, 6, 12 y 15 meses de edad. Para ello, se analizó el contenido lipídico total y se determinó la expresión hepática de las enzimas claves de la lipogénesis, de la beta-oxidación, así como de transportadores de membrana de ácidos grasos y glicerol.

En los ratones Ptn-/- tanto el peso del hígado como el contenido total de lípidos era significativamente inferior que en sus correspondientes controles. Por otra parte, en los ratones Ptn+/+ el envejecimiento se acompañó con un aumento en la lipogénesis hepática y en la síntesis de triacilglicéridos, mientras que la deleción de la pleiotrofina produjo una marcada reducción en la síntesis de ácidos grasos y su posterior esterificación. Asimismo, el análisis de la expresión de las enzimas clave de la oxidación de los ácidos grasos reveló una menor expresión en los animales en los que se había delecionado la pleitrofina (Ptn-/-). Finalmente, aunque no se vieron diferencias significativas en la expresión de los transportadores de ácidos grasos analizados, la expresión de la acuaporina 9, estaba claramente disminuida en animales Ptn-/-, lo que podría sugerir una menor captación de glicerol que en los ratones Ptn+/+.

Estos resultados son los primeros en evidenciar que la pleiotrofina participa en el control de la homeostasis lipídica, lo cual postula a la pleiotrofina como una potencial diana terapéutica para el tratamiento de diferentes alteraciones metabólicas tales como las dislipemias o la obesidad.

0086

El silenciamiento de la sirtuina 3 compromete el metabolismo mitocondrial y la respuesta antioxidante en línea celular de cáncer de mama MCF-7

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La sirtuina 3 (SIRT3) es una desacetilasa mitocondrial dependiente de NAD+ que se activa en condiciones de estrés oxidativo. Recientemente se ha sugerido que la SIRT3 es clave para la homeostasis de las mitocondrias, ya que entre sus dianas destacan enzimas clave del metabolismo mitocondrial y enzimas antioxidantes.

Nuestro objetivo fue evaluar si el silenciamiento estable de la SIRT3 mediante un shRNA en la línea celular de cáncer de mama MCF-7 podría comprometer su respuesta antioxidante y potenciar así el efecto del tamoxifeno. Para ello, se determinó la expresión de diversos genes relacionados con la biogénesis mitocondrial y enzimas antioxidantes, así como la viabilidad celular después del tratamiento con tamoxifeno (10 µM).

El silenciamiento de la SIRT3 disminuyó la expresión de los genes relacionados con el metabolismo mitocondrial y con la respuesta antioxidante. Además, el silenciamiento también provocó un descenso en la viabilidad celular, efecto que se vio potenciado en combinación con el tamoxifeno.

Estos resultados parecen indicar que la SIRT3 es clave para mantener la integridad mitocondrial y no sólo regula la acetilación de sus dianas, sino que podría estar interviniendo también en su expresión. Además, la SIRT3 podría ser un factor importante a tener en cuenta para mejorar la efectividad del tratamiento del cáncer mama.

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0088

Impaired acylglycerol metabolism in SND1 overexpressing hepatoma cells

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Staphylococcal nuclease domain-containing protein 1 (SND1) is a highly conserved protein. Among the multiple functions discovered for SND1, the regulation of gene expression is the most reported. SND1 is considered an oncogenic protein based on a variety of effects of differential expression of SND1. Regarding metabolic alterations in hepatocellular carcinoma, impaired cholesterol homeostasis is one of the most relevant characteristics; overexpression of SND1 deregulates the activation of the sterol regulatory element-binding protein (SREBP) 2, which results in the accumulation of cellular cholesteryl esters (CE). Given that acetyl-coenzyme A (CoA) is required for cholesterogenesis and fatty acids (FA) are the substrates of acyl-CoA:-cholesterol acyltransferase (ACAT) for the synthesis of CE, we hypothesized that alteration of cholesterol homeostasis would modify glycerolipid metabolism that might affect hepatoma cell growth when SND1 is overexpressed.

Quantification of cellular lipids and metabolic labelling using radioactive lipogenic substrates showed that hepatoma cells stably overexpressing SND1 have low triglyceride (TG) synthesis compared to control cells, but phospholipid biosynthesis or cell growth are not affected. NADPH generation capacity measured as glucose-6-phosphate dehydrogenase, malic enzyme and of isocitrate dehydrogenase activities in cytosolic extracts and acetyl-CoA availability are not limiting factors.

In this work, we demonstrate that the main factor limiting TG synthesis in SND1 overexpressing hepatoma cells is the utilization of FAs for cholesterol esterification. This metabolic adaptation is linked to high stearoyl-CoA desaturase (Scd1) expression, needed for the *de novo* production of oleic acid, the main FA used by ACAT. We conclude that high cholesterogenesis due to SND1 overexpression might determine the channeling of FAs to CEs.

0127-M

Hormetic effect of reactive oxygen species on the differentiation of brown preadipocytes.

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Increasing the mass of the thermogenic brown adipose tissue in adults is a promising tool to treat metabolic diseases, but the mechanisms of BAT differentiation are still unrevealed. In physiological concentration, reactive oxygen species (ROS) act as second messengers in many processes, such as insulin signaling or adipogenesis in white adipose tissue (WAT). Both infra and supra physiological concentrations have shown detrimental effects in the pathways under their influence. Our hypothesis is that, in a similar manner to what is already known in WAT, low amounts of ROS may promote adipocyte differentiation. We have used a murine line of brown preadipocytes (mBA) to test the effect of different concentrations of H2O2 in the differentiation process. Low concentrations of H202 (10nM) accelerated mBA differentiation, increasing the expression of feature markers such as Cidea, Pgc1a or Prdm16 between days 3 and 6 of differentiation. However, the addition of elevated concentrations of H2O2 (25 µM) slowed down the differentiation as inferred by the decreased expression of differentiation markers. We conclude that ROS act as hormetic modulators of brown adipocyte differentiation.

0157

Obesity-related changes in lipid homeostasis are associated with infertility in male mice.

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Objectives: 1) To study whether the dyslipemia associated with obesity may explain -in part - the well known relations between obesity and infertility in a mouse model fed with high fat diet (HFD) compared with low fat diet (LFD), and 2) to determine whether fat mobilization in obese mice with control diet or polyphenols (ellagic acid, EA), improves lipid parameters and reverses infertility. **Methods:** The experimental groups were: 1) LFD 10 weeks; 2) HFD 10 weeks; 3) LFD 18 weeks; 4) LFD 10 weeks and LFD + EA (100 mg/ml) 8 weeks; 5) LFD 10 weeks and LFD + EA (150 mg/ml) 8 weeks; 6) HFD 18 weeks; 7) HFD 10 weeks and HFD + EA (100 mg/ml) 8 weeks; 8) HFD 10 weeks and HFD + EA (150 mg/ml) 8 weeks; 9) HFD 10 weeks and LFD 8 weeks; 10) HFD 10 weeks and LFD + EA (100 mg/ml) 8 weeks.and 11) HFD 10 weeks and LFD + EA (150 mg/ml) 8 weeks. Plasma glucose, triglycerides, cholesterol, HDL and LDL were measured by colorimetric/enzymatic methods. Seminal fluid was obtained from epididymis for spermatozoa counts and motility. Plasma testosterone was measured by ELISA. **Results:** The mice fed with HFD have an increase in body weight, food efficiency rate and liver weight, and a decrease in % testis weight / body weight. These mice showed an increase in plasma cholesterol, HDL and LDL compared with mice fed with LFD. Finally, obese mice exhibited a decrease in sperm counts and plasma testosterone levels.**Conclusions:** The high fat diet decreases sperm count, causes hypogonadisms and alters lipids homeostasis that may underlie the infertility associated to obesity in male mice.

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0171

Mecanismos moleculares de acción de los agentes antiproliferativos EB-3D y EB-3P en células HepG2

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El cáncer es una de las principales causas de mortalidad y morbilidad en el mundo debido en parte a la resistencia a fármacos que el propio microambiente tumoral confiere. Por ello, y aunque es una enfermedad muy estudiada, se hace necesario buscar nuevas estrategias y nuevos agentes que la combatan de manera eficiente. En prácticamente todo tipo de tumores se observan elevados niveles de fosfatidilcolina (PC), debido principalmente a una mayor expresión de colina quinasa α (ChoKα), lo que se relaciona con un pronóstico desfavorable de la enfermedad. Por tanto, la ChoKα, además de convertirse en un marcador de la progresión tumoral, es una diana molecular en la terapia anticancerígena. En la última década se han sintetizado inhibidores de esta enzima, entre los que se encuentran los compuestos 1,1'-(((etano-1,2-diilbis(oxi))bis(4,1-fenileno))bis(metileno))-bispiridinio o -bisquinolinio, EB-3D y EB-3P respectivamente. Estos compuestos sintéticos inhiben la actividad de la ChoKα en un rango de concentración micromolar, mostrando además un efecto antiproliferativo sobre células de hepatocarcinoma humano HepG2. Además, los inhibidores producen una desregulación de las rutas de biosíntesis de PC y colesterol.

En este trabajo, nuestro grupo de investigación muestra que EB-3D y EB-3P disminuyen la expresión de los niveles proteicos de ChoKα, respaldando las nuevas estrategias que apuntan a que una inhibición en la expresión de la proteína inhibe el crecimiento de células tumorales. Además, mediante microscopía electrónica de transmisión confirmamos los estudios bioquímicos que revelan alteraciones mitocondriales, estrés de retículo endoplasmático y apoptosis.

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0183

La osteopontina desempeña un papel protector en el desarrollo de la enfermedad de hígado graso no alcohólica y las complicaciones metabólicas asociadas al envejecimiento

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La osteopontina (OPN), citoquina que forma parte del fenotipo secretor asociado a senescencia, está incrementada en distintas fases de la enfermedad de hígado graso no alcohólica (EHGNA). Nuestro objetivo fue investigar durante el envejecimiento, donde la prevalencia de síndrome metabólico aumenta, el papel de OPN en el desarrollo de la EHGNA y las complicaciones metabólicas asociadas. Para ello, se emplearon animales deficientes en osteopontina (OPN-KO) y sus controles (WT) de 3, 10 y 20 meses. Además, un grupo de animales de 16 meses fue alimentado con dieta rica en grasa (HFD) hasta su sacrificio a los 20 meses. Se realizaron análisis inmunohistoquímicos y de concentración lipídica, así como de flujos metabólicos. En animales WTs los niveles séricos y hepáticos de OPN aumentaron a 10 meses de edad y se mantuvieron elevados a 20 meses, edad en la que el incremento fue mayor cuando los animales se alimentaron con HFD. El número de hepatocitos senescentes, así como los niveles proteicos de p21 y E2F1 estaban incrementados en animales OPN-KO de 10 meses respecto a sus controles. La concentración hepática de triglicéridos (TG) y ésteres de colesterol, los flujos metabólicos implicados en la lipogénesis de novo y los niveles séricos de TG eran más elevados en los animales de 10 meses OPN-KO que en sus controles. En los OPN-KO de 20 meses la fibrosis estaba incrementada respecto a los WT. La ingesta de HFD en animales mayores indujo una mayor desregulación metabólica en los animales OPN-KO, a pesar de que se mantuviera el peso corporal como en los WTs. La HFD empeoró la sensibilidad a insulina, incrementó los niveles séricos de TG y la lipolisis del tejido adiposo y provocó el aumento de fibrosis y los niveles de F4/80. Sin embargo, no indujo el almacén lipídico observado en los animales WT. En conclusión, la deficiencia de OPN durante el envejecimiento induce un fenotipo metabólico asociado a envejecimiento de forma prematura. Por tanto, los resultados muestran que OPN desempeña un papel protector durante el envejecimiento.

0187

Complicaciones metabólicas de la obesidad: papel de E2F1

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La obesidad es un factor de riesgo para el desarrollo de la enfermedad del hígado graso no alcohólica, que la padecen entorno al 70% de los pacientes obesos. Se desconoce por qué en algunos pacientes el hígado graso progresa a etapas más avanzadas y agresivas de la enfermedad, como el carcinoma hepatocelular, y en otros no. Por ello, es necesario descubrir marcadores que permitan el pronóstico y diagnóstico precoz de la enfermedad. En este trabajo se ha estudiado el papel del factor de transcripción E2F1 en las alteraciones metabólicas asociadas al desarrollo del hígado graso durante la obesidad y en las alteraciones metabólicas asociadas. Para ello, se han utilizado ratones salvajes (WT) y ratones deficientes en E2F1(E2F1-/-), de 3 y 9 meses de edad, tratados con una dieta estándar (CD) o una dieta rica en grasa (HFD). Se han analizado los principales flujos metabólicos implicados en síntesis y oxidación lipídica hepática y de tejido adiposo blanco y pardo, así como la secreción hepática de triglicéridos (TG), su concentración en suero y el metabolismo de quilomicrones. Los resultados obtenidos revelan que la HFD no provocó en ratones E2F1-/- el aumento de peso corporal observado en ratones WT. Este hecho podría ser explicado con el aumento de la lipolisis del tejido adiposo blanco y el bloqueo de la síntesis de novo de TG que se observa en dicho tejido. Además, la ausencia en E2F1 confirió resistencia al desarrollo de la enfermedad del hígado graso no alcohólica, más notablemente a los 9 meses de edad, ya que la cantidad de lípidos hepáticos de los E2F1-/- es considerablemente menor que la de sus respectivos controles cuando son tratados con HFD. Mientras que la ausencia en E2F1 no alteró la secreción hepática de TG en forma de VLDL ni la síntesis de novo de TG, sí provocó el incremento en la β- oxidación hepática de ácidos grasos evitando así el acúmulo de lípidos. En conclusión, el factor de transcripción E2F1, implicado en obesidad y en las enfermedades metabólicas asociadas, controla la oxidación lipídica hepática. El incremento en la oxidación lipídica mitocondrial en hígado que induce su ausencia evita el acúmulo de la mayor llegada de lípidos procedentes del tejido adiposo.

0221

Efectos de la deficiencia postnatal de IGF1R sobre el metabolismo en un modelo animal de obesidad inducido por una dieta alta en grasa

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IGF1R (*Insulin-like Growth Factor type 1 Receptor*) es un receptor transmembrana de expresión ubicua con una gran similitud con el receptor de la insulina y con funciones biológicas esenciales. Pacientes con mutaciones alélicas en el gen IGF1R presentan alteraciones metabólicas y endocrinas. El objetivo del presente trabajo fue analizar metabólicamente un modelo animal de deficiencia de IGF1R alimentado con una dieta estándar o con una dieta alta en grasa (HFD). Se emplearon ratones mutantes *UBC-CreERT2; Igf1rfl/fl* con deleción de *Igf1r* inducida postnatalmente con tamoxifeno. Los ratones fueron sacrificados a los 12 meses de edad, 10 semanas después del inicio del estudio dietético. La sangre y los diferentes tejidos fueron extraídos para su análisis. La ingesta de una HFD aumentó el peso corporal mientras que la ausencia de IGF1R provocó una disminución significativa en el peso final y en la ganancia de peso corporal en ratones macho, tanto alimentados con la dieta estándar como con la HFD. Este efecto no fue observado en ratones hembra. La HFD incrementó los niveles plasmáticos de colesterol y de sus fracciones en ratones controles de ambos sexos. La deficiencia de IGF1R también incrementó estos niveles en ratones alimentados con una dieta control (machos y hembras), pero no en los ratones machos mutantes alimentados con HFD. La HFD provocó un incremento significativo de los niveles plasmáticos de glucosa, insulina y HOMA en ratones machos controles. Los machos mutantes no mostraron cambios significativos en los niveles de glucosa, aunque si una disminución significativa en la insulina y HOMA en comparación con los controles, tanto con la dieta estándar como con la alta en grasa. Ni la HFD ni la depleción de IGF1R se asoció con cambios en el metabolismo glucídico de las hembras. Finalmente, también se observaron efectos diferenciales de la carencia de IGF1R a nivel de tejido (grasa perigonadal *vs*. hígado). Los resultados muestran un claro dimorfismo sexual a nivel metabólico dependiente de IGF1R y sugieren que los ratones machos deficientes de este receptor son capaces de contrarrestar los efectos negativos inducidos por una dieta alta en grasa a nivel glucídico y lipídico.

0243

Cambios en la expresión de enzimas antioxidantes en estadios I, II, III y IV de cáncer de colon.

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El estrés oxidativo juega un papel muy importante en el desarrollo, progresión e invasión del cáncer colorrectal. Las especies reactivas de oxígeno (ROS) promueven la carcinogénesis mediante el daño en el DNA causando mutaciones y también modificando el estado oxidativo de proteínas activando vías de señalización proliferativas y antiapoptóticas. También influye en la metástasis promoviendo la angiogénesis y la infiltración de las células en los vasos sanguíneos. Ante un incremento de los niveles de ROS, las células incrementan sus defensas antioxidantes, induciendo, por ejemplo, la expresión de enzimas antioxidantes.

El objetivo de este estudio fue determinar los niveles proteicos de las enzimas antioxidantes MnSOD, Catalasa y CuZnSOD en tejido peritumoral y tumoral, en estadios I, II, III y IV mediante western blot.

En los tejidos peritumoral y tumoral, los niveles de las enzimas MnSOD, CuZnSOD y Catalasa se incrementan notablemente entre el estadio I y II. Por el contrario, en los estadios III y IV encontramos que dichas enzimas tienden a disminuir con respecto al estadio II. También se puede apreciar, en todos los estadios, que los niveles de estas enzimas antioxidantes son superiores en el tejido tumoral en comparación con el tejido peritumoral.

Los resultados obtenidos muestran que se producen cambios importantes en la respuesta antioxidante en el desarrollo del cáncer, y estos cambios podrían estar relacionados con los cambios que se producen en el desarrollo de esta patología. Así en el estadio II los elevados niveles de estas enzimas podrían ser, en parte, producto de la hipoxia, mientras que la disminución de la respuesta antioxidante observada en los estadios más avanzados podría ser uno de los rasgos característicos de la mayor malignidad del tumor.

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0252

Efecto de la activación de GPER sobre la función mitocondrial de adipocitos y macrófagos en condiciones de inflamación.

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En el estado obeso, se produce infiltración de macrófagos proinflamatorios (M1) en el tejido adiposo (TAB), que contribuyen a la inflamación del tejido y a su disfunción. Se ha descrito que el 17β-estradiol (E2) presenta efectos antiinflamatorios, no solo a través de los receptores clásicos, sino también a través del receptor de estrógenos asociado a proteínas G (GPER). Estudios previos de nuestro grupo han demostrado que el E2 induce la biogénesis mitocondrial en cardiomiocitos a través de GPER. El objetivo fue determinar la participación de GPER en los efectos de E2 sobre la biogénesis mitocondrial en TAB y en macrófagos en un entorno inflamatorio.

Se cultivaron explantes de TAB y macrófagos aislados de médula ósea de ratones C57BL/6J y se trataron con inductores de inflamación y G1 (agonista de GPER). Se determinaron marcadores de inflamación, de biogénesis y dinámica mitocondriales y producción de ROS. G1 incrementa la biogénesis mitocondrial en los explantes de TAB. En los macrófagos M1, la estimulación de GPER disminuye parcialmente los marcadores de inflamación y de biogénesis y masa mitocondrial.

Estos resultados ponen de manifiesto que la activación de GPER en un entorno inflamatorio tiene efectos opuestos en TAB y macrófagos, atenuando, en estos últimos, su actividad proinflamatoria.

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0268

Efecto de la genisteína sobre la biogénesis y función mitocondrial en distintas líneas célulares de cáncer de colon.

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La genisteína es un componente de la dieta presente en la soja que puede modular el metabolismo de la célula. La transformación tumoral supone un cambio en el metabolismo energético y el estrés oxidativo de la célula. Por ello nos hemos propuesto evaluar el efecto de la genisteína sobre la biogénesis y función mitocondrial en distintas células de cáncer de colon. Concretamente, se han utilizado las líneas HT29, derivadas de un tumor en estadio temprano, y SW620, derivadas de un tumor metastásico.

Se ha determinado la viabilidad celular, la producción de especies reactivas de oxígeno (ROS) y la expresión de genes relacionados con la biogénesis mitocondrial (SIRT1, PGC-1α, TFAM, NRF1, mtSSB, ERRα), la función mitocondrial (COXI y COXIV) después de un tratamiento con genisteína (100μM) durante 48 horas.

Los resultados sugieren que en la línea celular HT29, la genisteína disminuye la producción de ROS, mejora la viabilidad y disminuye la biogénesis mitocondrial (y que sucede con los COX). Por otra parte, existe una disminución de la expresión de COXIV, aunque hay un aumento de la replicación del genoma mitocondrial, posiblemente como mecanismo compensatorio. En el caso de SW620, la genisteína ejerce un marcado efecto citotóxico, disminuyendo la viabilidad celular y aumentando la producción de ROS. En esta línea, la expresión de los genes relacionados con la biogénesis y la función mitocondrial disminuyen.

Estos resultados muestran que la genisteína produce cambios sobre la biogénesis y la función mitocondrial de las células tumorales de cáncer de colon teniendo efectos distintos dependiendo de la procedencia de estas líneas celulares: tumor primario o metastásico. Este efecto diferencial de la genisteína podría deberse al también diferente nivel de estrés oxidativo en que se encuentran las células en distinto estadio tumoral.

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0282

Dimorfismo sexual en la respuesta hepática a la dieta hiperlipídica. Interacción del 17β-estradiol y la adiponectina

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La enfermedad del hígado graso no alcohólico (NAFLD) se ha convertido en uno de los principales factores de riesgo tanto de mortalidad general, como asociada a enfermedad hepática y cardiovascular. La prevalencia es mayor en hombres que en mujeres, lo que sugiere que, además de los hábitos y factores genéticos, las hormonas sexuales podrían jugar un papel importante. En este sentido, la adiponectina, cuyos niveles son mayores en mujeres, podría mediar esta protección a nivel hepático favoreciendo la oxidación de las grasas. En este estudio nos propusimos analizar las diferencias de sexo en la respuesta hepática a una dieta hiperlipídica (HL) y su relación con los niveles y la sensibilidad a la adiponectina. Como modelo experimental se utilizaron ratas Wistar de ambos sexos alimentadas con una dieta HL durante 16 semanas, y hepatocitos HepG2 tratados con palmitato (PA), 17β-estradiol (E2) y el agonista de la adiponectina AdipoRon (AdR). En ambos modelos se analizó la acumulación de triglicéridos, elementos de la vía de señalización de la adiponectina y enzimas clave del metabolismo lipídico y glucídico. En respuesta a la dieta HL, la lipemia y la acumulación hepática de lípidos fue mayor en las ratas macho. Este efecto hepatoprotector en las hembras, se asoció a unos mayores niveles de adiponectina circulante, así como a una mayor activación hepática de la AMPK, elemento clave de la vía de señalización de la adiponectina. Por otra parte, los experimentos in vitro en HepG2 mostraron que E2, además, puede potenciar los efectos de AdR sobre la oxidación de ácidos grasos. Estos resultados evidencian la existencia de una interacción estrógenos-adiponectina que podría explicar la menor prevalencia de NAFLD en el sexo femenino.

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0301-R/M

PAPEL DE LA PLEIOTROFINA EN EL METABOLISMO LIPÍDICO

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La pleiotrofina, es un factor de crecimiento de unión a heparina implicado en proliferación celular, angiogénesis y adipogénesis, aunque su papel en el metabolismo lipídico en el hígado es completamente desconocido.

El objetivo del presente estudio fue caracterizar el papel de la pleiotrofina en el metabolismo lipídico hepático en un modelo de ratón knockout para pleiotrofina (Ptn-/-) y en sus correspondientes controles (Ptn+/+) y que han sido alimentados con dieta rica en grasa (HF) o dieta normal (LF).

Como era de esperar, en el ratón Ptn+/+ alimentado con dieta HF se produjo un aumento en los lípidos hepáticos. Por su parte, la deleción de pleiotrofina se asoció a un menor contenido de lípidos en el hígado, y cuando estos animales fueron sometidos a una dieta HF, la acumulación de lípidos fue significativamente menor que en el grupo control. El estudio de expresión génica apunta que tanto la deleción de pleiotrofina como la dieta HF favorecen una disminución de la lipogénesis, así como de la entrada de glicerol y de la oxidación de ácidos grasos.

Estos resultados ponen de manifiesto que la deleción de pleiotrofina protege de la acumulación de lípidos en hígado y son los primeros en evidenciar su participación en el control de la homeostasis lipídica, postulándola como una potencial diana terapéutica para el tratamiento de alteraciones metabólicas como el hígado graso o la obesidad.

0303

Metabolic study of miR-29a/b-1-/- mice using Oxymax©/CLAMS

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In recent years, microRNAs have emerged as important regulators of human pathophysiology. The miR-29 family, encoded by the genomic clusters *miR-29a/b-1* and *miR-29b-2/c*, has been previously linked to metabolic regulation. To achieve a better understanding of miR-29 relevance in metabolism, we generated mice deficient in *miR-29a/b-1* and studied them using Oxymax©/CLAMS metabolic cages. Multiple metabolic variables of 6 *miR-29a/b-1*-deficient and 8 *wild-type* mice were recorded every 10 minutes for 48 hours, and graphical representations of variables over time and statistical analysis were performed using R-language. The results revealed increased O2 consumption, CO2 production and energy expenditure in *miR-29a/b-1-/-* mice compared with *wild-type* littermates. Respiratory exchange ratio (RER) showed an increased amplitude, characterized by a slight increase in mutant mice during the active phase and tenuous decrease during the resting phase. Interestingly, there also seems to be a phase shift in the metabolic activity of *miR-29a/b1-/-* mice, as the minimum of the RER takes place earlier in the resting phase. Interestingly, some studies propose that miR-29 family exhibits circadian expression and several clock genes are putative *in silico* targets of this family of miRNAs. In summary, our results show remarkable alterations in *miR-29a/b-1-/-* mice metabolism, as well as a possible alteration of circadian rhythms. Further experiments for longer periods and without light entrainment, as well as biochemical studies will help us deepening in the role of miR-29 in metabolism and its circadian behavior.

0317

Transgenerational epigenetic inheritance of diabetes risk by neonatal overfeeding

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Rapid weight gain during early life has been associated with several components of the Metabolic Syndrome. Previously we developed a mouse model of neonatal overfeeding and rapid weight gain by litter size reduction. Neonatal over-nutrition (ON) altered the metabolism of the exposed individuals (F0). Furthermore, offspring (F1) and grand-offspring (F2) of postnatal overfed male mice also developed metabolic complications during adulthood. Here, we hypothesized that epigenetic modifications, including DNA methylation, histone modifications, and noncoding-RNA, might be involved in the inheritance of diabetes risk in our model. Here we analysed sperm methylome of F0 and F1 generations, and of liver of 8-day-old mice of F1 and F2 generations. We found differentially methylated CpG sites when comparing control and ON mice throughout the three generations, and across the two tissues. Our results suggest that methylation of the male germ line, caused by nutritional challenges during early life, may carry information that influence metabolism across multiple generations.

0347

El metabolismo de la célula tumoral. Una diana para el diagnóstico y tratamiento del cáncer.

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El cáncer es una enfermedad multifactorial en la que las células pierden la capacidad de control de la proliferación celular y diferenciación. Uno de los factores que pueden influir en la aparición y progresión del cáncer es la reprogramación metabólica que sufren las células tumorales. Estas células se caracterizan por un incremento en la captación de glucosa generando mayoritariamente lactato, situación conocida como efecto Warburg. La desregulación del metabolismo energético de la célula tumoral se ha convertido en una característica distintiva de las células tumorales, y la mitocondria juega un papel central en esta regulación del metabolismo celular.

En los últimos años hemos estado investigando los efectos de las hormonas sexuales, los fitoestrógenos y la leptina sobre la funcionalidad mitocondrial y el estrés oxidativo. Los resultados obtenidos indicarían que la ratio de receptores de estrógenos α y β es un factor clave en la regulación de la funcionalidad y biogénesis mitocondrial.

Además, recientemente hemos estudiado el efecto de la sobreexpresión y/o silenciamiento de proteínas clave relacionadas con esta funcionalidad mitocondrial y la generación de estrés oxidativo en las células, como la UCP2, la SIRT3, el p53 y el PGC-1α. El objetivo es poder usar estas proteínas como posibles dianas para el diagnóstico y tratamiento en distintos tipos de cáncer. En efecto, la inhibición de UCP2 y de SIRT3 provocó un aumento de la eficacia de los tratamientos citotóxicos en células de cáncer de mama y colon, a través del incremento del estrés oxidativo y la disfunción mitocondrial. Asimismo, la expresión de p53 y PGC-1α afectó a la regulación de la biogénesis y función mitocondrial y la respuesta antioxidante en células de melanoma.

Para finalizar, actualmente estamos analizando el efecto anti-Warburg que se podría producir en el cáncer de colon. Los resultados preliminares muestran una recuperación de la maquinaria de la fosforilación oxidativa mitocondrial en muestras tumorales de cáncer de colon en estadios avanzados con respecto a aquellas de tumores de estadios más tempranos. Además, estamos poniendo en marcha un proyecto en el que pretendemos identificar marcadores relacionados con la funcionalidad mitocondrial y el estrés oxidativo que nos permitan una detección precoz del cáncer colorrectal.

0364

OBESITY AND ADIPOSE TISSUE REGULATION BY THE CDK2-P27 AXIS

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Obesity is a global health problem which affects millions of people worldwide and has nearly tripled its prevalence. It is characterized by an excessive accumulation of fat in adipose tissue, this increases the risk of suffering a series of metabolic diseases such as type 2 diabetes, dyslipidaemia an atherosclerosis. Recently, a family of protein kinases called cyclin dependent kinases (CDKs) has emerged as a possible mediator in obesity. CDKs and other members of the cell cycle machinery not only take part in cell cycle progression, but also in metabolic processes like insulin secretion in pancreas, gluconeogenesis in liver or insulin resistance in adipose tissue. The aim was to understand if the CDK2-p27 axis plays a function in the metabolism of mature adipocytes and consequently in obesity. An analysis of mRNA expression in human adipocytes isolated from subcutaneous adipose tissue shows that CDK2 expression is augmented with obesity. Moreover, in vivo studies in mice models indicated that CDK2 protein level is increase in diet-induced obese mice, thus, suggesting a likely function regarding metabolism. In fact, this role has been confirmed in genetic modified mice models, affecting the body weight, the adipose mass, and the adipose tissue function (white and brown). Furthermore, in vitro studies with cultured mature adipocytes and treated with a CDK2 inhibitor, showed that CDK2 was involved in regulation of insulin sensitivity, induction of glucose transport and glycerol release. Together these results indicate that the CDK2-p27 axis as a major regulator of the adipose metabolism which can be applied to optimize the design of clinical approaches and the discovery of new pharmacological treatments against obesity and its associated comorbidities.

0365

Targeting brown adipose tissue in obesity and diabetes

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Obesity is growing at an alarming rate and in parallel to the increase of its associated metabolic diseases such as type 2 diabetes, insulin resistance, cardiovascular disease and some forms of cancer. The discovery of active brown adipose tissue (BAT) in adult humans and that it is reduced during obesity and diabetes has put a spotlight on this tissue as a therapeutic target in obesity-induced metabolic disorders. As opposed to the energy-storing white adipose tissue (WAT), BAT utilizes fuels such as fatty acids to produce heat and control body temperature in a process called thermogenesis. The mechanisms involved in the development of obesity-induced metabolic diseases have been associated to WAT dysfunction such as fibrosis, apoptosis, inflammation, ER and oxidative stress. However, little is known of whether these processes are also present in BAT during obesity. Our aim was to characterize the BAT of obese and hyperglycemic mice treated with a high-fat diet (HFD) for 20 weeks. The hypertrophic BAT from obese mice showed elevated levels of inflammation, ER stress, ROS generation and antioxidant enzyme activity compared to lean controls. The response was attenuated compared with obesity-induced WAT derangements, which suggests that BAT is more resistant to the obesity-induced insult. In fact, mitochondrial

respiration measured with the Seahorse analyzer in BAT from obese mice was enhanced, with a 2-fold increase in basal oxygen consumption, through the upregulation of complex III of the electron transport chain and UCP1. In conclusion, our results show that obesity is accompanied by an increase in BAT mitochondrial activity, inflammation and oxidative damage.

R19 Señalización celular

0034

EFFECT OF SODIUM TUNGSTATE ON CELL PROLIFERATION AND SIGNALING IN DIFFERENT CELL TYPES

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EFFECT OF SODIUM TUNGSTATE ON CELL PROLIFERATION AND SIGNALING IN DIFFERENT CELL TYPES

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Compounds that regulate cell proliferation and differentiation through the modulation of specific signaling pathways constitute useful tools for the development of therapeutic agents. Sodium tungstate is an inorganic salt with antidiabetic properties, normalizing glucose metabolism in liver and muscle. In muscle, tungstate prevents protein degradation and stimulate protein synthesis and differentiation. These effects are produced by the activation of MAPK pathway, specifically ERK1/2, in liver and muscle. However, the over-activation of MAPK and Akt signaling pathways has been involved in cellular processes that lead to increase in cell proliferation and as a consequence to the development of pathologies. In this study, we have analyzed the modulatory effects of sodium tungstate on cell proliferation and signaling in different cell types, including non- and cancer-derived cells.

Our results indicate that sodium tungstate is able to modulate cell proliferation. These effects are due to a regulation of not only ERK1/2 signaling but also Akt dependent pathway.

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0048

COULD BE BREAST CANCER METASTASIS DISRUPTED BY MECHANOTRANSDUCTION?

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It has been seen that tumoral genotype triggers the transformation of the extracelular matrix (ECM) to an abnormal stiffer state. This stiff ECM, has then the ability to modify and orquestrate the pathological evolution of tumors1. One of the main ECM proteins in tumoral matrices is fibronectin (FN). Moreover, the presence of rich and stiff FN matrices correlate with less survival in invasive breast cancer. In this context, α5β1 integrin has been described as the only mechanoreceptor able to sense and transduce changes of ECM stiffness to the cell, by its interaction with its unique ligand, the RGD motif of FN, which is potentiated after binding the FN synergy site2. With our FNSYN/SYN mouse strain, we are studying *in vitro* and *in vivo* the outcomes of inactivating the synergy site of FN in mechanotransduction.

In the *in vitro* analysis we are determining the link between the different levels obtained for Yp397-FAK and the consequences for the HIPPO pathway dynamics by the immunofluorescence analysis of YAP in fibroblasts.

In vivo, we are working with transgenic FNWT/WT and FNSYN/SYN females aged of 60 and 100 days issued from the intercross between the MMTV-PyMT murine breast cancer strain and our FNSYN/SYN strain. Our goal here is to explore in those lungs and tumoral mammary glands the physical distribution of different processes associated with malignancy, such as neovasculogenesis (PECAM), neolymphogenesis (LYVE-1), desdifferentiation (Ki67), migration (aSMA) and proliferation (PIH3). We aim to find, in association with the FN matrix distribution in tumor and stromal microenvironment, a malignant functionality of the synergy site in those processes that would lack in our model. This missed functionality would represent a protective state against tumor stablishment and progression.

0072-R/M

Analysis of the interaction between C3G and proteins of the secretory machinery.

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C3G is a guanine nucleotide exchange factor (GEF) of several GTPases of the Ras family, including Rap1, Rap2 or R-Ras. C3G participates in the regulation of diverse cellular functions, such as adhesion, proliferation, differentiation or migration, and had also been associated with pathological processes such as cancer. Recent studies have attributed a role to C3G in platelet functions, including the regulation of angiogenesis and the promotion of tumor metastasis [1,2]. In particular, platelet C3G modulates, in a GEF-independent manner, the differential secretion of pro- and anti-angiogenic factors from different subpopulations of platelet alfa granules, both *in vivo* and *in vitro*. This is probably mediated by the interaction between C3G and VAMP7, which has been detected in activated platelets. To get a further insight into this new role of C3G, we have analyzed the interaction between C3G and proteins of the secretory machinery, including those involved in a-granule exocytosis, such as VAMP7 or VARP, and proteins involved in the regulation of the actin cytoskeleton, such as Arp2/3, WAVE or VASP. Co-transfection and co-immunoprecipitation experiments in HEK293T cells have uncover the existence of interactions between different domains of C3G and VAMP7, VARP, Arp2, Arp3 and VASP proteins. Immunofluorescence studies have also revealed a high colocalization of C3G with these proteins in activated HEK293T cells. Interestingly the interaction between C3G and VASP or Arp2 was also detected in transgenic C3G platelets stimulated with thrombin, but not in resting platelets.

Future studies will aim at characterizing the interaction of C3G with these proteins in platelets and their involvement in the differential secretion of the content of platelet a-granules.

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0073-R/M

Platelet C3G regulates ischemic-induced angiogenesis and early metastasis

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C3G is a Rap1 GEF whose expression in platelets promotes angiogenesis and metastasis [1]. Here, we examined whether C3G participates in the recruitment of BMDC (bone marrow-derived cells)-CXCR4+ to the sites of neovascularization, an important step in the formation of new vessels. We performed two hypoxic models, hind limb ischemia and tumor implantation, using two transgenic mice: tgC3G and tgC3GΔCat, (C3G mutant lacking the GEF domain), where the transgenes are under the control of the PF4 gene promoter. A faster recovery of the blood flow after hind limb ischemia was observed in the two transgenic models, that was accompanied by a higher platelet counts. However, contrary to what was expected, the levels of the CXCR4+ cells after both hypoxic stimuli were lower in the tgC3G mice than in their WT, while no differences were observed between tgC3GDCat and WT. This might be due to a failure in the release of SDF-1 (stromal derived factor 1, ligand of CXCR4) by the tgC3G platelets. These results are in agreement with a role of C3G in angiogenesis regardless of its catalytic activity, and indicate that the transgenic expression of C3G in platelets is capable of promoting neoangiogenesis independently of the recruitment of BMDC-CXCR4+ precursors.

On the other hand, our previous studies have shown that platelet C3G promotes melanoma metastasis in the lung 15 days after the injection of B16-F10 cells into the tail vein. To further investigate the role of platelet C3G in early metastasis, we have established a short-term metastasis experiment where B16-F10 cells, expressing EGFP, are injected into the retro-orbital sinus of our transgenic models. The presence of fluorescent cells in lungs, one hour after injection, was determined by flow cytometry. The number of EGFP+ cells was higher in both transgenic mice than in their WT controls, indicating the participation of C3G in the arrest of metastatic cells in the lungs by a mechanism independent of its GEF activity.

[1] Martín-Granado, V., et al (2017). Oncotarget 8(67): 110994-111011.

0083

Characterization of VRK1 mutants implicated in human neurodegenerative diseases

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VRK1 is a nuclear and chromatin Ser-Thr kinase that appeared late in evolution. VRK1 gene expression is ubiquitous, and it is highly expressed in proliferating cells, and in many tumours associated to a poorer prognosis. VRK1 participates in several processes that include regulation of cell cycle progression and proliferation, its implication on chromatin remodeling during transcription, replication and DNA repair, the regulation of transcription factors like p53, its implication on DNA-Damage Response, and the regulation of the Cajal Bodies dynamics and nuclear envelope assembly. Furthermore, VRK1 has a role both in the development and in the maintenance of the nervous system. Mutations in the human *VRK1* gene are associated to multiple neurodegenerative diseases, like Spinal Muscular Atrophy or Aminotrophic Lateral Sclerosis. In the present work, we have generated and characterized all known VRK1 mutants that have been identified in patients with neurodegenerative diseases ([R89Q], [H119R], [R133C], [G135R], [L195V], [V236M], [R321C], [R358X]). We determined the effect of the mutations on VRK1 stability and its autophosphorylation and transphosphorylation of its known substrates, such as p53, histone H3, 53BP1, NBS1 and coilin. VRK1 is a very stable protein. Some mutants have a significantly reduced half-life. Regarding to the kinase activity, some mutants have a reduced autophosphorylation but they maintain the phosphorylation of its targets. Others have altered the auto-phosphorylation and the substrate-specific phosphorylation activity. We conclude that the functional alteration of the VRK1 mutations and the impairment of its function contribute to the pathogenesis of the neurodegenerative phenotypes observed in patients.

0091-R/M

La membrana amniótica promueve la remodelación de los focos de adhesión estimulando la migración celular.

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Durante la cicatrización de una herida, la migración de queratinocitos sobre la matriz extracelular recién restaurada tiene como objetivo restablecer la continuidad de la epidermis. La aplicación de la membrana amniótica (MA) sobre heridas profundas, crónicas y no cicatrizantes, ha demostrado ser eficaz para estimular la reepitelización. Cuando se aplica en cultivos de células epiteliales, el tratamiento con MA consigue activar la ruta de las MAP quinasas, evidenciada por la fosforilación de ERK1/2 y de JNK1/2, acompañado de la sobreexpresión y fosforilación de c-Jun a lo largo del borde de la herida. El efecto de la MA sobre la remodelación de los focos de adhesión fue investigado mediante el estudio de proteínas críticas involucradas en este fenómeno: la quinasa de adhesiones focales (FAK), Paxilina y Vinculina. En células Mv1Lu y HaCaT, modelos validados para la migración celular y la cicatrización de heridas, la MA afectó la expresión y la activación de Paxilina, pero no tuvo ninguna consecuencia sobre la expresión de Vinculina; ambos factores se integran en las adhesiones focales. Además, el tratamiento con MA afectó la fosforilación de FAK, y por lo tanto, su actividad. Por último, identificamos que la regulación ejercida por la MA sobre los focos de adhesión implicaba la señalización de la ruta de MAPKs: JNK y MEK. Estos datos proporcionan las primeras evidencias sobre mecanismos moleculares inducidos por la MA que permiten entender su acción sobre aspectos moleculares de la migración celular. También permiten entender la organización y la dirección del movimiento de las células, que produce la MA, por la continua formación, maduración y reciclado de las estructuras de adhesión focal en el borde de migración. Más resultados serán discutidos en la presentación.

0101

Upregulation of downstream mitochondrial components of AMPK signaling by a combined docosahexaenoic acid (DHA) and triiodothyronine (T3) protocol.

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Combined omega-3 and thyroid hormone (T3) treatment prevents ische-

mia-reperfusion (IR) liver injury, with upregulation of peroxisome proliferator-activated receptor (PPAR)-α and fibroblast growth factor 21 (FGF21). These effects involve ligand activation of PPAR-α by DHA and enhanced expression of PPAR-α by T3, with consequent upregulation of PPAR-α controlled FGF21 and enhanced FGF21- AMP-activated protein kinase (AMPK) signaling1.

The present work was aimed to assess the influence of the DHA+T3 protocol on mitochondrial oxidative function through enhanced FGF21 signaling, which may involve the PPAR-α/AMPK activation of peroxisome proliferator activated receptor-γ-coactivator (PGC-1α) cascade. For this purpose, rats were subjected to DHA (300 mg/kg for 3 days), T3 (0.05 mg/kg at the fourth day), combined DHA+T3 and the respective vehicle control group. Studies assessed AMPK expression, in relation to those of PGC-1α and its controlled target nuclear respiratory factor 2α (NRF-2α), which in turn upregulate gene expression of downstream components of AMPK signaling, namely, cytochrome-c oxidase subunit-IV (COX-IV) and β-ATP-synthase.

Enhanced mRNA expression of AMPK was achieved by the DHA+T3 protocol, in concomitance with that of the AMPK targets PGC-1α and NRF-2α, in addition to that of the downstream components COX-IV and β-ATP-synthase. It is concluded that upregulation of PGC-1a through AMPK activation by combined DHA+T3 enhances mitochondrial electron transfer activity and oxidative phosphorylation capacity, supporting energy demands needed for liver preconditioning afforded by DHA + T3, which are of importance in conditions of liver injury.

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0107

Acciones de nitro-ácidos grasos sobre la activación de linfocitos T

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Los nitro-ácidos grasos (NAGs) derivan de la reacción no enzimática de especies reactivas del nitrógeno con ácidos grasos insaturados. Presentan acciones antiinflamatorias y antioxidantes, principalmente gracias a su carácter electrofílico, originando aductos con dianas nucleofílicas, como los residuos de cisteínas en ciertas proteínas, pudiendo modificar su localización y/o función.

En este estudio analizamos los efectos de NAGs derivados del ácido oleico (OA), como los ácidos 9- o 10-nitro-oleico (9NOA, 10NOA), en la activación de los linfocitos T. Mientras que el tratamiento con OA no afectó de forma significativa a ninguno de los parámetros estudiados, el tratamiento con 9NOA o 10NOA promovió una disminución en la proliferación de linfoblastos T humanos estimulados a través del receptor de la célula T (TCR), así como en la expresión en membrana de los marcadores de activación CD25 y CD71, sin modificar de forma significativa ni la viabilidad celularni la expresión del marcador de activación temprana CD69.El análisis de los efectos de los NAGs sobre la expresión génica, puso de manifiesto su efecto inhibidor sobre la expresión de citoquinas como TNFα, IFNγ oIL-2 en células T activadas. El estudio de la activación de los factores de transcripción implicados en la regulación de estos genes, permitió demostrar que, además de los efectos descritos en otros tipos celulares sobre NFκB, los NAGs también regulan negativamente la activación de NFAT. Mediante diferentes aproximaciones experimentales (inmunocitoquímica, western blot y ensayos actividad transcripcional), hemos determinado que estos compuestos reducen la de fosforilación y la translocación de NFAT al núcleo de linfocitos T activados.

Los resultados obtenidos permiten concluir que NAGs como el 9NOA y el 10NOA, a través de la regulación de la activación de NFAT, interfieren en la activación de los linfocitos T, un mecanismo que podría ayudar a entender mejor las acciones antiinflamatorias e inmunomoduladoras que estos agentes pueden ejercer durante la respuesta inmune.

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0116-R/M

La metformina inhibe la motilidad del espermatozoide humano, la señalización mediada por PKA y la fosforilación en tirosina, sin afectar la función mitocondrial.

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La metformina es una biguanida que actualmente es usada como fármaco para el tratamiento de la diabetes mellitus. Realiza su función en numerosos tipos celulares, aunque su acción concreta en el espermatozoide humano no ha sido investigada previamente.

Nuestra línea de investigación estudia la señalización intracelular del espermatozoide humano, centrada sobre todo en la vía de la proteína quinasa activada por AMP (AMPK). Por ello, dado que la metformina es un activador de la AMPK de amplio uso clínico, en este trabajo investigamos su efecto *in Vitro* sobre la función del espermatozoide humano.

Los espermatozoides fueron incubados en presencia o ausencia de metformina (10 mM) a diferentes tiempos de incubación (8h-20h). La motilidad fue evaluada mediante el sistema Integrado de Análisis de Semen (ISAS). Otros parámetros funcionales del espermatozoide se analizaron mediante citometría de flujo. Mediante Western Blotting estudiamos la señalización inducida por la proteína quinasa A (PKA) y la fosforilación en tirosina de proteínas espermáticas.

La metformina reduce significativamente el porcentaje de espermatozoides móviles, progresivos y rápidos a las 8h. A las 20h, además, disminuye todos los parámetros de velocidad espermática. El tratamiento con metformina no afecta a los parámetros funcionales de viabilidad, potencial de membrana mitocondrial o a la generación de anión superóxido mitocondrial.

Sin embargo, la metformina produce una clara inhibición de la vía PKA y de aquellas vías de señalización mediadas por la fosforilación en tirosina, que son esenciales para una correcta regulación de la función espermática.

Este estudio demuestra que la metformina tiene un claro efecto adverso en la motilidad de los espermatozoides humanos, y por ello pensamos que podría tener implicaciones en la reproducción de los pacientes tratados con este fármaco.

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0122-R/M

Targeting Ras-ERK pathway: an insight into scaffold proteins in thyroid cancer.

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An overwhelming body of data unquestionably links the Ras-ERK signaling pathway to cellular transformation and to the upbringing of human malignancies. Once this cascade is activated, the MAP Kinases phosphorylate and control the activity of key cytoplasmic molecules and nuclear proteins, which can regulate gene expression and participate in processes as important as proliferation, differentiation and cell survival. 70% of thyroid carcinomas have activating mutations in some of the components of this route, specifically Ras (20%) and BRaf (40%), so the inhibition of aberrant signaling through this cascade may be a valid therapeutic strategy for this type of tumors.

Scaffold proteins optimize Ras-ERK signals and modulate their intensity and spatial fidelity, acting as dimerization platforms. As such they could decisively intervene in signals conveyed through the RAS-ERK pathway to evoke thyroid tumors. We have used cellular models representative of different oncogenic driver lesions, namely BRaf, H/K/NRas and Ret/PTC, to study the role of scaffold proteins in thyroid tumorigenesis, in order to identify those scaffold essentials for the maintenance of the oncogenic phenotype. In addition, we have also investigated how either up- or downregulation of defined scaffold proteins affects the response of thyroid tumor cells to classical RAS-ERK pathway inhibitors depending on the driver lession.

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0124

LA INTERACCIÓN ENTRE SIRT1 Y SMAD2 MODULA LA REGULACION GENICA MEDIADA POR TGFß

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El factor de crecimiento transformante beta (TGFß) está involucrado en una gran variedad de procesos biológicos tales como la proliferación celular, la morfogénesis, la producción de matriz extracelular y la apoptosis. Los principales mediadores en la señalización de TGFß son las proteínas Smad, que orquestan la respuesta transcriptómica al TGFß y que varía según tipo celular y estado fisiológico o por presencia de otros estímulos, algo que complica la explicación de la diversidad de respuestas que esta citoquina puede generar.

La búsqueda de nuevas proteínas que interaccionan con Smad2 usando el sistema de levadura Cyto Trap Two Hybrid y una biblioteca de expresión preparada a partir de pulmón humano adulto, permitió detectar la interacción con Sirtuin 1 (Sirt1) . Sirt1 es una desacetilasa de histonas tipo III dependiente de NAD +, entre cuyos principales objetivos se encuentran varios factores de transcripción como p53 o FOXO. Así, Sirt1 actúa como un promotor tumoral en algunos tejidos como la próstata y el colon, a la vez que parece tener un papel supresor tumoral en otros tejidos como la vejiga y el ovario. Consecuentemente, se ha descrito que Sirt1 tiene un papel ambiguo en el cáncer, controversia que aún no se ha resuelto.

A nivel molecular, se ha encontrado que Sirt1 está involucrado en la desacetilación de Smad7, una Smad inhibidora. Curiosamente, Smad2, que está fosforilado por TGFβ, también se acetila en respuesta a esta citoquina, modificación necesaria para una respuesta transcripcional óptima. En nuestro laboratorio, utilizando diferentes modelos de cultivo celular, hemos confirmado la existencia de interacción entre Sirt1 y Smad2. Esta interacción pareció potenciarse mediante la activación de Smad2 después del tratamiento con TGFß y en relación con su estado de acetilación. Esta interacción parece ser parcialmente dependiente de la presencia de la actividad catalítica de Sirt1. Además, Sirt1 se aisló conjuntamente junto con las proteínas Smad de elementos de unión de Smad *in vitro*. La ocurrencia de esta interacción fue comprobada *in vivo*.

El estudio de la significacia de esta interacción para la modulación de la expresion génica regulada por TGFß, posibilita una mejor compresión de la patogénesis tumoral en aquellos casos en los es patente la alteración de la actividad Sirt1 y/o TGFß.

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0125-R/M

Involvement of TRIF in macrophage response to TLR2 ligands

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Toll-like receptors (TLR) are important to sense the invasion of pathogens as a first line of defense against infections triggering immune responses. TLR2 receptor is able to recognize a broad spectrum of pathogen-associated molecules. Until very recently, TLR2 ligands were considered to induce cytokine inflammatory responses only via MyD88 signalling pathway, whereas TLR4 activation triggers also the TRIF-dependent pathway, resulting in type I interferon (IFN) production. Here, we investigate the activation of the TRIF pathway after TLR2 stimulation and its role in the TLR2 ligand-mediated inflammatory responses. We observed that in TRIF-/- peritoneal macrophages there is a reduction in cytokine expression not only after TLR4 ligands stimulation but also in response to TLR2 ligands. Similar results were also obtained in murine RAW264.7 macrophage cell line with silenced TRIF expression by specific siRNAs. Moreover, LPS, PAM3CSK4 and FSL1-stimulated Raw264.7 cells were treated with incremental doses of blocking IFN-β antibody. Cytokine expression was found to be dose-dependently inhibited by the anti-IFN-β-Ab, further supporting that TRIF-IFNβ pathway is important for TLR4 and TLR2 later inflammatory response modulation. Further work will focus on elucidating the molecular mechanism governing the involvement of TRIF in TLR2 signaling.

0128

Scaffold proteins as modulators of RasERK pathway in cancer

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The RasERK pathway is noticeably linked to the development and progression of human malignancies. About 40% of human cancers harbor Ras and BRaf activating mutations. In addition to the main proteins of this signaling

pathway, there are several regulatory proteins, known as scaffold proteins, that modulate the intensity, amplitude and duration of ERK signals. These scaffold proteins can be proposed as antitumoral targets. Considering the hypothesis that scaffold proteins interact and regulate the signal transduction through the RasERK pathway in a coordinated manner we try to find out how these interactions are regulated and whether these interactions regulate the ability of each scaffold to activate ERK and promote specific cellular responses. Moreover, recent evidence has unveiled direct interactions among different scaffold protein species. These scaffoldscaffold complexes could constitute an additional level of regulation for ERK signals and may serve as nodes for the integration of incoming signals and the subsequent diversification of the outgoing signals with respect to substrate engagement. Focused on this interaction between ERK and scaffold proteins, especially KSR1, IQGAP1 and MP1, we have tested the different affinity of each scaffold protein to ERK what can point out to differences in regulation as well. To test if an incomplete scaffold complex (missing one or more kinases) could interact in trans with another type of scaffold to allow transphosphorylation we performed coinmunoprecipitation assays. Interestingly, we found that the association between activated ERK with a mutant scaffold unable to bind MEK suggests transphosphorylation as a signal integration mechanism between these scaffolds. Here, we propose that associations among different scaffold proteins will add one further degree of regulation for an already tightly regulated cascade and could provide a novel means for manipulating ERK signals, even with therapeutic purposes.

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0148-R/M

RAS activation at the Golgi Complex prevents tumourigenesis by inducing apoptosis via PTPRk-mediated inhibition of ERK activation

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IBBTEC Señalización Molecular y Celular

RAS GTPases are frequently mutated in human cancer. H- and NRAS isoforms are distributed over both plasma-membrane and endomembranes including the Golgi complex, but how this organizational context contributes to cellular transformation is unknown. Here, we show that RAS at the Golgi is selectively activated by apoptogenic stimuli and antagonizes cell survival by suppressing ERK activity through the induction of PTPRk, which targets CRAF for dephosphorylation. Consistently, RAS at the Golgi cannot induce melanoma in zebrafish. Furthermore, inactivation of PTPRk, which is frequently down-regulated in human melanoma, accelerates RAS- driven melanomagenesis in zebrafish. Thus, RAS oncogenic potential is strictly dependent on its sublocalization, with Golgi complex-located RAS acting as a tumor suppressor

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0152

Scaffold proteins and cytoskeletal dynamics as modulators of Ras-ERK pathway in cancer.

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An overwhelming body of data unquestionably links the Ras-ERK pathway to the upbringing and progression of human malignancies. About 40% of human cancers harbor activating mutations in proteins involved in this signaling cascade, in particular Ras and Braf. In addition to the main proteins of this signaling pathway, there are several regulatory proteins known as scaffold proteins, that modulate the intensity, amplitude and duration of ERK signals. Interestingly, it can propose the scaffold proteins as antitumoral targets. Considering the hypothesis that scaffold proteins interact and regulate the signal transduction through the Ras-ERK pathway in a coordinated manner we try to find out how these interactions are regulated. Ras proteins are distributed in different types of plasma membrane: microdomains and endomembranes. We have previously demonstrated that compartmentalization dictates Ras utilization of effectors, the intensity of its signals and the biological response of the cells. Cell migration is critical for many physiological processes and is often misregulated in developmental disorders and pathological conditions including cancer. MAPK signaling are known regulators of cytoskeletal dynamics during cellular processes such as cell adhesion and migration. Our hypothesis is that localized ERK signaling mediated through recently identified scaffold proteins and its interactions with the cytoskeleton may regulate specific cellular responses. Here we propose that associations among different scaffold proteins and tubulin will add one further degree of regulation for an already tightly regulated cascade and could provide a novel means for manipulating ERK signals, even with therapeutic purposes.

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0154-R/M

Understanding the role of Ras-ERK pathway scaffold proteins in melanoma

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The Ras-ERK pathway activates multiple cytoplasmic and nuclear substrates through which essential cellular functions, including proliferation, differentiation and transformation are regulated. Upregulation of ERK1/2 MAPK cascade has been reported in over 40% of all human cancers, with 15-20% of cutaneous melanomas harboring NRas mutations and oncogenic BRaf appearing in 50-60% of the cases.

ERK signalling is optimized by scaffold proteins that assemble pathway kinases into multi-enzymatic complexes whereby ERK signals intensity, amplitude, duration and localization are modulated. Accordingly, scaffolds under- or over-expression can trigger deep alterations in ERK signals and consequently on their transcriptional outputs and biological outcomes.

We hypothesized that differences in the MAPK scaffold usage may underlie the biochemical, biological and clinical divergences displayed by tumors of the same type but boosted by different Ras-ERK oncogenic signals. Therefore, we have screened the MAPK scaffold expression pattern in melanoma cell lines with a defined oncogenic signature, to determine if alterations in scaffold levels are preferentially associated to a certain driver oncogene.

As IQGAP1 and IQGAP2 are known to be frequently altered in melanomas, we are interested in how modifications in their expression levels, impact on melanomagenesis when induced by mutant BRaf and NRas and how this

can affect melanoma metastatic potential using chick embryo chorioallantoic membrane (CAM) xenografts in order to identify if these scaffolds are essential for the maintenance of the oncogenic phenotype.

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0160

Whi7 is an unstable cell-cycle repressor of the Start transcriptional program

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Start is the main decision point in eukaryotic cell cycle in which cells commit to a new round of cell division. It involves the irreversible activation of a transcriptional program by G1 CDK-cyclin complexes through the inactivation of Start transcriptional repressors, Whi5 in yeast or Rb in mammals. Here we provide novel keys of how Whi7, a protein related at sequence level to Whi5, represses Start. Whi7 is an unstable protein, degraded by the SCFGrr1 ubiquitin-ligase, whose stability is cell cycle regulated by CDK1 phosphorylation. Importantly, Whi7 associates to G1/S gene promoters in late G1 acting as a repressor of SBF-dependent transcription. Our results demonstrate that Whi7 is a genuine paralog of Whi5. In fact, both proteins collaborate in Start repression bringing to light that yeast cells, as occurs in mammalian cells, rely on the combined action of multiple transcriptional repressors to block Start transition.The commitment of cells to a new cycle of division involves inactivation of the Start transcriptional repressor Whi5. Here the authors show that the sequence related protein Whi7 associates to G1/S gene promoters in late G1 and collaborates with Whi5 in Start repression.

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0165-R

Modulación funcional por nitroalquilación con ácido nitro-oleico (NO2-OA) de la Peroxirredoxina 1 mitocondrial (Prx1p) de *Saccharomyces cerevisiae*

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Recientemente se ha demostrado la capacidad del óxido nítrico (NO·) y de algunos de sus derivados para reaccionar con lípidos insaturados, dando lugar a unas novedosas moléculas denominadas lípidos nitrados, nitrolípidos o nitroalquenos (NO2-FA). Estas moléculas presentan capacidad señalizadora, ya que pueden aductarse con residuos nucleofílicos de las proteínas dando lugar a una modificación post-traduccional (PTM) denominada nitroalquilación (1, 2).

En el presente trabajo se analizó la modulación funcional de la actividad enzimática antioxidante de la Peroxirredoxina 1 mitocondrial (Prx1p) de *Saccharomyces cerevisiae* (3) por ácido nitro-oleico (NO2-OA). Paralelamente, se identificaron los residuos diana de la nitroalquilación mediante técnicas de espectrometría de masas (nano-LC-MS/MS) y se realizó el análisis de modelización molecular *in silico* de la Prx1p.

Los resultados mostraron una disminución significativa de la actividad enzimática del sistema Prx1p dependiente de la concentración de lípido nitrado como consecuencia de la nitroalquilación con NO2-OA de residuos implicados en el mecanismo catalítico. En base a estos resultados, se puede concluir que la nitroalquilación de Prx1p por NO2-OA constituye un novedoso mecanismo de control de la actividad de este sistema antioxidante.

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0177

Targeting chondrocyte plasticity via connexin43 modulation attenuates cellular senescence in osteoarthritis

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Chondrocytes in articular cartilage undergo phenotypic changes and senescence, restricting cartilage regeneration and favoring osteoarthritis (OA) progression, a chronic disease characterized by degradation of articular cartilage. Like other wound healing disorders, chondrocytes from OA patients (OAc) show a chronic increase in the gap junction protein connexin43 (Cx43), which through the exchange or recruitment/release of signaling factors to the membrane regulates signal transduction. The aim of this study was to investigate the role of Cx43 in OA progression. Stem-like cells are found in cartilage from OA patients, yet their origin and role are unknown. Here we show that Cx43 acts as a positive regulator of the reversion of human chondrocytes to a less differentiated state. Overactive Cx43 maintains this stem-like phenotype by increasing the nuclear translocation of Twist-1 and increasing the expression of tissue remodelling and proinflammatory agents such as MMPs and IL-1β, which finally results in cellular senescence through upregulation of p53/p16INK4a and NF-κB contributing to the senescence-associated secretory phenotype (SASP). Downregulation of Cx43 leads to redifferentiation of OAc, decreasing MMPs, proinflammatory cytokines and cellular senescence. Collectively, these results identify a causal Cx43-sensitive circuit in chondrocytes that regulates de/re-differentiation events involved in wound healing and tissue repair. In addition, we show that chronic dedifferentiation drives catabolic processes and cellular senescence, which contribute to cellular reprogramming. Targeting Cx43 allows dedifferentiated osteoarthritic chondrocytes to revert to a chondrocyte-specific phenotype. These findings support the use of Cx43 as an appropriate therapeutic target to halt OA progression and to promote cartilage regeneration.

0193

Regulation of ERK dimerization and nuclear transport by novel phosphorylation events

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IBBTEC señalización molecular y celular

The MAP kinase cascade is a central signaling pathway that regulates a wide variety of stimulated cellular processes. ERK1/2 MAP kinases become activated in response to multiple stimuli, because of that, phosphorylated ERK monomers detach and may follow three destinies: (1) translocate as monomers to the nucleus where they activate nuclear substrates; (2) dimerize freely in the cytoplasm; free dimers could remain in the cytoplasm or (3) specific scaffolds acts as a platform in a sublocalization-specific fashion, where ERK2 dimers are assembled and the new complex can interact with specific substrates [1]. We had previously demonstrated the absence of ERK dimerization in chicken and this result prompted us to interrogate other species across the evolutionary scale. We found that dimerization only appears in mammals: mice, dogs and humans [2]. Next, we did the alignment of ERK2 sequences and we found a putative phosphorylation site Serine x of ERK2 is conserved only in mammalians. Using ERK2 mutants at this residue, native gels and a specific phospho Ser antibody, we demonstrated that phosphorylation at this Ser residue is required for ERK2 dimerization. Our results disclose a functional relationship between phosphorylation at Ser x and ERK2 dimerization and identify this specific phosphorylation at Ser x as a putative cytoplasmic localization of ERK2 as a dimer.

On the other hand, the phosphorylation at two Serine residues (SPS motif) in ERK1/2 allows it to bind Importin7, which escorts ERK molecules into the nucleus [3]. Based on this observation we would determine the complex IMP7- ERK2 structure, using electron microscopy, to demonstrate that ERK2 bind to IMP7 as a monomer allowing ERK2 translocation to the nucleus.

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0199

NOTCH3 MODULA LA RESPUESTA INFLAMATORIA EN MACRÓFAGOS ACTIVADOS POR TOLL-4 A TRAVÉS DE LA ACTIVACIÓN DE NFκB

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Múltiples trabajos han mostrado la participación de la vía de señalización de NOTCH en la regulación de la activación de los macrófagos por los receptores TOLL. Aunque en la mayor parte de los estudios se ha analizado la relevancia de los receptores NOTCH1 en estos procesos, debido al gran incremento de expresión de este receptor tras la activación de los receptores Toll, nuestros resultados muestran que es NOTCH3 el principal receptor NOTCH modulador de la activación de los macrófagos.

A diferencia de los otros receptores NOTCH, NOTCH3 se expresa de forma basal en los macrófagos diferenciados y su expresión se incrementa en las primeras horas tras la activación de TOLL4, disminuyendo posteriormente cuando la expresión del resto de receptores NOTCH aumenta. Un análisis de la translocación del dominio intracelular de estos receptores al núcleo muestra que NOTCH3 se transloca antes que NOTCH1. Nuestros resultados indican que NOTCH3 es el principal receptor NOTCH modulador de la actividad NFκB. En macrófagos activados con lipopolisacáridos bacterianos, la presencia de siRNAs específicos de NOTCH3 induce una disminución de la expresión de diversos genes pro-inflamatorios como el de la Óxido Nítrico Sintasa inducible, la IL-6 o el Interferón-β. Estos resultados muestran que NOTCH3 es el receptor NOTCH que modula las primeras etapas de activación de los macrófagos por el receptor TOLL4.

0229-R

Comparative Study on Whi7 and Whi5 Start Transcriptional Repressors

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Start is a key point of cell cycle control in eukaryotic cells, whose execution is an irreversible decision. In *Saccharomyces cerevisiae* it involves the activation of a transcriptional program by CDK-Cln complexes through the inactivation of the Start transcriptional repressor Whi5 (functional homologue of mammalian Rb). Very recently we have demonstrated that Whi7, a paralog of Whi5, also associates to G1/S gene promoters in late G1 acting as a repressor of SBF-dependent transcription (Gomar-Alba *et al.,* Nat Commun. 2017 Aug 24;8(1):329). In this work, we carry out a compared study of Whi7 and Whi5 proteins. First we search for differential determinants for Whi7 and Whi5 association to G1/S promoters. Binding analysis in the same cells demonstrated distinct preferences of both proteins for different G1/S genes. Moreover, whereas association of Whi5 to promoters is totally dependent of Swi6, Whi7 is able to bind promoters and the Swi4 protein in the absence of Swi6 protein. The analysis of the subcellular localization of Whi7 also revealed differences with Whi5. Although Whi7 localization is cell-cycle regulated in a similar way to that of Whi5, neither Kap95 nor Msn5 karyopherins are required for nuclear import or nuclear export, respectively, opposite to the case of Whi5. Finally, we described that Whi7 but not Whi5 is regulated by the PKC-pathway. All these observations support a specialization between Whi7 and Whi5 proteins.

0231

A cell cycle kinase-phosphatase module restrains PI3K-AKT activity in an mTORC1-dependent manner

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The AKT-mTOR signaling pathway is a central regulator of cell growth and metabolism. Upon sustained mTOR activity, AKT activity is attenuated by a negative feedback loop that restrains signaling via upstream elements of the PI3K-AKT-mTOR pathway. Despite the relevance of these feedback mechanisms, how cells control the signals that limit AKT activity is not fully understood. Here we show that MASTL/Greatwall, a cell cycle kinase that supports mitosis by phosphorylating the PP2A/B55 inhibitors ENSA and ARPP19, inhibits PI3K-AKT activity by sustaining mTORC1- and S6K1-dependent phosphorylation of IRS1 and GRB10. Genetic depletion of *MASTL* by RNAi or CRISPR/Cas9 techniques results in an inefficient feedback loop and AKT hyperactivity. These defects are rescued by expression of phosphor-mimetic ENSA/ARPP19 or inhibition of PP2A/B55 phosphatases. MASTL is directly phosphorylated by mTORC1, suggesting the presence of a parallel route that limits the PP2A/B55-dependent dephosphorylation of IRS1 and GRB10 adaptor proteins in the presence of strong mTORC1 signaling. Downregulation of *MASTL* in cells results in enhanced glycolysis and its genetic ablation in adult mice leads to increased glucose tolerance. These

findings establish the MASTL-PP2A/B55 kinase-phosphatase module as a major mechanism to control feedback regulation of AKT, thereby maintaining metabolic homeostasis.

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0244-R

Dual role of C3G in hepatocarcinoma tumor growth and progression. Implication in the HGF/c-Met signaling pathway

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C3G (RapGEF1) is a guanine nucleotide exchange factor for Rap1 and R-Ras. It is essential for embryonic development and regulates several cellular functions such as cytoskeletal remodeling, differentiation and cell death. However, its role in cancer is controversial acting either as a tumor promoter or suppressor. Using different public genomic data bases, we found that C3G expression increases in tumor samples from hepatocarcinoma (HCC) patients as compared to control livers, while it is reduced in metastatic samples. Accordingly, a Kaplan-Meir analysis revealed a shorter survival in patients bearing mutated C3G versus control patients. In agreement with this, we found that C3G protein levels are higher in HCC cells than in adult hepatocytes, as well as in mouse liver tumors generated by c-Met overexpression. Additionally, differences in the expression of C3G isoforms was detected.

Using different HCC cells (Hep3B and HLE), where C3G was down-regulated, a dual function of C3G was found. Hence, C3G promotes *in vitro* tumor growth by enhancing anchorage-dependent growth. In contrast, C3G inhibits migration and invasion. Moreover, *in vivo* tumor growth in nude mice was reduced upon C3G knock-down in Hep3B, while the dissemination of cancer cells to bone marrow increased, indicating a greater metastatic capacity. In contrast, the fibroblast infiltration in the tumor stroma was lower in this case based on alfa-SMA staining. Moreover, according to the increased expression of C3G in mouse liver tumors overexpressing c-Met, we found that activation of HGF/c-Met pathway was defective in HCC cells subjected to C3G downregulation. These data suggest that C3G is required for the full activation of c-Met and its downstream pathways.

0250-R/M

Role of platelet C3G in the regulation of the response to acute and chronic liver injury.

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C3G is a guanine nucleotide exchange factor (GEF) for members of the Ras family, mainly Rap1. However, C3G can also act through mechanisms independent of its GEF activity. Previous studies from our group demonstrated that C3G is involved in platelet activation and adhesion acting through Rap1. The bioinformatic analyses revealed the existence of binding sequences for the hematopoietic transcription factor GATA-1 in *C3G* gene regulatory regions. In addition, previous data from our group based on the overexpression approaches support that GATA-1, acting through C3G, induces the megakaryocytic/platelet differentiation process. The implication of GATA-1/C3G pathway in megakaryocytic differentiation has been demonstrated by knocking-down C3G in the promegakaryocytic Dami cell line.

Considering the relevance of platelets in inflammation and liver repair upon injury, we are using two transgenic mouse models with specific overexpression of full length C3G (tgC3G) or a truncated form of C3G (tgC3GDCat, lacking its GEF domain) in platelets, which were treated with CCl4. With these models we want to explore the function of platelet C3G in the regulation of liver inflammation, fibrosis and repair upon acute and chronic injury. Platelets accumulate at sites of liver injury and release pro-fibrogenic factors such as TGF-b or PDGF that activate hepatic stellate cells. In various mouse models of liver damage, platelets improve fibrosis through different mechanisms. Accordingly, our preliminary results showed an increase in the levels of HGF mRNA in livers of wt mice submitted to chronic treatment with CCl4. HGF expression was higher in the livers from tgC3G mice. However, there are not significant changes in TGF-b mRNA levels, as well as those of others pro-inflammatory cytokines. These data suggest that C3G overexpression in platelets might facilitate liver repair in response to damage induced by CCl4 through the stimulation of HGF release.

0251-R

Dissecting the role of C3G in oval cells physiology

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C3G is a guanine-nucleotide exchange factor (GEF) for some Ras family members such as Rap1, although it can act through GEF independent mechanisms. It is essential for embryonic development and it regulates several cellular functions such as migration, adhesion, invasion, cell death and differentiation.

Oval cells are bipotential transitory cells able to differentiate into hepatocytes and ductal cells. They have a high therapeutic potential, due to its capacity to regenerate the liver in response to chronic damage. However, they have been associated with the development of hepatocellular carcinoma and fibrosis. We have studied the role played by C3G in oval cells through gene silencing. We found that C3G knock-down increases migration and invasion, which correlates with morphological changes such as the presence of filopodia and lamellipodia and N-cadherin. These data suggest that C3G down-regulation promotes an EMT process that would enhance migration. In addition, C3G inhibits apoptosis in a dose-dependent manner. Hence, C3G down-regulation leads to a loss of cell viability. Moreover, a moderate reduction in C3G levels increased the number of foci in anchorage-dependent growth assays, while a higher down-regulation of C3G (70%) reduced foci formation capacity. However, oval cells were not able to grow in an anchorage-independent way, regardless of C3G levels. Therefore, C3G does not have any significant effect on the potential tumorigenic capacity of oval cells. On the other hand, C3G knock-down increases the levels of albumin, a marker of hepatocytes. However, it is unclear the regulation of other differentiation markers by C3G. Therefore, a deeper analysis is required to establish the function of C3G in this differentiation process.

0330

Mechano/osmosensitive ion channels in cancer invasion and metastasis

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Universitat Pompeu Fabra Ciencies Experimentals

The actin cytoskeleton is central to many cellular processes involving changes in cell shape, migration and adhesiveness. Therefore, there is a great interest in the identification of the signaling pathways leading to the re-

gulation of actin polymerization and assembly into stress fibers. However, to date it is not well understood how the mechanical interactions between cells and their environment activate the assembly of stress fibers. In this talk I will demonstrate that the mechanosensitive Piezo2 channel is required to sense physical cues from the environment to generate a calcium signal that maintains RhoA active and the formation and orientation of stress fibers and focal adhesions. Besides, this Piezo2-initiated signaling pathway has implications for different hallmarks of cancer invasion and metastasis. I will finish addressing the possibility that other mechano/osmosensitive ion channels may also contribute to the mechanobiological responses of by brain metastatic cells from breast cancer (MDA-MB-231-BrM2).

0358

Immunometabolism in Inflammatory diseases and Aging

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CSIC-UAM Centro de Biología Molecular Severo Ochoa

Mitochondrial dysfunction is a hallmark of aging. Our aim is to characterize how perturbations of mitochondrial function, specifically in cells of the immune system, affect organism homeostasis and lifespan. Using a mouse model whose mitochondrial function is compromised by deletion of mitochondrial transcription factor Tfam, specifically in CD4 immune cells. The absence of Tfam induces a severe decrease in mtDNA content resulting in severe mitochondrial dysfunction and impaired oxidative phosphorylation, thus forcing a metabolic switch towards glycolysis. Glycolytic T cells acquire proinflammatory features provoking a cytokine storm resembling inflammaging characterized by high serum levels of IL6, TNFa, and IFN-g. CD4Tfam-/- mice display reduced body weight, kyphosis, altered glucose homeostasis, sarcopenia, and cardiovascular alterations. On the whole, CD4Tfam-/- mice serve as an innovative genetic model for frailty and premature aging, reflecting the importance of tight immunometabolic control in preventing sterile inflammation and delaying aging and the onset of age-associated diseases

Keywords: Mitochondria function, inflammation, cytokines, senescence

0380

ERK dimerization and subcellular localization is determined by a novel phosphorylation site

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ERK 1/2 mitogen-activated protein kinases (ERKs) play a central role in the regulation of proliferative and survival processes in most cell types. It is well known that ERKs must be phosphorylated in their "canonical" TEY motif, in the activation loop, both to dimerize and to translocate to the nucleus, two essential events for ERKs functions. However, while absolutely necessary, this canonical phosphorylation is not sufficient to drive dimerization and nuclear translocation on its own. In this respect, we have unveiled a serine in the proximity of ERK1/2 S-P-S motif, whose phosphorylation is essential for dimerization and at the same time negatively regulates ERKs nuclear translocation.

RW2 3er Workshop Investigador Emergente

0021

Lysyl oxidase-like 3 is a novel druggable target in melanoma

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Lysyl oxidase-like 3 (LOXL3) is a member of the lysyl oxidase family comprising multifunctional enzymes with depicted roles in extracellular matrix maturation, epithelial to mesenchymal transition (EMT), development, differentiation and angiogenesis, as well as in distinct pathologies such as fibrosis and cancer 1-4.

Extensive work by our lab and others has established the deregulation of lysyl oxidases in cancer and their status has been associated with patient outcome in specific neoplasias 1,2. Our previous studies identified human LOXL3 as a modulator of EMT and Snail1 functional activity 5 and we have recently unveiled an unexpected contribution of LOXL3 to melanoma pathogenesis 6. Our studies reveal a role for LOXL3 in melanoma biology since

- Human melanoma cells are addicted to LOXL3 expression and LOXL3 knockdown halts cell proliferation and triggers apoptosis.

- LOXL3 cooperates to malignant transformation and contributes to melanomagenesis.

- LOXL3 binds to proteins that protect genome integrity while LOXL3 absence promotes a defective DNA damage checkpoint activation, deficient DNA repair and aberrant mitosis in melanoma cells.

Based on these results, we have generated a genetically engineered mouse model with conditional deletion of Loxl3. This inducible Loxl3 knock-out mouse was crossed to an established melanoma genetic model that recapitulates the biology of melanoma initiation, progression and metastasis mimicking the human disease 7. Ongoing experiments show that, in the absence of Loxl3, the onset of nevi is delayed while overall survival of mice is increased compared to control animals. Moreover, metastatic burden is lower in the absence of Loxl3 confirming that Loxl3 plays an important role in melanoma pathogenesis in vivo. Our data reveal an unexpected role for LOXL3 in the control of genome stability and melanoma progression, exposing its potential as a novel therapeutic target in malignant melanoma, a very aggressive condition yet in need for more effective treatment options.

0068

Inhibition of HER2 tumorigenesis by Beclin 1 and an autophagy-inducing peptide

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HER2 is a receptor tyrosine kinase whose gene is amplified in ~20% human breast cancer patients. Allelic loss of the autophagy gene, *beclin 1/BECN1*, is associated with HER2 amplification in breast cancer; low *beclin 1* mRNA expression is associated with increased risk of HER2-positive breast cancer; and overexpressed HER2 and Beclin 1 interact in cultured cells. However, the functional significance of HER2/Beclin 1 interaction and of altered autophagy in HER2-driven tumorigenesis and whether autophagy induction may be beneficial in preventing HER2-positive breast tumor growth is unknown. We explored the regulation of autophagy in breast cancer cells by HER2 *in vitro* and the effects of genetic and pharmacological approaches to increase autophagy on HER2-driven breast cancer growth *in vivo*. We show that endogenous HER2 interacts with Beclin 1 in HER2+ breast cancer cells and it inhibits autophagy. Mice with an increased basal autophagy due to a knock-in mutation in *Becn1* (*Becn1*F121A) that results in a decreased Bcl-2 binding to Beclin 1 are protected from mammary tumorigenesis when crossed with mammary-specific HER2 transgenic mice, and HER2 fails to inhibit autophagy in primary cells derived from these mice. Furthermore, HER2-positive human breast cancer xenografts treated with the autophagy-inducing peptide Tat-Beclin 1 inhibit tumor growth as effectively as a clinically used HER2 tyrosine kinase inhibitor (TKI). This inhibition of tumor growth is associated with a robust induction of autophagy, a disruption of HER2/Beclin 1 binding, and a transcriptional signature in the tumors that is distinct from that observed with HER2 TKI treatment.

Taken together, these findings indicate that the HER2-mediated inhibition of Beclin 1 and autophagy likely contributes to HER2-mediated tumorigenesis. They also suggest that strategies to block HER2/Beclin 1 binding and/or increase autophagy may represent a new therapeutic approach for HER2-positive breast cancers.

0096-R/M

RNA-dependent chromatin targeting of TET2 for endogenous retrovirus control in pluripotent stem cells.

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Ten-eleven translocation (TET) proteins play fundamental roles in regulating chromatin architecture and transcriptional events that define mammalian cellular identity, but the molecular underpinnings that contribute to stable TET occupancy at the chromatin remain poorly understood. Here we demonstrate that TET2 is recruited to chromatin in an RNA-dependent manner through Paraspeckle component 1 (PSPC1) protein. By genome-wide analysis, we show that PSPC1 binds both coding and non-coding RNAs, including endogenous retroviruses (ERVs). Consistent with this, PSPC1 depletion reduced TET2 binding to these abundant repetitive sequences and resulted in transcriptional deregulation of these parasitic mammalian elements. Notably, we discovered that early embryonic MERVL endogenous retrovirus expression is tightly regulated by dual TET2 independent functions: transcriptional repression via histone deacetylation and RNA destabilization via 5-hydroxymethylcytosine (5hmC) deposition. Our findings reveal a critical role for an RNA modulation of TET2 function at the chromatin level and provide evidences for a functional role of 5hmC RNA modification in posttranscriptional regulation of endogenous retroviruses.

Guallar, D. et al "RNA-dependent chromatin targeting of TET2 for endogenous retrovirus control in pluripotent stem cells" . Nature Genetics (2018)

0115

Dissecting the interactions between the tumour microenvironment and Oncostatin M pathway to find new therapeutic targets in advanced breast cancer

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Breast cancer is a highly prevalent health care problem. Although 70% of the cases are cured, metastasis is the major contributor to the death of patients. In this context, finding new therapeutic targets for the disseminated disease is an unmet need with high clinical impact. Cytokines are important players in inflammation, a process highly associated with tumour progression. Our results show that the pro-inflammatory cytokine Oncostatin M plays a key role in breast cancer metastasis and mediates the crosstalk between cancer cells and the tumour microenvironment. While the Oncostatin M receptor (OSMR) is expressed in cancer cells and cancer associated fibroblasts (CAFs), the main source of the ligand OSM are the macrophages, suggesting paracrine signalling in the tumour. OSMR activation in cancer cells induces the expression of pro-malignant genes such as VEGFA, Snail, IL6 and integrins. In addition, stromal fibroblast recruitment and activation to a cancer-associated fibroblast (CAF) phenotype has been implicated in cancer progression. OSM induces CAF proliferation and conversion by inducing expression of a-SMA and fibroblast contractility. Importantly, OSMR induces tumourigenesis and lung metastasis in orthotopic xenografts of human MDA-MB-231 cells. Accordingly, genetic depletion of OSMR in the transgenic model MMTV-PyMT decreases tumour onset and metastasis. Analysis of mRNA data from breast cancer databases (Metabric, TCGA) showed that OSM and OSMR are over-expressed in estrogen receptor negative tumours, where they associate with decreased survival. OSMR could be blocked by antibody based inhibition, strategy that has had a major impact on breast cancer. We recently showed that anti-OSM antibodies are effective in inhibiting metastasis in cervical cancer models (1, 2).

1. Caffarel MM, Coleman N (2014) Journal of Pathology 232:386-390 2. Kucia-Tran JA et al (2018) Journal of Pathology 244:283-295

0142

A crosstalk between bone and muscle endocrine functions favors adaptation to exercise

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The proximity of the two tissues and the fact that bone mass declines at the time muscle mass and functions also decrease have long suggested that a crosstalk between bone and muscle may exist. An additional reason to address this question is that circulating levels of the bone-derived hormone osteocalcin double during aerobic exercise, at the time muscle function needs to increase. In contrast, circulating levels of osteocalcin plummet early during adulthood at the time exercise capacity declines. Using mutant mice lacking either osteocalcin (*Ocn-/-*) or its receptor Gprc6a only in myofibers (*Gprc6aMck-/-*), here we show that osteocalcin signaling in myofibers is necessary for adaptation to exercise in adult mice because it promotes the uptake and catabolism of glucose and fatty acids. This function of osteocalcin also provide an explanation for why exogenous osteocalcin is sufficient to increase the exercise capacity of wild type (WT) mice. Indeed, acute or chronic delivery of osteocalcin can increase the exercise capacity of young adult mice and restore in 15 month-old mice the exercise capacity of 3 month-old mice. To deepen our understanding of the mechanisms of action of osteocalcin on muscle, we performed a transcriptomic analysis after exercise in muscles of

Gprc6aMck-/- and control littermate mice. This analysis showed that the expression of a single myokine, interleukin-6 (IL-6), was decreased in muscle of *Gprc6Mck-/-* compared to those of control mice. IL-6, one of the first myokine ever described, sees its circulating levels markedly increased during exercise. IL-6 then favors adaptation to exercise through several means one of them being an increase in the production of glucose and fatty acids. Moreover, IL-6 favors the generation of bioactive osteocalcin in part by increasing the expression of *Rankl* and decreasing the expression of *Osteoprotegerin* in osteoblasts. This regulation explains why bone resorption markers and circulating levels of bioactive osteocalcin increase after exercise in WT but not in *Il6*-deficient mice. Taken together these data reveal the existence of a positive crosstalk between bone via osteocalcin, and muscle via IL-6, that is necessary and sufficient to allow adaptation to exercise in the mouse.

Osteocalcin Signaling in Myofibers Is Necessary and Sufficient for Optimum Adaptation to Exercise. P. Mera et al. Cell Metabolism. Volume 23, Issue 6, p1078–1092, 14 June 2016

0155

Towards deciphering the architecture of CAD

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CAD is a multienzyme complex formed by hexameric association of a ~240 kDa polypeptide with three functional domains: glutamine-dependent carbamoyl phosphate (GLN-CPSase), aspartate transcarbamoylase (ATCase) and dihydroorotase (DHOase). Each of the domains catalyzes one of the initiating steps in *de novo* synthesis of pyrimidines and its activity is tightly controlled by allosteric effectors and by phosphorylation through different signaling cascades. The up-regulation of CAD activity is essential to fuel the high demand of pyrimidines during cell growth and proliferation. Thus, CAD is a potential target for the development of anti-tumoral drugs. Despite conservation of *de novo* pyrimidine pathway in all organisms, the fusion of the first enzymatic activities in a single polypeptide is unique to animals. In fungi, GLN-CPSase and ATCase are fused into a CAD-like polypeptide that contains a defective DHOase domain, and this activity is provided by a separated protein. In prokaryotes (and also archaea and plants) these activities are encoded as distinct monofunctional enzymes, for which structures are available. We reported the crystal structures of the DHOase and ATCase domains of human CAD; however, we still lack information about the architecture of the 1.5 MDa CAD hexameric complex. We have recently isolated full-length CAD from the fungus *Chaetomium thermophilum* and reported preliminary results on the visualization by electron microscopy. Our data support that CAD self-assembles as a "dimer of trimers", where the DHOase and ATCase domains form the core structural scaffold of the particle.

0214

Identification of the Tudor domain Royal family as a reader family of m6A-modified RNA

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Over 100 distinct RNA chemical modifications comprise the epitranscriptome. *N6*-methyladenosine (m6A) is the most prevalent internal modification of cellular mRNAs with essential roles regulating their turnover, processing and translation, adding another novel layer of gene regulation (1). Kaposi's sarcoma-associated herpesvirus (KSHV) is a double-stranded DNA virus responsible for several aggressive malignancies that have no effective treatment. Thus, we aimed to elucidate the KSHV m6A epitranscriptome and characterise m6A reader proteins that may play a key role in KSHV replication. Firstly, multiple m6A modifications in KSHV mRNAs were identified with the use of m6A-seq. Modifications found in the viral open reading frame 50 (ORF50) were of particular interest, as this RNA encodes the replication and transcription activator (RTA) protein. RTA protein is essential to switch latent cells into lytic replication with subsequent production of infectious virions. Then, through RNA affinity, proteins which bind selectively to three viral m6A modifications, two found in *ORF50* transcript, were investigated. Strikingly, mass spectrometry analysis revealed eight putative readers that belong to the Tudor domain Royal family, thus all share an aromatic cage implicated in binding methylated residues in proteins which is structurally similar to the aromatic cage used by the only other family reported to date that directly binds m6A (2) (3). Electromobility shift assays using these Royal domains demonstrate their ability to specifically bind m6A in a RNA secondary structure-dependent manner. One such reader, SND1, was further characterised using RIP-seq. This technique confirmed that SND1 is a *bona fide* RNA-binding protein that targets m6A-modified RNAs inside cells, including *ORF50* transcript. Importantly, SND1 depletion resulted in a dramatic decrease in the stability of *ORF50* transcript and consequent impairment of KSHV lytic replication, revealing SND1 as an essential m6A reader that could be targeted for novel antivirals.

1. Meyer KD, Jaffrey SR. Rethinking m6A Readers, Writers, and Erasers. Annual Review of Cell and Developmental Biology. 2017;33(1):319-42. 2. Chen C, Nott TJ, Jin J, Pawson T. Deciphering arginine methylation: Tudor tells the tale. Nat Rev Mol Cell Biol. 2011;12(10):629-42. 3. Liao S, Sun H, Xu C. YTH Domain: A Family of N6-methyladenosine (m6A) Readers. Genomics, Proteomics & Bioinformatics. 2018.

0230

El análogo de las estrigolactonas GR-24 induce cambios en el citoesqueleto de células tumorales y endoteliales y modula su balance redox

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Las estrigolactonas son hormonas vegetales con estructura terpenoide, implicadas en el control de una serie de procesos que incluyen la inhibición de la ramificación de la raíz y el brote. Recientemente, se ha descrito el potencial de algunas fitohormonas en la prevención y el tratamiento de distintos tipos de cáncer. Entre ellas, diferentes análogos sintéticos de estrigolactonas, como el GR-24. Nuestro grupo ha caracterizado la actividad inhibidora de la angiogénesis in vitro e in vivo de GR24. Con la intención de caracterizar el mecanismo de acción de su actividad biológica, hemos estudiado el efecto del compuesto sobre la organización del citoesqueleto, implicada en muchos de los pasos de la angiogénesis, así como sobre el balance rédox de las células endoteliales y tumorales, que coexisten en el microambiente angiogénico. Nuestros resultados refuerzan la posibilidad del uso de GR24 como candidato a fármaco para el tratamiento del cáncer y otras enfermedades dependientes de la angiogénesis.

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0320-R/M

Blocking of translation by C9ORF72 dipeptides repeats as a therapeutic target to alleviate toxicity in ALS disease.

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The expansion of GGGGCC repeats within the first intron of *C9ORF72* constitutes the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Through Repeat-associated non-ATG (RAN) translation, these expansions are translated into dipeptide repeats proteins (DPRs), among these, poly-proline-arginine (poly-PR) and poly-glycine-arginine (poly-GR) peptides present higher neurotoxicity. It is already known that DPRs accumulate at nucleoli, impairs translation, and lead to cell death. In order to better understand the mechanism by which DPRs inhibit translation, we have performed proteomics after immunoprecipitation of the Ribosomal Protein S9 (RPS9). Our data demonstrate that cells treated with poly-PR suffer an imbalance between 40S and 60S ribosomal subunits, that was confirmed by the presence of halfmers observed by polysome profiles. *In vitro* experiments demonstrate that both, initiation and elongation of translation, are blocked after poly-PR treatment and that such inhibtion is rescued by impairing the binding of poly-PR to the mRNA. Since inhibition of translation is contributing to the neurotoxicity in C9ORF72/ALS, our data could help to design therapeutic approach to alliviate neurotoxicity in patients with C9ORF72/ALS.

0361-R/M

p38gamma is essential for cell cycle progression and liver tumourigenesis

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Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid Papel de las quinasas activadas por el estrés en el desarrollo de enfermedades cardiovasculares, diabetes y cáncer

The cell cycle is a tightly regulated process orchestrated in mammals by sequential activation of cyclin-dependent kinases (CDKs). Here, we found that a stress-activated kinase, p38g, functions as a parallel CDK route to activate the cell cycle. Upon stress stimuli, p38g is activated and phosphorylates retinoblastoma protein (Rb), releasing hepatocytes from quiescence and initiating the cell cycle. p38g compensate the loss of the cyclin-dependent kinases CDK1 and CDK2, inducing hepatocyte proliferation and liver regeneration after partial hepatectomy (PHx). p38g thus plays a central role in the transition of hepatocytes from quiescence to proliferation by regulating cell cycle progression, similarly to CDKs. This finding has clinical implications since we showed that p38g expression is increased in human hepatocellular carcinoma (HCC) and is required for HCC cell proliferation. Genetic deletion or pharmacological inhibition of p38g resulted in a lower tumour burden and extended the lifespan of mice with chemically-induced liver tumours. Our results establish the importance of p38g in HCC initiation and development and strongly suggest that inhibition of p38g could provide therapeutic benefit in HCC.

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