SEBBM

LIBRO DE RESÚMENES / BOOK OF ABSTRACTS

46º Congreso de la Sociedad Española de Bioquímica y Biología Molecular

A Coruña

3 al 6 de septiembre 2024

CINBIO Universidade de Vigo

USC Universidade de Santiago de Compostela

Universidade da Coruña

CSIC Consejo Superior de Investigaciones Científicas

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BIENVENIDA

Queridos y queridas colegas

En nombre del Comité Organizador os damos la bienvenida y os animamos a participar activamente en el 46º Congreso de la SEBBM que tendrá lugar en el palacio de congresos PALEXCO en el centro de la ciudad de A Coruña del 3 al 6 de septiembre de 2024.

Durante el Congreso, disfrutaremos de un amplio abanico de Conferencias Plenarias, Simposios, entre ellos el Simposio científico Hispano-Luso y el de Educación, Reuniones de los Grupos científicos de la SEBBM, Sesiones de posters y la Exposición Comercial de las empresas colaboradoras. Podremos disfrutar de actividades satélites como el Curso de Iniciación a la Investigación en Bioquímica y Biología Molecular, el Foro de Desarrollo Profesional para Jóvenes Investigadores, y las actividades de Bioquímica en la ciudad, con exposiciones, espectáculos científico-artísticos, mesas redondas y talleres científicos.

Las conferencias plenarias serán impartidas por investigadores e investigadoras relevantes de diferentes áreas de la bioquímica. El programa final estará disponible en la web del congreso en unas semanas. Los Simposios presentarán las investigaciones más actuales y contarán con la colaboración de diferentes empresas o fundaciones. Las sociedades bioquímicas de Argentina, México y Chile estarán representadas por sus respectivos ponentes durante estas conferencias y con la colaboración de la PABMB.

Animamos a los congresistas a visitar los estands de las empresas patrocinadoras a las que agradecemos su apoyo y también a participar en las diferentes convocatorias de Premios de la SEBBM. La entrega de premios tendrá lugar durante la sesión de clausura del Congreso.

¡Os esperamos en A Coruña en septiembre del 2024!

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(Presidenta del Comité Organizador)

Isabel Varela Nieto
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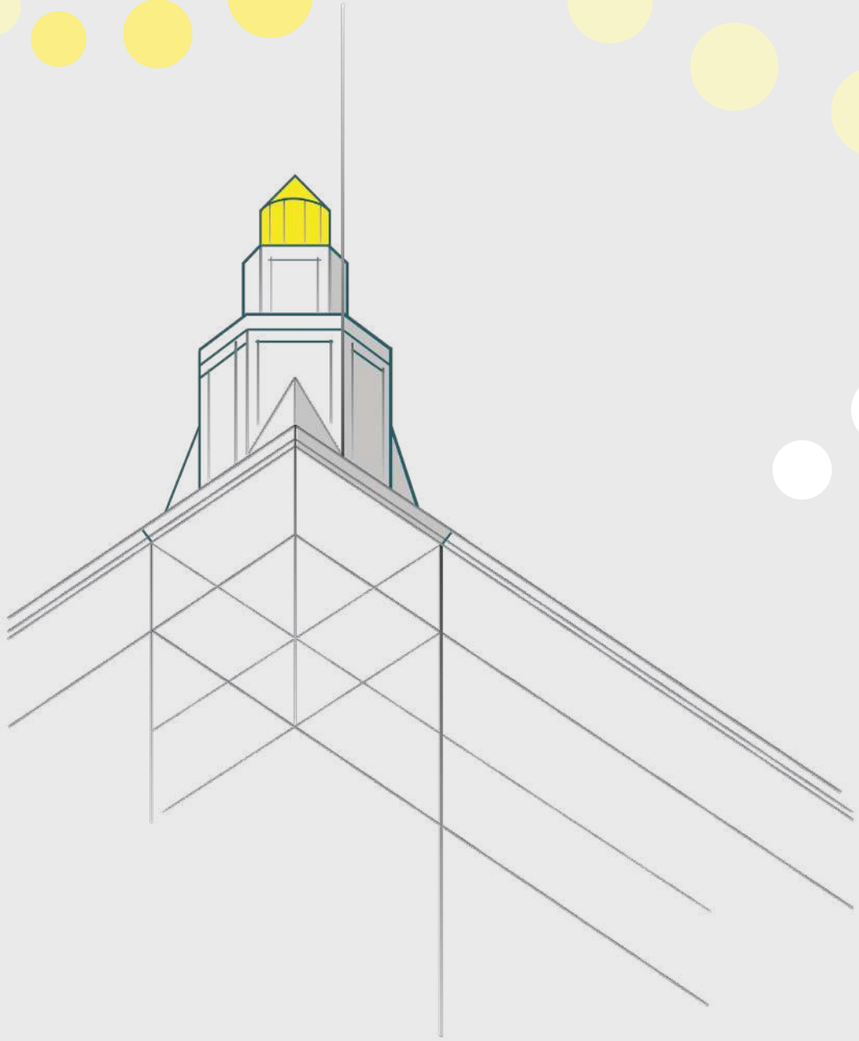

CONFERENCIAS PLENARIAS

PL - 01

Metabolic and Neural Regulation of Immunity and Cancer

TAK W. MAK

Princess Margaret Cancer Centre, University of Toronto and Centre of Oncology and Immunology, University of Hong Kong

Interest in the theme of connections between metabolic regulation of immune responses and cancer development has recently accelerated, making this topic an area of intense research in the fields of both immunology and cancer biology. It has become clear that not only multiple molecular mechanisms, both intrinsic and extrinsic, but also numerous metabolites converge to alter core cellular metabolism during tumorigenesis. This state of affairs has made it difficult to analyse precisely the roles that key metabolites play in immune cell functions and/or cancer. Fortuitously, the finding that certain mutations in particular metabolic enzymes, such as isocitrate dehydrogenase, can give rise to malignancies such as gliomas, acute myeloblastic leukemia (AML) and angioimmunoblastic T cell lymphoma (AITL) give us avenues to comprehending metabolic and epigenetic pathways that are important in both in tumorigenesis and immune responses. Another metabolite that plays an important linking role between a seemingly unconnected biological process and immunity is acetylcholine. Although choline has been known for decades to be involved in neurotransmission, its deficiency has been shown to cause liver cancer. Our laboratory has reported several findings supporting the intriguing proposition that neurotransmitters like norepinephrine and acetylcholine may be key components that tie the neuronal system to the regulation of immune responses. In this presentation, I will discuss our findings dissecting the roles of important metabolic elements and neurotransmitters in immune cell regulation and cancer development.

PL - 02

Cellular functionality and plasticity in the senescence spectrum

MASASHI NARITA

CRUK, Cambridge, UK

Cellular senescence can be induced by various stimuli, leading to characteristic morphological changes and a stable exit from the cell cycle. While senescent cells may play a role in physiological tissue homeostasis, their accumulation in vivo contributes to individual ageing and age-related diseases such as cancer. The adverse effects of senescent cells are often attributed to the loss of original cellular functions. However, the acquisition of new functions by senescent cells is also critical. Cellular function is largely defined by tissue/lineage-specific gene expression patterns, representing cellular diversity, and is closely associated with 3D epigenetic configuration. I will discuss senescence as a dynamic process involving a shift in the functional identity of cells, introducing the emerging concept of the senescence spectrum.

PL - 03

Evolution as a lens into lncRNA functionality

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Long non-coding RNAs (lncRNAs) have recently emerged as prominent elements of the regulatory transactions of eukaryotic genomes. Many of the known regulatory functions of lncRNAs in both animals and plants rely on the rearrangement of chromatin through direct interactions or recruitment of chromatin-modifying elements. In this talk, I will discuss the difficulty in identifying evolutionary conservation in lncRNAs, and how we characterize these evolutionarily volatile elements in the context of their role as regulators of the three-dimensional conformation of nuclear chromatin. I will also discuss how such techniques have vast potential to illuminate biomedically relevant lncRNAs when analyzed from a comparative genomics perspective.

PL - 04

Polypharmacological Modulation of Ion Channels Associated Diseases

WENDY GONZÁLEZ

University of Talca, Chile

Our research group has been working for about 15 years in ion channel pharmacology, focusing on the structural basis for the modulation of these proteins, which play a key role in many diseases. In this lecture, I'll present our progress in studying the polypharmacological modulation of ion channels associated with atrial fibrillation (AF), the most common arrhythmia in the Western world. During AF, electrical remodeling occurs, involving ion channels like $Na_V1.5$, $K_V1.5$, and TASK-1. A promising AF treatment encompasses inhibiting these channels. In this study, acetamide compounds were designed based on the local anesthetic pharmacophore as potential $Na_V1.5$, $K_V1.5$, and TASK-1 inhibitors. Compound **6f** emerged as the most potent in the series, with IC_{50} values determined in *Xenopus* oocytes of approximately 0.3 µM in TASK-1, 81.5 µM in $K_V1.5$, and 21.2 µM in $Na_V1.5$. Unexpectedly, **6f** activated at 100 µM another cardiac K_2P channel (TASK-4) by about 40%. Next, we performed patch clamp experiments of human atrial cardiomyocytes from sinus rhythm (SR) or AF patients. In SR **6f** reduced action potential amplitude (indicating $Na_V1.5$ block) while in AF it increased action potential duration (APD), reflecting high affinity for TASK-1. Additionally, a hyperpolarization in resting membrane potential occurred in AF cardiomyocytes by **6f**, consistent with the TASK-4 activation we observed. In a mathematical whole-atria model, **6f** prolonged APD and tissue refractoriness, proving efficacious both for AF prevention and cardioversion. Favorable pharmacokinetic properties of **6f** *in silico*, including good absorption and low toxicity, as well as its lack of cytotoxicity in a hemolytic assay, suggest its potential as an AF treatment.

PL - 05

Autophagy to rule them all

MARINA GARCÍA-MACIA[1,2]

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[2] *Department of Biochemistry and Molecular Biology, University of Salamanca, Salamanca, Spain*

Autophagy is the cellular clearing and recycling program that degrades cytoplasmic content in lysosomes. This process takes place in all cells and is further induced during cellular stress. Besides its crucial role as a quality control mechanism, autophagy is also involved in a host of other functions such as growth and differentiation, metabolic regulation and as an alternative energy source through a type of selective autophagy called lipophagy. This is the most effective way to mobilize fatty acids, preventing their toxicity, and is the preferred mechanism to generate energy during starvation Furthermore, lipophagy is involved in the control of the central nervous system over peripheric organs, as liver or brown adipose tissue. Disruption of this brain-to-periphery axis leads to diseases like obesity, metabolic disorders and even cancer. The lack of knowledge about specific receptors and the exact lipophagy pathway hinders the creation of efficient therapies. Currently, we are studying how the disturbed brain-to-periphery axis may produce the muscular symptoms in a neurodegenerative disorder. Besides, the main purpose of Macphagy lab is to decipher the role of lipophagy in the immune system. Particularly, we are modulating autophagy/lipophagy to switch macrophages activation. This will help to ameliorate the chronic inflammation in diseases steatosis or obesity, but also will trigger the immune system to fight against tumoral cells.

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PL - 06

PCK1 neddylation: a new mechanism in the control of hepatic glucose production

MARÍA JESÚS GONZÁLEZ RELLÁN.

CiMUS, Molecular Metabolism Group, Departamento de Fisiología de la Universidad de Santiago de Compostela

La producción exacerbada de glucosa hepática es una característica común en los pacientes con diabetes, contribuyendo a la hiperglucemia y la resistencia a la insulina que caracteriza la enfermedad. Esta desregulación está en gran medida relacionada de forma directa con la actividad de PCK1, la enzima principal involucrada en el proceso de producción de glucosa. Este trabajo describe por primera vez un mecanismo implicado en la regulación de la actividad gluconeogénica de PCK1 a través de la modificación postraduccional de neddilación. Específicamente, descubrimos que PCK1 necesita ser neddilada en los residuos K278, K342 y K387, un proceso que induce un cambio conformacional en la enzima esencial para su funcionalidad. En condiciones hiperglucemiantes, PCK1 está hiper-neddilada, y la inhibición específica de este proceso con modelos genéticos, virogenéticos o con el uso del fármaco MLN4924, inhibidor específico de la neddilación, reduce la producción de glucosa hepática y disminuye los niveles de glucosa en sangre, ofreciendo un enfoque innovador en el control de la producción hepática de glucosa.

PL - 07

The Emerging Role of Mitochondrial Metabolism in Parkinson's Disease

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disease in the world. There is, however, no disease-modifying therapy available. Emerging research highlights the pivotal role of mitochondrial metabolism in PD pathogenesis, with mitochondrial complex I (MCI) disruption being particularly significant. Using intersectional genomics, we developed MCI-Park mice by deleting the gene Ndufs2, which encodes a catalytic subunit of MCI. About a month after deletion, mitochondria became net consumers of ATP to maintain their membrane potential by running complex V in reverse. As mitochondrial OXPHOS declined, SNc dopaminergic neurons upregulated glycolysis genes and downregulated mitochondrial ATP-producing genes, undergoing a Warburg shift. At the same time, there is a shift away from the pentose phosphate shunt, decreasing the glutathione redox couple. Neuronal survival was enabled by this metabolic shift, but triggered a progressive loss of the dopaminergic phenotype that was first evident in nigrostriatal axons. This axonal deficit was accompanied by motor learning and fine motor deficits, but not by levodopa-responsive parkinsonism. In contrast, older MCI-Park mice cannot sustain this metabolic demand. In the three months following the deletion of MCI, SNc dopaminergic neurons downregulated glycolysis genes as well as genes that produce ATP. It is at this stage that MCI-Park mice show a progressive, human-like parkinsonism in which nigral dopamine release makes a critical contribution to motor dysfunction. Our findings underscore the importance of the bioenergetic status of SNc dopaminergic neurons in PD progression, suggesting that targeting MCI impairment may offer a promising therapeutic strategy for PD.

PL - 08

Beyond the molecular mechanisms of regulation of protein kinases downstream of insulin and growth factors

RICARDO M. BIONDI

Instituto IFIBYNE (UBA-CONICET), Buenos Aires, Argentina

The protein kinases involved downstream of insulin and growth factors were identified 25 years ago. I will present the molecular mechanisms of regulation, substrate specificities, and the application of this knowledge for drug discovery. The phosphoinositide-dependent protein kinase 1 (PDK1) phosphorylates multiple protein kinases, all belonging to the AGC kinase group. Some substrates of PDK1, such as PKCs, are phosphorylated constitutively after synthesis, while others, like Akt/PKB, S6K, SGK, and RSK, are phosphorylated by PDK1 following insulin/growth factor and PI3-kinase activation, using different mechanisms. Our investigations focus on using small compounds to stabilize different conformations of PDK1. PDK1 is now a comprehensive model for an allosteric protein modulated bidirectionally with small compounds. One of the compounds we developed, PS48, which binds to the PIF-pocket of PDK1, was recently shown to improve memory and learning in a mouse Alzheimer's model. Very selective allosteric drugs in clinical trials stabilize the PIF-pocket in the inactive conformation of Akt/PKB. PDK1 homologues and AGC kinases are present in all eukaryotes. While some central mechanisms are conserved, secondary mechanisms involving the conformations of full-length PDK1 differ in more distant organisms, suggesting that these differences may be targeted for drug discovery against infectious organisms.

PL - 09

Adventures in Adipose Tissue Biology

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Harvard Medical School. Broad Institute of MIT and Harvard

White adipose tissue (WAT) has long been neglected by scientists because of the perception that it is bland and uninteresting. In recent years, however, there has been growing appreciation for the complexity of this tissue and the fundamental role that it plays in a wide variety of physiological and pathophysiological processes. I will discuss recent advances in the biology of WAT on two separate fronts. First, I will present the analysis of WAT composition at the single cell level across multiple axes, including species (human and mouse), body weight, depot, and sex. These studies reveal new adipocyte subpopulations with distinct relationships to human disease. Second, I will discuss novel findings related to the control of lipolysis, a critical process localized primarily to WAT, specifically focused on a role for the hormone oxytocin. Taken together, these studies provide insight into the basic biology of fat as well as the pathogenesis and treatment of metabolic disease.

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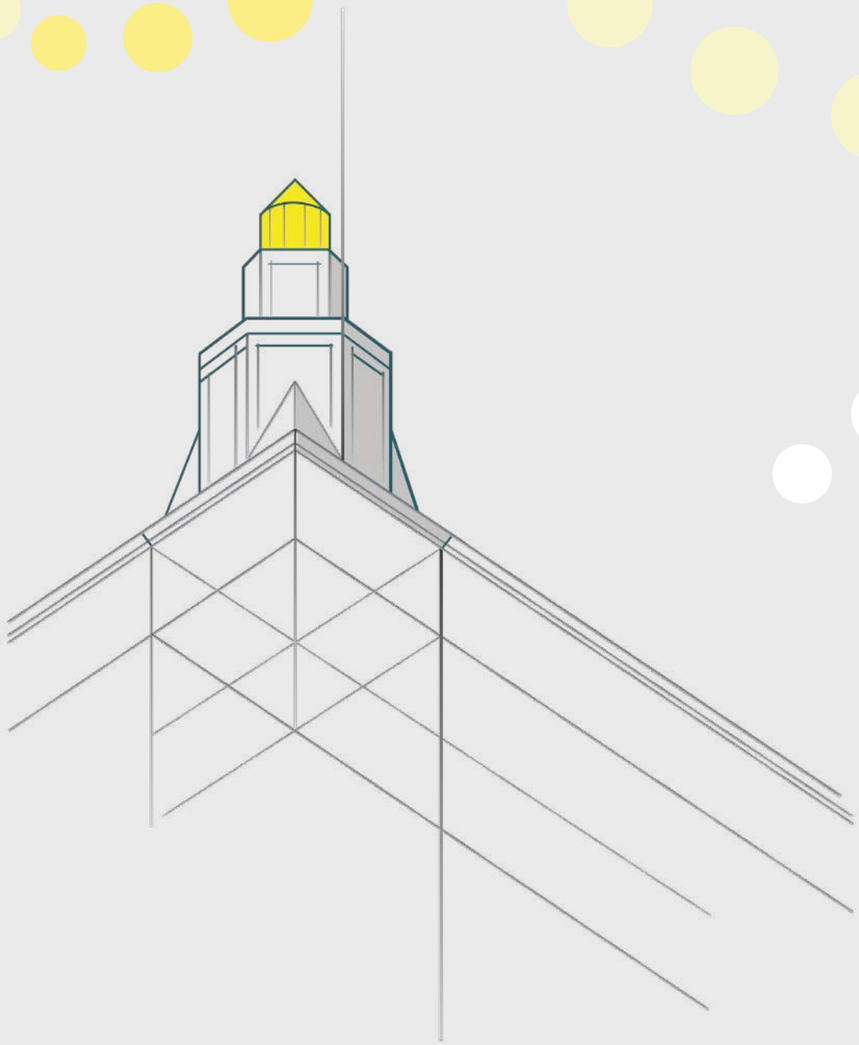

SIMPOSIO DE EDUCACIÓN

S. ED - 366 - O

Resumen y conclusiones del Simposio

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El Simposio de Educación es una actividad que se celebra en el marco del Congreso de la SEBBM enfocada en dar a conocer algunos de los abordajes educativos más novedosos que se están aplicando en asignaturas de Grados universitarios relacionados con el ámbito de la Bioquímica y la Biología Molecular, con el fin de establecer una reflexión y discusión activa y enriquecedora entre los participantes del Simposio. En esta ponencia se hará un resumen de las principales estrategias expuestas en dos conferencias invitadas, centradas en aspectos tan importantes para la formación integral de nuestros estudiantes, como son el fomento y el análisis del aprendizaje activo, la evaluación colaborativa, así como el compromiso social de los mismos, mediante el desarrollo de metodologías de aprendizaje y servicio o aprendizaje basado en investigación. Por otra parte, al final de esta ponencia se resumirán las conclusiones más relevantes extraídas, no sólo de las conferencias, sino también de la discusión suscitada como consecuencia de las mismas. Con todo ello, se persigue aumentar la motivación de la comunidad universitaria para implementar actividades de mayor calidad formativa para nuestros estudiantes.

Keywords: Simposio De Educación

S. ED - 364 - O

Transformando la Enseñanza en Bioquímica y Biología Molecular: Un Viaje a Través de Metodologías Docentes Innovadoras

MANUEL BECERRA FERNÁNDEZ

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En esta ponencia, se examinará el impacto de diversas metodologías docentes innovadoras en la enseñanza de la Bioquímica y Biología Molecular. Iniciaremos explorando el uso estratégico de *screencasts* como herramientas para fomentar el aprendizaje activo, permitiendo a los estudiantes revisar conceptos complejos a su propio ritmo y reforzar su comprensión mediante ejemplos visuales dinámicos.

A continuación, se discutirá la incorporación de la evaluación colaborativa mediante rúbricas electrónicas, que no solo facilita una evaluación más objetiva y transparente, sino que también promueve la autoevaluación y la retroalimentación constructiva entre los estudiantes. Este enfoque ha demostrado mejorar significativamente la calidad del aprendizaje y el desempeño académico.

La ponencia también abordará la implementación de la metodología de aprendizaje y servicio (ApS), donde los estudiantes aplican sus conocimientos en proyectos que benefician a la comunidad, permitiéndoles desarrollar un sentido de responsabilidad social.

Finalmente, se analizará el uso de la analítica de aprendizaje como una herramienta para la mejora continua del proceso educativo. La analítica de aprendizaje permite monitorear el progreso de los estudiantes, identificar áreas de dificultad y ajustar las estrategias de enseñanza en tiempo real, optimizando así los resultados educativos.

A través de estas experiencias, se demostrará cómo la combinación de estas metodologías ha transformado la educación en Bioquímica y Biología Molecular, proporcionando una educación más dinámica, participativa y orientada a la práctica. La integración de estas innovaciones no solo enriquece el aprendizaje, sino que también prepara a los estudiantes para enfrentar los desafíos del mundo real con una base sólida de conocimientos y habilidades.

Keywords: Metodologías Innovadoras, Aprendizaje Activo, Evaluación Colaborativa, Aprendizaje Y Servicio, Analítica De Aprendizaje

S. ED - 365 - O

El Aprendizaje Basado en Investigación: una herramienta eficaz para impulsar la competencia Compromiso Social en estudiantes de Biociencias

L. RUTH MONTES

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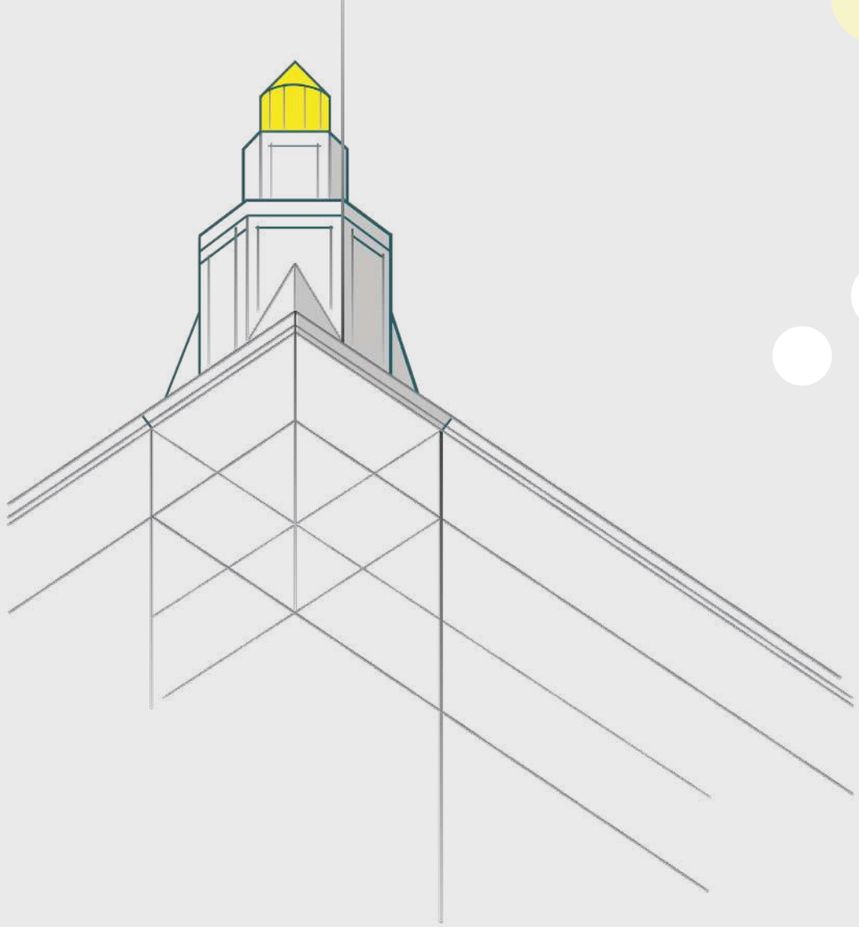
La competencia transversal "Compromiso social" es una de las competencias menos desarrolladas en los grados de Biociencias. El proyecto de innovación educativa i³KD "Bi³oGela" tiene entre sus objetivos la alfabetización del alumnado en el área de los Objetivos de Desarrollo Sostenible (ODS) a través de la sinergia entre dos asignaturas (Tecnología del DNA Recombinante y Técnicas Instrumentales) de los grados en Bioquímica y Biología Molecular y Biotecnología. Mediante el Aprendizaje Basado en Investigación, el estudiantado diseña un proyecto de investigación para llevar a cabo la sobreexpresión y purificación de una proteína cuyo uso responda a un reto social. El proyecto debe estar enmarcado de forma razonada en uno o varios ODS, por lo que el alumnado, además de recibir la formación estrictamente curricular de las asignaturas, se forma también en aspectos relativos a la Agenda 2030 y los ODS. Una prueba piloto previa a la implantación del proyecto "Bi³oGela" realizada durante el curso 2022-2023 mostró que el alumnado, a lo largo del curso, adquiere un mayor conocimiento y concienciación sobre los ODS y la Agenda 2030 y es capaz de implantar este conocimiento en las tareas formativas de su grado. Todo ello invita a pensar que la sinergia entre asignaturas de un mismo grado y el abordaje de los ODS mediante un Aprendizaje Basado en Investigación constituye una herramienta eficaz para trabajar la competencia transversal "Compromiso social" en Biociencias.

Keywords: Compromiso Social, Objetivos De Desarrollo Sostenible (ODS), Sobreexpresión, Purificación Proteica, Aprendizaje Basado En Investigación.



SIMPOSIO 1

S1.1. SIMPOSIO HISPANO MEXICANO EN METABOLISMO

S1.1 - 1

Metabolic Drivers of Cancer and Obesity-related Diseases

GUADALUPE SABIO

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Hepatocellular carcinoma (HCC), the predominant form of primary liver cancer, is the third most lethal cancer globally. With the escalating obesity epidemic, the incidence of HCC is projected to increase by 55% by 2040. In the context of obesity, where HCC incidence is fourfold higher, there is a critical need to predict which individuals will progress to liver cancer or develop non-alcoholic steatohepatitis (NASH), a significant precursor to HCC. Stress-activated protein kinases (SAPK) including p38 and JNK are important player in its regulation. Several studies have tried to unravel their role; we will discuss the role in inmmune system and in hepatocytes.

In this talk, I will present how obesity is trigger of cancer through the dysfunction of adipose tissue that alterations in the secretion of adipokines. This positions AT as a pivotal endocrine organ with a profound impact on whole-body metabolism and liver cancer development. In this context, we have identified specific adipokines correlated with a predisposition to cancer.

These findings hold promise for advancing our understanding of the link between obesity and liver cancer and identifying new therapeutic targets in the ongoing battle against HCC.

Keywords: Obesity Cardiac Disese Cancer Adipose Tissue

S1.1 - 2

Investigating the HIF-mitochondria interface in health and disease

AURORA GOMEZ-DURAN

CIMUS, Santiago de Compostela

Mitochondrial DNA (mtDNA) variants influence the risk of rare and late-onset human diseases, but the reasons for this are poorly understood. Interestingly, the same variant exert a great variability in disease penetrance in each individual, which suggests the existence of a complex system that does not necessarily imply the dysfunction of the energy synthesis. In here, through the combination of multi-omics approaches on several human models under normoxic and hypoxic conditions, we will describe how variations in oxidative phosphorylation system capacity driven by the mtDNA variants modulate oxygen driven response and their role in cancer and mitochondrial diseases.

S1.1 - 3

Through the pregnane X receptor (PXR), dengue virus modulates the immune response and lipid metabolism in mouse peritoneal macrophages

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Dengue virus is the main mosquito-transmitted virus in the world, causing 600,000 people annually to develop severe forms of the illness, such as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). The latter is the result of reinfection with a different serotype, which makes the preparation of vaccines difficult. In recent years, a substantial increase in cases has been observed, presumably due to climate change. Among the strategies to treat this disease are those that interfere with the signaling pathways that the virus uses to infect its target cells such as macrophages and dendritic cells. We have identified that, in mouse peritoneal macrophages (MF), dengue virus serotype 2 (DENV2) activates the Pregnane X Receptor (PXR). PXR is a ligand-dependent transcription factor involved in cell protection from toxic insults by inducing the expression of drug metabolizing enzymes. It also promotes lipid metabolism and has been identified as a negative regulator of the inflammatory response. We also uncovered that, activation of PXR in MF by DENV2 results in an increased droplet lipid biogenesis together with an inhibition of the inflammatory response, essential conditions for virus replication. In contrast, pharmacological inhibition of PXR results in a decrease in lipid droplets, required for virus assembly and restored the inflammatory cytokine levels decreasing virus replication. These results point to PXR as a therapeutic target to treat this disease.

S1.2. MOLECULAR REGULATION OF QUIESCENCE IN ADULT STEM CELL POPULATIONS

S1.2 - 1

A full, dynamic view of neural stem cell quiescence

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Abstract

Neurogenesis continues throughout life in most mammals, but only in specific brain regions or neurogenic niches. There, adult neural stem cells (aNSCs) are found in a distinct and reversible G0 metabolic state known as quiescence. Once activated, aNSCs generate new neurons that integrate into existing mature circuits to modulate their function. However, they do not self-renew very efficiently, linking activation to depletion. My group explores how aNSC quiescence is regulated by intrinsic and extrinsic factors, and how these factors affect the rate and maintenance of adult neurogenesis in the hippocampal niche. Based on our own work and the literature, we propose that rather than a linear transition from quiescence to activation, aNSCs exist in a complex and fluid cloud of states which increases their heterogeneity. Heterogeneity is in fact key for aNSC function, as these cells need to respond to extremely diverse cues and stimuli as a population but should also preserve their pool to sustain neurogenesis during the whole lifespan of the organism. In my talk, I will summarize our efforts to build an aNSC states matrix based on single sequencing data. I will also present our recent data on the effects of intermittent fasting on aNSCs and neurogenesis.

S1.2 - 2

Dynamics of muscle stem and niche cells in homeostasis and regeneration

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The quiescent state of muscle stem cells (MuSCs) is intricately controlled by multiple signaling pathways, making MuSCs one of the most extensively studied populations of cells in G0 phase. While a universal molecular signature for stem cell quiescence across various tissues has yet to be identified, certain common traits such as diminished cytoplasmic and mitochondrial content and reduced metabolic activity are universally observed, suggesting a protective role for this cell state. Pathological conditions that cause acute or chronic inflammation, such as influenza and cancer cachexia, generate systemic signals with far-reaching impacts on several tissues and organs. For example, although influenza primarily affects the respiratory system, it can lead to muscle weakness, while cancer cachexia causes significant muscle and fat loss. Both conditions stimulate a host immune response and widespread inflammation. The effects of these pathological states on tissue-specific quiescent stem cells have been largely unexplored. We show that quiescent MuSCs experience alterations in cell size and downregulation of key regulators of quiescence, yet cell cycle entry, commitment, and differentiation processes are disrupted. These changes lead to impaired and delayed muscle regeneration post-injury. Notably, despite not being directly exposed to pathogens or cancer cells, MuSCs display immune and stress-related responses. We propose that exposure to pathology-induced systemic inflammation prompts MuSCs to enter a novel quiescent state, significantly altering their molecular and functional characteristics.

S1.2 - 3

Notch trans-activation to cis-inhibition switch underlies hematopoietic stem cell aging

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Abstract

Notch signaling plays a key role during the emergency of hematopoietic cells from the aorta-gonad-mesonephros but, already after embryonic day 11.5, hematopoietic stem cells (HSCs) reduce Notch activity, hindering its involvement during adulthood and aging. While the expression of Notch ligands in the bone marrow (BM) niche is necessary for adult hematopoiesis, the impact of Notch signaling for adult and aged HSC function remains controversial.

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Here we show that in the BM of young mice Notch activation is not homogeneous within the HSC pool and depends on the sinusoidal expression of the Notch ligand Jagged2 (Jag2). Deleting sinusoidal Jag2 decreases Notch activity in HSCs, alters their localization at sinusoids and affects the frequency and the quality of stem cell divisions, impairing HSC regenerative potential. Strikingly, Notch activity in HSCs is strongly reduced upon aging and like in sinusoidal Jag2 knock-out mice, aged stem cells upregulate Jag2, which promotes HSC clustering by Notch signaling cis-inhibition.

Collectively, these findings highlight a critical role for sinusoidal Jag2-dependent Notch activation in preserving quiescence and regenerative potential of adult HSCs and disclose a Notch trans-activation to cis-inhibition switch underlying HSC aging.

S1.3. WHEN MOLECULAR BIOLOGY EMBRASES NANOTECHNOLOGY: THE RNA THERAPIES ERA

S1.3 - 1

Impact of Nanomaterials in the development of new RNA Vaccines and Advanced Therapies

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Nanomaterials hold great promise in advancing precision medicine by enhancing drug access to their intended therapeutic targets. Additionally, nanotechnology plays a pivotal role in the development of RNA-based therapies and vaccines. Our laboratory has actively contributed to advancing targeted drug delivery of biological drugs, notably polynucleotides. Starting in the 90's with the encapsulation of DNA in polymeric particles and, recently moving into polymer/lipid hybrid nanocarriers, we have been exploring the potential of our nanocarriers in a variety of disease areas, including cancer, ocular diseases, brain diseases and fibrotic malignancies.

More information about these projects and associated publications can be found at:

http://www.usc.es/grupos/mjalonsolab/

My plan is to moderate a session, in which we can disclose the potential of a variety of nanoparticles for the treatment of a variety of diseases as well as the main challenges ahead for the targeted delivery of RNA molecules and gene editing.

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S1.3 - 2

Emerging nanocarriers for RNA Delivery

CRISTINA FORNAGUERA

NanoTherapies Lab (NanoTher), Grup d'Enginyeria de Materials (Gemat), Institut Químic de Sarrià (IQS), Universitat Ramon Llull (URL)

Emerging (nano)therapies are revolutionizing the field of healthcare, with RNA therapies at the forefront. These therapies, which are becoming the next-generation standard of care, are addressing a multitude of unmet medical needs, including neural diseases, rare disorders, and tumor therapeutic vaccination. However, the delivery of these therapies presents challenges. RNA, namely mRNA, is susceptible to degradation by nucleases and easily recognized and cleared by the immune system due to its large size. To overcome these issues, the development of nanometric carrier systems is crucial.

Our proprietary oligopeptide end-modified poly (beta-aminoester) polymers (pBAE), a type of biocompatible, biodegradable, robust, and versatile polymers have emerged as promising carriers for the electrostatic complexation of RNAs. They offer a novel platform for the protection of the RNA and for safe, targeted, and efficient *in vivo* delivery. We demonstrated pBAE nanoparticles ability to selectively target RNAs to specific cells of interest, such as tumor cells, and to cross biological barriers using non-invasive delivery routes, such as the blood-brain barrier. Here, after a deep description of these polymers, we present groundbreaking examples at the intersection of emerging RNA therapies and nanomedicine. Through the different uses, including a gene therapy for a rare neurological condition, and melanoma cancer vaccination, we demonstrate how pBAEs are opening new avenues for addressing unmet medical needs.

Keywords: RNA therapeutics; polymeric nanoparticles; poly(beta aminoester) polymers; targeted delivery

S1.3 - 3

Lipid Nanoparticles for Overcoming Biological Barriers to mRNA Delivery

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Recent years have witnessed tremendous developments and breakthroughs in the field of RNA-based therapeutics and vaccines. The distinct mechanisms of exogenous RNAs and analogs, including messenger RNAs, small interfering RNAs, microRNAs, and antisense oligonucleotides, have brought them unprecedented potential to treat a variety of pathological conditions. However, the widespread application of RNA therapeutics and vaccines is hampered by their intrinsic features (e.g., instability, large size, and dense negative charge) and formidable host barriers. Development of safe and efficient vectors is key for successful delivery and translation of RNA therapeutics and vaccines. In this talk, I will discuss our efforts towards the development of lipid nanoparticle (LNP) platforms that enable the delivery of RNA therapeutics and vaccines to a range of target cells and tissues in the body. Furthermore, I will describe new therapeutic strategies utilizing these LNPs including (i) in vivo reprogramming of immune cells for cancer immunotherapy and vaccination, (ii) in utero gene editing for treating disease before birth, and (iii) mRNA prenatal therapeutics for treating pregnancy disorders such as pre-eclampsia.

S1.3 - 4

Computational approaches in improving RNA nanocarriers

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Delivery is the major hurdle thwarting the therapeutic potential of RNA medicines.[1] While all siRNA drugs on the market target the liver, the lung offers a variety of currently undruggable targets which could be treated with RNA therapeutics. Hence, my lab **rationally designs inhalable and biocompatible nanocarriers** for efficient siRNA **delivery to the lung**.

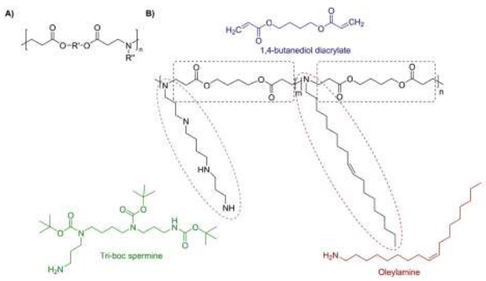

Figure 1: A) Poly(*β*-amino ester)s (PBAEs) and **B**) synthesis of amphiphilic, spermine-based PBAEs by Michael addition polymerization and boc-deprotection with trifluoro acetic acid.

Poly(beta-amino ester)s (Fig. 1) are biodegradable cationic polymers capable of promoting nucleic acid delivery *in vitro* and *in vivo*[2-4] and can be tailormade to investigate structure–function relationships.[5] While biomaterials are commonly synthesized in large libraries6 and optimized empirically via one-variable-at-a-time experimentation, we combine Design-of-Experiments (DoE) with Molecular Dynamics Simulations and Machine Learning (ML) to accelerate the discovery and optimization process of polycationic siRNA nanocarriers at reduced wet-lab resources.[7] siRNA encapsulation by polycations bears the disadvantage of a less defined morphology of the resulting nanoparticles in comparison to LNPs which has been reported to lead to polydisperse particle size distribution and poorly reproducible formulations.[8] By microfluidic assembly, both disadvantages can be addressed efficiently, however, for the formulation of defined and reproducible RNA nanocarriers.[9] Sophisticated *in vitro* and ex vivo models of the lung are available to screen therapeutic efficacy against respiratory viral infections,[10] against transcription factors upregulated in asthma[11] and COPD or against mutated oncogenes[12] in

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the presence of relevant biologic barriers.[13]

Our previous results show the feasibility of synthesizing oleylamine-modified spermine-based poly(β-amino ester)s (PBAEs) that efficiently encapsulate siRNA into nanoparticles <100 nm at low polymer excess ratio (N/P 5-10) for up to 95% gene silencing *in vitro*. This finding is very important to decrease the excess of polymer in the nanoparticle formulation to avoid problems of toxicity and polymer-induced side effects,[14] while literature commonly describes 30- to 900-fold PBAE weight excess over siRNA, corresponding to similarly high N/P ratios.[2-4] The PBAE-based polyspermines successfully delivered siRNA for gene silencing in 2D cultures and Transwell® air-liquid-interface cultures.[15] Additionally, Boc-protected PBAE-based polyspermines mediated therapeutic gene silencing of mutated KRAS12 resulting in impaired cell migration.[16]

In an effort to compare polymer backbones, polyacrylamide (PAA)-based polyspermines were synthesized and resulted in more efficient siRNA delivery and gene silencing in Transwell® air-liquid-interface cultures compared with Lipofectamine but had a much more favorable safety profile *in vitro* and *in vivo*. After intratracheal administration to mice, the PAA-based polyplexes were efficiently taken up by Type II pneumocytes and successfully evaded recognition by macrophages in the lung.[17]

Additional *in vivo* efficacy experiments are currently under way. Based on our preliminary data, polyspermines seem to be very promising candidates for RNA formulation and delivery with an excellent safety profile in the lung.

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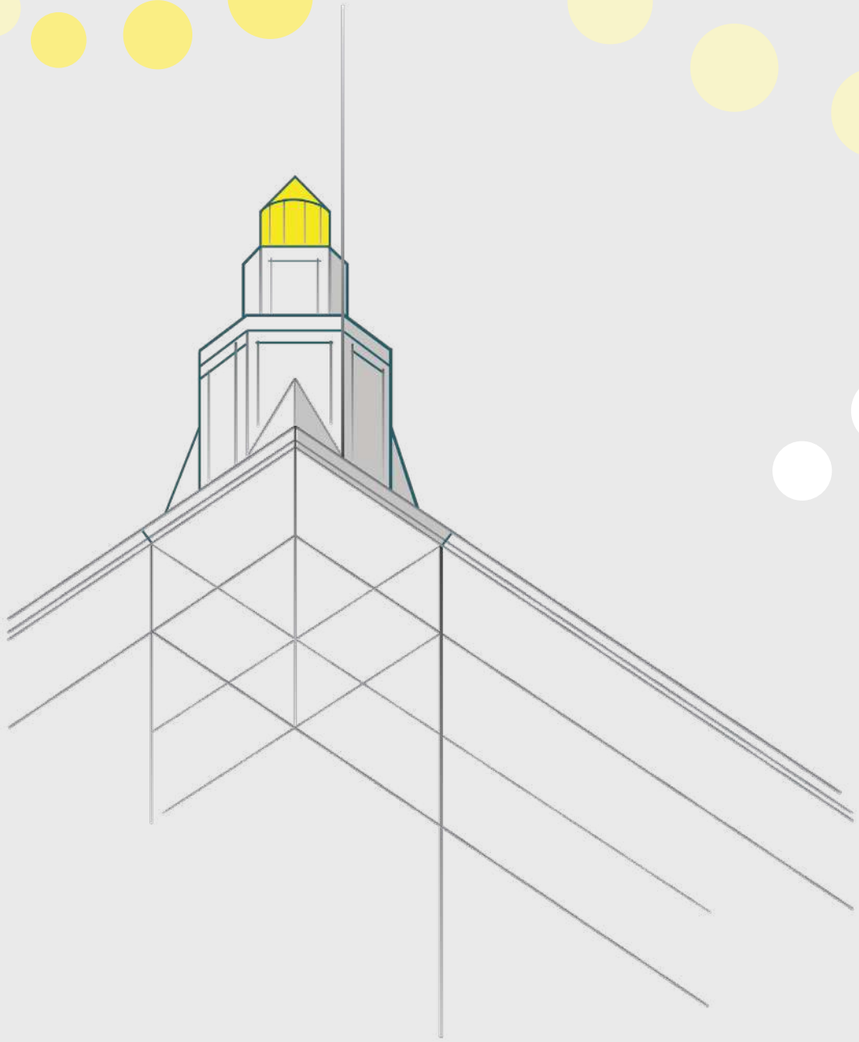

SIMPOSIO 2

S2.1. TARGETING MYELOID CELLS IN SOLID TUMORS: WHERE ARE WE?

S2.1 - 1

Mining immunomodulatory myeloid programs in cancer

MARIA CASANOVA-ACEBES

CNIO, Madrid, Madrid, España

Recent advances in tumor immunology have uncovered a tremendous complexity of myeloid cellular states. However, before trying to design therapeutic strategies targeting these pathogenic states, we need a deeper understanding of the biology of myeloid cells in solid tumor microenvironments by functional means.

As myeloid cells represent the most abundant and diverse compartment across all leukocytes, our goal is to identify and manipulate innate leukocytes as a first approach to harness anti-tumor immunity.

In this talk, I will cover the latest advances from my laboratory, in which we particularly focus: *(1)* on the temporal timing of myeloid-focused immunotherapies; *(2)* the identification of novel innate biomarkers in lung metastasis, and *(3)* how we envision cancer evolution, taking advantage of molecular tools to trace and map clonal co-evolution between tumors and the immune microenvironment at metastatic sites.

Keywords: Myeloid Cells, Solid Tumors, Heterogeneity

S2.1 - 2

Leveraging innate immunity to improve cancer control and immunotherapy response.

HIND MEDYOUF

Georg Speyer Haus, Institute for Tumor Biology and Experimental Therapy, Germany

Over the last decades, our understanding of cancer has undeniably evolved from a tumor cell centric view towards a more comprehensive “tissue-like” picture integrating extrinsic cues from the surrounding tumor microenvironment (TME) to shape a local ecosystem that favors the acquisition of the so called “hallmarks of cancer”. This has fueled the idea that deciphering the TME signals cancer cells rely on, could help unravel tumor vulnerabilities and pave the way to new treatment opportunities. However, such niche-dependencies do not follow a “one size fits all” pattern but are rather “made to tailor” as they can be highly dependent on the cancer type, disease stage, organ site as well as dynamically evolving niche functions. In this meeting, I will discuss our ongoing efforts to map organ specific TME contributions that promote cancer progression and limit the benefit of adaptive immune checkpoint therapies by specifically hindering the early steps of the “cancer immunity cycle”, both in hematological malignancies and metastasis. I will also share how we leverage the gained knowledge for the rational design of new therapeutic combinations that could benefit patients.

S2.1 - 3

Epigenetic co-option of epithelial-immune crosstalk in pancreatic cancer initiation

DIRENA ALONSO CURBELO

Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology

Cancer results from a complex interaction between genetics and environmental insults that triggers changes in cell and tissue state. These changes are highly reminiscent of regenerative inflammatory processes yet, paradoxically, contribute to cancer development and metastatic progression. To understand how oncogenic mutations rewire physiological inflammatory responses to drive tumorigenesis, we integrated single-cell profiling methods and functional genomics tools to characterize and perturb molecular and cellular networks defining normal, inflamed, pre-malignant, and malignant tissues in autochthonous models of pancreatic cancer. We uncovered aberrant chromatin states in the pancreatic epithelium that are uniquely induced by a cooperative interaction between inflammation and oncogenic KRAS, and which distinguish neoplastic transformation from normal regeneration (Alonso-Curbelo et al. Nature 2021). These early epigenomic alterations endow discrete KRAS-mutant epithelial cells with an enhanced capacity to drive and sense inflammation and establish feedback communication loops with immune cells in their environment that define and direct tumorigenesis (Burdziak*, Alonso-Curbelo* et al. Science 2023). Building upon this experimental and conceptual groundwork, our ongoing studies are dissecting aberrant epithelial-immune crosstalk programs at the onset of tumorigenesis, aiming to define how these reprogram both tumor and immune cell states in pre-malignancy and cancer. We propose that a better understanding of how regenerative immune responses go awry early during tumor development will uncover new concepts that may be exploited to prevent and intercept inflammation-driven tumorigenesis.

S2.2. THE IMPACT OF INTEGRATIVE RESEARCH INFRASTRUCTURES IN STRUCTURAL BIOLOGY

S2.2 - 1

The Spanish Instruct-ERIC Center: Our current access offer and our vision of the future

JOSÉ MARÍA CARAZO, MARCOS GRAGERA, ROBERTO MERELO, CARLOS OSCAR SORZANO, JAVIER CHICHÓN, ROCIO ARRANZ, MARIA TERESA BUENO-CARRASCO, JOSÉ MARÍA VALPUESTA

Spanish National Biotechnology Center CNB-CSIC, Madrid, Madrid, España

Research infrastructures (RI) are facilities that provide resources and services for research communities to conduct research and foster innovation. At the European level, they are often organized as European Research Infrastructure Consortia (ERIC), as it is the case for Instruct, the RI addressing Integrative Structural Biology. Instruct-ERIC services/technologies cover sample preparation, biomolecular and structural analysis (X-ray, NMR, cryoEM). By providing access to cutting-edge structural biology services, technologies and expertise in 11 centers across Europe, Instruct has been involved in almost 2000 publications in the past 15 years, paving the way for researchers to accomplish structural studies when the lack of expertise, time or resources becomes the limiting factor.

The Instruct-ERIC Spanish Center (Instruct-ES) is currently focused on Cryogenic Electron Microscopy (cryoEM), with two facilities, one being the leading-edge instrument microscopy facility localized at the CNB-CSIC (cryoEM CNB-CSIC), while the second one is the single image processing facility of Instruct, referred to as the Instruct Image Processing Center (I2PC). The two facilities are very popular in terms of being demanded by Instruct-ERIC users, at the same time that Spanish scientists are one of the most active communities in accessing other facilities throughout Europe.

As the future of Structural Biology widens, Instruct-ES also evolves, with the prospect to include in the near future NMR facilities, new cryoEMs and some beamlines of the Spanish synchrotron ALBA.

ACKNOWLEDGEMENTS: We acknowledge the financial support from the Ministry of Science, Innovation and Universities (BDNS n. 716450) as part of the Spanish participation in Instruct – ERIC

Keywords: Structural Biology, Infrastructures

S2.2 - 2

Deciphering the structure of the Vault particle of *Dictyostelium discoideum*

MARÍA GONZÁLEZ-ÁLAMOS, PABLO GUERRA, ROBERTO MELERO AND NÚRIA VERDAGUER

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Vault particles (~10 MDa) are cargo delivery nanodevices, with ample phylogenetic distribution across eukaryotes, however, their biological function or payload specialization is not completely understood [1]. The vault shell is organized in two symmetrical moieties, each consisting of 39 copies of the Major Vault Protein (MVP), which assemble to form a barrel-like cage with an enormous interior volume [2-3]. Using a combination of vault recombinant reconstitution, biophysics and cryo-electron microscopy (cryo-EM) techniques, we have previously characterized the structural dynamics of rat vaults, providing a mechanistic model for the vault opening-closing cycle with functional implications for cargo encapsulation, transport, and delivery [4].

Surprisingly, given the high conservation of vault particle among species, it is remarkable that *Dictyostelium discoideum* is the only organism described so far possessing two different MVP isoforms: MVPα and MVPβ and the presence of both proteins are essential for vault assembly. This fact raises the biological question whether *Dictyostelium* vaults are hetero-oligomers formed by MVPα and MVPβ and how they are organized in the structure, considering the odd number of MVPs that make up the complete vault. To address this challenge, we developed a strategy involving the design of a vault-nanobody complex, relying on the selective recognition of nanobodies bound specifically to MVPβ, which contained a myc-tag artificially inserted in an exposed loop of the R6 domain. Through cryo-EM and single-particle analysis, performed in part at the high-performance cryo-EM image computing center at CNB-CSIC (Madrid), we structurally characterized the vault-nanobody complex resulting in 3D maps where the nanobody densities were clearly visible. This approach revealed valuable insights into the organization of MVPα and MVPβ subunits in *Dictyostelium* vaults that will be discussed in this talk.

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S2.2 - 3

Decoding Chikungunya Virus Membrane-Associated Replication

JUAN REGUERA

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Positive-sense single-stranded RNA viruses, such as coronaviruses, flaviviruses and alphaviruses, carry out transcription and replication inside virus-induced membranous organelles within host cells. The remodeling of the host-cell membranes for the formation of these organelles is coupled to the membrane association of viral replication complexes and RNA synthesis. These viral niches allow for the concentration of metabolites and proteins for the synthesis of viral RNA, which prevents the detection of this RNA by the cellular innate immune system. I will present the cryo-electron microscopy structures of non-structural proteins 1 and 3 (nsP1 and 3) of the alphavirus chikungunya virus, which are responsible for RNA capping and membrane binding of the viral replication machinery (nsP1) and the interaction with host factors and generation of cytoplasmic non-membranous organelles (nsP3). nsP1 structure shows the enzyme in its active form, assembled in a monotopic membrane-associated dodecameric ring. The structure shows how the complex formation couples the membrane binding, oligomerization and allosteric activation of the capping enzyme. The nsP3 structure shows tubular macro-assemblies present in both viral replication complexes and in honeycomb-shaped cytoplasmic granules which gather host and viral proteins and RNA. Our results provide high-resolution information about the membrane association of the replication machinery of positive-sense single-stranded RNA viruses, and the structural basis of the generation of membrane and non-membrane viral organelles which control the spatiotemporal organization of viral action for taking over the cell and lead to a productive infection. Here we provide a new understanding of alphavirus infection opening up new avenues for the generation of antivirals.

S2.3. EN LA FRONTERA DE LA REGULACIÓN GÉNICA: AVANCES EN EPITRANSCRIPTÓMICA

S2.3 - 1

Exploring the impact of RNA modifications in pluripotency and ageing

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Post-transcriptional modifications can modulate the structure and function of different RNA types. In recent years, RNA modifications (also referred to as “the epitranscriptome”) and the writers, readers, and erasers that fine-tune these marks have been implicated in the regulation of physiological and pathological processes, including age-related diseases. In our laboratory, we aim to characterize the epitranscriptomic pathways involved in the maintenance of the youthful cellular state which are altered during ageing. Epigenetic alterations are well correlated with ageing and DNA methylation can be used to predict the chronological age. However, how RNA modifications, in particular RNA methylation, are affected during ageing and whether they contribute to the ageing process itself remains greatly unexplored. To address the role of 5-methylcytosine (m^5C) on RNA during cellular ageing, we performed bisulfite-sequencing (BS-seq) on rRNA-depleted total RNA isolated from fibroblasts derived from “young” (2 months-old) and “old” (28 months-old) mice. Transcriptome profiling of these cells indicated that the ageing signature described in vivo is greatly maintained in our *in vitro* system. Importantly, BS-seq analysis revealed a modest increase of m^5C global levels in old mice samples. Mapping of m^5C-modified sites resulted in the identification of several hundred sites in messenger RNAs, some of which change in an age-dependent manner. Our work uncovers for the first time a correlation between ageing and m^5C modification in mRNA and highlights the importance of investigating ageing from an epitranscriptomic perspective.

S2.3 - 2

The role of adenine mRNA methylation in cancer

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N6-methyladenosine (m^6A) is the most abundant internal modification of eukaryotic mRNAs. m^6A affects the fate of its targets in all aspects of the mRNA life cycle and has important roles in various physiological and pathophysiological processes. METTL3 is the major writer of N6-Methyladenosine (m^6A) and has been associated with controversial roles in cancer. This is best illustrated in urothelial carcinoma of the bladder (UCB), where METTL3 was described to have both oncogenic and tumor-suppressive functions. Our results show that *METTL3* knockout reduced the oncogenic phenotype and m^6A levels of UCB cell lines. However, expression and survival analyses of clinical data revealed a highly complex role for METTL3 in UCB, with decreased m^6A mRNA levels in UCB tumors. To avoid this complexity, we have focused our current research the m^6A *reader* YTHDC1, which is downregulated in muscle-invasive bladder cancer and negatively correlates with the expression of epithelial-mesenchymal transition genes. Integrative analysis of multi-modal sequencing datasets provided detailed insights into the molecular mechanisms mediating YTHDC1-dependent phenotypes and identified *SMAD6* as a key transcript involved in the invasiveness of UCB. Notably, *SMAD6* showed reduced colocalization with YTHDC1 in tumoral tissues compared to paratumoral tissues, indicating disrupted binding during cancer progression. Our findings thus establish YTHDC1-dependent m^6A reading as a critical epitranscriptomic mechanism regulating UCB invasiveness

S2.3 - 3

Presenting: Targeting tRNA methylases in cancer

SANDRA BLANCO

Centro de Investigación del Cáncer, CSIC -University of Salamanca, 37007 Salamanca, Spain.

Non-mutational events, particularly RNA post-transcriptional modifications, are emerging as key players in tumour development and progression in several cancer types. Emerging evidence show that self-renewal, survival and migration are regulated by epitranscriptomic marks, which may be a potential therapeutic target to specifically eliminate cancer cells.

By using genomic screenings, epitranscriptomic tools, CRISPR/Cas9 technology, proteomics, cell and mouse models and patient samples we aim to decipher the epitranscriptome in prostate cancer in order to implement novel therapeutic strategies. We found that overexpression of novel transfer RNA (tRNA) methyltransferases correlated with poor prostate cancer prognosis. Loss-of-function analyses resulted in reduced protein synthesis. Mechanistically reduced tRNA methylation resulted in reduced global protein synthesis of cell cycle and metabolic genes, but increased expression secretion and interferon signalling pathways regulators, resulting in increased infiltration of pro-inflammatory immune cells in tumours, cell proliferation and invasion *in cellulo*, and tumour formation and metastasis *in vivo*. In summary, we find that tRNA modifications regulate cell proliferation and invasion, metabolic pathways and the tumour microenvironment crosstalk by adapting the translational machinery.

Targeting tRNA methylation is emerging as an effective therapeutic tool to eliminate cancer cells. Whether targeting tRNA methylation alone, or in combination with agents targeting metabolic pathways or immunotherapy can be used as effective therapeutic tools needs further validation.

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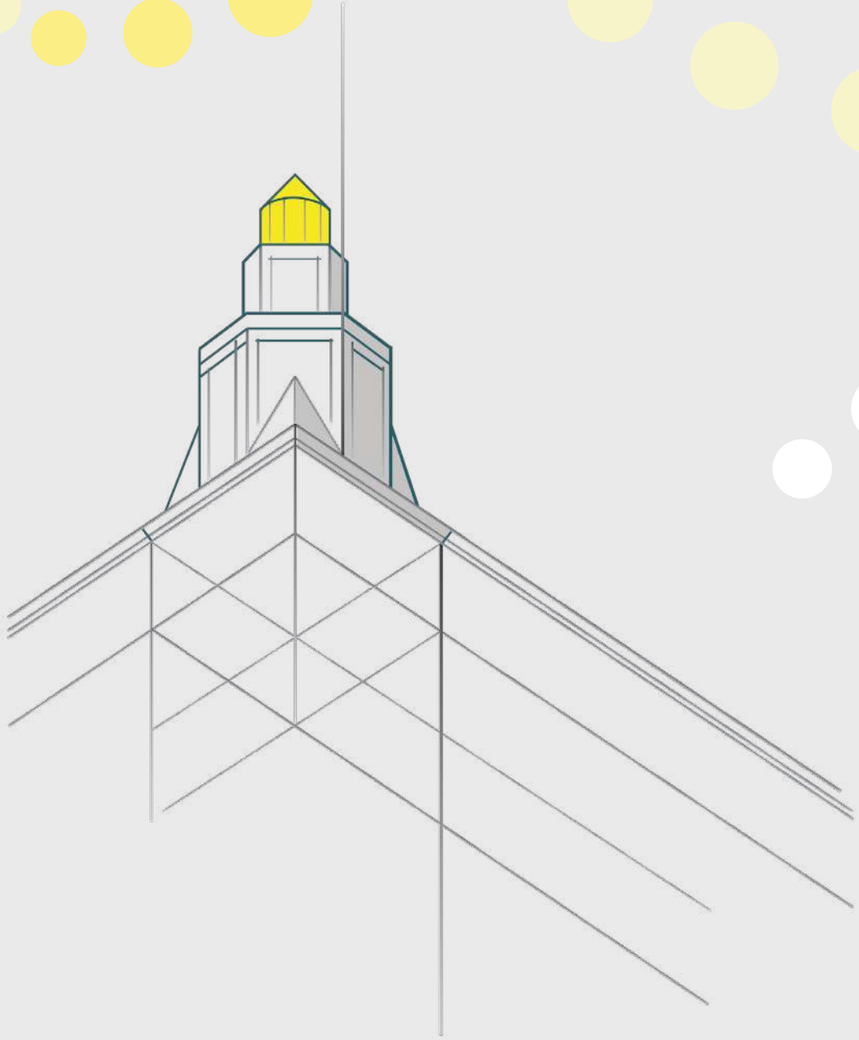

SIMPOSIO 3

S3.1. THE FUNCTIONAL DIVERSITY OF THE STRUCTURAL DISORDER

S3.1 - 1

Molecular ordering of intrinsically disordered domains controls the dynamics of cellular condensates.

IRENE DÍAZ-MORENO

Institute for Chemical Research – Centre of Scientific Research Isla de la Cartuja (IIQ – cicCartuja), University of Seville – CSIC, Spain

Use protein compartmentalization by liquid-liquid phase separation (LLPS) to control biochemical reactions spatially and temporally. Among them, nucleolar trafficking and arrest of mRNA translation in cytoplasmic stress granules (SGs) occur by biomolecular condensation. Within this frame, nucleophosmin (NPM1) is a histone chaperone involved in nucleolar ribosome synthesis and chromatin remodeling, while T-cell intracellular antigen 1 (TIA-1) is the main SGs nucleator.

In the nucleolus, NPM1 harbors cytochrome *c*—which translocates from mitochondria to the nucleus in response to DNA damage—in the cavity formed by its arms, the disordered acidic middle regions of NPM1. Cytochrome *c* does trigger an extended-to-compact conformational change in NPM1 so as to drive the release of the ARF tumor suppressor from the condensate[1].Nuclear cytochrome *c* thus emerges as a small globular protein with sequence-encoded heterotypic phase-separation properties, with its lysine-rich clusters being responsible for controlling the trafficking and availability of nucleolar proteins[2,3].

Regarding TIA-1, phosphorylation triggers the disorder-to-order transition resulting from the assembly of a β-hairpin at the intrinsically disordered domain, thereby facilitating self-association into SGs[4]. Since several mutations in the unstructured domain of TIA-1 have been linked to aberrant SGs and neurodegenerative diseases, a better understanding of the molecular mechanisms underlying the liquid de-mixing of TIA-1 may provide valuable data for the development of novel therapeutic treatments.

References:

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4. Velázquez-Cruz *et al.* (2024) *Nat. Comm. submitted*

S3.1 - 2

Kinetic stabilization of translation-repression condensates by a neuron-specific microexon

XAVIER SALVATELLA

ICREA and IRB Barcelona

The inclusion of microexons by alternative splicing is frequent in neuronal proteins. The roles of these sequences are in most cases unknown, but changes in their degree of inclusion are associated with neurodevelopmental diseases. We recently found that the decreased inclusion of a 24-nucleotide neuron-specific microexon in CPEB4, an RNA-binding protein that regulates translation through cytoplasmic changes in poly(A) tail length, is linked to idiopathic autism spectrum disorder (ASD). Why this microexon is required and how small changes in its degree of inclusion generate a dominant-negative effect on the expression of ASD-linked genes is not clear. Here we show that neuronal CPEB4 forms condensates that dissolve upon depolarization, a transition associated with a switch from translational repression to activation. Heterotypic intermolecular interactions between the microexon and a cluster of histidine residues kinetically stabilize the condensates by competing with homotypic interactions between clusters, that otherwise lead to the irreversible aggregation of CPEB4. We conclude that the microexon is required in neuronal CPEB4 to preserve the reversible regulation of CPEB4-mediated gene expression in response to neuronal stimulation.

S3.1 - 3

Towards a mechanistic understanding of phase transitions driven by protein homorepeats

JAVIER OROZ

Científico Titular del CSIC, Grupo de RMN de Proteínas, Instituto de Química Física Blas Cabrera, Madrid

Abnormal trinucleotide repeat expansions alter protein conformation causing malfunction and contribute to a significant number of incurable human diseases. Due to the repetitive, polymorphic and dynamic nature of expanded homorepeats, their structural study is highly challenging and only sparse structural insights are available on homorepeat-expanded proteins related with disease. This lack of knowledge is detrimental towards the design of effective therapeutics. For the essential transcription factor PHOX2B, polyAlanine repeat expansions in a 20-alanine tract are associated with Congenital Central Hypoventilation Syndrome (CCHS), a rare incurable disease affecting mainly children. PHOX2B aggregation triggered by long polyAlanine expansions is one of the fundamental mechanisms proposed to explain PHOX2B dysfunction in CCHS. However, the structural basis of the aggregation-prone PHOX2B mutants is still unknown. To help understand the pathogenic nature of polyAlanine expansions in PHOX2B, we have determined the structure and dynamic properties of PHOX2B C-terminal fragment by Nuclear Magnetic Resonance spectroscopy (1). Interestingly, while polyalanine expansions do not affect PHOX2B main structural conformations, they promote nascent conformations that prompt a length-dependent phase transition towards small, solid condensates that capture wild-type PHOX2B. I will show that there is a direct correlation between increase in disorder and phase transitions, since HSP70 and HSP90 chaperones specifically seize PHOX2B alternative conformations blocking de-mixing. The direct observation of the nascent polymorphs in expanded PHOX2B leads us to propose a structural basis for the unbalanced phase separation, interpreted as a novel pathophysiological mechanism in homorepeat expansion diseases.

References:

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S3.2. AI IN SERVICE OF MOLECULAR BIOLOGY: PERSPECTIVES AND CHALLENGES

S3.2 - 1

AI in Service of Molecular Biology: Perspectives and Challenges

CARLOS FERNANDEZ-LOZANO

Universidade da Coruña, Centro de Investigación en Tecnologías de la Información y Comunicación (CITIC), A Coruña

The symposium “AI in Service of Molecular Biology: Perspectives and Challenges” explores the transformative impact of artificial intelligence (AI) on the understanding and advancement of molecular biology. This interdisciplinary event focuses on the application of data integration techniques, machine learning, and AI to address the growing challenges in biomolecular research.

The use of AI has revolutionized the interpretation of large biological datasets, accelerating the analysis of genetic sequences, predicting molecular interactions, and facilitating the identification of subtle patterns. Experts in AI and molecular biology will discuss how these advanced technologies can predict protein structures, identify disease biomarkers, and optimize drug research.

In particular, Dr. Susana Ladra will address the fundamentals and applications of AI in molecular biology, highlighting how AI algorithms can recognize complex patterns and make meaningful predictions from biological data. Dr. Martín Garrido Rodriguez-Cordoba will present how knowledge-assisted machine learning enhances omics data analysis, utilizing prior knowledge to develop detailed models of cellular signaling and facilitate the interpretation of spatial data. Meanwhile, Dr. Oscar M. Rueda will explore the integration of philosophies between classical statistical models and AI, focusing on the development of modern prognostic models for breast cancer.

In addition to scientific advancements, the symposium will also address ethical and regulatory challenges associated with the implementation of AI in molecular biology. This event provides a crucial platform for collaboration among researchers, data scientists, bioinformaticians, and molecular biology professionals, promoting new avenues for the treatment and understanding of diseases at the molecular level.

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S3.2 - 2

AI Fundamentals and Applications to Molecular Biology

SUSANA LADRA,

Universidade da Coruña

Artificial Intelligence (AI) has revolutionized the way we interpret and analyze vast biological datasets. AI has proven to be a very useful tool in the interpretation of massive biological data, accelerating the analysis of genetic sequences, predicting molecular interactions and facilitating the identification of subtle patterns in large data sets.

In this talk, we explore the transformative role of AI in the field of molecular biology, focusing first on its foundational principles and then on its practical applications in biological research.

We begin by outlining the basic concepts of AI, including machine learning, neural networks, and deep learning. Understanding these core principles is essential for grasping how AI algorithms can be trained to recognize patterns, make predictions, and derive insights from complex biological data.

Next, we review some applications of AI in molecular biology. One of the best-known applications consists in the interpretation of large-scale genomic data more, uncovering genetic markers and mutations that may be linked to diseases. AI has also shown its great potential in predicting molecular interactions, such as protein-protein interactions and drug-target interactions, which are critical for drug discovery and development.

However, the use of AI in this context presents several challenges, including ethical issues, data quality, and transparency, among others. Addressing these challenges requires a collaborative effort between AI experts and molecular biologists.

In conclusion, AI is a very powerful tool for the field of molecular biology. It is vital that researchers understand its fundamentals, so they can bring out its full potential, being able to develop innovative solutions to complex biological problems.

S3.2 - 3

Enhancing Omics Data Analysis with Knowledge-Assisted Machine Learning

MARTÍN GARRIDO RODRÍGUEZ-CÓRDOBA

Institute for Computational Biomedicine, Heidelberg University. Research Area Leader, Saez-Rodriguez Group

In recent years, machine learning has become essential in biology, particularly in areas like imaging and structural proteomics. This progress is due to factors such as large sample sizes, well-defined learning objectives and metrics, and limited solution spaces.

In omics data analysis, even with advances in spatial and single-cell technologies increasing the number of observations, we often lack predefined objectives and metrics. Typically, large-scale molecular readouts are used to generate hypotheses about the mechanisms behind biological states, like diseases. Therefore, omics data analysis cannot rely solely on black-box predictive models.

To improve the analysis and interpretation of omics data, and to address the scarcity of observations across studies, we can use prior knowledge. This prior knowledge helps narrow down the potential hypotheses, identify cooperation mechanisms between molecules, and sometimes provide insights into the causes of specific phenotypes.

In this talk, we will showcase a variety of tools and methods for analyzing different types of omics data using existing prior knowledge. We will first introduce our meta-resource OmniPath, which includes diverse categories of molecular information such as gene regulation, ligand-receptor interactions, and phosphorylation/dephosphorylation events. Then, we will demonstrate how to use these resources alongside various computational approaches to understand cell-cell communication, integrate different types of omics data, and develop detailed models of signaling. Additionally, we will highlight how, in certain scenarios like spatial omics data analysis, combining machine learning methods with prior knowledge can enhance result interpretability.

S3.2 - 4

Modern prognostic models for breast cancer: combining classical statistics and machine learning ideas.

ÓSCAR RUEDA

MRC Biostatistics Unit, University of Cambridge. Programme Leader

The increasing availability of complex data types available in biomedical studies require a balanced data analysis approach that maintains the good properties of classical statistical methods and the predictive ability of modern machine leaning techniques. In this talk we will describe several examples that illustrate this approach, with applications to prognosis in breast cancer, drug response prediction in pan cancer studies and gene expression profile denoising. These approaches place great emphasis in model interpretability and good performance.

S3.3. RECONSTITUTING BIOLOGY: TOWARDS BUILDING LIFE-LIKE SYSTEMS FROM THE BOTTOM UP

S3.3 - 1

S9: Reconstituting biology: towards building life-like systems from the bottom-up

GERMÁN RIVAS

CIB Margarita Salas - CSIC, Madrid

The design and synthesis of biological (or bio-inspired) systems that exhibit valuable functions, even those that do not occur in nature, have accelerated over the past decades and matured into synthetic biology. This field, which introduces the engineering perspective (mastering complexity; understanding by building) to study biological processes and design systems with programmable functionality, has brought about significant benefits. It has fostered an increasingly larger scientific community to join efforts to master the intrinsic capabilities of molecular and biological systems. This fundamental understanding of how life works is being translated into resources for novel solutions to outstanding green- and health-related problems, thereby demonstrating the potential impact of synthetic biology.

The symposium will focus on bottom-up synthetic biology (synthetic cell engineering) that aims to redesign and reconstruct biological parts, devices, and systems with increasing levels of complexity toward a minimal cell-like scaffold. The speakers will describe the progress made in the reconstitution of essential cellular machines from complementary approaches (systems biochemistry, macromolecular chemistry, biophysics, and physiology of minimal cell systems) from their molecular building blocks. This progress will facilitate the integration of the individual molecular systems into functional modules toward building a synthetic cell from the bottom up, revealing the boundaries of life.

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S3.3 - 2

Building a cellular architecture from scratch

THOMAS SURREY

Centre for Genomic Information, Barcelona, Spain

The microtubule cytoskeleton is important for intracellular organization, for organizing intracellular trafficking, and for correct cell division. At different stages of the cell cycle and in different differentiated cells, the microtubule cytoskeleton adopts distinct architectures required for distinct functions. How do these different architectures self-organize? Cell biological experiments have identified the important players: microtubule nucleators, regulators of microtubule dynamics, microtubule crosslinkers, molecular motors, kinases and phosphatases. How do these players work together to control the generation of distinct organizations of the cytoskeleton. We use bottom-up reconstitution approaches combining some of the key components of the cytoskeleton to build minimal architectures resembling the real cytoskeleton in cells. We observe the formation of these reconstituted cytoskeletal architectures and their dynamic properties using fluorescence microscopy. Our aim is to understand the minimal requirements for cytoskeleton organization and to elucidate the design principles controlling the formation of distinct architectures. In this lecture, I will present recent examples of our research illustrating how active cytoskeleton network formation is controlled by the biochemical and biophysical properties of the network's constituents.

S3.3 - 3

Supramolecular Dynamic Chemistry for Membrane Transport and Biomimetic Systems

JAVIER MONTENEGRO[1]

Universidad de Santiago de Compostela

CIQUS - Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares

[1]Jenaro de la Fuente, s/n 15705 Santiago de Compostela

javier.montenegro@usc.es

Our research group is interested in the application of supramolecular chemistry to understand and manipulate biology. [1,2] Our work philosophy is based in the importance of weak and non-covalent forces to control the shape and the topology of biomolecules, which are governed by the principles described by supramolecular chemistry. These supramolecular lessons can then be applied to control the properties and function of biomolecules. We believe that by modulating the shape we can mimic, control and improve functional behaviour. With focus in supramolecular interactions for artificial membranes and tubular composites, we investigate the construction of synthetic systems for controlling and emulating biology and life-like soft systems. [3-4]

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S3.3 - 4

Essential physiological processes in a genomically minimal cell

JAMES F. PELLETIER[1], LIJIE SUN[2], KIM S. WISE[2], NACYRA ASSAD-GARCIA[2], BOGUMIL J. KARAS[3], THOMAS J. DEERINCK[4], MARK H. ELLISMAN[4], ANDREAS MERSHIN[5], NEIL GERSHENFELD[5], RAY-YUAN CHUANG[2], SAÚL ARES[1], GERMÁN RIVAS[6], JOHN I. GLASS[2], AND ELIZABETH A. STRYCHALSKI[7]

[1] Centro Nacional de Biotecnología (CNB-CSIC), Spain

[2] J. Craig Venter Institute, USA

[3] The University of Western Ontario, Canada

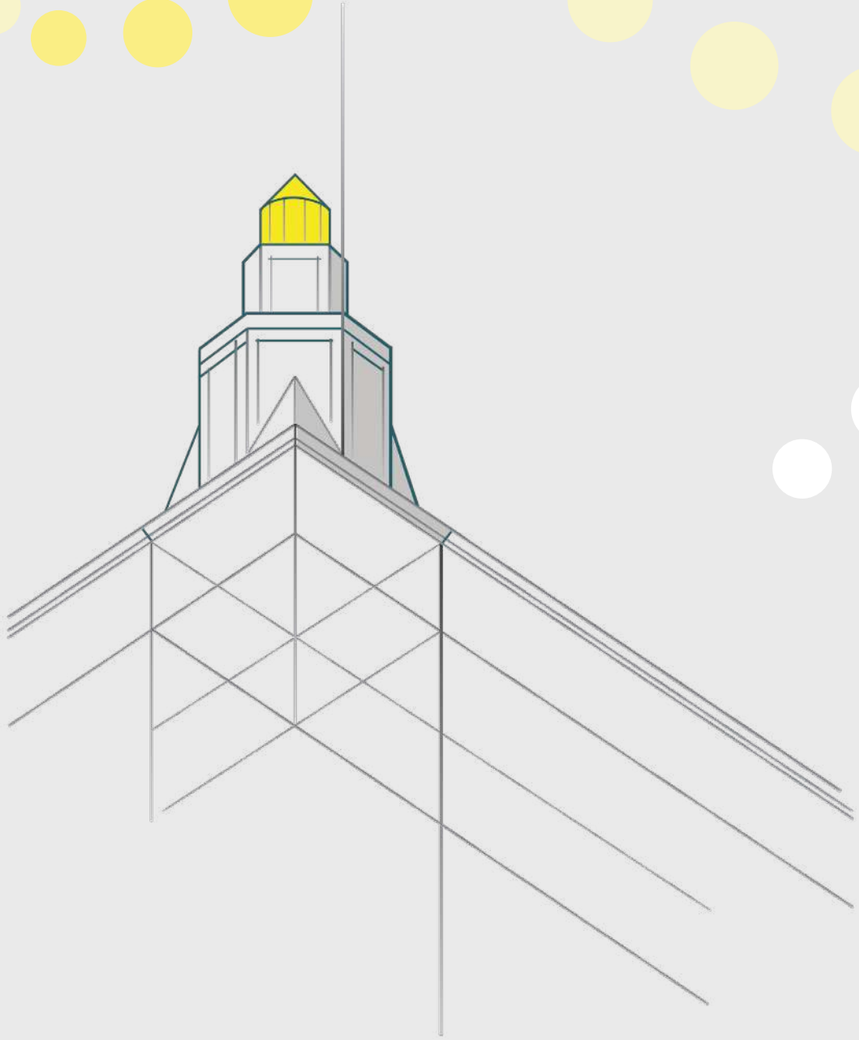
[4] The University of California San Diego, USA

[5] Center for Bits and Atoms, Massachusetts Institute of Technology, USA

[6] Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC), Spain

[7] National Institute of Standards and Technology, USA

Essential physiological processes in a genomically minimal cell Engineered to have as few genes as possible, genomically minimal cells offer simplified systems to study essential physiological processes. JCVI-syn3A has the smallest genome of any known cell capable of autonomous self-replication, retaining 493 of the 901 genes that comprise the naturally reduced genome of *Mycoplasma mycoides*. Even in the minimal genomic context of JCVI-syn3A, fundamental physiological processes such as cell division oden depend on many genes of known and unknown function. JCVI-syn3A exhibits binary ission, resulting in round cells less than one micron in diameter. Deletion of some genes of,unknown function perturbs cell division, generating a fraction of large cells greater than several microns in diameter. JCVI-syn3A retains the highly conserved gene *ftsZ*, which encodes a bacterial tubulin homolog that can assemble into a constricting ring at the division site in most bacteria. Surprisingly, deletion of *ftsZ* from some strains does not produce large cells, suggesting other forces contribute to constriction during cell division in JCVI-syn3A. As a reference, physical models that describe shape transformations in vesicles can predict spontaneous constriction in the absence of a constricting ring, for certain values of the surface-area-to-volume ratio and the preferred membrane curvature. We are characterizing the genetic bases for these physical properties and studying how FtsZ may act in the biophysical context of a membrane curved by other forces. This physical view provides a quantitative framework to compare mechanisms of cell division and extends to other essential physiological processes.



CONFERENCIAS PATROCINADORES

CP01 DELTALAB GROUP

LVL Technologies: Tubos Safe 2D con triple codificación y máxima calidad.

IRENE RUIZ CORRAL

Product manager Deltalab Group

El almacenamiento de muestras a medio o largo plazo para fines de investigación y diagnóstico requiere cumplir requisitos relacionados con el recipiente, manipulación, almacenamiento trazabilidad, etc. La prioridad es la mejor conservación de la calidad y la cantidad de la muestra. Por lo que se deben garantizar tres aspectos: **seguridad, identificabilidad y ahorro de espacio**.

La gama de **Tubos Safe 2D de LVL tech con triple codificación grabada a laser** (imborrables) y tapón sellado que cumple normativa 100% IATA y test de CO_2 es la mejor opción para Biobancos, gestión de compuestos, criopreservación, estudios de población, medicina transfusional y permite la procesabilidad automatizada en el laboratorio, no existe una alternativa mejor para el almacenamiento moderno de muestras.

¡Olvídate de las etiquetas y de los problemas de almacenamiento! **WELCOME TO NEXT LVL**

CP02 CERTEST BIOTEC

Certest, en la búsqueda de soluciones biotecnológicas para la investigación en diagnóstico y terapia.

JORGE GÁMIZ ÁLVAREZ

Raw Materials Export Area Manager, Certest

Certest Biotec es una empresa fundada en 2002 con sede en San Mateo de Gállego, (Zaragoza) enfocada en la investigación y fabricación de productos de diagnóstico in vitro.

La pandemia marcó un antes y un después para Certest, haciendo que el volumen de trabajo creciera drásticamente, lo que desencadenó la búsqueda de nuevas soluciones y la necesidad de adaptarse al mercado, respondiendo a este en tiempo récord y llevando a la empresa a ser, actualmente, una de las más punteras del país en el sector biotecnológico.

Pero, Certest no es solo diagnóstico, más allá de las PCR, los *Rapid Test* y los test de CLIA y turbidimetría, Certest está centrada en el desarrollo de nuevas tecnologías para el desarrollo de soluciones terapéuticas y la liberación de fármacos, como moléculas de siRNA y lípidos ionizables.

Al mismo tiempo, Certest pone a disposición del mundo de la investigación todo su *know-how* en diseño y purificación de proteínas y enzimas, desarrollo de anticuerpos monoclonales, y síntesis química para la elaboración de sondas fluorescentes, para contribuir al desarrollo del conocimiento científico y contribuir al bienestar de la sociedad colaborando con numerosos centros de investigación y universidades públicas.

Gracias al crecimiento experimentado por Certest, la división de *Raw Materials* es capaz de proveer con sus materiales, no solo a la división de diagnóstico, sino a cualquier colaborador externo o cliente al que pueda aportarle valor para su actividad.

CP03 CONDALAB

¿Estrés oxidativo post-vacacional? Soluciones para estudiar marcadores de oxidación celular.

LAURA FERNÁNDEZ,

Jefa de Producto, Condalab.

El estrés oxidativo celular está en el punto de mira de numerosas investigaciones, por la relación que existe entre el daño oxidativo y enfermedades o procesos de envejecimiento. En esta sesión conocerás nuestras propuestas para estudiar marcadores de oxidación y capacidad antioxidante, que te permitirán identificar y comprender los procesos oxidativos y los mecanismos de defensa frente a los mismos.

CP04 ILUMINA

Empowering Biological Insights and Discovery with Multiomics

VIVEK MISHRA

Senior Marketing Manager, Illumina

The presentation will explore how multiomic research approaches can unlock new insights and drive innovative breakthroughs and how Illumina's cutting-edge technologies fit into this spectrum.

CP05 TALLER CONTROLTECNICA

Taller de electroforesis capilar en gel

COORDINADORA:

LOLA LLORENTE

Especialista de producto de Biología Molecular, Controltecnica

¿Cansado de perder tu tiempo con cubetas de geles de agarosa?

¿Te gustaría tener tus resultados en mucho menos tiempo?

¿Cansado de perder tu dinero enviando muestras a secuenciar sin la calidad mínima requerida?

¿Cansado de electroforesis que corren mal y con baja resolución de fragmentos?

Los bioanalizadores de fragmentos de Bioptic son un sistema totalmente integrado simplificando el procedimiento de análisis por electroforesis en gel ya que ahorra la preparación del gel, carga de las muestras, electroforesis y procesado de las imágenes. Estos equipos ofrecen una tecnología en electroforesis capilar en gel con detección por fluorescencia al alcance de todos permitiendo analizar muestras de DNA, RNA y proteínas de forma rápida y sencilla, garantizando una máxima precisión, sensibilidad y reproducibilidad de sus resultados.

Trae tus propias muestras y prueba la última tecnología patentada de electroforesis capilar en gel. Bastará con 2 minutos para conocer tamaño, concentración e integridad de tus muestras.

CP06 MDPI

Open Access Publishing through MDPI.

ANGELA TORIBIO

Bacteria Journal Relations, MDPI

Open Access publishing, a cornerstone of MDPI's philosophy, aims to remove barriers to scientific information so that scientific articles may reach a wider audience including researchers, students, and the general public. MDPI's passion for Open Access is highlighted in its initiatives that facilitate Open Science (for example Sciforum, Scilit, and Encyclopedia) and its extensive portfolio of peer-reviewed journals (such as Cells, Biomolecules, and Genes) covering diverse disciplines. Through Open Access and serving scholars, Cells, Biomolecules and Genes empower researchers to disseminate their findings without restrictions, accelerating the pace of scientific discovery. This approach fosters collaboration and innovation across disciplines, facilitating interdisciplinary research and breakthroughs, aspects that the journals are committed to maintain year by year.

CP07 APLITECH BIOLAB

De la poyata a la automatización 100%: cómo los robots *liquid handling* pueden ayudarte en el laboratorio.

ELISABETH CASADO

Product Manager, Aplitech Biolab

La automatización de los protocolos de pipeteo del laboratorio puede causar respeto si es la primera vez que nos enfrentamos a la idea de trabajar con un robot *liquid handling*. En Aplitech Biolab contamos con especialistas con más de una década de experiencia ofreciendo soluciones automatizadas 100% para protocolos NGS, qPCR, extracción de DNA/RNA/cfDNA, purificación de proteínas, ensayos celulares, etc.

En esta conferencia discutiremos cómo estos equipos son una herramienta útil para cualquier laboratorio, y fáciles de usar, a través de las diferentes aplicaciones en bioquímica y biología molecular

CP08 PHCBI

Live Cell Metabolic Analyzer: Continuous monitoring of energy metabolism.

ISAAC BLANCO

Product Manager, PHCbi

Precise measurement of glucose and lactate turnover provides deep insight into glycolysis and general energy metabolism. PHCbi´s in-line sensor technology offers a unique sample-free method to follow glucose and lactate levels in the cell culture medium up to 10 days continuously. By maintaining the cells in their common cell culture environment, the LiCellMo (Live Cell Metabolic Analyzer) delivers unperturbed results with high resolution. Sala 9+10

ÍNDICE

CP09 LI-COR BIOSCIENCES

Enhancing Reproducibility in Western Blot: Unlocking Reliable Data for Accelerated Research.

ANTONIO PETEIRA MARTÍNEZ, LI-COR BIOSCIENCES.

Technical Sales Consultant (Spain & Portugal), LI-COR Biosciences.

Accurate quantification of protein expression changes is essential for target and biomarker identification as well as functional characterization. However, researchers often face obstacles that compromise reproducibility and data integrity. Alarmingly, 80% of scientists acknowledge the reproducibility crisis as a significant barrier in their work. This recognition has prompted us to investigate the key factors and best practices necessary for dependable protein abundance quantification.

Scientific journals and funding agencies have responded to this challenge by publishing increasingly stringent Best Practices and Guidelines, often curated by active scientists. These guidelines aim to establish rigorous requirements for techniques, detection methods, imaging parameters, and analysis.

In this talk, we will highlight these scientific requirements, offering insights and recommendations to enhance the reproducibility, cost-effectiveness, and efficiency of your Quantitative Protein Assays. By following these guidelines, researchers can focus more on their core biological research and less on troubleshooting experimental inconsistencies. Our aim is to equip researchers with the knowledge and tools to overcome reproducibility challenges, obtain reliable data, and accelerate scientific discovery.

CP10 AGILENT TECHNOLOGIES SPAIN

From Hypothesis to Publication: Leveraging Advanced Cell Analysis Technologies for Comprehensive Cellular Insights.

MIRIAM CONTRERAS MOSTAZO[1], ALFREDO CARO MALDONADO[2]

[1] *Cell Analysis Product Specialist for Cell Analysis, Agilent Technologies Spain*

[2] *Product Specialist of Agilent Seahorse and xCELLigence in Agilent Technologies Spain*

In cellular biology, transitioning from hypothesis to publication requires precision and a multifaceted approach to data collection and analysis. This abstract outline a robust workflow using state-of-the-art cell analysis technologies: Seahorse XF Analyzers, xCELLigence Real-Time Cell Analysis (RTCA), Cytation Imaging Systems, and Novocyte Flow Cytometers. These technologies collectively offer a comprehensive view of cellular functions and responses.

Introduction: The research begins with formulating a hypothesis addressing critical questions in cellular biology. Testing this hypothesis involves employing a suite of advanced tools to gather detailed data on cellular metabolism, dynamic events, imaging, and flow cytometric profiling.

Methods:

1. **Seahorse XF Analyzers:** These analyzers measure metabolic parameters such as oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), providing insights into cellular energy production and metabolic shifts.
2. **xCELLigence RTCA:** This technology enables real-time, label-free monitoring of cell proliferation, morphology, and viability through impedance measurements, offering dynamic insights into cellular behavior and treatment responses.
3. **Cytation Imaging Systems:** Combining automated digital microscopy with multi-mode detection, Cytation systems facilitate high-content imaging and quantitative analysis of intracellular processes and protein expression.
4. **Novocyte Flow Cytometers:** These flow cytometers provide high-throughput, multi-parametric analysis of cell populations. By examining cell surface markers, intracellular proteins, and cell cycle status, they offer detailed phenotypic and functional characterizations.

Conclusion: Employing Seahorse XF, xCELLigence, Cytation, and Novocyte flow cytometers offers a powerful framework for cellular research. This integrated approach ensures a seamless transition from hypothesis testing to data analysis, leading to high-quality publications that advance cellular biology understanding.

Keywords: Cellular analysis, Seahorse XF, xCELLigence, Cytation, Novocyte flow cytometer, metabolic profiling, real-time cell analysis, high-content screening, flow cytometry.

CP11 KUHNER SHAKER INC.

Review of the Application of Shake Flasks and Multiwell Plates.

TIBOR ANDERLEI

CSO of Kühner AG

Even though shaken bioreactors like flasks and multiwell plates have been applied for a long time in the cultivation of mammalian cells and microorganisms the knowledge of influencing engineering parameters is limited. Therefore, this presentation will describe the importance of e.g. shaking diameter, flask material, and closures, and measuring data/results will be shown. Furthermore, online measuring technology will be introduced to determine cells' oxygen consumption and CO_2 production (OTR/CTR).

CP12 PARALAB SL

One Step Closer to In Vivo Models: Life-Cell Label-Free Imaging, Intravital Microscopy, and 3D Bioprinting.

ALBERTO FERNÁNDEZ OLIVA

Product Specialist, Paralab Bio

The field of biomedical research increasingly relies on precise and minimally invasive techniques to study living organisms. Here, we will discuss the integration of advanced technologies that bring us closer to authentic in vivo models. Label-free imaging of live cells allows for real-time, high-resolution observation of cellular dynamics without the interference of external labels, maintaining the natural state of cellular function. Intravital microscopy is a transformative tool that enables the direct visualization of biological processes within living organisms, providing unprecedented insights into tissue and organ physiology. Additionally, we will explore the advancements in 3D bioprinting, a technology that fabricates complex, biologically functional tissues, replicating the structural and functional properties of native tissues. Overall, these methodologies enhance our ability to study living systems in their native context, facilitating significant advancements in translational research and therapeutic development.

CP13 DREAMGENICS

Aspectos clave en el diseño de un proyecto NGS de investigación biomédica

JORGE PALOMINO

Sales Manager Dreamgenics

En esta charla veremos diferentes ejemplos de análisis de datos NGS y discutiremos la importancia de contar con una empresa especializada a la hora de diseñar correctamente cada una de las etapas de un proyecto, centrándonos en la secuenciación masiva y el análisis bioinformático de los datos generados.

CP14 CYTIVA

Tangential Flow Filtration: Basics and applications.

XÈNIA MASSANA MUÑOZ

Bioprocess Specialist, Cytiva

Tangential flow filtration (TFF), sometimes called cross-flow filtration, is a separation technique commonly used in biopharmaceutical applications and life science research. This filtration technique derives its name from the tangential feed flow across a surface filter. The primary applications for TFF are concentration, diafiltration (desalting and buffer exchange), and fractionation of large from small biomolecules. You can also use TFF to clarify and remove cells or cellular debris from cell culture or fermentation media.

Let's discuss and learn together the basics of TFF, understanding both the science and the applications.

CP15 PROQUINORTE S.A.

Single Cell at SCALE: Discover Biology at Single Cell Resolution

RAVI GIRDHAR

Director de Ventas y Soporte para EMEA, Scale Biosciences.

ScaleBio technology for single cell sequencing enables unprecedented sample indexing and cell throughput across a broad range of genomic, proteomic, and multi-omic applications to enable researchers to discover biology at scale. Our single cell technology platform uses combinatorial bar-

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coding of analytes within cells themselves (cDNA, DNA, Protein) which eliminates the requirement for specialised instrumentation. The technology is scalable, profiling 100's of samples and 100,000 to millions of cells per experiment. Flexible, fixation enables storage and batching of samples. This instrument-free workflow only requires reagent kits, basic laboratory equipment, and simple plastics.

CP16 TALLER DREAMGENICS

Plataforma Genome One Reports para la visualización de datos NGS

JORGE PALOMINO[1], ALBERTO GONZÁLEZ[2]

[1] *Sales Manager Dreamgenics*

[2] *Key Account Executive*

En esta actividad los asistentes podrán utilizar nuestra plataforma Genome One Reports con la que entregamos a nuestros clientes los resultados del análisis bioinformático de datos NGS. En particular, veremos cómo se visualizan los resultados de análisis genómicos, RNA-Seq, Single-cell RNA-Seq y de integración de datos multiómicos.

CP17 TALLER PROQUINORTE

Meet QF-Pro® and Violet 3.0 - the new spatial biology standard with proven clinical value.

JAMES MILES

Product Manager, HAWK Biosystems

A short presentation on our QF-Pro® technology which quantifies protein-protein interactions and protein post-translational modifications directly with fixed patient samples, including how the technology works and a notable case study. This will be followed up with a demo of our new Violet 3.0 platform, an all-one bench-top microscopy device that has been designed from the ground up to run QF-Pro® assays and analyses.

CP18 BIO-RAD LABORATORIES S.A.

Western cuantitativo, variables críticas y soluciones propuestas por Bio-Rad.

ENRIQUE OROZCO

Field Application Specialist. Bio-Rad Laboratories, S.A.

La transferencia de proteínas a membrana es una de las técnicas más utilizada para identificar y cuantificar la expresión de proteínas. Sin embargo, a menudo los resultados son inconsistentes y poco robustos en gran medida por las limitaciones vinculadas a esta técnica. En esta presentación se abordarán los principales problemas asociados a la cuantificación de un western y las soluciones propuestas por Bio-Rad.

CP19 ECOGEN S.R.L.

Resultados directos basados en técnicas qPCR sin necesidad de extracción de DNA.

SAMI IRAR MARTINEZ

Comercial director, ECOGEN

Los nuevos productos de Meridian Bioline ODX permiten realizar técnicas basadas en qPCR sobre muestras sin necesidad de hacer la extracción de DNA. Estos nuevos productos (son varios) están dirigidos al diagnóstico molecular permitiendo una rápida identificación molecular en muestras como sangre, orina, heces, biopsia líquida etc. Dado que las biopsias líquidas permiten realizar pruebas de cáncer a partir de muestras clínicas no invasivas, proporcionan acceso al ADN libre de células (cfDNA), lo que permite detectar rápidamente células cancerosas, favoreciendo y acortando tiempos de monitorear la recurrencia de la enfermedad, selección de tratamientos y su efectividad, así como identificación de los marcadores moleculares claves para estas patologías.

CP20 CONTROLTECNICA S.L.

Fundamentos y aplicaciones de la electroforesis capilar en gel.

LOLA LLORENTE

Especialista de producto de Biología Molecular, Controltecnica

La electroforesis capilar es una técnica que permite separar diferentes moléculas atendiendo a su relación masa/carga, a través de un capilar de pequeño diámetro al someterlas a un alto voltaje. Los bioanalizadores de fragmentos Qsep1 y Qsep100, son equipos automatizados basados en la tecnología de electroforesis capilar y detección por fluorescencia. Estos equipos unifican el proceso de preparación de gel, carga de muestras, electroforesis y toma de imagen en un único paso proporcionando toda la información relativa a las muestras tales como concentración e integridad en forma de electroferograma y gel. Las ventajas que supone el uso de estos bioanalizadores frente al método convencional de electroforesis son múltiples: requieren poco volumen de muestra, ofrecen alta resolución y reproducibilidad de los resultados y ahorran valioso tiempo y dinero. Los campos en los que habitualmente se emplea esta técnica son los de biotecnología y biología molecular, industria farmacéutica, diagnóstico clínico y laboratorios forenses. Entre las numerosas aplicaciones del equipo se encuentra la tipificación del virus del papiloma humano (VPH), genotipado animal y vegetal, detección de microsatélites, estudio de control de calidad post-PCR, control de calidad de preparación de librerías para secuenciación, diagnóstico de microdeleciones, visualización de amplificaciones de PCR con heterodímeros o evaluación de calidad de muestras de DNA/RNA previa secuenciación entre otras.

Capillary electrophoresis is a technique that allows different molecules to be separated according to their mass/charge ratio, across a small diameter capillary by subjecting them to a high voltage. The Qsep1 and Qsep100 Bio-Fragment analyzer are automated equipments based on capillary electrophoresis technology and fluorescence detection. These equipments unify the process of gel preparation, sample loading, electrophoresis and imaging in a single step, providing all the information related to the samples such as concentration and integrity in an electropherogram and gel. The advantages of using these bioanalyzers over the conventional electrophoresis method are multiple: they require low sample volume, offer high resolution and reproducibility of results, and save valuable time and money. The fields in which this technique is commonly used are biotechnology and molecular biology, the pharmaceutical industry, clinical diagnosis, and forensic laboratories. Among the numerous applications of the equipment are the typing of the human papillomavirus (HPV), animal and plant genotyping, microsatellites detection, quality control post-PCR, quality control of library preparation for sequencing, microdeletions diagnosis, visualization of PCR amplifications with heterodimers or quality evaluation of DNA/RNA samples prior to sequencing, among others

CP21 INTEGRATED DNA TECHNOLOGIES (IDT)

The Use of the CRISPR for genome editing.

MARTA OÑÓS MENÉNDEZ

Iberia & UK Representative Integrated DNA Technologies BVBA

The Use of the CRISPR for genome editing has been a major technological breakthrough, making genome modification in cells or organisms faster, more efficient, and more accurate than previous genome editing methods. The Alt-R CRISPR Systems were developed through comprehensive research on each component of the CRISPR-driven, double-stranded break generation critical for gene disruption (knock-out) and DNA insertion by homologous recombination (knock-in by HDR). IDT Alt-R Cas enzymes are designed to improve on-target CRISPR editing and are available in a variety of format, from the widely adopted S. pyogenes Cas9 to our proprietary engineered mutants. In addition to Cas9 and Cas12a systems, we also offer chemically synthesized and modified custom guide RNAs for specialized research applications. Alt-R Custom Guide RNAs are ideal for prime editing (pegRNA) projects, CRISPR-Cas13 applications, and most alternative CRISPR-Cas systems. All our CRISPR guide RNAs are available with chemical modifications that increase stability, potency, and/or nuclease resistance.

To identify unintended CRISPR effects, IDT developed a quick and efficient solution based on RNase H2-dependent PCR technology generates amplicon libraries for targeted sequencing on Illumina® NGS platforms to scan targets of interest. The rhAmpSeq CRISPR Analysis System allows accurate quantification of CRISPR-Cas edits.

CP22 TEBUBIO

Unlocking the Future of RNA based vaccines: Tebubio's production solutions.

XAVIER WARNET .

Project Manager -mRNA specialist

Since the Covid-19 pandemic, the application of RNA vaccines for cancer treatment has represented significant hope, although clinical effectiveness remains to be demonstrated. To achieve this, researchers require high-quality production of vaccine mRNA and accurate, cost-effective testing tools tailored to oncology research.

At Tebubio, we have developed a complete pipeline to meet researchers needs. Our Contract Research Services offer customizable RNA production solutions, providing small quantities (from 100 µg to 2 mg) of Research-Grade mRNAs. Complemented by a formulation and RNA delivery platform using lipid nanoparticles (LNPs) and cellular tests with biomarker analysis.

Through concrete examples, such as the analysis of the stability and expression of the tumor antigen p53 in antigen-presenting cells (APCs), or data on mRNA encapsulation within LNPs and expression analysis in different cellular models, find out how Tebubio can help you speed up your research projects.

CP23 CULTEK SL

High-Resolution Single-Cell Multiomic Analysis with BioSkryb Genomics' Innovative Solutions.

LUCA MAZZITELLI

Channel Manager EMEA BioSkryb

The adoption of new sequencing technologies is advancing rapidly, with the next stage of molecular characterization focusing on more sensitive, multiomic approaches. ResolveOME, powered by Primary Template-directed Amplification (PTA), provides superior whole-genome coverage and uniformity. In addition, the full-length transcriptome may be observed from the same single cells, enabling gene expression characterization, cell identification and cell state, gene isoform detection, copy number analysis, structural variant detection, and single nucleotide variant detection from the same single cell.

This comprehensive single-cell multiomic data analysis enables researchers to resolve tumour heterogeneity or unravel somatic mosaicism and cell type identification in neurodegenerative diseases.

Publications using BioSkryb technologies will also be discussed

CP24 CYMIT QUÍMICA SL

Desbloqueando el potencial online: Estrategias eficientes para ahorrar en tus compras de laboratorio.

LIA ÁVILA[1], AROA CHANS[2]

[1] *Departamento de Life Science, Cymit Química.*

[2] *Departamento de Life Science, Cymit Química*

Una charla diseñada para mostrar las ventajas de comprar online con nosotros. Exploraremos cómo nuestra plataforma facilita la comparación de precios, la disponibilidad y la transparencia en la selección de productos de alta calidad. Además, explicaremos cómo nuestras herramientas online optimizan el proceso de compra, permitiendo gestionar pedidos e inventarios de manera eficiente. El objetivo siempre es optimizar su presupuesto, reducir costos y simplificar sus operaciones de laboratorio. ¡Descubra cómo transformamos sus compras en una experiencia eficiente y económica!

CP25 INSTITUTO DE QUÍMICA MÉDICA

Conexión Nanomedicina, la medicina del futuro.

ANA V. VILLLAR

Prof. Contratada Doctora Universidad de Cantabria

Distintas soluciones farmacológicas nanoterapéuticas que se desarrollan en los grupos de la Conexión Nanomedicina CSIC a la vez de mostrar las aplicaciones biomédicas de las mismas.

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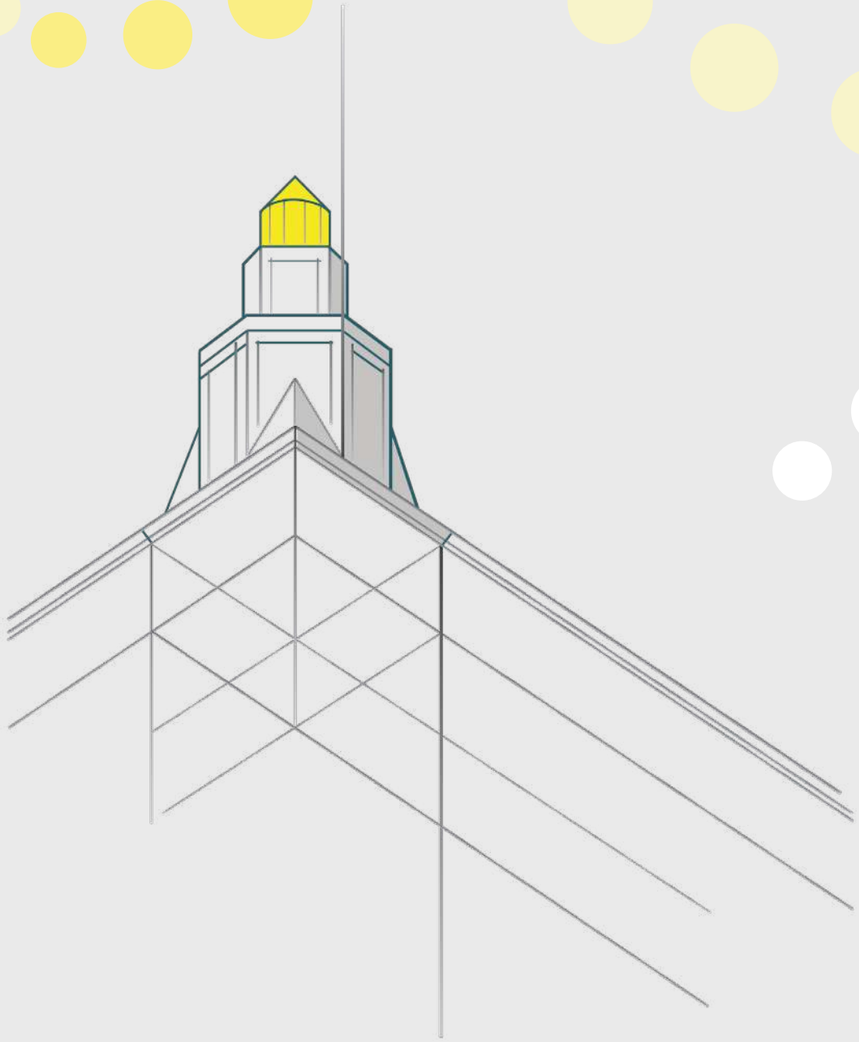

GRUPOS CIENTÍFICOS DE LA SEBBM

G01: Bases moleculares de la patología

G01 - 10 - P

Lung abnormalities resembling human alveolar proteinosis are present in the ARF-deficient mouse model

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Alveolar macrophages (AM) perform important functions in lung homeostasis, representing not only the first line of defence against invading pathogens, but also regulating the uptake, storage and catabolism of surfactant lipids. Impaired function of AM affects lipid metabolism and pulmonary surfactant turnover, leading to the intracellular lipid accumulation and the development of lung pathologies as pulmonary alveolar proteinosis (PAP) or interstitial lung diseases (ILD). In this study, we provide evidences implicating the tumor suppressor ARF in the maintenance of lung homeostasis and regulation of surfactant clearance. We describe a murine model in which ARF-deficiency provokes dysfunction of AM resulting in the development of a phenotype similar to human PAP. In line with the findings in PAP patients, lungs from ARF-deficient mice show abnormal accumulation of surfactant lipids and proteins in the alveolar spaces as revealed by histological analysis and BALF turbidity. Moreover, AM are enlarged and highly vacuolated, exhibiting a foamy appearance. Interestingly, ARF deficiency also induces some detrimental effects on pulmonary function, causing a restrictive phenotype compatible with the pattern observed in hPAP. ARF-/- mice showed a significant decrease in inspiratory capacity (IC) and a clear elevation in the respiratory system elastance (E), tissue elastance (H) and tissue damping (G), suggesting a reduced total capacity and an increased stiffness of the lungs.

These observations indicate that ARF may be a critical factor for the maintenance of lung homeostasis and provide new insight into the potential issues involved in the development of pathogenesis of PAP-like diseases.

Grants: Thanks to ISCIII for support to S.H. (PI20CIII/00018) and A.L (PI18CIII/040)

Keywords: Alveolar Macrophages, Inflammation, Lipids, Alveolar Proteinosis, ARF-/-

G01 - 15 - O

Remodeling p38 signaling in muscle controls locomotor activity via IL-15

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Physical inactivity and changes in dietary habits have increased the prevalence of obesity in contemporary societies, creating a major health problem. Therefore, understanding the mechanisms that lead to the development of obesity comorbidities is paramount to decrease their incidence and mortality. p38MAPK pathway has been shown to be an important modulator of homeostasis, and excessive activation of this cascade could be responsible of diseases so important as cancer and diabetes. Although that p38 function in obesity and its associated pathologies is starting to be addressed, its role in interorgan communications in the context of obesity and its associated diseases remain unsolved. Using a mouse model lacking p38α in striated muscle we found that p38α deletion protects mice against high-fat diet (HFD)-induced obesity by increasing energy expenditure and skeletal muscle metabolic remodelling. This phenotype is accompanied by an hyperactivation of p38γ, which improves glucose and energy homeostasis through an increase in the voluntary locomotor activity observed in our conditional mouse models and in human samples after acute exercise.

We also identified myokines released by skeletal muscle that mediate the high voluntary locomotor activity in our models and we demonstrated which specific motor brain areas control this mechanism. This effect decreased the risk of diabetes and obesity revealing a new muscle-brain crosstalk and identified this p38 pathway as a new therapeutic approach to the current obesity and metabolic disorders.

Keywords: Obesity, P38s, IL-15, Skeletal Muscle, Locomotor Activity, Motivation, Energy Balance

G01 - 23 - P

Noise-induced hidden hearing loss affects neural coding in the inferior colliculus of the rat

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Many people with normal audiograms report difficulties understanding speech in noisy environments, a condition called hidden hearing loss (HHL). Cochlear synaptopathy, the loss of synapses between inner hair cells and the afferent fibers of the cochlea nerve exclusive of hair cell loss, is often proposed as a primary cause of HHL. This pathology can be induced by acoustic overexposure, aging, or both. Much of the neuronal research thus far has investigated how HHL affects the response characteristics of individual fibers in the auditory nerve, instead of exploring whether it induces functional changes in the central nervous system. Human models show that the stochastic nature of action potentials in the auditory nerve, combined with a reduced number of afferent fibers, degrades the quality of the neural representation of the sound waveform.

We have developed a rat model of noise-induced HHL and cochlear synaptopathy and presented stimuli that varied in frequency, intensity, and duration. We then investigated how HHL affects the neuronal responses to these stimuli in the inferior colliculus of rats. Finally, we show how shorter stimuli are encoded less effectively by the auditory midbrain than longer stimuli, and how this could lead to a clinical test for HHL.

Work supported by the Consejería de Educación y Cultura

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Keywords: Cochlear Synaptopathy, Hearing Loss, Inferior Colliculus, Noise Exposure, Single Unit Electrophysiology

G01 - 25 - O

Galectin-3 depletion tames pro-tumoural microglia and restrains glioblastoma and brain metastasis

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Galectin-3 (Gal-3) is a multifunctional protein that plays a pivotal role in the initiation and progression of several central nervous system diseases, including cancer. Although the involvement of Gal-3 in tumour progression, treatment resistance and immunosuppression has long been studied in various cancers, mainly outside the central nervous system, its elevated expression in myeloid and glial cells underlines its profound impact on the immune response in the brain. In this context, microglia and infiltrating macrophages, the predominant non-cancerous cells in the tumour microenvironment, play a critical role in establishing an immunosuppressive milieu in various brain tumours. Using primary cell cultures and immortalised microglial cell lines, we have elucidated the central role of Gal-3 in promoting cancer cell migration, invasion and immunosuppressive microglial phenotypic activation. Furthermore, using two different in vivo models of primary (glioblastoma) and secondary (breast cancer brain metastasis) brain tumours, our histological and transcriptomic analyses show that Gal-3 depletion triggers a robust pro-inflammatory response within the tumour microenvironment, particularly based on interferon-related pathways. Interestingly, this response is prominently observed in tumour-associated microglia and macrophages (TAMs), resulting in the suppression of cancer cell growth

Keywords: Cancer, Therapy, Immune Response

G01 - 29 - P

Titin mechanical knock-out triggers muscle disease with myonuclei internalization and sarcomere-free myofibers

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Mechanical proteins are typically large, multifunctional and involved in disease development by complex mechanisms that remain poorly understood. The study of these proteins has been hampered by the lack of tools to uncouple their mechanical and non-mechanical functions *in vivo*. Here, we exploit site-directed protein cleavage to trigger mechanical knock-out of otherwise intact titin, the elastic shaft of muscle sarcomeres. Skeletal muscle titin cleavage in homozygosis induces a severe form of myopathy characterized by marked atrophy and depressed force generation. Affected fibers, although persistent, shrink and display progressive sarcomere depletion that correlates with altered levels of titin interactors including rapid upregulation of MuRF1 and active ANKRD1, as well as mitochondria aggregation, mislocalized intermediate filaments and internalized nuclei. Titin mechanical knock-out in heterozygosity causes milder phenotypes resembling human myopathy caused by titin mutations. Our results demonstrate that knocking out force transmission across a mechanical protein is sufficient to trigger disease.

Keywords: Mechanobiology, Skeletal Muscle, Titin, Muscle Stiffness

G01 - 30 - P

Validation of Tumoral Dissemination Markers in Metastatic Colorectal Cancer: TMOD2 characterization

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Colorectal cancer (CRC) is the third most diagnosed cancer worldwide. Among the different metastatic niches common in CRC, liver represents the one with the highest death rate.

In previous studies, TMOD2 was identified as a dysregulated protein in highly metastatic-to-liver CRC cells (KM12SM) in contrast to low-metastatic isogenic cells (KM12C). In parallel, mRNA and protein levels of TMOD2 in tumoral tissue were higher than in paired normal tissue, and higher protein expression levels of TMOD2 were observed by tissue microarrays and immunohistochemistry in metastatic tissue. This evidence led us to associate TMOD2 overexpression to metastasis in CRC. In this work we aimed to analyse its functional role in CRC metastasis. We performed a stable TMOD2 overexpression in established isogenic KM12 and SW cell models of CRC metastasis.

The effect of TMOD2 overexpression in the tumorigenic and metastatic capacities of CRC cells was evaluated by *in vitro* and *in vivo* functional assays. Additionally, the interactome and proteome associated to TMOD2 were investigated by quantitative proteomics through label free quantification to identify TMOD2 interacting proteins and dysregulated proteins associated to its overexpression. Based on changes on the tumorigenic and metastatic capacities induced by TMOD2 overexpression, we evaluated changes in focal adhesions by immunofluorescence assay.

After validation by western blot, the correlation between interactors and dysregulated proteins and TMOD2 was confirmed. Subsequently to the focal adhesions analysis, alterations in the cytoskeleton were observed after TMOD2 overexpression.

Validation of these results allowed us to conclude in a potential role of TMOD2 as biomarker of CRC and in the involvement of CRC progression and metastasis.

Keywords: TMOD2, Proteomics, Colorectal Cancer, Metastasis, Interactome, Biomarker

G01 - 34 - P

Towards new migrastatic therapies targetting specific pools of actin

IRENE OLIVAS CANO, M. ÁNGELES JUANES

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Metastasis is the main cause of death from cancer. The first step of this process is tumor cell invasion, which involves the generation of actin-rich protrusions that extend from the leading edge of cells and steer their migration emanating from the tumor mass toward neighboring tissues. The actin cytoskeleton is central to these processes and therefore the prime target of drugs known as migrastatics (anti-invasive or anti-metastatic). However, current drugs inhibiting overall actin dynamics disrupt many other biological functions (e.g., cell division, organelle or molecule transport) and prompt undesired side effects that limit their therapeutic use. Then, targeting specific pools of actin may lead to more effective and less damaging cancer treatments.

The tumor supressor Adenomatous polyposis coli (APC), considered as the gatekeeper of colorectal cancer, is a potent actin nucleator. We generated a separation-of-function mutant of APC, known as APC-m4, that specifically disrupts its actin nucleation function, and showed that APC-m4 impairs cell adhesion and consequent invasion. These findings prompt us to use biochemistry, cell biology and artificial intelligence approaches to design a “drug-like particle” that mimics the APC-m4 behaviour to ultimately block tumor invasion. This drug-like particles would be used as pharmacologic treatment to inhibit specifically the pool of actin filaments nucleated by APC, rather than overall actin nucleation and/or other functions of APC. Our findings may open avenues to develop more personalize antimigratory therapies.

Keywords: Metastasis, Actin, APC, Migration, Migrastatic

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G01 - 38 - P

CREB induces a transforming-neuroendocrine molecular landscape to promote merkel cell carcinoma development and dissemination.

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Merkel Cell Carcinomas (MCCs) are malignant skin tumors with an increasing incidence and the highest mortality rate amongst skin cancers. The molecular pathogenesis of MCCs is not completely understood as reflected by the lack of effective targeted therapies for advanced cases. MCCs, display a neuroendocrine phenotype and constitute two types: Merkel cell polyomavirus (MCP+) cases (55-90% of the total) and non-viral cases with UV-derived mutational signatures, high mutational burdens and lower survival (UV+). Activated CREB is associated with fatal outcomes (HR=5.6) independently of multiple clinical variables and MCP status. Thus here, we studied the biology and the molecular mechanisms controlled by CREB in MCCs using a combination of cell cultures, in vivo, CAM assays and a cohort of FFPE cases. CREB was inhibited using genetic and pharmacological approaches.We identified CREB target genes using comparative mRNA-seq and Nanostring. Activation of CREB in MCC cells was triggered by cAMP-PKA signaling and by EGFR and FGFR. Inhibition of CREB impaired MCC cell proliferation, tumor growth and metastasis. Transcriptomic analyses enabled the identification of multiple genes and gene-sets commonly regulated by CREB in MCP+ and in UV+ cells. These hits display known malignant activities like metastasis (i.e. CXCR4), proliferation (i.e. AURKB) or transcription (i.e. RUNX1T1), and included neuroendocrine markers like CHGA or SSTR2. In MCC cases, CREB hits were differentially expressed when compared tocontrols. RUNX1T1 and AURKB were significantly deregulated in MCP+ vs. UV+ cases. Thus, we studied MCCs in the context of CREB and identified multiple molecular effectors, including specific targets for therapy to explain important malignant activities associated to MCCs.

Keywords: Merkel Cell Carcinoma, Transcriptomics, CREB, Neuroendocrine, EGFR, FGFR, Metastasis.

G01 - 40 - P

AI-predicted drug as a promising therapy for Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis (FHHNC)

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FHHNC is an ultra-rare renal disease caused by mutations in *CLDN16* or *CLDN19,* and characterized by urinary Mg2+/Ca2+ wasting and progression to kidney failure. In Spain, 90% of patients carry the *CLDN19* p.G20D mutation. Currently, there are no specific treatments available, highlighting the need for therapeutic targets and prognostic biomarkers. Our lab has employed artificial intelligence (AI) algorithms, combining clinical information and differential urinary miRNAs profiles from patients with different FHHNC progression, to predict possible repositioning drugs. To validate the efficacy of these AI-predicted therapies, we have generated and characterized FHHNC cell models stably expressing either the WT or p.G20D CLDN19 forms. Our results have showed that, contrarily to what it is observed for the CLDN19 WT form, p.G20D mutation causes an intracellular retention of the protein, impairing its trafficking, processing and release to the extracellular medium. Besides, we found that ATF6 expression, a protein involved in unfolded protein response, and *CLDN16* levels are altered in cells carrying the CLDN19 p.G20D form. We have used these features as read-outs that clearly distinguish between WT and mutated CLDN19 cell lines to assess the effect of the AI-predicted repositioning drugs. We have found that drug number 1 rescues CLDN19 p.G20D release to the extracellular medium and improves its trafficking to the plasma membrane. In conclusion, our findings reveal an AI-predicted drug as a promising compound for recovering CLDN19 p.G20D localization and function, therefore suggesting it as a possible therapeutic strategy for FHHNC.

Keywords: Rare Disease, Claudin-19, Artificial Intelligence

G01 - 48 - P

Integration of single-cell immunoproteomic data in a multiomic stydy of patients with IDIC-15 syndrome, a rare disease of neurodevelopment

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The Idic15 syndrome is a rare neurodevelopmental disease caused by duplications in the q11-q13 region of chromosome 15. We are performing a multi-omic study in a cohort of young Spanish patients with Idic-15, aimed to fingerprinting the patients of this complex condition on the basis of genomic, proteomic and metabolomic parameters. As in other neurological conditions, we have found immune alterations and enhanced susceptibility to infections in many patients. In order to assess the relevance of single-cell proteomic markers of immune-cell maturation and function we have applied polychromatic flow cytometry (FCM) to quantifiy the expression of 20 relevant surface proteins (CD markers) in distinct subpopulations of immune cells. By standard statistical analysis we have found several significant immune alterations in patients, including evidences of accelerated immunosenescence, a finding already described in other neurological diseases. Immunoproteomics revealed further differences among patients when stratified according the extension of the genomic lession, clinical signs and susceptibility to repeated infections. In addition, unsupervised bioinformatic analysis has shown that immunoproteomic data by themselves may separate patients into different clusters with interesting associations with gender, genetics, neurological symptoms and susceptibility of infections. Moreover, including immunoproteomic data parameters with other omic data potentiates the clustering of patients according to their risk of infection. Sponsored by donations to the "One House One Life" Initiative promoted by Great Chance SLU. JEO is a member of the Spanish Network of Inflammatory Diseases (REI-RICORS: RD21/0002/0032), Institute of Health Carlos III, Madrid, Spain

Keywords: Enfermedades Raras, Citometría, Neurodesarrollo, Inmunofenotipo, Infección

G01 - 59 - P

Long non-coding RNAs Amplification and Metabolic and cardiovascular Disorders in the Dominican Republic

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Introduction: The relationship between type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) is increasingly evident and represents a growing challenge for global public health. This situation exposes a particularly vulnerable population and poses significant challenges for healthcare. The underlying factors of this complex association include both environmental influences and genetic and epigenetic predispositions. Among the latter, long non-coding RNAs (lncRNAs) have emerged as significant players in the pathophysiology of both T2DM and CVD. **Objectives**: Analyze the gene expression of 4 lncRNAs in 26 patients with DM who did not present CVD and 26 patients with type 2 DM with the presence of CVD, and to compare the expression of these 4 lncRNAs in both groups. **Materials and Methods**: A literature search was conducted for 4 lncRNAs linked to T2DM and CVD, and their expression in participants was analyzed using real-time Polymerase Chain Reaction (qPCR). Data were analyzed using GraphPad Prism 10.2.[3] **Results**: Among the 4 lncRNAs analyzed (CDKN2BAS1, KCNQ1OT1, MIAT, MALAT 1), significantly increased expression of KCNQ1OT1 and MIAT (p value=0.05) was observed in patients with type 2 DM linked to CVD. **Conclusions**: The results obtained highlight that the lncRNAs KCNQ1OT1 and MIAT may play an important role as potential biomarkers and therapeutic targets in the combined management of type 2 DM and CVD. Additionally, they suggest the need to study the population genetics of Dominicans in the context of mixed populations to understand the dimensions of association between these and diseases.

Keywords: Metabolic Disease, Cardiovascular Disease, Type 2 Diabetes Mellitus,, Long Non-Coding RNAs.

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G01 - 61 - O

Metabolic heterogeneity as a driver of stem cell fate and tumorigenesis in the intestine

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Reprogramming of cellular metabolic pathways is key in most cancer cells. Despite the many efforts made to elucidate the metabolic adaptations of cancer cells and their contribution to tumour progression, little is known about the role of metabolic reprogramming during early stages of transformation and the specific metabolic properties of tumour initiating cells. Although adult stem cells have been described as key elements in cancer development and progression, the metabolic control of stem cell self-renewal, differentiation and lineage commitment has been poorly addressed. In this context, identification of metabolic pathways controlling the fate of these cells will help in designing new therapeutic approaches.

Using the intestine as a model tissue, our lab has unveiled a metabolic heterogeneity among intestinal epithelial cells and adenomas, where we have uncovered a population of quiescent highly glycolytic cells with stem cell potential. These cells, identified to be differentiated enteroendocrine cells (EECs), depend on active glucose metabolism to decrease oxidative metabolism and ROS production. In order to further analyse the role of glucose metabolism on EECs fate and stem cell potential, we have developed novel mouse models carrying genetically-encoded metabolic reporters that will enable the direct visualisation, tracing and functional characterisation of these cells during homeostasis and tumorigenesis. Our preliminary results show that highly glycolytic EECs possess stem cell potential *in vitro* and *in vivo* and participate in tumour initiation. Our data suggests that glucose metabolism regulates stem cell activity and tumour initiation in the intestine and that metabolism can contribute to cancer heterogeneity.

Keywords: Cancer Metabolism, Stem Cells, Cancer

G01 - 68 - P

Peptide-mediate inhibition of pericyte chaperone-mediated autophagy promotes glioblastoma cell elimination

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Lack of knowledge of the pathogenesis and mechanisms of progression of glioblastoma (GB), the most aggressive brain tumor, leads to ineffective therapeutic strategies. Our team has demonstrated that GB cells induce an aberrant upregulation of chaperone-mediated autophagy (CMA) in pericytes (PCs), main component of the neurovascular unit. This CMA upregulation in pericytes is beneficial for GB as it ablates the PC anti-tumor immune responses to eliminate tumor cells. In this work, we wanted to determine if a peptide known to selectively inhibit CMA activity in other cell types could prevent the aberrant GB-induced upregulation of CMA in PCs and promote the PC anti-tumor functions, as a possible potential treatment against GB. We studied the PC-GB interactions, GB proliferation and viability of different GB cell lines that were co-cultured with peptide-pretreated PCs. We analyzed the aberrant upregulation of GB-induced CMA in PC. Pretreated-PC or the peptide alone were intravenously injected as different therapeutical strategies in a GB mouse model. We found that in cell co-cultures, the peptide prevents the aberrant GB-induced CMA in PCs, reducing the GB-PC cell interaction and thus, affecting GB cell proliferation and survival. In vivo intravenous injection of peptide-pretreated PCs was efficient to promote tumor cell elimination. The effects of direct administration of the peptide in vivo were dose-dependent and less inflammatory. Treated mice showed remaining tumor associated-immune populations with hardly detection of immunosuppressive FOXP3+ T cells. We confirmed that the peptide reached the tumor areas in the brain parenchyma and affected the host CMA in PCs. Our results identify a CMA inhibitory peptide as possible effective treatment for GB progression.

Keywords: Glioblastoma, Pericytes, Autophagy, Chaperone-Mediated Autophagy, Glioblastoma Therapy

G01 - 69 - P

Estudio de la gramática molecular de los gránulos de estrés en proteostasis y la miopatía distal de Welander

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TIA1 es una proteína de unión a ARN con tres dominios de reconocimiento de ARN (RRM) y un dominio C-terminal de baja complejidad (LCD), implicados en la formación y el ensamblaje de los gránulos de estrés (GEs). El parálogo de TIA1 es TIAR/TIAL1 (*TIA1-related/like protein*), cuya estructura y función es similar a la de TIA1. Ambas proteínas son componentes claves de los GEs, que son condensados no membranosos de ARN y proteínas formados bajo condiciones estresantes y cuya dinámica se encuentra alterada en la miopatía distal de Welander (MDW). La MDW es una distrofia muscular de aparición tardía cuya causa última se ha correlacionado con una mutación de cambio de nucleótido (p.E384K) en el gen que codifica la proteína TIA1. En esta comunicación abordamos el estudio del mecanismo de formación de los GEs analizando el funcionamiento del dominio LCD de TIA1 a través de un análisis exhaustivo de mutagénesis dirigida de la posición 384, y de su parálogo TIAR. Este estudio con las variantes de TIA1 y TIAR ha permitido comprobar, confirmando observaciones previas, que es necesaria y suficiente la presencia de una carga positiva en la posición 384 de TIA1 para que se altere la dinámica de formación de los GEs dependientes de TIA1, mientras que en TIAR la presencia de una carga positiva no parece desempeñar un papel determinante. Los resultados también apuntan un papel relevante de otros residuos aminoacídicos en la dinámica de ensamblaje de los GEs. En resumen, esta comunicación ilustra el comportamiento distinto de TIA1 y TIAR en el contexto de la mutación de la MDW en la formación de los GEs en condiciones de estrés celular. Este trabajo ha sido financiado por el MICINN/AEI/FEDER/UE a través del proyecto PID2021-1261520B-I00.

Keywords: TIA1, TIAR, Gránulos De Estrés, Miopatía Distal De Welander.

G01 - 70 - P

Connecting different metabolic alterations in Idic15 Syndrome, a rare neurodevelopmental disease

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Variable duplications in the 15q11-q13 region cause Idic15, a rare neurodevelopmental syndrome not explored at the metabolic level. In our previous results, a significant increase in the urinary levels of polyamines (PAs) and related metabolites in Idic15 patients was observed. PAs are ubiquitous polycations with key roles in development. So, the critical homeostasis of their levels is sustained by a robust metabolon in which converge multiple and strict regulatory mechanisms. To evaluate the origin and significance of the dysregulation of PAs´s metabolism in Idic15 we have analyzed its relationship with the length of duplication (BP1-BP3 or BP1-BP5) and other PAs related metabolites. Two different patterns of changes were identified. One associated to the BP1-BP3 duplication characterized by elevated serum spermine (40.56 vs 26.67 nM; P=0.043) and folic acid (11.51 vs 7.81 ng/mL; P=0.033), decreased urinary spermine (0.04 vs 0.14 nmols/mg Cr; P=0.014), and

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increased urinary excretion of GABA (1.25 vs 0.67 nmols/mg Cr; P=0.016). The BP1-BP5 duplication associates to higher urinary excretion of spermine and putrescine (1.57 vs 0.66 nmols/mg Cr; P=0.038), and higher serum homocysteine (11.56 vs 9.10 mM; P=0.039). Moreover, homocysteine related positively to urinary spermine (r=0.580; P<0.001) and folate inversely to the excretion of acetylated derivatives of PAs (P<0.01). The relationship of PAs metabolism with the folate and SAM cycles opens the possibility that dysregulation of PAs metabolism play a primary or secondary role in the etiopathogenesis and/or symptomatology of Idic15 syndrome. (PAs analyzed at the COS Joint Unit of the Universitat Rovira i Virgili and Eurecat). Work sponsored by donations to the “One House One Life” Initiative promoted by Great Chance SL.

Keywords: Idic15 Syndrome, Polyamines, Metabolic Analysis

G01 - 72 - P

Inflammatory and cardiovascular biomarkers to monitor Fabry disease progression and treatment monitoring

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Fabry Disease is an invalidating multisystemic disorder affecting α-Galactosidase, a rate-limiting hydrolase dedicated to lipid catabolism. Non-metabolized substrates, such as Globotriaosylceramide and its derivatives trigger the direct or indirect activation of inflammatory events and endothelial dysfunction. In spite of the efficacy that enzyme replacement therapy, or pharmacological chaperones, demonstrated to delay disease progression, only a few studies analyzed whether these treatments can improve the pro-inflammatory state of FD patients. Therefore, the aim of this work was to assess cytokines cardiovascular risk-related proteins and autophagy related biomarkers detectable in plasma from FD patients or mice models, whether treated or not with ERT or pharmacological chaperons, to evaluate the reliability of these markers in monitoring disease stage and treatment effects. We identified (ELISA, Wb, Inmunofluorescence) inflammatory endothelial dysfunction and autophagy related markers (ADAMTS-13, TNF-α, GDF-15, MIP-1β, VEGFA, MPO, MIC-1, LAMP, LC3B) that cooperates in a common pathway and which are increased in FD patients' plasma samples or mice model tissues. As shown by the assessment of these proteins over the time, they can help to evaluate risk of higher severity in FD, as well as treatment effects. Even though the analyzed proteins cannot be considered as proper biomarkers due to their non-specificity to FD, taken together they can provide a signature of reference molecules with prognostic value for early diagnosis, evaluation of disease progression and treatment efficacy, using blood samples.

Keywords: Lysosomal Function, Inflammation, Autophagy, Fabry Disease

G01 - 79 - P

Effects of diet on spatial memory and metabolic/oxidative markers in a high fat diet model in rats

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Metabolic Associated Fatty Liver Disease (MAFLD) is characterized by fat accumulation in the liver (>5% of hepatocytes) affecting up to 30-40% of the adult population. It can alter cognitive functions and induce inflammation and damage in the tissues. There is no pharmacological intervention against the pathology, so efforts are made to resolve the side effects and to recommend lifestyle changes. The objective was evaluating the effects of diet in cognition and different markers in a rat high fat diet (HFD) model in both sexes. Three rat groups fed a HFD three months, then one group changed to standard pellet diet and one to antioxidant high diet during two months; the third one continued with a HFD. Another group fed pellet (5 months, control). Radial maze was evaluated at 3 and 5 months to evaluate spatial working memory. Once sacrificed, lipidic damage and antioxidant enzymes in liver, and glucose, advanced glycation end products (AGEs), polyphenols and interleucine-6 in plasma were measured. More time was only needed for males in the spatial memory test at the end of the experiment in the HFD group. For both sexes, an increased lipidic damage accompanied by a reduced activity of catalase and superoxide dismutase were observed in the HFD group respect the control group with a partial recovery for the groups that changed the diet at both 3 and 5 months. The glucose, AGEs and IL-6 were also higher in the HFD group. The higher polyphenolic content was found in the group that changed to a diet rich in those. In conclusion, spatial memory worsens in HFD in a sex-dependent manner, and the diet intervention in both male and female rats showed an improvement of the biomarkers, glycemic and inflammatory markers.

Keywords: Fatty Liver, Cognition, Oxidative Stress, Inflammation

G01 - 103 - P

Analysis of the expression profile of miR-146a and miR-155 and their interplay with inflammation in Age-related Macular Degeneration.

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Age-related macular degeneration (AMD) causes central vision loss in older adults. There are two subtypes of the disease: dry (dAMD) and wet (wAMD), the latter being the most severe form of the disease. Only wAMD is treated with limited success. The exact pathophysiology of AMD is unknown, but inflammatory and angiogenic imbalances have been observed. Both processes are modulated by microRNAs (miRNAs). Several miRNAs have been reported to be deregulated in different pathologies where inflammation is constant, being associated with the development of the disease.

In AMD, some miRNAs such as miR-146a and miR-155 and others, are also deregulated, but their contribution to AMD is unclear. In this work we investigate the expression profiles of these miRNAs, as well as cytokines to elucidate their relationship with the progression of AMD.

Changes in the expression of miRNAs and cytokines were analyzed in serum of subjects with AMD and controls by RT-PCR and multiplex-flow cytometry, respectively. For the analysis, subjects with AMD were grouped into wAMD and dAMD. Our results showed different profiles between the study groups. While miRNAs were downregulated, pro-inflammatory cytokines and VEGF were increased in AMD with greater deregulation in wAMD. Altogether, the results showed that deregulation in the expression of miR146a and miR-155 negatively impacts inflammatory and angiogenic pathways, favoring disease progression.

Keywords: AMD, Inflammation, MicroRNA,

G01 - 105 - P

ITLN1-Associated Transcriptome in Human Epicardial Adipose Tissue

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Omentin-1 (*ITLN1*) is an adipokine predominantly found in epicardial adipose tissue (EAT). *ITLN1* expression has been associated with cardiovascular disease risk, although it remains unclear whether it functions as a protective or detrimental factor within EAT. Thus, the objective of this work was to gain insight into the role of *ITLN1* in human

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EAT. EAT samples from 30 patients (aged 65-75 years) without coronary lesions undergoing surgery for aortic or mitral valve disease were obtained. The gene expression profile of EAT was analyzed using microarray technology.

A positive correlation between **ITLN1** levels and hypertrophy was observed. Patients were stratified into high (n=15) and low (n=16) **ITLN1** expression groups. The low-**ITLN1** group was mostly composed by women (68.8%, n = 11), whereas high-**ITLN1** group showed a more balanced distribution (8 men, 6 women). We found 146 differentially expressed genes (DEGs) based on FDR < 0.2. Among them, 136 showed an upregulation in the high-**ITLN1** group. According to Gene Ontology classification, **ITLN1** co-expressed genes showed association with regulation of transforming growth factor beta production, intermediate filament organization and cardiac ventricle morphogenesis. The top DEGs were mainly constituted by markers of epicardium (**UPK3B**, **SLPI**, **MSLN**, **ALOX15**) and mesothelium (**PRG4**, **CLDN1**, **CDLN15**). These markers have been associated with an impairment of the epithelial-to-mesenchymal transition linked to myocardial regeneration. Our results suggest that **ITLN1** from EAT may be associated with the myocardial regenerative capacity of the epicardium.

Keywords: Omentin, Epicardial Adipose Tissue, Microarray

G01 - 109 - P

Collagen Triple Helix Repeat Containing 1 (CTHRC1) is a prostate cancer prognosis biomarker regulated by androgen receptor (AR) activity

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Prostate cancer (PCa) is the second most common cancer in men and the sixth leading cause of cancer death worldwide. Androgen deprivation therapy (ADT), which targets androgen receptor (AR) activity, is the standard of care for PCa; however, 15% of patients relapse and develop metastases, making it important to identify molecular markers for prognosis.

Transcriptomic data from cancer samples are used for classifying, stratifying, and identifying clinically relevant genes in tumors. Deregulation of gene expression is key in cancer pathogenesis and progression. Cancer cells communicate via secreted molecules and their transcriptional deregulation can affect cancer progression

Our research focused on secretome-related genes in PCa to improve prognosis, progression, and therapy. Through computational screening, we identified Collagen Triple Helix Repeat Containing 1 (CTHRC1) as a candidate gene for PCa prognosis. CTHRC1 expression is increased in PCa lesions compared to benign ones, at both mRNA and protein level

We investigated the molecular mechanisms underlying this increased expression. CTHRC1 mRNA expression consistently and inversely correlated with three independent AR transcriptional signatures. Further studies indicated that CTHRC1 expression is negatively regulated by AR activity, possibly through disruption of the TGFβ signaling pathway.

Our findings suggest that increased CTHRC1 expression has strong prognostic potential in PCa and is associated with reduced AR transcriptional activity. We propose the integration of CTHRC1 expression monitoring into PCa precision medicine strategies to provide prognostic information and guide ADT response. The AR-TGFβ crosstalk may support novel therapeutic strategies targeting both pathways in aggressive, CTHRC1-high PCa tumors

Keywords: Prostate Cancer, CTHRC1, Precision Medicine

G01 - 111 - P

Transcriptomic Analysis of Epicardial Adipose Tissue in Coronary Artery Disease Patients Reveals Elevated Expression of Endothelial Adhesion Molecules

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Epicardial adipose tissue (EAT) is recognized for its potential impact on cardiac function via the release of paracrine signals. Due to its distinctive transcriptome and anatomical proximity to the myocardium and coronary arteries, EAT has been suggested as a significant risk factor in the development of cardiovascular diseases. This study aimed to identify potential biomarkers of inflammation in EAT that could contribute to increased cardiovascular risk. The EAT transcriptome of 15 subjects with coronary artery disease (CAD) and 13 subjects with isolated valvular disease without coronary lesions (NCAD) was analyzed using microarray technology. The selection of patients was based on age (65-75 years) and clinical criteria. Differentially expressed genes (DEGs; p-value < 0.05; FC > 1.5) of potential interest were selected and validated by RT-qPCR in EAT samples. Out of a total of 232 DEGs, 161 genes were upregulated in CAD patients. GSEA analysis revealed notable differences in the expression profile of genes involved in inflammatory processes, lipid synthesis, atherosclerosis, and endothelial dysfunction. Accordingly, ORA analysis (FDR < 0.2) showed an overrepresentation of processes involving cytokine production and hyaluronan metabolism. The most significant differences between CAD and NCAD patients were observed in the expression of selectin E (SELE). In order to validate microarray data, a set of inflammatory and endothelial dysfunction markers, including *SELE*, *SERPINE1*, *CCL2*, *IL6*, *VCAM1* and *ICAM1* were validated by RT-qPCR in EAT samples. Overall, these results highlight the profound impact of the EAT transcriptome in CAD patients and suggest *SELE* as a potential biomarker of EAT inflammation status.

Keywords: Coronary Artery Disease, Epicardial Adipose Tissue, Edothelial Adhesion Molecules

G01 - 116 - P

Dietary polyphenols curcumin, epigallocatechin gallate, oleuropein and quercetin are potential therapeutic agents in non-small cell lung cancer

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Non-small-cell lung cancer (NSCLC) is a major public health problem due to the long-term ineffectiveness of available therapies. Thus, it is necessary to identify novel compounds that could provide the basis for future therapeutic options in NSCLC. Interest in the use polyphenol-rich natural products has increased considerably in recent years. In this regard, the polyphenols curcumin, epigallocatechin gallate (EGCG), oleuropein and quercetin, present in fruits and vegetables, have been suggested to be promising anticancer agents. We investigated the anticancer activity of these polyphenols against H1299 and A549 human NSCLC cell lines. Specifically, curcumin showed the highest cytotoxic activity against these cell lines (12.89 µm, H1299 and 24.78 µm, A549) followed by EGCG (23.81 µm, H1299 and 31.55 µm, A549), quercetin (56.52 µm, H1299 and 104.2 µm, A549) and oleuropein (54.10 µm, H1299 and 102.3 µm, A549). Noteworthy, these polyphenols decreased the colony formation, cell migration, invasion and adhesion in H1299 and A549 cell lines. To complement this data, we performed immunofluorescence stainings using these polyphenols and we observed that these compounds increased apoptosis, senescence, DNA damage and telomeric damage, as well

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as decreased proliferation and expression of Telomerase (TERT), an enzyme that is reactivated in malignant cells. On the other hand, we performed human NSCLC xenografts in athymic nude mice, in which we observed that these polyphenols reduced tumor growth. Our results indicate that these compounds could be promising therapeutic agents in NSCLC.

Keywords: Polyphenols, Curcumin, EGCG, Oleuropein, Quercetin, NSCLC, Anticancer Agents, Telomeric Damage, Telomerase (TERT)

G01 - 124 - P

Sex-specific Insights into Rosiglitazone's Therapeutic Effects on Rat White Adipose Tissue

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Obesity and type 2 diabetes mellitus are linked to white adipose tissue (WAT) dysfunction and exhibit clear sexual dimorphism in their clinical manifestations. Rosiglitazone (RSG), a potent antidiabetic drug from the thiazolidinedione class, acts as a PPARγ agonist, promoting healthy expansion of WAT, as well as through MitoNEET modulation, enhancing mitochondrial function. Its use was banned due to increased cardiovascular risks. However, new therapeutic strategies that specifically target RSG to WAT while minimizing adverse effects have renewed its therapeutic potential. Therefore, the aim of this study was to investigate whether a sexual dimorphism exists in the insulin-sensitizing effects of RSG on WAT during obesity and inflammation.

Wistar rats of both sexes were fed either a control or a high-fat diet (HFD, 22.5% fat content) for 16 weeks. Two weeks prior to sacrifice, a group of HFD rats were treated with RSG at a dosage of 100 mg/kg of diet per day. In the retroperitoneal WAT, we measured parameters related to mitochondrial function, insulin sensitivity, inflammation, apoptosis, hypoxia, and lipid mobilization.

Our results demonstrated that in response to the HFD, male rats developed a greater degree of insulin resistance compared to females. RSG treatment was especially effective in males and significantly improved insulin sensitivity in this sex by increasing adiponectin levels, promoting PPARγ expression, enhancing mitochondrial function, as well as reducing lipemia, inflammation markers, and apoptosis.

Therefore, a sexual dimorphism exists in the antidiabetic effects of RSG on WAT, possibly associated with the worse metabolic profile observed in males. These findings underscore the necessity of tailoring therapeutic treatments according to sex.

Keywords: Rosiglitazone, White Adipose Tissue, Sex Dimorphism

G01 - 129 - P

Dietary anthocyannins as potential therapeutic agents in non-small cell lung cancer

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Non-small-cell lung cancer (NSCLC) is a leading cause of cancer death due to the long-term ineffectiveness of current therapies. Thus, there is an urgent need to identify novel therapeutic strategies for NSCLC patients. Interest in the use of complementary and alternative medicine, particularly polyphenol-rich natural products has increased considerably in the past two decades. Noteworthy, anthocyanins (ACNs), the most abundant polyphenols, have been suggested to be promising anticancer agents. Specifically, delphinidin, cyanidin, malvidin, pelargonidin, peonidin and petunidin are the most common ACNs available in vegetables and fruits. We investigated the anticancer properties of these ACNs against H1299, A549 and H358 human NSCLC cell lines. Specifically, delphinidin and petunidin showed the highest cytotoxic activities against H1299 (13.05 and 24.64 µm), A549 (25.56 and 58.98 µm) and H358 (50.26 and 105.1 µm) cell lines. Moreover, ACNs showing a considerable cytotoxic activity (IC50 lower than 106 µm) reduced the colony formation, cell migration, invasion and adhesion in all human NSCLC cell lines. Next, we performed immunofluorescence stainings using the most promising ACNs (delphinidin and petunidin) and we observed that these compounds increased apoptosis, senescence, DNA and telomeric damage, as well as decreased proliferation and expression of Telomerase (TERT), an enzyme that is reactivated in malignant cells. To complement this data, we performed human NSCLC xenografts in athymic nude mice, in which we observed that both delphinidin and petunidin reduced tumor growth. Our results point out that these compounds could be promising therapeutic strategies in NSCLC.

Keywords: Polyphenols, Anthocyanins, NSCLC, Anticancer Agents, Telomeric Damage, Telomerase (TERT)

G01 - 133 - P

Functional characterization of oncogene-transformed hepatic progenitor cells. Role of HGF and TGF-β signalling pathways

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Adult hepatic progenitor cells, also known as oval cells in rodent models, are bipotential cells that participate in liver regeneration after chronic injury. Both Hepatocyte Growth Factor (HGF) and Transforming-Growth Factor-beta one (TGF-β1) are key signals involved in liver regeneration and are key oval cells regulators. However, these factors also play a role in promoting tumour development and progression. In this study, we have investigated the effects of both factors, either alone or in combination, on the behaviour and properties of oval cells transformed with v-H-Ras oncogene.

Our results reveal that transformed oval cells gain proliferative and clonogenic growth capacity, as well as migratory and invasive capacity, as compared to parental oval cells. Signalling and mitogenic response to HGF is maintained *in vitro*, and a trend to enhanced tumour growth capacity *in vivo* is also seen with HGF treatment. TGF-β1, on the other hand, has a clear inhibitory effect on clonogenic growth, as well as on spheroid formation in non-attachment culture conditions. Moreover, transformed oval cells chronically treated with TGF-β1 were generated. Interestingly, despite losing epithelial features, these cells display similar clonogenicity, and tumour growth capacity *in vivo*, to the untreated cells, suggesting a loss of TGF-β1 inhibitory effects in chronically treated cells. Loss of some HGF stimulatory effects is also seen in TGF-β1-treated transformed oval cells.

Overall, our results reveal relevant changes in the functional properties of oval cells, together with some alterations in the response to HGF and TGF-b, after an oncogenic transformation.

Keywords: Hepatic Progenitor Cell, HGF, TGF-ß1, Tumorigenic Potential

G01 - 135 - P

Rosiglitazone and sex-specific improvements in hepatic insulin sensitivity of rats

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The dysfunction of white adipose tissue in the obese state is directly linked to the development of insulin resistance by promoting, among others, an ectopic fat deposition in the liver. Rosiglitazone (RSG), an antidiabetic PPARγ agonist, en-

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hances insulin sensitivity by mitigating steatosis. Given the pronounced sexual dimorphism observed in obesity-related pathologies, our hypothesis suggests that a sexual dimorphism exist in the anti-diabetic effects of RSG in the liver.

Wistar rats of both sexes were subjected to either a control or a high-fat diet (HFD, 22.5% fat content) for 16 weeks. During the two weeks preceding sacrifice, half of the HFD-fed animals received RSG treatment (100 mg/kg diet). Hepatic levels of markers of insulin sensitivity, adiponectin pathway activity, mitochondrial function, and lipid metabolism were assessed.

Our findings revealed that male rats exhibited a greater degree of insulin resistance in response to HFD compared to females, along with a more pronounced response to RSG. In males, RSG improved insulin sensitivity by attenuating gluconeogenesis and hepatic steatosis, accompanied by a reduced lipolysis and augmented fatty acid oxidation in the liver. Conversely, in females, RSG ameliorated steatosis by stimulating VLDL assembly and secretion.

This study underscores the sex-dependent nature of RSG effects, involving the modulation of different molecular targets that enhance hepatic insulin sensitivity in both sexes. Furthermore, it emphasizes the need for tailored therapies that account for sex as a variable in drug selection.

Keywords: Liver, Sex Dimorphism, Rosiglitazone, Insulin Sensitivity

G01 - 137 - P

Sexual Dimorphism in ARNTL Expression in Human Epicardial Adipose Tissue

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There are significant sex differences in the prevalence of cardiovascular diseases, with men showing a higher risk of myocardial infarction. Considering that adipose tissue function is influenced by sex, the aim of this study was to analyze gene expression differences in epicardial adipose tissue (EAT) between men and women. EAT samples were obtained from 13 men and 17 women (aged 65-75) without coronary lesions who were undergoing surgery for aortic or mitral valve disease. EAT expression profile was assessed using microarray analysis. Genes of interest were validated by RT-qPCR both in the group analyzed by microarray and in an independent group of patients (17 men, 10 women, aged 31-60 years). Additionally, genes of interest were evaluated in three white adipose tissue (WAT) depots of male and female rats. A total of 70 differentially expressed genes (DEGs) were identified in human EAT (FC > 1.5; p-value < 0.05). Among these genes, we focused on Aryl Hydrocarbon Receptor Nuclear Translocator-Like Protein 1 (ARNTL) gene due to its association with hypertension, type 2 diabetes mellitus, and myocardial infarction. EAT *ARNTL* expression was higher in men compared to women (FC = 2.29; p-value = 0.004). Moreover, this difference was confirmed in both patient groups as well as in the retroperitoneal and mesenteric WAT depots of Wistar rats, with a similar trend observed in the gonadal depot. In conclusion, differences in *ARNTL* expression in EAT could help explain disparities in cardiovascular risks between men and women, suggesting new avenues for sex-specific treatments.

Keywords: White Adipose Tissue, Aryl Hydrocarbon Receptor Nuclear Translocator Like, Cardiovascular Risk

G01 - 142 - P

Molecular pathophysiology of Hereditary Tyrosinemia Type I and molecular mechanism of NTBC

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Hereditary Tyrosinemia Type I (HT1) consists in an autosomal recessive rare disease caused by the deficiency of the last enzyme in the tyrosine catabolism pathway, fumarylacetoacetate hydrolase (FAH). FAH absence leads to the accumulation of toxic by-products, such as succinylacetone (SA), which provokes liver and kidney failure, as well as mental impairment. On the other hand, there are biochemical features whose metabolic origin is not well known, such as hypoglycemia, hyperinsulinemia, and hyperaminoacidemia.

The only treatment for HT1 is nitisinone (NTBC), which inhibits the enzyme 4-hydroxyphenyl pyruvate dioxygenase (HPD). This way, the second enzymatic step in the degradation of tyrosine is blocked, preventing the accumulation of toxic metabolites. Despite this, the effect of NTBC in other metabolic areas is still unknown.

Understanding the mechanisms that cause the central metabolism impairments and NTBC side effects, is crucial to comprehend the pathophysiology and to complement the current treatment.

We characterized an HT1 mouse model with G337S mutation in *fah* gene, using proteomics, genomics and metabolomics. We leveraged this model to analyse the metabolic changes that occur within the first month of the disease.

Our model resembles the main pathophysiological features of HT1. Therewith, we discovered the role of microRNA-539 in the downregulation of tyrosine catabolism pathway. Also, homozygous animals showed a decrease in epinephrine synthesis that enhanced hypoglycemia and amino acid

accumulation. In this way, NTBC restores euglycemia, but increases fumarate anaplerosis, leading to mitochondrial respiration problems.

These results suggest that NTBC treatment should be optimized, and this mouse model can be the subject for further investigations.

Keywords: Tyrosinemia, Liver, By-Products, Succinylacetone, FAH, Metabolism, Catecholamines, Epinephrine

G01 - 145 - P

Succinate receptor SUCNR1 in macrophages regulates the development of NAFLD-related HCC in mice

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Hepatocellular carcinoma (HCC) represents a major global health problem, with high incidence and mortality worldwide. Nowadays, non-alcoholic fatty liver disease (NAFLD) is becoming a dominant cause of HCC. In recent years, it has been observed that the tricarboxylic acid cycle intermediate succinate is elevated in the context of cancer. Succinate is considered an oncometabolite, for its involvement in the regulation of cancer development, enhancing tumor growth and metastasis, acting both in an autocrine and a paracrine manner by activating its receptor SUCNR1. However, the role of succinate/SUCNR1 axis specifically in the development of HCC remains unknown. In our study, taking in consideration that myeloid cells play a critical role in the tumor microenvironment, we used mice with myeloid-specific deficiency of *Sucnr1* and control mice to evaluate the impact of *Sucnr1* ablation in HCC development. Both groups were fed a choline deficient L-amino acid-defined high-fat diet for 36 weeks to induce NAFLD-related HCC. Mice lacking *Sucnr1* in myeloid cells presented a smaller liver with less macroscopic nodules than the control group, but larger in size. Regarding gene expression, the liver of mice lacking *Sucnr1* in myeloid line exhibited a higher expression of the tumoral markers *Myc* and *Yap1*, lower expression of *Col1a1*, which encodes the collagen type I alpha 1 chain, and higher expression of the anti-inflammatory genes *Arg1* and *Mrc1* than control mice. Additionally, mice with *Sucnr1* deletion in macrophages have reduced body weight and smaller adipocytes in subcutaneous white adipose tissue, with more infiltration of immune cells, than the control group. In summary, our results suggest a protector role of SUCNR1 in macrophages in the development of NAFLD-related HCC in mice.

Keywords: SUCNR1, HCC, NAFLD, Macrophages

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G01 - 148 - P

In vivo and *in vitro* deciphering of tumor-associated macrophage heterogeneity in experimental liver cancer

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Among several factors, inflammation is a key crucial of liver cancer. Most primary tumors grow within an inflammatory milieu in patients with fibrosis, but many aspects of the interaction between immune and cancer cells are unknown. We aimed to evaluate tumor-associated macrophage (TAM) gene expression in mouse liver tumors and in different *in vitro* models of macrophage and cancer cell interaction. Orthotopic liver tumors were generated in non-fibrotic or fibrotic BALB/c mice (fibrosis induced by i.p. CCl_4) by intrahepatic injection of BNL liver cancer cells. CD11b+ TAM were isolated by magnetic cell sorting from tumor (T) and non-tumor (NT) areas. Anti-inflammatory (ARG1, IL10, CD36) and TAM-specific (ferroportin-1, VEGFA) genes were analyzed by qPCR. Macrophage and cancer cell interaction was analyzed *in vitro* in RAW macrophages cocultured with BNL or RAW stimulated with BNL conditioned media (CM). Liver tumors were found in 90% of fibrotic mice but only in 50% of non-fibrotic mice. In fibrotic mice, the expression of anti-inflammatory genes and VEGFA was higher in TAM from T area compared to NT (ARG1, 5.7 vs. 13 fold change (fc) $p<0.05$; IL10, 1 vs 3.8 fc $p<0.05$; CD36, 1.8 vs 16 fc $p<0.05$; VEGFA, 1 vs 4.4 fc $p<0.05$). In contrast, ferroportin-1 decreased in T area (2.7 vs 1 fc, $p<0.01$). In non-fibrotic mice, different expression patterns between T and NT were only found in ferroportin-1 (2 vs 0.1 fc, $p<0.05$). Ferroportin-1 also decreased in RAW cocultured with BNL compared to RAW stimulated with CM. Overall, TAM showed different phenotypes depending on the presence of a fibrotic milieu or their interaction with cancer cells. This work provides insights into TAM regulation by tumor environmental factors and highlights ferroportin-1 as a potential target to modulate TAM.

Keywords: Liver Cancer, Fibrosis, Tumor-Associated Macrophages

G01 - 149 - P

Establishment of a murine model of localised advanced ovarian cancer to test novel intraperitoneal drug delivery systems

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Ovarian cancer (OC) is often diagnosed at an advanced stage, resulting in widespread peritoneal tumors and a poor prognosis. Despite treatment advances, further research is needed on novel intraperitoneal drug delivery systems (iDDS) to target microscopic tumor remnants after cytoreductive surgery. To succeed in translational research, well-characterized animal models of advanced OC are essential.

In this study, we aimed to establish and characterise a localised advanced OC mouse model using luciferase-transduced OVCAR-3 cells, to evaluate novel local iDDS therapies in future studies.

After genetically engineering OVCAR-3 to stably express the firefly luciferase gene (OVCAR-3Luc), luciferase activity was confirmed post-transfection. Mycoplasma absence was assessed using PCR. OVCAR-3Luc cell suspensions, with or without basement membrane extract (BME), were implanted into the preperitoneal space behind the posterior sheath of the anterior rectus abdomen in athymic nude mice. This location was chosen as free peritoneal cancer cells exhibit a preference for attachment here, facilitating tumor growth and invasion. Tumor progression was monitored weekly using bioluminescence in vivo imaging. Animals were sacrificed at various time points, and tumor tissue, plasma, and ascitic fluid were collected for histological, immunohistochemical and biochemical analyses.

Bioluminescent imaging confirmed a localised tumor formation. Cell concentration (1 vs 4x106 cells) did not impact tumor volume, whereas using BME with 1x106 cells significantly increased it. Furthermore, histopathology images revealed peritoneal infiltration and a papillary pattern.

In conclusion, the described murine xenograft model offers a valuable platform for testing novel local iDDS therapies.

Keywords: Localised Ovarian Cancer, Intraperitoneal Therapy, Drug Delivery System, Animal Model

G01 - 153 - O

C3G is a new regulator of liver and hepatocarcinoma metabolism. Impact on PKM2 expression

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The liver is a vital organ with multiple functions, playing a central role in the regulation of metabolism, xenobiotic detoxification, and biliary excretion. C3G is a guanine nucleotide exchange factor (GEF) for members of Ras superfamily, which regulates several cellular functions, through mechanisms dependent or independent of its GEF activity. C3G expression is high in hepatic progenitor cells and neonatal hepatocytes, but low in adult hepatocytes, being upregulated in hepatocarcinoma (HCC). Based on this, we have studied its function in the liver by generating a hepatocyte C3G deficient mouse (Alb-C3GKO). These Alb-C3G-KO mice showed mild liver damage, an impaired full liver maturation and an altered metabolic function. Under feeding conditions, serum glucose levels were reduced, and the hepatic expression of enzymes involved in glucose metabolism was altered in Alb-C3GKO mice, increasing PKM2 isoform expression. C3G deletion also enhanced the expression of lipogenic and ketogenic enzymes. Under fasting, *Pepck* and *Hmgcs2* mRNA levels were higher in Alb-C3G-KO mice. In addition, we have evaluated whether C3G regulates PKM2 expression through modulation of alternative splicing factors (PTBP1, SRSF2/3) using Hep3B and HLE HCC cell lines with permanent C3G silencing and C3GKO hepatocytes. The results suggest that C3G regulates the expression of PTBP1. In addition, we are evaluating how C3G silencing and C3GKO impact on insulin and glucagon response in hepatocytes and HCC cells.

In summary, our data support a role for hepatocyte C3G in the regulation of liver metabolism and glucose homeostasis, controlling the expression of several metabolic enzymes and PKM2 alternative splicing.

Keywords: Liver; C3G; Metabolism

G01 - 162 - P

Using CRISPR screenings to discover essential genes governing the progression of localized prostate cancer to metastasis

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Metastatic prostate cancer patients (mPCa) have a reduced survival mainly due to the inefficacy of the available treatments. Therefore, it is crucial to find new therapeutic targets to increase survival. Therefore, we sought to identify the most relevant regulators of mPCa onset by performing high-throughput CRISPR/Cas9 screenings in mPCa cell lines to identify new biomarkers and novel therapeutic targets to improve patient diagnosis and, potentially, survival.

This approach, followed by *in vitro* and *in vivo* characterization, has enlightened the intricated biological processes implicated on metastasis establishment allowing us to successfully identify genes and biological processes that significantly promote the invasion capacity of PCa cells like *PRMT7, SYCP3 and TECPR1*. To understand better the molecular mechanism governing the metastatic phenotype, we have also developed Gain of function screenings in low metastatic prostate cancer cells to identify new factors that promote the acquisition of the metastatic phenotype. This new approach has led to the identification of a new set of factors involved on the progression of PCA cells like (*PRKAR1B* or *RNF8*)

Nevertheless, these screenings rely on *in vitro* systems narrowing the screening windows to specific process of the metastatic cascade. To overcome these limitations, we have developed an *in vivo* genome wide screening using orthotopic inoculation of prostate tumor cells. This *in vivo* screening has allowed us to study the complete metastasis mechanism and facilitate the identification of different tropisms depending on the acquired abilities of the metastatic cells paving the way for the discovery of new prognosis biomarkers and/or therapeutic targets that could be of great value to the treatment of mPCa.

Keywords: Prostate Cancer, Metastasis, CRISPR/Cas9, Invasion, High-Throughput Screenings

G01 - 165 - P

Role of eIF5A in maintaining proteostasis during Heat Stress, Starvation, and Aging

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The eukaryotic translation Initiation Factor 5A (eIF5A) is an essential protein highly conserved throughout evolution and the only one known modified by hypusination, which is necessary for its activity. Hypusinated-eIF5A binds to ribosomes to facilitate the translation of specific peptide motifs containing consecutive prolines or combinations with glycine and charged amino acids. eIF5A has also been linked to various diseases, including cancer, diabetes and aging. Recent studies have shown that hypusinated eIF5A levels decrease with age and are associated with premature brain aging and a decline in mitochondrial functionality; while an increase improves cognitive functions and longevity. Aging, heat stress and starvation are well-known conditions that challenge protein homeostasis (proteostasis). Under these conditions, molecular chaperones, such as heat shock proteins (HSPs), play crucial roles in protein refolding, aggregation/disaggregation and degradation to ensure the maintenance of cellular proteostasis. We found that eIF5A is induced upon heat stress, and its depletion compromised yeast growth under heat stress and starvation. Moreover, temperature-sensitive eIF5A yeast mutants showed reduced translation of chaperones mRNAs (Hsp70 family: Ssa1/2/3/4; Hsp12/26/30) upon stress, dysfunctional protein aggregation/disaggregation kinetics and a shorter chronological lifespan (CLS). Thus, our results suggest that eIF5A plays a role in maintaining cell proteostasis by modulating the synthesis of specific chaperones during stress and aging, and therefore highlight eIF5A as a potential target for combating age-related disorders, such as Alzheimer's disease and Parkinson's disease, and promoting healthy aging.

Keywords: EIF5A, Proteostasis, Chaperones, Aging, Stress, Translation

G01 - 174 - P

NRP2 promotes disseminated tumor cells escape from dormancy and progression to metastasis

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Metastasis is the main cause of cancer-specific death, so deciphering its mechanisms is of vital importance. The main source of metastases are disseminated tumor cells (DTCs). These cells, before generating secondary tumors, go through a latency state called "cell dormancy" characterized by activation of quiescence and survival signals. The tumor microenvironment (TME) plays a fundamental role on regulating DTC's fate. Our study is focused on the role of nervous system mediators in this process. We have previously described that the neurogene Neuropilin 2 (NRP2) acts as a biomarker of poor prognosis in breast and head and neck cancer. Our results show that NRP2 promotes cancer cells proliferation, survival and invasion. Moreover, pluripotency assays revealed that depletion of NPR2, reduces tumor cells cancer stem cells capabilities as well as downregulates pluripotency genes expression. Furthermore, the absence of NRP2 also induces the acquisition of a mixed epithelial-mesenchymal phenotype, as well as reduces adhesion to type IV collagen-rich matrices. In agreement with this, analysis of TCGA patient data has shown that high expression of NRP2 correlates with extracellular matrix remodeling pathways suggesting that NRP2 is necessary for DTCs crosstalk with the stroma and that this could regulate DTCs fate. Moreover, NRP2 depletion induces a quiescent state in lung DTCs through the induction of p21 and p27 and inhibits lung metastases formation. Therefore, NRP2 seems to be a key dormancy awakening inducer, which makes it a promising predictive biomarker of metastasic recurrences and a potential therapeutic target for metastasis prevention.

Keywords: Dismenitated Tumor Cells, Metastasis, Neuropirin 2, Stem Cells, Stemness

G01 - 185 - P

Effect of stromal Gαq on tumor vascularization in Head and Neck Squamous Cell Carcinoma

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Tumor angiogenesis and lymphangiogenesis are two essential features of tumor progression and metastasis. They are especially critical in Head and Neck Squamous Cell Carcinoma (HNSCC), which has a high degree of irrigation, particularly from lymphatic vessels. Despite their great importance, the molecular mechanisms that control these processes are poorly understood.

The intricate interplay between the cellular microenvironment and tumor progression in HNSCC is a subject of intense research. CAFs are a pivotal component of tumor stroma since they are activated fibroblasts capable of influencing tumor cells through extracellular matrix deposition and remodelling, exosome secretion, and metabolic regulation via the autophagic process. Our recent findings show Gαq as a regulator of autophagic flux and a crucial role in modifying the tumor microenvironment in HNSCC by controlling fibroblast plasticity and functioning. Fibroblasts lacking Gαq expression exhibit CAF-like features, encouraging high collagen matrix deposition and ECM remodelling. Furthermore, exosomes released by these fibroblasts promote aberrant tumor growth.

Based on these precedents, we focused this project on understanding how the mechanical and matrix composition changes caused by the absence of Gq at the stromal level, as its secretome, influence the formation of the characteristic blood and lymphatic vessels of the most aggressive HNSCC tumors. Our findings, which combine 2D/3D culture systems, high-resolution microscopy, exosome biology, and mechanobiology, suggest a critical role of stromal Gαq in the modulation of these processes by influencing vascular architecture and endothelial functionality in response to mechanical stress, highlighting the potential of Gαq as a novel therapeutic target.

Keywords: Endothelial Cells, Tumor Vascularization, Gaq, CAFs, HNSCC, Mechanical Stimuli

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G01 - 191 - P

Assessment of migration capacity in KO models of ciliary syndromes

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Rare diseases manifesting abnormalities in the structure or function of cilia are grouped under ciliopathies. Bardet-Biedl Syndrome (BBS) stands out, with retinal dystrophy, obesity, polydactyly, renal-urogenital abnormalities, and cognitive impairment as the main diagnostic features. Previous proteomic analyses have identified an enrichment of proteins related to TGF-β signaling and the epithelial-mesenchymal transition (EMT) process. The TGF-β pathway is a key signaling pathway involved in different cellular processes such as cell migration and EMT. This study aims to demonstrate whether stimulating the TGF-β pathway in Bardet-Biedl knockout models leads to an increase in the activation of the EMT process and cell migration. For this purpose, three clones of BBS1 KO and three clones of BBS4 KO were analyzed using EMT-specific markers by qPCR. Additionally, a cell migration experiment using transwell and a wound healing assay were performed. When the TGF-β pathway is stimulated, there is an alteration in the expression of EMT markers, as well as an increase in cell migration and an increase in the speed of wound healing. Therefore, it can be concluded that the TGF-β pathway actively participates in the EMT process in the BBS1 and BBS4 KO models.

Keywords: Bardet-Biedl Syndrome, EMT, Ciliopathy, TGF-ß

G01 - 192 - P

The action of trastuzumab-deruxtecan beyond HER2-positive breast cancer

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Life expectancy and prognosis of HER2 positive breast cancer patients has significantly improved with targeted therapies. But the emergence of resistances has pushed the development of new drugs as the antibody-drug conjugates (ADCs), designed to selectively direct a chemotherapeutic agent to cells decorated with a specific cell surface molecule. Two of them, T-DM1 and more recently T-DXd, have reached the clinic.

T-DXd was initially approved to treat advanced HER2+ breast cancer, but recent studies have demonstrated its effect also on tumors expressing low or extra-low levels of HER2. Given the clinical impact of this discovery, we evaluated the effect of T-DXd on cell lines with different levels of HER2. T-DXd was effective in HER2 overexpressors at low concentration but was also effective on cells with low or null HER2 when treated at higher concentrations. We next investigated the effect of the free payload (DXd) on those cells and observed that all of them were similarly sensitive to the drug. In addition, we evaluated the levels of the final target of the ADC, TOPO1, involved in DNA damage repair and no correlation between TOPO1 levels and T-DXd effect was observed.

Besides, immunofluorescence experiments designed to analyze T-DXd internalization in the different cells, demonstrated that cells lacking HER2 were able of internalizing the drug through unknown mechanisms independent of the receptor, pointing to a mechanism of nonspecific internalization, possibly related with fluid-phase endocytosis, that we are currently investigating.

In conclusion, our data indicate that the presence of HER2 is important for T-DXd action, but also point to other factors beyond the presence of the receptor that are key in this response and need to be further investigated.

Keywords: Breast Cancer, HER2, ADC, T-DXd

G01 - 194 - P

Lung fibroblasts in secondary microenvironments regulate disseminated tumor cells fate and progression to metastasis through FGF5/FGFR2 pathway

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Metastasis is one of the main causes of death among solid tumor patients. Disseminated tumor cells (DTCs) travel to secondary organs where they remain in a reversible quiescent state called dormancy, before reactivating and giving rise to metastasis. Metastases diverge from their primary tumors and hence, are usually resistant to treatments. The microenvironment in the secondary organ is known to be an important mediator of dormancy, so studying the molecular mechanisms is key for developing new therapies.

In this context, we have studied the lung stroma as a regulator of the dormant to proliferative transition. We had previously seen that lung fibroblasts secretion of TGFß1 upregulates NRP2 expression *in vitro,* a protein that is implicated in tumor progression and metastasis. Moreover, we have seen that treatment with lung fibroblasts conditioned medium increases tumor sizes *in vivo* and activates ERK *in vitro*. Additionally, we had previously shown that FGF5 and FGFR2 regulate fibroblasts mediated treatment resistance in breast cancer. Accordingly, we have analyzed FGFR2 expression in our cell lines and discovered that it is overexpressed in proliferative lung DTCs. Furthermore, pharmacologic or genetic inhibition of FGFR2 in lung DTCs derived cells induces cell cycle arrest in G1 phase and reduces tumor size *in vivo*.

We propose that lung fibroblasts secretome promotes DTCs release from dormancy which can progress into lung metastasis. Additionally, we suggest that lung stroma regulation of lung DTCs might be mediated through FGFR2 pathway.

Keywords: Metastasis, Dormancy, Fibroblasts, FGFR2

G01 - 199 - P

Mangostanin as an antimicrobial and biocompatible topical antiseptic for skin and O tissues

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Skin and O tissue infections are prevalent global health issues. While various effective antiseptics against diverse bacteria exist, concerns regarding toxicity and resistance have arisen. Therefore, it is necessary to find novel antiseptic alternatives. This study aimed to determine the antibacterial activity and biocompatibility of mangostanin (MGTN), a xanthone present in the fruit of *Garnicia mangostana*, using both, different bacterial and cellular *in vitro* models and a periodontitis rat model.

Different concentrations of MGTN were evaluated in solution, using chlorhexidine (CHX) as control, to assess its antimicrobial activity against different skin and O bacteria. We also assessed its biocompatibility on conventional 2D cultures of human gingival fibroblasts and its ability to prevent plaque biofilm formation using pooled human saliva. Furthermore, the biocompatibility of MGTN gel formulation was tested on 3D models of human epidermis and O epithelium and examined its antimicrobial activity, compared to commercial antiseptic formulations. Subsequently, the study examined the effects of the MGTN gel formulation compared to a CHX-containing commercial gel on rats with induced periodontitis.

MGTN exhibited remarkable antimicrobial effectiveness against all the skin and O pathogens examined while displaying no cytotoxicity on human gingival fibroblasts neither

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on 3D human epidermis and O epithelium compared to the gold standard CHX. It also effectively reduced plaque biofilm formation from saliva. In the in vivo model, the MGTN gel reduced inflammation and influenced O microbiota and histomorphometry of gingival tissue.

MGTN is proposed as a promising antiseptic for skin and O tissue infections.

Keywords: O And Skin Diseases, Antiseptic, Xanthone

G01 - 201 - P

Mutational characterization of the *CORO2B* gene and its implication in Bardet-Biedl Syndrome

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Cilia are conserved structures projecting from the surface of nearly all vertebrate cell types. In mammals, cilia function as a signaling center essential for the reception and transmission of extracellular signals. Ciliary malfunction causes ciliopathies, including Bardet-Biedl Syndrome (BBS). Mutations in the coronin-2b (CORO2B) gene have been identified in BBS individuals. This protein, part of the actin-regulating family, is involved in multiprotein complex assembly, and its malfunction is linked to various diseases. This study aims to determine its relationship with ciliopathies.

To assess CORO2B's role in BBS, we performed qPCR to compare mutant and wild type (WT) expression, and examined *CORO2B* gene expression across different cell types. Additionally, Western Blot (WB) was used to analyse CORO2B protein expression. Our results indicate that CORO2B mutations exhibit differential expression compared to WT, with significant gene expression variations among the analysed cell types. WB data supported these findings at the protein level.

Our research suggests that CORO2B mutations affect expression at both RNA and protein levels, potentially contributing to ciliopathies like Bardet-Biedl Syndrome. These findings lay the groundwork for future studies on CORO2B's role in cilia biology and ciliopathic diseases.

Keywords: CORO2B, Actin, Mutations, Ciliopathies, BBS

G01 - 202 - P

Investigating the role of pulmonary ionocytes in cystic fibrosis through transcriptomic analysis and hiPSC-derived models

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Human pulmonary ionocytes are a rare cell type within the airway epithelium, notable for their rich expression of ion transport channels, including the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Mutations in the CFTR gene are the cause of cystic fibrosis (CF), a severe genetic disorder affecting the respiratory and digestive systems. Thus, ionocytes may play a key role in CF pathophysiology, possibly by affecting disrupted ion transport mechanisms.

Single-cell RNA sequencing (scRNA-seq) technology has enabled detailed transcriptomic profiling of individual cell types, including ionocytes. However, knowledge of how ionocyte expression profiles are altered in CF remains limited. To address this, we explored publicly available scRNA-seq data to identify transcriptomic differences between ionocytes from healthy individuals and those from CF patients, aiming to uncover specific disease-associated changes.

Next, we used human induced pluripotent stem cell (hiPSC)-derived airway epithelial cells (AECs) to experimentally validate our observations. We differentiated AECs from CF patient-derived hiPSCs and their corrected isogenic counterparts and characterized them phenotypically and functionally. Importantly, CF hiPSC-AECs displayed CFTR dysfunction, but the presence of ionocytes was confirmed in both genotypes. Through immunocytochemistry and gene expression assays, we assessed the expression of key proteins and obtained quantitative data on differences between WT and CF conditions.

Our study offers insights into the potential role of ionocytes in the respiratory complications of CF, which could pave the way for the identification of novel molecular pathways for therapeutic intervention for patients suffering from this debilitating disease.

Keywords: IPSC, Cystic Fibrosis, Ionocytes, Transcriptomics

G01 - 207 - P

Deciphering the regulation of the biology of TDP-43 by different diets in the brain

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TDP-43, a nuclear protein encoded by the *TARDBP* gene, accumulates in cytoplasmic inclusions in several neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43 has a central role in the regulation of the cellular RNA metabolism, and when this protein is dysregulated, as in ALS, it causes aberrant splicing in thousands of target genes. The mouse model carrying a physiological point mutation in the endogenous *Tardbp* gene, the M323K mutation, develops cognitive alterations and progressive motor impairment with TDP-43 mislocalization, and alteration in the splicing of its targets. It is not known whether and how changes in systemic metabolism could affect the regulation of brain TDP-43 and in its function. Here, we use different lifelong diets in wildtype and TDP-43 M323K mice to evaluate the effect of the diet-induced metabolic changes in the regulation of TDP-43 in the frontal cortex of the brain. We performed RNA sequencing, with both short and long reads technologies, and subsequent bioinformatics analyses of the transcripts and splicing events obtained in the different groups, followed by multiple validations using PCR and agarose gel electrophoresis.

Our results might have a major impact on elucidating how different metabolic lifestyles could be modifying the appearance of brain proteinopathies.

Keywords: TDP-43, Diets, Splicing

G01 - 215 - P

Characterization of extracellular vesicles secreted by *Salmonella*-infected intestinal cells and their ability to modulate the immune response of adjacent cells

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Extracellular vesicles (EVs) play a crucial role in bacterial infections, acting as versatile communicators that influence disease progression, and offering insights for new therapeutic strategies. However, the communication mechanism facilitated by host-derived EVs during bacterial infection is unclear. This study aimed to characterize EVs released by intestinal cells during *Salmonella* infection and analyze their impact on neighboring cells. Intestinal epithelial cells were infected with *Salmonella*, and EVs were isolated from the culture media. Remarkably, infected cells produced more EVs than control cells, suggesting a cellular response that could contribute to immune defense and modulate neighboring cell responses. Proteomic analysis identified 2073 proteins in EVs, with 403 showing differential expression, and 157 markedly overexpressed in EVs from infected cells. Key clusters of host cell proteins were highlighted: those involved in leukocyte function (FLOT1, FYN, LYN), activation of antigen-presenting cells (JAK1, NOTCH2), and transferrin proteins (TF, LTF) linked to angiogenesis and inflammatory pathways. EVs also contained numerous pathogen-derived molecules including outer membrane proteins (OmpA) iron transport proteins (IroN, FepA, CirA), glycolysis-related proteins (pgk, pykF), and lps assembly proteins. To assess the impact of EVs from infected cells on neighboring epithelial cells, we conducted an EV uptake assay, which led to a substantial upregulation of IL8, TNFα, CCL2, and ICAM1, indicating a robust activation of the inflammatory response pathway. This study clarifies the role of EVs in *Salmonella* infection, highlighting their ability to transport both host and pathogen proteins and to impact neighboring cells by activating the immune response cascade

Keywords: Salmonella Infection, Extracellular Vesicles (EVs), Immune Response,Intercellular Communication

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G01 - 223 - P

Deciphering the effect of different lifelong diets in the motor and cognitive alterations caused by TDP-43 dysregulation

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It is unknown whether and how different metabolisms affect the onset of neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The cytoplasmic aberrant accumulation of the TDP-43 protein is a hallmark of ALS and many FTD cases. Mutations in *TARDBP*, which encodes the TDP-43 protein, cause ALS and, less frequently, FTD in patients. Metabolic alterations play a crucial role in the pathophysiology of these complex disorders, but it is unknown how.

We conducted a comprehensive study using a mouse model with a point mutation in the *Tardbp* gene (*Tardbp*$^{M323K/M323K}$), which develops both cognitive and motor alterations with cytoplasmic mislocalization of TDP-43. These mice were put under three different diets from 5 weeks until 12 months of age. We analysed a battery of motor and cognitive tests longitudinally to evaluate the impact of the diets on the disease progression. We performed protein analyses by western blot and immunohistochemistry of neurodegeneration markers, including the nuclear-cytoplasmic mislocalization of TDP-43 in different parts of the nervous system. We implemented a multi-omic approach, transcriptomics and lipidomics, to uncover the main metabolic pathways involved, as well as their functional relationship with brain metabolic studies by glucose positron emission tomography (^{18}F-FDG PET).

The results revealed that the different diets were able to modify the effects of the mutation on brain alterations and we identified potential pathways responsible for the observed modifications.

This study represents an entry point to understand how diets can affect brain function, including the modification of alterations caused by mutations, which has major implications in the field of neurodegenerative diseases.

Keywords: TDP-43, Diets, Cognition, ALS, FTD, Mouse

G01 - 226 - P

Characterization of brown adipose tissue during the early development of type 1 diabetes in rodents

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Type 1 diabetes mellitus (T1D) is a chronic and autoimmune disease characterized by an inflammatory condition that contributes to destroy insulin-producing cells, leading to hyperglycemia and other associated diseases. Normalizing glucose homeostasis is the main target to prevent T1D's side effects. Brown adipose tissue (BAT) has recently emerged as a relevant player involved in both glucose and lipid metabolism. BAT utilizes glucose and lipids to produce heat and maintain body temperature through thermogenesis. This is possible thanks to its large number of mitochondria and specific uncoupling protein 1 (UCP1) expression. BAT activity is reduced in some metabolic diseases such as type 2 diabetes and obesity. However, little is known about the pathophysiology of BAT in T1D. Here, we aim to characterize BAT during the early development of T1D.

We used the BB rats as a T1D model. BAT was dissected from BB rats at 6 and 7 weeks of age, in a state of preclinical diabetes. BAT's morphology and inflammation were studied histologically. The expression of markers of inflammation, thermogenesis, lipid and glucose metabolism, mitochondrial fusion and reticulum stress was analyzed by qRT-PCR.

Our results indicate that BAT from T1D BB rats is smaller and exhibits increased macrophage infiltration, without changes in endoplasmic reticulum stress. Despite this, thermogenesis markers show that is more active. We conclude that BAT appears to buffer the deleterious effects of T1D at an early stage of the disease when normoglycaemia is still maintained. Further studies include the characterization of BAT at later time points (10 and 11 weeks of age) when T1D is fully established.

Keywords: BAT, BioBreeding Rat, Hyperglycemia, T1D, Thermogenesis, Ucp1

G01 - 232 - P

Sexually dimorphic effect of TGFβ3 in determining mitochondrial function in the development of fibrosis in kidney

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Transforming growth factor β1 (TGFβ1) is associated with renal fibrosis; however, TGFβ3 is not so clear. We have previously demonstrated that deficiency of TGFβ3 is implicated in renal fibrosis and the dysregulation of lipid metabolism in males, but not in females. Thus, we aimed to unravel the sexually dimorphic effect of TGFβ3 in the pathophysiology of the kidney.

GTT, ITT and adiponectin and PAI-1 were measured in male and female 16-week-old heterozygous (HZ) TGFβ3 and the wild-type (WT) littermate mice. Seq RNA, RT-PCR and analysis of mitochondria by Seahorse was performed.

Lack of TGFβ3 did not affect body weight, insulin sensibility, serum PAI-1 or adiponectin levels in both genders. However, we found decreased estradiol levels in HZ compared to WT male mice (34.6 *vs*. 66.9ng/ml, P≤0.05), but not in females. HZ renal cortex from males showed an altered respiratory capacity compared to WT. KEEG pathways from seqRNA analysis showed a downregulation of oxidative phosphorylation and an alteration in AMPK signaling pathways in HZ males regarding their WT littermates, with a decrease in β-oxidation (*PPARα, PGC1α, PGC1β and CPT1*, *P≤0.05*) and mitochondria genes (*Mfn1* and *Opa1)* in the HZ male mice. These alterations did not occur in female kidneys. Although androgen receptor (AR) was significantly increased in HZ compared to WT male mice, no major changes were observed in estradiol receptor alpha (ER-α) or ER-β in any genotype neither male nor female mice. Metalloproteinases (MMP) 2 and 9 genes were altered in female HZ compared with WT kidneys. Altogether, these data suggest an important role of TGFβ3 in the sexually dimorphic effect of mitochondria function alterations in the development of renal fibrosis.

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Keywords: Kidney, Fibrosis, TGFbeta3, Motochondria, Chronic Kidney Disease

G01 - 233 - P

Vessel co-option can be inhibited by using b1-integrin inhibitors in experimental lung metastases

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Tumor vascularization is essential for solid cancers to grow. Tumor blood vessels provide oxygen to cancer cells. Vascularization mechanisms can be divided into angiogenic or non-angiogenic. In tumor angiogenesis, new blood vessels are formed from pre-existing ones. Vessel co-option (VCO) is a non-angiogenic mechanism in which cancer cells hijack pre-existing blood vessels of the affected tissue. Non-angiogenic tumors can arise intrinsically or as therapeutic resistances, especially against anti-angiogenic treatments, and present a worse prognosis. During VCO cancer cells are adhered to basement membranes of pre-existing blood vessels through integrins such as b1.

The objective of this study is to promote the predominance

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of VCO tumors by inhibiting b1 integrin. Experimental models of lung metastases were performed from a primary breast tumor (4T1 cell line) in female BALB/c mice, which were treated with β1 integrin inhibitors: ATN-161 and c(phg-isoDGR-(NMe)k)TFA, and in combination therapies with carboplatin. Lung samples were analyzed using various histological procedures to study lung size, hypoxia, extracellular matrix fibers, vascular network, and immune cell infiltration; to characterize the changes produced and the tumor microenvironment.

Our results indicated that VCO can be inhibited by targeting b1-integrin and that this inhibition generates a favorable tumor microenvironment and enhance chemotherapy efficacy.

Keywords: Vessel Co-Option, Lung Metastases, B1 Integrin, Cancer

G01 - 239 - P

Study of enfocytic pathway in Bardet Biedl syndrome: TGF-β as a model

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Ciliopathies are a group of diseases characterized by an alteration in the function or structure of the primary cilium, a cellular organelle responsible for signal transduction. One of these pathologies is Bardet-Biedl syndrome (BBS), which is distinguished by mutations in genes that encode the proteins that form the BBSoma. This is a protein complex that, among other functions, participates in regulating ciliary vesicular traffic. An alteration in these proteins could produce an alteration in the movement of vesicles, preventing correct transduction of external signals, regulation of ciliary activity, stimulation of cell growth or cell death (apoptosis).

The TGF-β pathway is an essential pathway involved in various cellular processes. In this project, the endocytic pathway of the RI receptor (TGFBRI) of this pathway is studied through immunofluorescence as well as the colocalization between different endosomal markers with the BBSomal proteins BBS1 and BBS4. Furthermore, transferrin is used as a comparison to TGFBRI trafficking since it is a glycoprotein with a membrane receptor with ciliary transport. For this, a model with a wild phenotype, a **BBS1** KO and **BBS4** KO model, was used.

With the results obtained, we theorize the possible function outside the BBSome of the BBS1 and BBS4 proteins in endocytic trafficking given the different significance between both KO models and the WT.

Keywords: Bardet-Biedl Syndrome, Ciliopathies, Endocytosis, TGF-ß

G01 - 241 - P

Co-opted tumor blood vessels can be normalized by using a combination of losartan and a BMP9-ALK1 targeting antibody

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During tumor development, primary tumors and metastases undergo different vascularization strategies to obtain the necessary nutrients and oxygen. The most studied mechanism is angiogenesis, the formation of new blood vessels from pre-existing ones. However, some tumors grow through non-angiogenic vascularization strategies, such as vascular co-option (VCO), hijacking pre-existing blood vessels in the tissues. These tumors appear to have a worse prognosis. Co-opted blood vessels show quiescent phenotype.

To reverse the VCO phenotype of tumors, we inhibit vascular quiescence, promoted by the cytokine BMP9 via the activin receptor-like kinase 1 (ALK1) receptor. By inhibiting this BMP9/ALK1 pathway, we aim to activate blood vessels and promote angiogenesis. In this way, we aim reverse the VCO phenotype and develop a new mature network of blood vessels with better prognosis for future anti-tumor treatments.

We performed injectable lung metastases in BALB/c mice with the 4T1 breast cancer cell line. These lung metastases undergo VCO. To inhibit the BMP9/ALK1 pathway, we used the BMP9-ALK1 inhibitor PF-03446962 (PF). Our *in vitro* studies demonstrate that treatment with PF increases the proliferation of endothelial cells (EA.hy-926). Treatment with PF at high dose results in an increase in tumor size, as well as causing various changes such as vessel regression, increased tumor hypoxia and extracellular matrix deposition. We believe that new deficient blood vessels are being generated that promote tumor growth. Losartan treatment normalizes these blood vessels and controls tumor growth.

Our results demonstrate that VCO can be inhibited by a combination of losartan and PF, generating a fine mature blood vessel network that promotes a favorable tumor microenvironment.

Keywords: Vessel Co-Option, Metastasis, Angiogenesis, Tumor Vascularization

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Aged microglia promote pro-inflammatory microenvironment and tumour growth arresst

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Ageing is biological process characterised by cellular senescence and altered immunity cells response. In that vein, microglia are the primary innate immune cells of the brain. Once they are activated, their phenotype fluctuates from a pro- to an anti-inflammatory state. In brain cancer, tumour-associated microglia and macrophages (TAMs) play a major role during disease progression. One of the most deleterious events in brain tumours is the strong immunosuppressive microenvironment. Ageing switches microglia homeostatic state into a “priming” (pro-inflammatory) phenotype, which may counteract the immunosuppressive microenvironment found in primary (glioblastoma) and secondary brain tumours (brain metastases).

Previous research shows the negative correlation between advanced age and the onset of brain tumours. Thus, the aim of this study is to determine if aged microglia create a pro-inflammatory environment that may boost the brain immune response against cancer cells.

To achieve this goal, we performed cell culture techniques using BV2 (microglia), EO771 (breast carcinoma) and GL261 (glioblastoma) murine cell lines, to study their senescent (β-gal and p16) and inflammatory (iNOS and Arg1) levels. In vivo approaches in different age mice also evaluated the microglial role in tumour conditions. The participation of other molecules as interleukin-34, a cytokine released by neurons whose function is crucial in the growth and survival microglial process in the grey matter, was also analysed by using a murine knockout model.

Our results showed that aged microglia exhibit a pro-inflammatory state, both in vitro and in vivo. This senescent-related activation reduced the immunosuppressive tumour microenvironment, supporting brain tumours growth arrest.

Keywords: Ageing, Microglia, Brain Tumour

G01 - 256 - P

Deciphering social jet lag chronodisruption effects on the liver

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Social jet lag quantifies the dissonance that often emerges between our internal circadian rhythms and external societal schedules, resulting in behavioral changes. Previous research in humans has established a connection between social jet lag, a mild type of chronodisruption, and an elevated risk of obesity. Epidemiological studies have also linked chronic chronodisruption to an increasing incidence of specific human malignancies such as prostate, colorectal and breast cancer.

In this study, we delve into the hepatic changes induced by social jet-lag chronodisruption. Our preliminary data suggest that the stress induced by social jet lag triggers a dynamic response within the hepatic tissue, as well as in secreted exosomes. Within the liver, we have identified disparities in clock gene expression and a more pronounced impact on the overall transcriptome. Among the most differentially expressed pathways, we have observed an upregulation of *de novo* lipogenesis, which aligns with the development of hepatic microsteatosis in mice exposed to social jet lag and differences in mitochondria bioenergetics biomarkers.

Also, we propose to rely on non-invasive techniques for both early detection and monitoring of liver disease progression associated to chronodisruption by deciphering the liver´s secretome, represented by liver exosomes (hepatosomes) under these pathological situations. Hepatosomes analyzed through mass spectrometry reveal that exosomes from the liver are loaded in a circadian manner, possibly serving as a means of communicating temporal information and responding to chronodisruption on a hepatic level, which may have broader implications for the entire organism.

Keywords: Social Jet Lag, Chronodisruption, Circadian Rhythm, Liver, Disease

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G01 - 260 - P

Immune cell dynamic barcoding for clonal resolution of the metastatic tumour microenvironment

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Single-cell (sc) technology has refined our view of immune cells. While this *terminal snapshot* reveals local cellular states, it often overlooks that hematopoiesis is a clonal process. Perturbations in peripheral organs can shift bone marrow hematopoietic balance into an emergency state, leading to novel clones. **Metastasis** is one such perturbation, causing a local increase in myeloid cell output and a decrease in lymphoid compartment diversity, critically reducing T cell clonality. Whether this reflects increased myeloid progenitor diversity and lymphoid depletion remains unknown. We hypothesize that *clonal myeloid-derived progeny at metastatic sites mirrors increased clonal diversity in the bone marrow (BM), outcompeting B and T cell progenitors, thus restricting lymphoid* **anti-tumor immunity** *and enabling metastases to expand*. We will test this using the triple-negative breast cancer (TNBC) metastasis models in mice. To trace clonal relationships between hematopoietic and stem cell progenitors (HSPC) and their progeny, we will use **lineage tracing** systems based on **dynamic barcoding**. Sequential and heritable accumulation of barcodes will be generated using CRISPR-Cas9 in HPSC. scDNA/RNA-sequencing of the CD45 compartment will help us infer a phylogenetic tree, mapping immune relationships with clonal states at metastatic organs. Our preliminary data suggest progressive immune lineage selection in metastatic organs. Initial lentiviral (LV)-based tracing of murine HSPC showed a myeloid bias, favoring *in vivo* monocyte differentiation. We are optimizing our HSPC infection protocol for mice reconstitution to allow complete immune cell tracing. The final goal is to identify clonal relationships in metastatic TNBC that can be targeted by current therapies e.g., immunotherapy.

Keywords: Lineage Tracing, Dynamic Barcoding, Metastasis, Tumour Microenvironment, Tissue Immunity.

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BMAL1 controls circadian glucose metabolism in breast tumors and lung metastasis

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Circadian rhythms (CRs) are rhythmic processes occurring every 24 hours due to the earth's daily rotation and are synchronized by stimuli called *Zeitgebers* (ZT). CRs control physiological processes such as sleep-wake cycles, metabolism and immunity. At the molecular level, CRs are controlled by a set of transcription factors (TF), which activate or repress clock-controlled genes (CCGs) to drive 24-rhythmic transcriptional programs. BMAL1 is the main TF orchestrating CRs, and it's binding to E-box regulatory regions in the genome sets the expression of CCGs. Importantly, while disruption of the circadian clock is associated to cancer, a physiological understanding of CRs in the tumor microenvironment remains elusive.

Here, we aim to investigate cell-intrinsic programs controlled by BMAL1 in two preclinical models of cancer: primary breast tumors (E0771) and lung metastasis (KP). We *hypothesize that BMAL1 expression in cancer cells controls daily pro-tumoral programs.*

To test this, we first assessed whether time-dependent changes in the host drive differences in tumor burden. We found increased burden in mice injected in the morning (ZT5, or noon) as opposed to the ones injected at night (ZT13, 8pm). Next, to determine which molecular programs underlie preferential diurnal growth, we performed bulk transcriptomics on tumoral KP cells from mice harvested at different ZTs. Interestingly, we identified a time-dependent metabolic program enriched in glucose metabolism, hypoxia and mTORC1 signalling. To investigate which daily tumor metabolites promote growth, we set to perform mass spectrometry-based metabolomics along 2-ZT.

Our work suggests that cancer cell metabolic rhythmic programs can be harnessed therapeutically, and open new venues for combination chronotherapies.

Keywords: Cancer, Circadian, BMAL1

G01 - 271 - P

Chemotherapy resistance mechanisms in colorectal cancer cells

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Our aim is to determine the underlying mechanisms involved in resistance to conventional chemotherapy treatment that is responsible for most of the deaths associated to colorectal cancer as it eventually leads to metastasis. For this purpose, our first objective has been the establishment of resistant cancer cell lines. Cells are incubated over short-periods of time with a chemotherapy agent, which is then removed to let the cells recover. This process is repeated over a long time period with increasing concentrations of the chemotherapy agent. To assess drug-resistance, cells are tested in a dose-dependent manner to evaluate differences in cell viability compared to parental cells that have not received previous treatment. Comparison of IC50 values can then used as a parameter to determine whether the cells are resistant. Moreover, distinct cell morphology is also observed in the resistant cell line. A molecular marker (the Multi-Drug Resistance protein MDR1) was also evaluated using anti-bodies labeled with a f luorescent probe, to determine differences in expression using fluorescence microscopy. For the next stages of our project, we sought to perform proteomic analyses that would reveal differentially expressed proteins between the resistant cells and the parental cells, to uncover not-yet described regulators in the acquisition of chemotherapy resistance. A bioinformatic analysis of data deposited already in databases is also included as a preliminary study to reveal potential targets and that will at least validate candidate proteins found in our study.

Keywords: Colorectal Cancer, Drug-Resistance

G01 - 272 - P

PD-1/PD-L1 functional engagement quantified by QF-Pro® in NSCLC is a strong predictor of immune checkpoint inhibitors response

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Poster abstract: 60-80% of ICI-treated NSCLC patients do not respond to treatment, thus PD-L1 biomarker fails to correctly stratify. Nevertheless, in 188 NSCLC tumour samples we identified with high significance ICI responders by measuring high levels of PD 1/PD L1 interaction with QF-Pro®. These responses were irrelevant of PD-L1 expression status (PD-L1 TPS <1%).

Keywords: PD-L1, Biomarker, Inhibitors, NSCLC, Tumors

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G01 - 282 - P

Evaluation of the antineoplastic effect of CBD-loaded nanoemulsions in canine mammary carcinoma

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El cáncer mamario es altamente prevalente en perras que no están esterilizadas o que fueron esterilizadas después de los 2 años de vida. Una posible alternativa terapéutica involucra terapias con cannabinoides como el cannabidiol (CBD), ya que, diversos estudios muestran que puede inhibir la progresión tumoral de algunos tipos de cáncer mediante la detención del ciclo celular, inducción de apoptosis e inhibición de la migración, sin embargo, por su baja solubilidad acuosa posee una baja compatibilidad biológica. Una alternativa que podría mejorar su dispersibilidad en medio acuoso es su incorporación en nanoemulsiones del tipo aceite en agua (O/W). Actualmente existe escasa evidencia acerca del efecto de nanoemulsiones de CBD (CBD-nem) sobre células de carcinoma mamario canino, por esta razón, se incorporó CBD en nanoemulsiones para evaluar el efecto antineoplásico sobre una línea de carcinoma mamario canino (CF41.Mg), y su seguridad sobre una línea celular de epitelio renal canino (MDCK). Se sintetizaron nanoemulsiones las cuales fueron caracterizadas obteniendo tamaño nanométrico (150 -170 nm), con una alta eficiencia de encapsulación (99%). En los ensayos de viabilidad se observó que CBD-nem induce un efecto en la viabilidad celular en células de carcinoma, aunque de menor intensidad que con CBD en etanol (CBD-E), al igual que en las células de epitelio renal. Se determino también una disminución significativa en la capacidad de migración e invasión de las células CF41.Mg, tanto con 20 nM de CBD-E, como con 20 y 50 nM de CBD-nem. Esta es la primera investigación que observa efectos antineoplásicos en esta línea celular, por lo que, es necesario continuar con la investigación en el área.

Keywords: Nanoemulsions, Canine Mammary Carcinoma, Cannabidiol

G01 - 283 - P

Las células supresoras derivadas de mieloides (MDSC) están disminuidas en el trofoblasto de la placenta en diabetes gestacional. Posible papel de la leptina

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Introducción: La diabetes mellitus gestacional (DMG) es la patología más frecuente del embarazo, aumentando la morbi-mortalidad materno-fetal. La inflamación es una característica común en la DMG. Las células mieloides supresoras (MDSC) son células innatas inmunosupresoras que pueden participar en la tolerancia fetomaterna. Así, se han encontrado niveles elevados de MDSC en la sangre periférica y el cordón umbilical en embarazos normales. Nuestra hipótesis fue que el trofoblasto de la placenta de la DMG podría tener niveles más bajos de MDSC, y esto podría estar mediado por la leptina sobreexpresada en el trofoblasto de DMG.

Material y Métodos: Analizamos placentas (5) de embarazos con DMG y controles sanos (10) obtenidas mediante cesárea programada, para estudiar el número de MDSC y estudiamos los efectos in vitro de la leptina en MDSC en leucocitos aislados de donantes sanos en presencia o no de inhibidores de MAPK (PD98059) y PI3K (wormannin) durante 24 h. Las muestras se analizaron por citometría de flujo de las MDSC. El análisis estadístico se llevó a cabo por un test de ANOVA seguido de Bonferroni.

Resultados: Observamos que el trofoblasto de la placenta de la DMG contiene un porcentaje más bajo (70%) de MDSC en comparación con el trofoblasto control. Además, observamos que, in vitro, la leptina disminuyó el número de MDSC en los leucocitos de la sangre periférica, de forma dependiente de la dosis, con un efecto máximo a 100 nM (25% disminución). Además, encontramos que este efecto es dependiente de MAPK, pero independiente de PI3K.

Conclusión: Las MDSC están disminuidas en el trofoblasto de la placenta de la DMG, facilitando la inflamación y la leptina sobreexpresada, a través de MAPK podría mediar en este proceso.

Keywords: Diabetes Gestacional, Placenta, MDSC, Leptina

G01 - 284 - P

Isolation and preliminary characterization of IGF1R-deficient murine embryonic and adult lung fibroblasts

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Fibroblasts are the most common cell type of the connective and the principal source of extracellular matrix proteins, such as collagens and fibronectin. Reciprocally, these proteins can promote the differentiation of profibrotic myofibroblasts through positive feedback regulation during fibrosis. In the lung, fibrosis is a growing clinical problem involved in many respiratory diseases including idiopathic pulmonary fibrosis, asthma, COPD, lung cancer and during clearance of COVID-19. Insulin-like Growth Factors (IGF) act as profibrotic in the lung, and IGF receptor (IGF1R) deficiency reduces lung fibrosis in mouse models of bleomycin (BLM)-mediated fibrosis, asthma and lung cancer. In order to understand the molecular mechanisms behind this action we aimed to isolate and characterize IGF1R-deficient murine embryonic fibroblasts (MEFs) and IGF1R-deficient adult murine lung fibroblasts (AMLFs). Although naïve *Igf1r-/-* MEFs did not display evident differences in growth rate or morphology respect to controls, they showed a significant decrease in ATP production–linked mitochondrial respiration in either presence or absence of pyruvate. After a BLM challenge, IGF1R deficiency normalized cell and mitochondrial morphology as well as mitochondrial homeostasis. In addition, *Igf1r-/-* MEFs were protected from BLM-mediated DNA damage and nuclear impairment, senescence and production of ROS. Normal and IGF1R-deficient AMLFs were successfully isolated and grown in plastic-, and even more efficiently in collagen-coated plates.

Keywords: Fibroblasts, IGF1R, Lung

G01 - 285 - P

Transcriptome profiles in acute injured lungs reveals IGF1R action on metabolic reprogramming, mitochondrial homeostasis, cellular senescence and epigenetics

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Acute lung injury and acute respiratory distress syndrome (ARDS) usually cause a “cytokine storm”. IGF1R (Insulin-like growth factor receptor 1) is a tyrosine kinase with pleiotropic cellular functions. IGF activity maintains lung homeostasis and is broadly implicated in respiratory diseases including ARDS and COVID-19. In mice, IGF1R deficiency counteracts respiratory inflammation and damage after bleomycin (BLM)-induced lung injury. To explore the molecular mechanisms mediated by IGF/IGF1R signaling in pulmonary homeostasis after the BLM challenge, we performed RNA-seq in lungs of IGF1R-deficient and control mice three days after BLM or saline instillation identifying a large number of differentially expressed genes. As expected, functional enrichment detected biological processes and signaling pathways involved in the pathobiology of acute lung injury. Differential gene expression due to IGF1R depletion in BLM-challenged mice revealed reversal of a large part of the transcriptional changes triggered by BLM mainly related of the inflammatory “cytokine storm” profile. RNA-seq data mining also identified changes in the expression of gene clusters with key roles in metabolic reprogramming, mitochondrial homeostasis, cellular senescence and epigenetics. Further exploration of these transcriptomic functional categories, together with additional validation studies, provide new insights into the molecular mechanisms caused by IGF1R deficiency on acute lung injury and inflammation.

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These findings allow a more complete view of IGF1R signaling at the transcriptional level, reinforcing its importance in promoting acute lung inflammation and senescence, modulating metabolism, and postulating it as a global epigenetic regulator.

Keywords: Transcriptome Profiles, IGF1R, Acute Lung Injury

G01 - 291 - P

Voltage-gated Ca2+ channels and Ca2+-dependent K+ channels promote glioblastoma cell growth: do they act in concert?

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Glioblastoma multiforme (GBM) is the most frequent malignant primary tumour of the central nervous system. GBM standard treatment combines tumour resection, radiotherapy and chemotherapy with temozolomide. However, it only provides limited benefit to a specific group of patients, being the overall survival less than two years. In the search for novel therapeutic targets, our previous studies have demonstrated that knocking down voltage-gated Ca2+ channels (VGCCs) of the Cav3 family dramatically reduce the viability of GBM cell lines. However, the modus operandi of VGCCs in GBM cells is counterintuitive, given that their plasma membrane is steadily depolarized and most VGCCs would be in the inactivated state. Here we have studied the expression of VGCC and KCacs in U87-MG GBM cells, and the effects of their gene silencing by lentiviral-driven expression of shRNAs. Our data shows that the expression of some channel subtypes is cross-regulated, and that their gene silencing or pharmacological targeting reduces the proliferation index and rises the membrane potential. We propose that VGCCs and KCa form functional tandems that hyperpolarize the membrane to increase the driving force for Ca2+ entry through multiple channel types, thus effectively activating pro-proliferative and pro-survival pathways necessary for GBM growth.

Keywords: Glioblastoma, Calcium, VGCC, KCa

G01 - 297 - P

Exploring the effect of TAT-Cx43$^{266-283}$ in lung cancer brain metastasis models

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Brain metastasis is the most common type of brain cancer and metastasis accounts for 90% of cancer deaths. Lung cancer is the most common primary tumor to generate metastasis in the brain. Cancer stem cells have been characterized as key target for metastasis therapy, being able to acquire invading properties, migrate to other tissues and proliferate. Our laboratory developed an antitumoral peptide based on Cx43 sequence, TAT-Cx43266-283, which inhibits the oncoprotein Src activity, reducing proliferation, migration and invasion properties and improving survival in glioblastoma models. Given the relevance of Src for lung cancer brain metastasis, we analyzed the potential of TAT-Cx43266-283 as a treatment for this disease. In vitro models showed how TAT-Cx43266-283 impaired migration and invasion, and reduced cell viability of human and mouse lung cancer cell lines. Using a murine in vivo model of lung cancer brain metastasis based on the intracranial implantation of mouse lung cancer cells, we showed that TAT-Cx43266-283 improved mouse survival. To identify the molecular mechanism of TAT-Cx43266- 283 effect in these models, we relied on phosphoproteomics. With this approach, we unveiled ERK as a key mediator of TAT-Cx43266-283 effect in lung cancer brain metastasis, which included changes in cytoskeleton and vascularization. We also studied the effect of TAT-Cx43266- 283 in combination with inhibitors of MEK1/2, PKC, GSK-3ß and CaMKII showing promising results. Overall, the results presented in this work support further research to advance in the proposal of TAT-Cx43266-283 as a candidate for brain metastasis treatment, alone or in combination with other therapies.

Keywords: Metastasis, Lung Cancer, Cx43, Src, ERK, Phosphoproteomics

G01 - 304 - P

Global methylation profiling of uterine aspirates identifies the GABAergic pathway as a factor in cancer aggressiveness

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Endometrial cancer (EC) is the 6th most common tumor in females, with both incidence and mortality on the rise. Despite generally high survival rates, a subgroup of patients presents advanced disease and faces significantly lower survival rates due to limited treatment options. Although 40% of these patients have serous histology, this alone does not account for all cases of disease progression, indicating a need for deeper understanding of the underlying biology of EC progression. This study aims to explore the epigenetic differences among endometrial cancer patients to identify novel pathways involved in patient survival.

57 uterine samples from EC patients across various hospitals were collected and processed for DNA extraction. The methylome profile obtained through the Infinium MethylationEPIC Array revealed a predominantly hypomethylated profile in patients with serous histology, linked to GABAergic signaling. This was validated with pyrosequencing. In silico pathway analysis with TCGA data showed overexpression of GABA receptors and metabolic enzymes in cancer samples. Further analysis ELISA analysis with a cohort of 66 patient samples revealed a correlation between GABA secretion levels and survival rates, independent of histological markers.

Methylome analysis of endometrial cancer patients highlighted a global methylation profile associated with serous histology and identified the GABAergic signaling pathway as a novel factor in endometrial cancer. In silico studies confirmed the association between GABAergic markers and cancer, and patient studies further validated their link to survival rates. This points to GABAergic components as a novel molecular pathway influencing endometrial cancer behavior.

Keywords: Cancer, Endometrial Cancer, Molecular Pathways, Epigenetics, Methylation Profiles

G01 - 310 - P

The inhibition of different domains of Hsp90 as a key to determining the key populations of profibrotic fibroblasts in fibrotic disease

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The chaperon Hsp90 is a ubiquitous protein that carries out an important role in cell fibrosis. Since the targeting of the complete protein can result in dire consequences, the possibility of targeting only one domain of the chaperone is alluring. To evaluate the key domains related to fibrosis of the protein Hsp90 the presence of stablished pro-fibrotic biomarkers and the deletion and inhibition of the domain under study, we use the main cell source of fibrosis production, fibroblasts.

To study the chaperone, wild type cells, as well as cells with a direct mutation in the C-terminal domain of the Hsp90α, will be used. Concurrently, the cells will be treated with three different drugs that act in specific domains of Hsp90. The treatments use to inhibit Hsp90 are three: 17-AAG directed to the N-terminal and Epigallocatechin-3-Gallate (EGCG) directed to the C-terminal, the third treatment is an experimental approach developed by the group directed to the C-terminal called CTPR390.

Thus far, the results are encouraging. At a cellular level, the reduced protein expression of stress fibers α-SMA appeared to confrere a phenotypic change, between the fibrotic WT fibroblasts, and the 17-AAG-treated cells whiles markers such as fibronectin kept its protein expression. When cells were treated with Epigallocatechin-3-Gallate (EGCG), morphological changes of fibroblasts were observed without losing α-SMA expression. These analyses were achieved through immunohistochemical assays, and high-throughput microscopy. We conclude that, inhibition of key ATPase-domains of Hsp90 lead to different phenotype changes, pointing to a crucial methodology for future segregation of fibroblast population within fibrotic tissue.

Keywords: Cardiac Fibrosis, Hsp90, Chaperone, Fibroblasts

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G01 - 317 - P

Role of perlipin-1 in the differentiation of myofibroblasts in lung fibrosis

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Fibrosis appears in the lung as a consequence of a chronic damage to the airways. Myofibroblasts, the cells responsible for producing extracellular matrix components, primarily derive from fibroblast activation, though their exact origin it´s still a matter of debate. In recent years, lipofibroblasts, fibroblasts filled with lipid droplets, have been postulated as a precursor of myofibroblasts. Perilipins (PLINs 1-5) are proteins associated with intracellular lipid droplets and involved in lipid metabolism and storage. Our hypothesis is that PLINs have a main role in the differentiation of lipofibroblasts to myofibroblasts and the progression of fibrosis. Using human primary fibroblasts from both healthy donors and patients with idiopathic fibrosis (IPF), we found that PLIN1, PLIN2, PLIN3 and PLIN4 are expressed in both cells, being PLIN1 and PLIN2 downregulated in fibrotic fibroblasts compared to control ones. Moreover, the expression of both PLIN1 and PLIN2 decrease after adding TGFb, a fibrotic stimulus, to healthy fibroblasts. Notably, PLIN1 (but not PLIN2) translocates to the nucleus following a fibrotic stimulus, suggesting that it may have a nuclear function repressing the differentiation to myofibroblasts and the expression of profibrotic genes. Indeed, PLIN1 inhibition increases the expression of genes associated with myofibroblasts transition and extracellular matrix components. Finally, while PLIN1 doesn´t affect the proliferation of either healthy or fibrotic fibroblasts, it does impact their migration. In summary, we propose that PLIN1 plays a novel role as a transcription factor regulating the lipofibroblast to myofibroblast transition in lung fibrosis.

Keywords: Myofibroblasts, Fibroblasts, Perlipin, PLIN, Fibrosis

G01 - 318 - P

Estudio de la Interacción entre Probióticos, Metabolismo Lipídico y Microbiota en Estados Postprandiales

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Introducción: La microbiota intestinal interviene en la regulación de enfermedades metabólicas (resistencia a insulina, diabetes, obesidad). En este trabajo, estudiamos el efecto de una suplementación probiótica en la regulación de metabolitos en suero y su posible influencia en el estado postprandial tras una sobrecarga grasa.

Metodos: 26 pacientes recibieron un placebo (n=12) o un probiótico (Lactobacillus) (n=14) durante 2 meses. Se les administró una sobrecarga grasa antes y después del estudio. Se tomaron muestras de suero en ayunas al inicio, a 1 mes, a 2 meses y 3 horas después de cada sobrecarga grasa. Las muestras fueron analizadas por resonancia magnética nuclear (RMN) para determinar metabolitos relacionados con la inflamación, el metabolismo lipídico, aminoácidos y metabolitos derivados de la microbiota intestinal.

Resultados: Se observó un aumento de los marcadores inflamatorios GlycA, GlycB y GlycF 3 horas tras la sobrecarga grasa. El tratamiento con probiótico disminuyó estos mismos marcadores y la insulina. La sobrecarga grasa aumentó el hidroxibutirato (derivado de la microbiota) y disminuyó el lactato. Este incremento de hidroxibutirato fue mayor al final del estudio en el grupo probiótico. También se observó un aumento de ARAEPA y acetato al mes de inicio del estudio y de glicerol al final del estudio solo en el grupo probiótico. Además, se vio una disminución de glutamato y tirosina (al mes) y w3 (a los 2 meses) solo en los pacientes con el suplemento probiótico.

Conclusiones: Este estudio evidencia que el probiótico Lactobacillus puede tener efectos significativos sobre el metabolismo lipídico, la inflamación y los metabolitos derivados de la microbiota, especialmente en estados postprandiales.

Keywords: Probiótico, Microbiota, Metabolismo Lipídico, Inflamación

G01 - 321 - P

Adenovirus Entry and Disassembly Explored by Fluorescent Microscopy

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Adenoviruses are pathogens responsible for a wide range of human diseases, from mild respiratory infections to severe illnesses like pneumonia. Beyond their role as infectious agents, adenoviruses have been extensively studied and utilized as viral vectors in gene therapies and vaccines due to their ability to infect a diverse array of cell types and their genetic stability. Here, we study the entry of human adenovirus 5 (Ad5), a double-stranded DNA virus, into the host cell and it subsequent journey to the nucleus.

This work investigates the entry process of Ad5 in U2OS cells to understand its interaction with various key cellular structures, including the plasma membrane, endosomes, and microtubules. Advanced confocal microscopy and Stimulated Emission Depletion (STED) microscopy were employed to visualize these interactions at a high subcellular resolution. The application of these techniques has deepened our understanding of the virus-host interactions in Ad5 infection, highlighting specific points of interaction with cellular structures and suggesting potential alternative viral entry pathways.

Keywords: Adenovirus, Disassembly, Fluorescent Microscopy, Entry, Virus-Host Intractions

G01 - 322 - P

Development of an ex vivo pulmonary fibrosis platform for identifying personalized therapies

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La fibrosis pulmonar idiopática (FPI) es una enfermedad rara y progresiva que deteriora la salud y genera altos costos médicos, con una supervivencia mediana de 3 a 5 años tras el diagnóstico. Los tratamientos actuales, como la pirfenidona y el nintedanib, ofrecen beneficios limitados y tienen efectos secundarios graves, lo que lleva a altas tasas de abandono. El trasplante de pulmón es la única solución a largo plazo, pero es invasivo y no accesible para la mayoría de los pacientes. Por ello, es urgente desarrollar terapias innovadoras.

Este proyecto propone crear una plataforma de cribado masivo de medicamentos aprobados por la FDA para identificar nuevas moléculas con propiedades antifibróticas. Hemos optimizado un ensayo automatizado basado en microscopía de fluorescencia para medir la deposición de matriz extracelular inducida por TGF-beta en fibroblastos pulmonares humanos en placas de 384 pocillos. En esta primera fase, presentamos los desafíos enfrentados al desarrollar el ensayo y los marcadores más efectivos en nuestro modelo de inducción fibrótica.

Keywords: Pulmonary Fibrosis, Extracellular Matrix, Drug Dyscovery

G01 - 324 - P

El crecimiento angiogénico tras la inhibición de la integrina a5b1 se asocia a una respuesta a la quimioterapia

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El cáncer de pulmón es una de las principales causas de muerte en los países desarrollados. El tratamiento actual se basa en la quimioterapia con inmunoterapia, que tiene muchas limitaciones. Una de las limitaciones principales es la resistencia a los tratamientos de quimioterapia que experimentan las células tumorales.

El pulmón es un órgano altamente vascularizado donde los tumores pueden crecer mediante patrones de crecimiento no angiogénicos secuestrando los vasos sanguíneos pre-existentes, en un fenómeno conocido como cooptación vascular (del inglés *vessel co-option, VCO*). Estos tumores tienen un peor pronóstico.

Nuestro objetivo ha sido inhibir el crecimiento de VCO bloqueando la adhesión entre las células tumorales y los vasos sanguíneos pre-existentes, empleando un inhibidor de la integrina a5b1. Esta integrina estaría expresada en la célula tumoral y se uniría a la fibronectina de la membrana basal de los vasos sanguíneos pre-existentes. Empleando distintos tipos de quimioterapia (Paclitaxel-carboplatino, oxaliplatino-ciclofosfamida y cisplatino) encontramos

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que los tumores ofrecen resistencia a estos tratamientos modificando la matriz extracelular. En concreto la combinación de Paclitaxel-carboplatino produce un microambiente inmunosupresivo que se caracteriza por un aumento de colágeno. El tratamiento con el inhibidor de integrina a5b1 ATN-161 en combinación con esta quimioterapia, indujo un descenso del tamaño tumoral. Sin embargo, estos tumores con tamaño más reducido mostraron un incremento en la angiogénesis tumoral y proteínas de matriz extracelular.

Estos resultados indican que el crecimiento angiogénico y depósito de la matriz extracelular están relacionados con una respuesta a los tratamientos de quimioterapia en tumores de pulmón no angiogénicos.

Keywords: Integrinas, Angiogénesis, Co-Optación Vascular

G01 - 325 - P

p38 activation in cancer surveillance

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Hepatocellular carcinoma (HCC) is closely linked with chronic liver inflammation, often developing in the context of cirrhosis and influenced by multiple risk factors. Late-stage diagnosis restricts therapeutic options, with curative treatments available to only a third of patients. Immunotherapy has emerged as a promising treatment, yet tumor resistance remains a significant challenge. This study focuses on the role of CD8+ T cell exhaustion in HCC progression and examines the activation of p38 MAPK, a key player in hepatic inflammatory responses, in this context. Using a MKK3/6CD4-KO mouse model, we investigated the impact of p38 kinase activity on T cell exhaustion during HCC development. Our findings indicate that p38 activation in CD8+ T cells is crucial for their function, with chronic stimulation under hypoxic conditions in vitro showing increased exhaustion when p38 is inactive. MKK3/6CD4-KO mice demonstrated greater tumor growth compared to controls, as confirmed by histological and flow cytometry analyses of exhaustion markers. Furthermore, the transfer of CD8+ T cells from RAG2-/- OT-I MKK3/6CD4-KO mice to a RAG2-/- model resulted in enhanced tumor growth, underscoring the significant impact of these cells in HCC progression, independent of other immune cells and tumor microenvironment variations. Our results underscore the critical role of p38 kinase in modulating the antitumor immune response. Lack of p38 activation in CD8+ T cells leads to their pronounced exhaustion, creating a harmful tumor microenvironment and accelerating tumor growth. These findings position the p38 MAPK pathway as a potential therapeutic target in HCC treatment, offering new paths for immunotherapy to enhance antitumor immune responses and control liver cancer progression

Keywords: T Cell CD8+, T Cell Exhaustion, P38 MAPK, Hepatocellular Carcinoma.

G01 - 346 - O

he Rag GTPase - mTORC1 axis, metabolic driver of cancer and aging

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Mutations in components of the Rag GTPase pathway are puzzlingly low in human cancer, with the exception of GDP-like, activating mutations in RagC in B-cell lymphomas. We have knocked-in in mice some of these mutations and full-body RagCmut/+ mice have accelerated lymphomagenesis when bred to the follicular lymphoma prone strain VavP-Bcl2. Heterozygous RagC mutations confer only a mild increase in nutrient signaling to mTORC1 that results in anomalous B-cell activation upon antigen stimulation. Cells are only permissive to a subtle increase in Rag GTPase signaling, while massive deregulation of the pathway is deleterious, consistently with the absence of mutations leading to overt activation of the pathway in cancer. Without a lymphoma-prone genetic background, RagCmut/+ mice exhibit a striking reduction in spontaneous tumorigenesis at old ages, and a shortened longevity with multiple features of premature aging. The accelerated aging of RagCmut/+ is not driven by increased Rag GTPase signaling in bone marrow-derived cells, as reconstitution of the BM with RagCmut/+ in wt mice does not result in compromised longevity; whereas the reciprocal reconstitution of wild-type cells in a RagCmut/+ host does result in premature aging. However, acute control of myeloid inflammation in aged RagC-mutant mice reverts some of the premature aging features, and extended elimination of myeloid cells extends the longevity of mice with increased nutrient – Rag GTPase signaling. We provide the first genetic proof of increased nutrient signaling and mTORC1 driving aging in mammals, and support a two-component model in which increased nutrient signaling drives autonomously parenchymal damage, and myeloid inflammation further precipitates organ deterioration and accelerated aging.

Keywords: Cancer, Nutrients, Aging

G01 - 353 - O

Altered RNA metabolism in liver diseases

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The liver, a critical organ for metabolic homeostasis, is vulnerable to a broad spectrum of pathological conditions, including viral hepatitis, steatotic liver diseases, cirrhosis, and hepatocellular carcinoma. The multifaceted nature of these diseases demands an understanding of the underlying cellular and molecular processes, where RNA metabolism stands as a critical regulatory mechanism. This talk will provide a general overview of the dysregulation of RNA metabolism in liver pathologies, focusing on splicing, nonsense-mediated decay (NMD), RNA-exosome activity, and tRNA biogenesis and metabolism. We will explore how aberrations in RNA splicing contribute to the misexpression of liver-specific isoforms and how this mis-splicing relates to disease progression. The talk will then delve into the role of NMD in hepatic cells, elucidating its dual role in both safeguarding against transcripts with premature stop codons and modulating the abundance of specific RNAs, which can be disrupted in liver disease. The RNA-exosome's involvement in the turnover of defective RNAs and its implication in maintaining hepatic homeostasis will be examined, alongside evidence of its dysregulation in liver pathology. Furthermore, we will discuss the perturbations in tRNA biogenesis and function, highlighting how alterations in tRNA-associated enzymes are implicated in liver disease. The talk will also include an overview of the therapeutic potential of targeting these specific aspects of RNA metabolism, offering insights into their translational application in treating liver diseases. Through the detailed exploration of these key RNA metabolic processes, we aim to show their roles in the etiology of liver pathologies and their potential as novel targeted therapeutic strategies.

Keywords: RNA, Splicing, NMD, RNA-Exosome, MASLD, HCC

G01 - 357 - O

Mitochondria-associated ER membranes (MAM) contributes to the regulation of metabolic flexibitliy: Relevance for amyotrophic lateral sclerosis (ALS)

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Mitochondrial function is modulated by its functional interaction with the endoplasmic reticulum. Recent research indicates that these contacts are disrupted in familial models of amyotrophic lateral sclerosis. We report here this impairment in the crosstalk between mitochondria and the endoplasmic reticulum impedes the use of glucose-derived pyruvate as mitochondrial fuel, causing a shift to fatty acids to sustain energy production. Over time, this deficiency alters mitochondrial electron flow and the active/dormant status of complex I in spinal cord tissues, but not in the brain. These findings suggest MAM plays a crucial role in regulating cellular glucose metabolism and that its dysfunction may underlie the bioenergetic deficits observed in ALS.

Keywords: MAM, Mitochondria, ALS, Glucose

G01 - 360 - O

Novel roles for endothelial cell autophagy during inflammation

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A key feature of infection and inflammation is tissue infiltration of neutrophils, a response that requires breaching of endothelial cells (ECs) that line the vascular lumen and must be tightly regulated to avoid excessive tissue damage. The role of autophagy as an essential regulator of immunity is well accepted, and defects in autophagy are linked to numerous inflammatory conditions. However, while there is ample evidence of immune cell autophagy-related genes regulating inflammation, less is known about the role of EC autophagy in this context. Here, we explored the role

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of microvascular EC-autophagy in neutrophil trafficking in response to acute inflammation.

To investigate autophagy in ECs *in vivo*, we detected LC3-punctae in postcapillary venules using GFP-LC3 transgenic mice. With this approach, we found that inflamed venular ECs exhibited enhanced levels of LC3-puncta that localised exclusively at EC contacts. Furthermore, mice with selective EC deletion of the key autophagy gene *Atg5* exhibited increased neutrophil extravasation in multiple inflammatory models, including pre-clinical models of sepsis. Real-time and high-resolution analysis of neutrophil-EC interactions by 4D confocal intravital microscopy revealed significantly exaggerated and faster neutrophil transendothelial migration across autophagy deficient ECs, while pharmacological induction of autophagy inhibited neutrophil migration. Mechanistically, autophagy machinery regulates the remodeling of EC junctions and expression of key EC adhesion molecules, facilitating their intracellular trafficking and degradation.

Collectively, our results identify EC autophagy as an essential cellular process to limit physiological neutrophil trafficking during acute inflammation.

Keywords: Inflammation, Endothelial Cell, Autophagy, Neutrophil

G02
Biología del desarrollo y modificación genómica

G02 - 55 - P

Search for players in intussusceptive angiogenesis using the chick embryo chorioallantoic membrane model

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During embryonic development, the vasculature can expand through the intussusceptive angiogenesis (IA) process. IA begins with the invagination of endothelial cells into the vascular lumen, leading to the formation of intravascular pillars. In our laboratory we study the role of matrix metalloproteinases (MMP) in vascular remodeling. We have recently identified MT1-MMP as a new player in IA during induced colitis in mice (Esteban *et al.*, 2020). We have shown that MT1-MMP cleaves thrombospondin-1 (TSP1), allowing its binding to integrin αVβ3 and thus activating endothelial nitric oxide synthase (eNOS). The levels of nitric oxide increase and triggers vasodilation promoting IA. We are interested in testing whether this molecular pathway relevant for IA in the inflammatory context in mice is conserved in IA during development.

To this end, we have used the chicken CAM model in which vascular expansion occurs mainly by sprouting angiogenesis at E6 and by IA at E10 and onwards. We have analyzed that pathway by qPCR and by quantitative proteomics at E3, E6, E10 and E12. The analysis shows an over-representation of functional categories related to the extracellular matrix, integrins and eNOS as well as increased abundance of proteins related to the described molecular pathway in stage E10 *vs* E6. We are currently immunostaining the CAM with anti-smooth muscle actin and *Sambucus nigra* lectin to visualize and quantify the vessels and intravascular pillars at these different stages.

Our data demonstrate that CAM can serve as an experimental model to identify signaling pathways that may constitute targets for the treatment of diseases in which IA is involved such as cancer, lung conditions and inflammatory bowel disease.

Keywords: Chorioallantoic Membrane, Chick, MT-MMPs, CAM, ECM, SMA, Ve-Cadherin, ENOS, MT1-MMP, MT4-MMP

G02 - 102 - O

Use of human mesenchymal stem/ stromal cells to rescue the functional defects of cells from Rett syndrome patients

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Rett syndrome (RS) is a progressive and severe neurodevelopmental disorder, discovered by Andreas Rett in 19661, which affects mainly females and for which there is currently no cure. It is caused mainly by mutations in the X-linked CpG-binding protein 2 (MECP2) gene, which is involved in neuronal development and maturation2. Effective therapeutic strategies are necessary, especially since it has been shown in mice that SR could be reversible3.

The objective of this project is to test a cell therapy *in vitro* in an experimental cell model prior to a possible preclinical trial that may improve or reverse RS pathology.

As a cellular model, we have used mesenchymal stem/stromal cells (MSCs) derived from periodontal ligament and/or human dental pulp (PDL/DP) obtained from deciduous teeth of patients with SR in collaboration with the Spanish and Catalan Associations of SR. These tissues contain neural crest progenitors with the potential for neural differentiation4.

As a result: 1) we have created the first biobank of SR cells with capacity of neural differentiation; 2) we have derived neurons from PDL/PL MSCs; and 3) we have successfully demonstrated the *in vitro* treatment of cells from SR patients with molecules secreted by human adipose tissue-derived MSCs isolated from healthy donors. These secreted molecules were able to normalize the activity of the patients' cells, reducing the oxidative stress that characterizes them and increasing their survival in culture. Furthermore, this normalization includes an increase in the expression of BDNF in the patients' cells, a gen directly affected by MECP2 and necessary for memory, learning and neuronal maturation in general, indicating that the treatment could contribute to correcting or even reversing the SR pathology.

Keywords: Rett Syndrome, Human Mesenchymal Cells, Cell Therapy

G02 - 113 - O

Enhancing Gene Delivery to Human Mesenchymal Stem Cells: Functionalizing Polyethyleneimine with Aldehydes for Improved Transfection Efficiency and Biosafety

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Polyethyleneimine (PEI) stands out as a potent cationic polymer for the transfection of human mesenchymal stem cells (hMSCs), mainly due to its buffering properties. Nevertheless, unmodified PEI faces significant challenges, including cytotoxicity, aggregation under high ionic strength, and susceptibility to opsonization. To address these issues and enhance transfection efficiency and biosafety, this study explores the functionalization of PEI using various aldehydes.

The characterization of functionalized PEI was performed at various PEI concentrations and compared with that obtained with the commercially available transfectant lipofectamine (LPF). The free amine groups of PEI were quanti-

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fied using fluorimetric methods. PEI was then modified with guanidinium and hydrophobic aldehydes (octanal and dodecanal) in a 0.1M acetic acid solution, using different guanidinium/hydrophobic aldehyde ratios (70/30, 87.5/12.5, 93/7). Transfection efficiency of primary bone marrow hMSCs was assessed using reporter *lacZ* and GFP gene plasmids and measured by determining *lacZ* luminescence and GFP fluorescence. Cells transfected with LPF and untransfected cells were used as positive and negative controls.

Characterization of the polyplexes demonstrated their effectiveness in gene delivery. Transfection experiments pointed out concentrations between 5 and 7.5 µg/ml as the most optimal for gene delivery and 70/30 as the most effective functionalization ratio for transfecting hMSCs.

These results denote that the functionalization of PEI with selected aldehydes significantly enhances its potential as a transfection agent for hMSCs. Further studies in a 3D *in vitro* culture model using chondrogenic genes will elucidate the efficiency of these formulations in promoting hMSCs chondrogenesis.

Keywords: Gene Therapy, Primary Cell Culture, Cationic Polymers

G02 - 166 - O

Innovative Dual-Targeted Therapies for Overcoming Chemoresistance in Pancreatic Cancer

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This research focuses on developing new treatments for Pancreatic ductal adenocarcinoma (PDAC), a highly aggressive and often treatment-resistant cancer. The study aims to inhibit the Hippo pathway, specifically targeting YAP-1 and FOSL-1, using lipoplexes (liposomes delivering siRNA). Additionally, it explores combining epigenetic inhibitors like histone deacetylase inhibitors (HDACi) with standard chemotherapy.

The lipoplexes were formulated using cationic liposomes composed of dioctadecyldimethylammonium bromide (DODAB) and 1-monooleoyl-rac-glycerol (MO). The chemotherapy included Entinostat, a class I HDACi, combined with low doses of Gemcitabine. The combination therapy was tested in human and mouse PDAC organoids and in mouse xenograft models. Organoid viability was assessed via immunofluorescence, and tumor growth was monitored daily post-treatment. Histological analyses (paraffin/frozen), RT-PCR, and Western blotting were performed to assess stromal formation and gene/protein silencing.

The results showed that treatment with lipoplexes targeting YAP-1 and FOSL-1 significantly reduced tumor size and collagen content within the tumor stroma, primarily by targeting cancer-associated fibroblasts (CAFs). The combination therapy with Entinostat and Gemcitabine further decreased tumor volume compared to either treatment alone.

In conclusion, the combined therapy reduced cell proliferation and stromal content in PDAC models, potentially enhancing the delivery and efficacy of chemotherapy. Inhibiting the Hippo pathway and reversing epigenetic changes with HDACi show promise in overcoming PDAC's chemoresistance, offering a strategy for improving patient outcomes. This research presents a promising therapeutic avenue for PDAC by targeting specific molecular aberrations.

Keywords: Pancreatic, Cancer, Organoids, Mice, Epigenetic, SiRNA

G02 - 188 - P

Zebra Fish model for Hutchinson-Gilford Progeria Syndrome. New therapeutic strategies targeting senescence.

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Hutchinson-Gilford Progeria Syndrome (HGPS) is a very rare and fatal disease, characterized by premature aging and death of patients before reaching puberty. In HGPS, accumulation of a truncated form of the lamin A precursor, progerin, occurs, causing structural defects in the nuclear lamina, as well as in the differentiation and proliferation of mesenchymal stem cells (MSCs). To this day, we maintain at CICA a mutant zebra fish line for the *zmpste24* gene, which we will use as a HGPS model as well as senescence pathology model. These animals are transparent for a large part of the time of their development, which allows the study of their organs in a visual and minimally invasive way. In addition, they have a great capacity to regenerate parts of their body, females can produce hundreds of embryos every week and these embryos develop very quickly, which allows for very agile research, especially interesting in the study of our pathology. We have carried out functional studies in the zebra fish HGPS model, such as identification of markers of senescence (*p53, MDM2, LMNA, P18, p27 and CDKN2A/B*), oxidative stress and purine metabolism (*CD13*, *ENO* and *PRPS1*) by genetic and proteomic studies. The senescence markers are statistically significant increase in our HGPS model *vs* wild type. This is the first step towards our goal, which is to use this animal model to refine microRNA microinjection protocols into larvae and to perform shotgun proteomic studies to discover the pathways involved in MSC aging, and therefore that symptoms associated with HGPS and aging are reduced providing better quality of life to these patients.

Keywords: Zebra Fish, Hutchinson-Gilford Progeria Syndrome, Senescence

G02 - 262 - O

Proteostatic regulation of the actin-associated protein LUZP1 in the context of a rare disease, Townes-Brocks syndrome

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SALL1TRUNC (a truncated form of SALL1 transcription factor) causes Townes-Brocks Syndrome (TBS), a rare autosomal dominant genetic disorder with ciliopathy-like phenotypes. LUZP1 (leucine zipper protein 1), an actin-associated protein which negatively regulates ciliogenesis, interacts with SALL1TRUNC. We have previously shown that, in TBS-patient-derived fibroblasts, SALL1TRUNC promotes degradation of both stress-fiber-associated and centrosomal LUZP1 via the ubiquitin proteasome system (UPS). SALL1TRUNC may interfere with LUZP1 proteostasis by either activating E3 ligase(s) that destabilize LUZP1 or, by inactivating DUBs (deubiquitinating enzymes) that stabilize LUZP1. Focusing on centrosomes, we show here that the E3 ligase MIB1 (Mindbomb1) and deubiquitinase USP21, can both interact with LUZP1 and influence its stability. Furthermore, levels of centriolar MIB1 and PCM1 (pericentriolar material 1; a key regulator of centriolar satellite integrity and potential LUZP1 interactor) are reduced in TBS model cells, suggesting a complex regulatory network for LUZP1 proteostasis in the context of the disease

Keywords: SALL1, Townes-Brocks Syndrome, LUZP1, Proteostasis, USP21, MIB1, Centrosome

G02 - 356 - P

DNMT2-dependent m^{5}C on RNA promotes MET during somatic cellular reprogramming

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The generation of induced pluripotent stem cells (iPSCs) from somatic cells holds significant therapeutic potential and provides a valuable tool for investigating the molecular mechanisms underlying cell fate transitions. Although previous efforts have focused on understanding the epigenetic and transcriptional changes involved in this process, how RNA modifications contribute to overriding somatic cell identity is incompletely understood. Here we investigate the role of DNMT2, the most conserved and enigmatic member of the DNA methyltransferase family that acts by catalyzing m5C on RNA species, during pluripotency acquisition through somatic cellular reprogramming. We found that DNMT2 loss-of-function abrogates epithelial cell specification during somatic cell reprogramming, in a catalytic-dependent manner. Specifically, lack of DNMT2-dependent m5C impedes mesenchymal-to-epithelial transition (MET). Thus, our study establishes a critical role for m5C in cell fate transitions during reprogramming, which delineates a novel regulatory layer underlying MET control for efficient reprogramming.

Keywords: RNA,M5C,Reprogramming,IPSCs,DNMT2

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G02 - 361 - O

The role of pioneer neurons in the organization of the statoacoustic ganglion

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Bipolar neurons of the inner ear are in the statoacoustic (SAG) ganglion and connect inner ear hair cells with the corresponding neurons of the brainstem. To address how neuroblasts migrate, coalesce and establish proper innervation patterns, we have imaged neurblasts at single-cell resolution by photoconversion and photoablation experiments and disrupted candidate molecules using both Gal-UAS and CRISPR Cas9/Cas13. Delaminated neuroblasts migrate towards a group of pioneer neurons, where they coalesce to organize the anterior SAG lobe. They migrate non-collectively and actively as they require RhoGTPases. Interestingly, pioneer cells extend pioneer axons targeting a posterior set of hair cells. These axons act as scaffolds for neuroblasts crawling and organization of the posterior SAG lobe. Ablation of pioneer cells causes defects in cell migration, axogenesis and neuroblast crawling, indicating a relevant role of pioneer cells in the sculpturing of the SAG. Finally, we have identified the chemokine Cxcl14 as a novel pioneer axon guidance and/or fasciculation cue. A lack of Cxcl14 due to a CRISPR/Cas9 KO results in defasciculation of pioneer axons, incorrect targeting to hair cells and malformation of the posterior lobe. We also find defects on SAG and lateral line axons entering the hindbrain in mutant embryos, as Cxcl14 is also expressed in two spots adjacent to the hindbrain. Our results indicate that Cxcl14 plays a key role in regulating the correct targeting of SAG towards otic hair cells but not hair cells of the lateral line. Altogether, we propose a model of SAG shape-to-function acquisition directed by the role of pioneer neurons and their axons.

Keywords: Inner Ear, Sensory Neurons, Axon Guidance, Migration, Zebrafish, Chemokine

G03: Biología molecular ómica y bioinformática

G03 - 50 - O

Investigation of the differential proteome associated to CRC recurrence by proteomics analysis of FFPE tissue samples and plasma extracellular vesicles

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Colorectal cancer (CRC) is the second leading cause of cancer-related deaths and the third most common type of cancer worldwide. CRC is usually detected in advanced stages of the disease, when the tumour has already spread to other parts of the body, reducing the therapeutic options and survival rates. Thus, early detection, and prognostic monitoring through biomarkers, and the identification of new targets of intervention would contribute to improving patients' survival.

In this work, we aim to compare the protein expression profile of tumoral tissue from recurrent and no-recurrent stage II CRC patients during follow-up. The objective was to identify dysregulated proteins in tumoral tissues that might be related to the development of the disease and could act as diagnostic/prognostic biomarkers of the disease. For such a purpose, we analyzed paired protein extracts from FFPE tissue samples and extracellular vesicles (EVs) from plasma of recurrent and non-recurrent stage II CRC patients by quantitative proteomics using Tandem Mass Tag (TMT) 10-plex and an Orbitrap Exploris 480 mass spectrometer equipped with FAIMS Pro Duo Interface.

From the analysis of the two TMT proteomics experiments using MaxQuant and R programme, we identified and quantified a total of 343 proteins from the EVs and 642 from FFPE tissues. In total, we identified and quantified 25 and 59 dysregulated proteins in plasma EVs and tissue samples, respectively, with a fold change ≥ 1.5. Dysregulation of the proteins was validated via western blot using cell extracts (from the isogenic CRC models KM12 and SW) and paired healthy/tumoral tissue protein extracts and via ELISA using plasma samples of CRC patients and healthy individuals.

Keywords: Proteomics, Colorectal Cancer, Biomarkers

G03 - 67 - P

Identification of autoantibodies with diagnostic ability in non-seroreactive colorectal cancer patients for the development of diagnostic approaches

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Colorectal cancer (CRC) has an estimated incidence of 447 per 100,000 new cases per year in Europe, constituting the second most common cancer type. It is also the second most lethal after lung cancer, since many patients are diagnosed at advanced stages and relapses occur even in patients diagnosed early. In this context, it is necessary to improve the diagnostic tools to timely treat the disease.

Previous studies have identified a panel of tumour-associated antigens (TAAs) present in CRC patients, and their corresponding autoantibodies have displayed high diagnostic ability. However, a subset of patients is non-seroreactive to these TAAs, and therefore they would be missed on screening approaches based on autoantibodies. Thus, in this study we have focused on identifying TAAs and their corresponding autoantibodies in this subset of non-seroreactive patients to complete the previously described diagnostic panel. Using plasma samples, we have performed an immunoprecipitation with IgGs from non-seroreactive CRC patients coupled with LC-MS/MS analysis to identify new TAAs. Then, we have synthesized and purified the candidate proteins and performed indirect ELISAs with plasma samples to confirm the presence of autoantibodies against them. The most promising proteins have been incorporated to the previous panel and evaluated altogether for their prognostic ability. These results open the door to develop an easy and accessible diagnostic approach for the detection of these autoantibodies using blood samples to evaluate the status of the CRC by liquid biopsy.

Keywords: Cancer, Autoantibodies, Proteomics

G03 - 75 - P

Synthesis of prebiotics by hydrolysis of wheat straw using novel thermoenzymes from geothermal metagenomic libraries.

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There is currently a growing interest in the additional effect of certain foods, known as "nutraceuticals", in the promotion of physical health and disease prevention. Within this category of foods, there is abundant evidence from *in vitro* and *in vivo* animal and human studies on the prebiotic effect of low molecular weight oligosaccharides generated by endogenous or exogenous hydrolysis of the hemicellulosic fraction of cereals, as well as the health benefits associated with their consumption.

Thermostable enzymes have generated great interest due to their potential applications in various industrial processes. In this study, we employed a metagenomic approach to explore the genetic diversity of a thermal metagenome, with the aim of discovering novel thermostable xylanases. Xylanases, in particular endo-1,4-β-xylanases, together with β-xylosidases, play a crucial role in the complete hydrolysis of xylan to xylose, a major component of plant cell walls, which makes them valuable in the food industry, such as in the production of prebiotics.

Functional metagenomics of soil samples identified a gene encoding an endo-xylanase, while genomic mining of a bacterial isolate detected a gene encoding a beta-xylosidase. Both recombinant enzymes were obtained from the microbiome of the Río Caldo geothermal spring, with an upwelling temperature of 77°C, located in the province of Ourense (Spain), and demonstrated high thermal stability in a proof-of-concept experiment to produce prebiotic xylooligosaccharides by hydrolysis of pretreated wheat straw.

References: Saavedra-Bouza, A., EscuderRodríguez, J.-J., deCastro, M.-E., Becerra, M., & González-Siso, M.-I. Xylanases from thermophilic archaea: A hidden treasure. *Current Research in Biotechnology*, ***5***, 100116 (2023)

Keywords: Hemicellulose, Prebiotics, Thermophilic Enzymes, Metagenomics

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G03 - 104 - P

Multi-omics profiling of gut microbiota and fecal metabolome throughout three generations: infants, mothers, and grandmothers.

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The human gut microbiota undergoes substantial changes throughout life. After a first microbial seeding coming predominantly from the maternal side (mode of birth, breastfeeding), the first year is crucial for the maturation and stabilization of the microbiota. In this study, we conducted a comprehensive multi-omics study with three generations, collecting fecal samples from 200 subjects – 69 infants, their 67 mothers, and 64 maternal grandmothers. We performed metagenomics (16S rRNA and shotgun metagenomics sequencing) and multiplatform metabolomics, and the results were integrated using mixOmics package. Infants showed significant differences in the fecal microbiome and metabolome compared to elders, with minor differences between mothers and grandmothers. The microbiota of infants exhibited lower richness and α-diversity, with an increase in Actinobacteria and Proteobacteria phyla, while Firmicutes were the main phylum in adults. In addition, over 50% of the infant microbiota comprised *Bifidobacterium*, *Escherichia/Shigella,* and *Veillonella* genera, while adults showed higher abundances of *Faecalibacterium, Blautia,* and *Roseburia*. Regarding metabolomics, infants showed higher levels of acetate, γ-aminobutyric acid, and glucose, whereas butyrate and branched-chain fatty acids (BCFA) were decreased in this group. In line with this, multi-omics correlations between microbial composition, functionality and metabolic profile showed that carbohydrate metabolism was increased in infants, while polyamine signaling and BCFA metabolism was enriched in adults. This is, to our knowledge, the first longitudinal multi-omics study including fecal samples from three generations of the same family, and was recently published in Nature Communications (10.1038/s41467-024-47182-y).

Keywords: Genomics, Metabolomics, Aging, Biomarkers

G03 - 107 - O

Complete Bioinformatics Single-cell RNA-seq Analysis

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Recent advancements in next-generation sequencing (NGS) and high-throughput technologies have significantly enhanced our understanding of biological systems, human diversity, and disease pathology. These innovations encompass a range of methodologies such as genomics, transcriptomics, and multi-omics approaches, which are increasingly being leveraged for single-cell analysis. Single-cell sequencing methodologies afford the ability to comprehensively characterize complex cellular populations while preserving cellular heterogeneity, thus facilitating the elucidation of gene regulatory networks and providing insights into cellular diversity and evolutionary dynamics. The versatility of single-cell analysis extends to diverse fields, including oncology, neurology, immunology, reproductive biology, etc., positioning it as an indispensable tool in biomedical research. Among the latest advancements in this domain is single-cell RNA sequencing (scRNA-seq), which enables the profiling of the transcriptome of individual cells. However, the large amount of data generated by scRNA-seq poses challenges in data analysis and interpretation, which emphasizes the importance of the analytical methodologies shown in this poster.

Keywords: Bioinformatics, Sequencing, NGS, Single Cell, ScRNA-Seq

G03 - 108 - P

Transcriptomic and translatomic analysis of the *Mycobacterium tuberculosis* response to capreomycin

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Mycobacterium tuberculosis is the causative agent of human tuberculosis (TB), one of humankind's deadliest diseases. This bacterium can persist in an individual host for decades switching between replicating and non-replicating states. TB remains a threat to the health of people worldwide, with 7.5 million new cases and 1.30 million deaths in 2022. In addition, more than a quarter of global deaths due to antimicrobial resistance are due to drug-resistant *M. tuberculosis*. Most clinically used antibiotics target the bacterial ribosome and the process of protein synthesis. Amongst these, capreomycin is a cyclic peptide antibiotic that is used to treat multi-drug resistant and persistent TB infections. How *M. tuberculosis* responds to capreomycin exposure and how specifically this antibiotic blocks translation is currently understudied. To answer these questions, we have challenged *M. tuberculosis* for 45 minutes under two different capreomycin concentrations above the minimum inhibitory concentration and performed parallel RNA sequencing and ribosome profiling. As expected, upon exposure to capreomycin the most represented functional category amongst down-regulated genes corresponds to ribosomal protein synthesis and modification. By further integrating the transcriptomic and translatomic response we are able to better define the initial *M. tuberculosis* gene regulatory program in response to capreomycin treatment.

Keywords: Tuberculosis, RNA-Seq, Ribosome Profiling, Drug Resistance, Capreomycin, Gene Regulation

G03 - 130 - P

Integration of Multi-Omics Layers Empowers Precision Diagnosis through Unveiling Pathogenic Mechanisms on Maple Syrup Urine Disease

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Maple syrup urine disease (MSUD) is a rare inherited metabolic disorder characterised by deficient activity of the branched-chain alpha-ketoacid dehydrogenase complex, required to metabolize the amino acids leucine, isoleucine and valine. Despite its profound metabolic implications, the molecular alterations underlying this metabolic impairment had not yet been elucidated. We performed a comprehensive multi-omics integration analysis, including genomic, epigenomic and transcriptomic data from 10 fibroblasts derived from a cohort of MSUD patients and 5 unaffected controls. MSUD patients exhibit a defined episignature that reshapes the global DNA methylation landscape, resulting in the stimulation of HOX cluster genes and the impairement of cell cycle gene-related signatures. Subsequent data integration revealed the impact of AP1-related and CEBPB transcription factors on the observed molecular reorganisation, with MEIS1 emerging as a potential downstream can-

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didate affected by robust epigenetic repression in MSUD patients. Moreover, the integration of multiple -omic layers facilitated the identification of a strong epigenetic repression in the *DBT* promoter in a patient wherein no BCKD pathogenic variant was detected, thereby unveiling alternative modes of disease inheritance.

Keywords: Epigenomics, Transcriptomics, Rare Diseases, MSUD

G03 - 144 - P

Age-dependent Effect of Environmental Stimulation on the 3D Chromatin Interactome of the Mouse Hippocampus

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Aging is a multifactorial biological process resulting in physiological and cellular decline. However, our understanding of age-related changes across various molecular layers, as well as the impact of external interventions on this process, remains limited. We characterized changes that occur at 3D chromatin interactome upon aging, using the low input Promoter Capture Hi-C (liCHi-C) on hippocampal neurons from young and old mice. To identify functional implications of these changes at the transcriptome level, we also performed RNA-seq analysis and integrated this expression data with promoter interactions. Furthermore, we assessed the effect on the hippocampus of exposing young and old mice to environmental stimulation (ES). Remarkably, our findings revealed an age-dependent modulation of promoter interactions and expression with ES. Namely, in response to ES, aging-like changes were induced in young mice, while age-related alterations were partially reverted in old mice, leading to a partial rejuvenation of aged mouse hippocampi.

Keywords: Chromatin Interactome, Aging, Hippocampus, Environmental Stimulation, Transcriptome

G03 - 147 - P

FiCRoN, a deep learning-based algorithm for the automatic determination of intracellular parasite burden from fluorescence microscopy images

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Intracellular protozoan parasites are responsible for dramatic infectious diseases such as malaria, leishmaniasis, Chagas disease, toxoplasmosis, etc., which threaten the lives of billions of people around the world. Among these diseases, leishmaniasis has become the second most common cause of human death after malaria. Leishmaniasis is caused by the amastigote form of *Leishmania*, which resides within the macrophages of the infected host. The quantification of intracellular parasites is a fundamental step in the validation of a new treatment, drug susceptibility, assessment of clinical strains and target validation using genetically modified parasites. This task is routinely performed by manually counting on Giemsa-stained slides or fluorescence microscopy images, which is a very time-consuming and laborious process that may generate errors due to inaccurate counts. Therefore, the development of automatic counting methods derives from the need to overcome the subjectivity of the counting process, rendering it reproducible and efficient. We propose a novel method FiCRoN, based on fully convolutional regression networks (FCRNs), as a promising new tool for estimating intracellular parasite burden. This estimation requires three values, intracellular parasites, infected cells and uninfected cells. FiCRoN solves this problem as multi-task learning: counting by regression at two scales, a smaller one for intracellular parasites and a larger one for host cells. Linear regression reveals an excellent correlation coefficient between manual and automatic methods. FiCRoN is an innovative freedom-respecting image analysis software based on deep learning, designed to provide a fast and accurate quantification of parasite burden, also potentially useful as a single-cell counter.

Keywords: Deep Learning Fluorescence Imaging Heme Intracellular Parasites Leishmania

G03 - 154 - P

Analysis of the non-coding RNA transcriptional landscape in Ovarian Epithelial Cancer

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Ovarian Epithelial Cancer (OEC) is a major cause of cancer-related mortality among women, largely due to its late diagnosis and complex molecular characteristics. (1) Non-coding RNAs (ncRNAs), such as long non-coding RNAs, are crucial regulators of gene expression and have been implicated in various aspects of cancer biology, including OEC.(2) This study aims to explore the ncRNA transcriptional landscape in OEC, using high-throughput RNA sequencing technologies to comprehensively profile the ncRNA expression in OEC tissues and compare it to normal ovarian tissues. Examining the differential expression patterns of these ncRNAs could provide insights into novel potential biomarkers for early diagnosis and targets for therapeutic intervention.(3) To achieve this, in this work epithelial cells are isolated from healthy and tumor ovarian tissue using REAleaseTM Immunomagnetic Separation Technology. The cells are then subjected to Sequential Cell Sorting and once separated, RNA-Seq is performed to obtain their ncRNA transcriptional profile. The results obtained here, analyzed in combination and comparing with the data available from previous studies, allow a better understanding of the role of the non-coding transcriptome in the development of the disease.

Previously published in: (1) Torre, L. et al. (2018) Cancer J Clin 68, 284–296; (2) Salamini-Montemurri, M. et al. (2020) Cancers 12; (3) Toden, S., Goel, A. (2022) Br J Cancer 126, 351–360

Keywords: Ovarian Epithelial Cancer, Non-Coding RNA, RNA Sequencing, Transcriptional Profile

G03 - 157 - P

Profiling of Tumour-Infiltrating Lymphocyte Landscape in Ovarian Epithelial Cancer

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Ovarian cancer, with Epithelial Ovarian Cancer (EOC) as the most prevalent subtype, remains a significant clinical challenge. It is the fifth leading cause of cancer-related deaths in women. Standard treatments, including surgery and chemotherapy, face high recurrence rates (75-85%), underscoring the need for new approaches.(1) Immunotherapy has shown promise in various malignancies but faces challenges in EOC due to the cold nature and the reduced mutational load. The role of tumour-infiltrating lymphocytes (TILs) is increasingly recognized for their potential in improving cancer outcomes. CD8+ TILs, crucial for anti-tumor responses and associated with better prognoses in various cancers, have been reported to recognize neoantigens presented by MHC-I in EOC patients.(2) This work aims to profile CD8+ TILs in EOC to understand their characteristics and therapeutic potential. This is achieved through mechanical and enzymatic dissociation of tumor ovarian tissues and healthy tissues, followed by enrichment for CD3+ T cells using REAlease MicroBead Technology, enhancing the sensitivity of single-cell immune profiling. The enriched cells are then analyzed using the Chromium Single Cell Immune Profiling Solution by 10x Genomics. Future steps involve producing in vitro TCRs (from the most expanded clones in tumour samples, selecting those absent in healthy samples) and the screening of these against peptide-human leukocyte antigen (pHLA) libraries using Yeast

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Surface Display (YSD) to identify high-affinity HLA-peptide complexes, thereby facilitating the identification of novel neoantigens.

References:
(1) Torre, L. A. et al. CA. Cancer J. Clin. 68, 284–296 (2018) 10.3322/caac.21456

(2) Bobisse, S. et al. Nat. Commun. 9, 1092 (2018) 10.1038/s41467-018-03301-0

Keywords: Epithelial Ovarian Cancer (EOC), Tumor-Infiltrating Lymphocytes (TILs), Single-Cell Sequencing, T-Cell Receptor Sequencing

G03 - 190 - P

Serum Proteome Responses in Sea Bass (Dicentrarchus labrax) Following Immunization

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Finding correlates of protection after fish vaccination is highly dependent on factors such as genetics, immunological history, type of vaccine...

As immunization is the main preventive strategy used in aquaculture, defining the traits that provide protection could be a stepping stone towards the rational design of vaccines, as well as the monitoring of fish immune status, which would lead onto the improvement of vaccine efficiency.

In the present study, the serum protein response after immunization with a Photobacterium damselae subsp. piscicida vaccine was analyzed in individually marked sea bass. Fish were finally challenged against the pathogen. Fish sera of immunized and control groups were classified depending on fish survival. Groups were compared by qualitative and semi-quantitative (label- free liquid chromatography mass spectrometry) proteomic analysis.

A great variation in serum proteins was observed. This correlates with heterogenity of fish populations.

Protein groups specific of fish survival in immunized fish were identified. Besides, protein groups present on survivor fish, regardless of being vaccinated, were also identified as traits of fitness. These proteins clustered into groups showing different responses to vaccination, with identification of protein groups belonging to classical immune system, acute phase molecules, other proteins, (many related to immune system) and some uncharacterized proteins (which shows the status of the lack of information contained in fish protein databases).

Overall, this study provides new knowledge of fish serum proteome improving our underestanding of sea bass immunization and providing prospective paths of study for correlates of protection identification, as well as for vaccine development.

Keywords: Proteomics, Serum, Seabass, Photobacterium, Vaccine

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Identification of immunogenic proteins in the pathogen Aeromonas salmonicida for inclusion in a subunit vaccine.

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Furunculosis is one of most common fish diseases on aquaculture, and it is caused by Gram-Negative bacterium *Aeromonas salmonicida*. *A. salmonicida* causes septicemia and wounds in the musculature, called furuncles, increasing the probability of colonization by opportunistic pathogens, worsening the infection. Although furunculosis affects different fish species, in this study we focused on its occurrence in turbot, since currently, the number of proteins studied related to the adhesion of *A. salmonicida* to this host is scarce. Expanding the number of known proteins found within the infectious pathway of this bacterium would help to develope a subunit vaccine, which roughly speaking, is a vaccine designed from virus or bacterial components, usually proteins. In this study, a search for outer membrane proteins of A. salmonicida was carried out for subsequent incorporation into a subunit vaccine effective against different isolates of this pathogen in turbot. For this purpose, we approached a strategy based on bioinformatics, a second based on proteomic analysis, and finally, a third based on a literature search, including in our list of antigens several *A. salmonicida* proteins whose expression on the outer surface of the bacterium is well established. Thus, by combining these three strategies, we tried to maximize the accuracy in the search for these membrane antigens.

Keywords: Shaving, Inmunoinformatics, Vaccines, Aeromonas, Turbot

G03 - 249 - P

Evaluation of DNA-metabarcoding as a new approach for honey's botanical authentication

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Honey is one of the foods most prone to be adulterated, with botanical origin mislabelling being one of the most frequent frauds. Honey's botanical origin is traditionally determined by microscopic analysis of pollen grains, a laborious method requiring expertise and often providing only family-level identification. Pollen identification by DNA-metabarcoding would allow for a faster, simpler, and more accurate determination. However, the reliability of using the number of sequences reads to estimate pollen percentages in honey remains unclear.

To address this issue, the number of pollen grains per mg in 13 pollen samples was determined using a Neubauer chamber. Then, four pollen mock mixtures were created, two containing 5 species and two 13 species. In each case, one of the mock mixtures was prepared with an equal mass of each pollen species (corresponding to varying amounts of grains), and the other was prepared using a similar percentage of each pollen species. Each mock mixture was also added to agave syrup (naturally pollen-free) to simulate the honey matrix. The pollen and agave mock mixtures were subjected to DNA extraction, PCR and ITS2-metabarcoding, and parallelly to pollen microscopic analysis.

DNA-metabarcoding results aligned well between the pollen mixtures and the agave syrup mixtures. In both cases, a few species were overrepresented and others underrepresented but, in general, the quantitative profile was according to the expected. In contrast, microscopy results closely matched the expected composition of the pollen mock mixtures, but significant discrepancies were observed in the agave samples.

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Keywords: DNA-Metabarcoding, Honey, Authentication

G03 - 250 - P

Comparison of different sequencing approaches to uncover botanical adulteration in herbal products

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Due to the increasing demand for popular medicinal plants, herbal products are prone to botanical adulteration. Aiming for fraud detection, DNA-barcoding emerges as one of the most suitable approaches for species identification in medicinal plants.

In this study, 58 commercial herbal products labelled with plant species traditionally used for cognitive, mood, or sleep improvement were submitted to DNA extraction and PCR targeting three barcodes. MatK and rbcLa amplicons were sequenced by Sanger, while ITS2 amplicons were sequenced by NGS for plant species identification. The Sanger sequences were identified by BLAST using a custom script. The NGS sequences were identified using a bioinformatic pipeline that included three ITS2 custom databases: one containing sequences of plants traditionally used for improving brain health, one containing medicinal plant sequences, and the third was a curated global database containing 307,977 sequences representing 111,382 species of vascular plants. NGS results were analysed by a script that sequentially went through these databases to ensure that a maximum number of sequences were identified as accurately as possible. Of the evaluated samples, MatK was able to correctly identify 62% labelled samples, rbcLa 76%, and ITS2 68%, with only 28% of the samples being correctly identified by the three barcodes simultaneously. NGS revealed that 29% of samples with a correct identification by Sanger sequencing were in fact mixtures of species, with the labelled one being the most representative. Overall, only 34% of samples conformed to the label, while 56% were mixtures containing the labelled species.

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Keywords: DNA Barcoding, Botanical Adulteration

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Role of the bacterial ribosome in the response to stress

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The ribosome has an essential role in the adaptation to environmental stress as a checkpoint to detect changes in temperature and nutrient levels ((VanBogelen & Neidhardt, 1990) (Cashel, Gentry, Hernandez, & Vinella, 1996) (Ashe, De Long, & Sachs, 2000). In previous work, we studied the effect of the several 16S rRNA mutations that conferred resistance to the antibiotic kasugamycin (Ksg) in *Escherichia coli (E.coli)* (Vila-Sanjurjo, Squires, & Dahlberg, 1999) (Schuwirth et al., 2006). These mutations, namely, A794G, G926A and A1519 mapped to universally conserved sites in 16S rRNA stronlgy suggesting the importance of these sites in protein synthesis (Vila-Sanjurjo, Squires, & Dahlberg, 1999). While the reason for the survival of the mutants was obscure, in light of their expected functional importance, we have recently observed that some of these mutants grow poorly at low temperatures, lose viability in stationary phase, and even lose the resistant phenotype when exposed to these conditions. As it has been shown that bacterial cells under a particular stress become cross protected against additional stresses, we named this phenotype loss of stress cross protection.

To understand this phenotype, we decided to identify the genes potentially involved in the stationary-phase-dependent cold sensitivity and loss of Ksg resistance. Part of the project consisted in properly defining the response of the cells to ksg. Here, we present these results for the wild type and resistant mutants. We believe that these results will help us in our way towards the elucidation of the mechanism of stress cross protection. Understanding this mechanism could have important effects in our ability to target bacterial cells when they are most resistant to environmental stress.

Keywords: Ribosome, Escherichia Coli, Kasugamycin

G03 - 266 - P

Técnica y casos prácticos de aplicaciones de la electroforesis capilar con detección por fluorescencia

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La electroforesis capilar es una técnica que permite separar diferentes moléculas atendiendo a su relación masa/carga, a través de un capilar de pequeño diámetro al someterlas a un alto voltaje. Los bioanalizadores de fragmentos *Qsep1* y *Qsep100*, son equipos automatizados basados en la tecnología de electroforesis capilar y detección por fluorescencia. Estos equipos unifican el proceso de preparación de gel, carga de muestras, electroforesis y toma de imagen en un único paso proporcionando toda la información relativa a las muestras tales como concentración e integridad en forma de electroferograma y gel. Las ventajas que supone el uso de estos bioanalizadores frente al método convencional de electroforesis son múltiples: requieren poco volumen de muestra, ofrecen alta resolución y reproducibilidad de los resultados y ahorran valioso tiempo y dinero. Los campos en los que habitualmente se emplea esta técnica son los de biotecnología y biología molecular, industria farmacéutica, diagnóstico clínico y laboratorios forenses. Entre las numerosas aplicaciones del equipo se encuentra la tipificación del virus del papiloma humano (VPH), genotipado animal y vegetal, detección de microsatélites, estudio de control de calidad post-PCR, control de calidad de preparación de librerías para secuenciación, diagnóstico de microdeleciones, visualización de amplificaciones de PCR con heterodímeros o evaluación de calidad de muestras de DNA/RNA previa secuenciación entre otras.

Keywords: ELECTROFORESISCAPILARENGEL, ECG, QSEP, BIOPTIC, FLUORESCENCIA

G03 - 267 - P

Improving colorectal cancer bacterial biomarkers: ONT *vs*. Illumina

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Background

This study aimed to evaluate the capabilities of full length V1V9 16S ribosomal RNA (16S rRNA) sequencing through Oxford Nanopore Technologies (ONT)'s new duplex chemistry, in comparison to Illumina's gold standard V3V4 16S rRNA sequencing for the characterization of the human gut microbiota, mainly for the discovery of colorectal cancer biomarkers. This was achieved through the analysis of feces from 123 subjects, comparing both approaches (Illumina-V3V4 vs. ONT-V1V9), multiple basecalling models (fast, hac and sup with dorado duplex) and multiple databases (SILVA vs. rrnDB+NCBI 16S RefSeq).

Results

Genera and species were reliably identified through ONT-V1V9, whereas

Illumina-V3V4 remained mostly at the genus level. Basecalling models broadly

resulted in similar results, although they influenced the number of observed features, with the worst model obtaining significantly more, and had disagreements for the identification and abundance of rare species. Database choice influenced the identified species greatly, with rrnDB+NCBI obtaining significantly higher diversity and identification than SILVA. More specific bacterial biomarkers for colorectal cancer than those obtained with Illumina were found, such as *Parvimonas micra, Fusobacterium nucleatum, Peptostreptococcus stomatis, Peptostreptococcus anaerobius, Gemella morbillorum, Clostridium perfringens, Bacteroides fragilis* and *Sutterella wadsworthensis*.

Conclusion

Full 16S rRNA sequencing through Oxford Nanopore achieves accurate species-level bacterial identification, facilitating the discovery of more precise colorectal cancer biomarkers and increasing the taxonomic fidelity of future microbiome analyses.

Keywords: Colorectal Cancer, Biomarkers, High-Throughput Sequencing.

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Scaling Single-Cell Sequencing: Multiplexing Innovations for Cost-Effective High-Throughput Profiling

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Single-cell sequencing technology has vastly enriched our understanding of biology across various domains. However, its widespread application in high-throughput endeavors such as screens, atlases, and large cohorts has been hindered by the high costs associated with instrumentation and reagents, along with challenges related to throughput and sample diversity. Addressing these constraints, combinatorial indexing exploits the inherent characteristics of individual cells as reaction compartments, allowing sequential barcoding of samples within a plate-based workflow.

Scale Bio offers flexible avenues for sample multiplexing, accommodating a broad range of cell inputs into both the fixation workflow and the 3-level plate based single-cell RNA workflow. The Scale low cell input fixation protocol accommodates up to 1 million cells in a 1.5mL tube, streamlining the fixation process. Complementing this capability, ScalePlex enables further multiplexing with hash oligos during the fixation protocol upstream of the Scale scRNA workflow.

The modular scRNA plate-based workflow incorporates 96 unique barcodes in its initial step, theoretically allowing for 96 distinct fixed samples. Additionally, the Scale scRNA kit can efficiently handle cell inputs ranging from 2,500 to 10,000 cells per well, ensuring robust downstream cell recovery and sequencing metrics. Augmenting throughput capacity, Scale introduces extended throughput kits, enabling recovery of up to 500,000 cells throughout the assay.

Keywords: Single Cell, Mulpiplex, ScRNA, Barcodes

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A panel of bacterial biomarkers for the diagnosis of colorectal cancer using fecal samples.

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Colorectal cancer (CRC) is one of the most common types of cancer worldwide, after breast, prostate, and lung cancers. The global increase in CRC incidence underscores the importance of early detection in order to reduce mortality. Thus, there is an urgent need for new noninvasive CRC biomarkers. Different interesting studies have identified disruptions in the O and gut bacterial communities of CRC patients, along with a harmful disruption in the gut vascular barrier. The present study revealed the microbial composition from saliva, crevicular fluid, feces, and both non-neoplastic and tumor intestinal tissue samples of 93 CRC patients and 30 healthy individuals without digestive disorders (non-CRC) using 16S rRNA metabarcoding techniques. The results indicated a significant over-representation of *Parvimonas, Fusobacterium* and *Bacteroides fragilis* in the stool samples of CRC patients, while *Faecalibacterium* and *Blautia* were notably more abundant in the non-CRC group. Additionally, tumor tissues were found to be enriched with well-known periodontal anaerobes, such as *Fusobacterium*, *Parvimonas*, *Peptostreptococcus*, *Porphyromonas*, and *Prevotella*. These O microorganisms co-occurred in the subgingival pockets and tumor tissues of CRC patients, correlating with other gut microbes like *Hungatella*. This work suggests that O pathobionts, typically found in subgingival pockets, may migrate to the colon and form synergistic consortia with other aerobic bacteria. Finally, we propose an interesting noninvasive fecal test for early CRC detection, utilizing a combination of *Fusobacterium*, *Parvimonas, Bacteroides* and *Faecalibacterium*. This method could potentially enhance the reliability of current CRC screening tests, which are based on fecal occult blood analysis.

Keywords: Biomarkers, Colorectal Cancer, Microbiome

G03 - 298 - P

Unraveling pine curvature genetics

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The maritime pine, *Pinus pinaster*, is of significant ecological and economic importance due to its substantial presence in the timber industry, producing quality products. To enhance yield, straight growth without curvature is preferable, as this avoids the formation of compression wood. Climate change anticipates an upsurge in extreme wind occurrences, which may potentially induce undesirable tree curvature and consequent compression wood production.

This study proposes an experimental design to assess the genetic underpinnings of *Pinus pinaster* curvature, complemented by a phenotypic investigation. Gene validation will entail the identification of homologous genes in Arabidopsis thaliana, given that the species under study has not yet been completely sequenced. The outcomes of this research will provide a more comprehensive genetic understanding of *Pinus pinaster*.

Keywords: Pinus Pinaster, Abiotic Stress, Gene Expression

G03 - 302 - P

Deciphering the protein profile of circulating human exosomes as a possible diagnostic tool in patients with pancreatic ductal adenocarcinoma (PDAC)

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Exosomes (<150nm) are extracellular vesicles released from tumoral and healthy cells which contain genetic material, proteins and metabolites. The high lethality of pancreatic ductal adenocarcinoma (PDAC), expected to be the second leading cause of cancer-related mortality worldwide by 2030, is due to late detection and ineffective therapies. Exosomes are very valuable biomarkers because they can be collected by using non-invasive methods.

Aiming to identify blood protein biomarkers for early detection, we isolated exosomes in blood samples from PDAC patients (n=12) and healthy donors (n=11). Isolation was done by ultracentrifugation and their size distribution was confirmed by imaging flow cytometry (ISX, Amnis) and dynamic light scattering (DLS). After the extraction, we analysed their protein profile by Mass Spectrometry-based proteomics and differences were considered positive with a fold change ≥±2 and $p<0.01$.

We identified eight proteins showing significant changes among healthy and PDAC individuals. TTR and Apo-A1 decreased in serum samples from patients compared to donors (-2.3 and -2.7-fold, respectively). Gelsolin levels were lower in serum and plasma patient samples compared to control samples (-23.0 and -43.0-fold). In plasma samples ITIH2, ApoH and Apo-A2 decreased in patient samples (-6.4, -3.7, and -2.1-fold, respectively). Finally, LRG2 and serpin-3 increased in patients (+8.4 and +3.5-fold).

We are currently investigating the utility of these proteins to conform a diagnostic panel for PDAC by liquid biopsy, with or without the previous isolation of exosomes.

Funded by GR21037 (Junta de Extremadura-FEDER) and by EQC2019-006152-P (MICINN-FEDER). C.O.P. was awarded a grant of AECC.

Keywords: Exosomes, PDAC, Pancreatic Cancer Diagnosis, liquid biopsy, Biomarkers, Proteomics, Gelsolin

G03 - 312 - O

Improving Proteomic Analysis with Advanced Software Utilizing Shared Peptides. Implication for protein quantification

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Bottom-up proteomics is based on the identification of peptides by mass spectrometry. However, a considerable proportion of the identified peptides belongs to several proteins, complicating protein identification. In spite of its importance, the problem of peptide redundancy has not been thoroughly resolved.

We previously proposed an algorithm to calculate exact protein identification probabilities from peptide probabilities. However, this algorithm was developed for unique, non-shared peptides.

In this work, we present a new software that includes a refinement of the previous algorithm, capable of estimating protein probabilities from the probabilities of peptides that are shared by more than one protein. This new algorithm weights the peptides based on the probability that the candidate protein originates such peptide. We have demonstrated that the protein probability estimated by the improved algorithm agrees very accurately with the observed probability in a variety of cases. Besides, protein identification performance surpasses that achieved by using unique peptides or assigning shared peptides to their razor proteins.

In addition, our new software allows to improve quantification analysis, by discarding excessively shared peptides which could introduce distortions in the quantification process. By using the Generic Integration Algorithm, we demonstrate that the new algorithm improves the quantification accuracy

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of peptides when compared to that achieved when peptides are assigned to their razor protein. This software will contribute to optimizing the peptide information available for quantitative proteomics.

Additionally, a custom node for Proteome Discoverer will also be published, facilitating the incorporation of the new algorithm into the analysis workflows.

Keywords: Proteomics, Shared-Peptides, Quantification, Bioinformatics

G03 - 326 - O

Identificación de nuevas variantes genéticas en neoplasias mieloproliferativas crónicas Filadelfia-negativas mediante secuenciación de exoma y genómica computacional

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En este trabajo se muestra el proceso de búsqueda e identificación de nuevas variantes genéticas en un grupo de cánceres hematológicos a partir de la secuenciación de exoma completo de muestras biológicas procedentes tanto de pacientes como de familiares sanos mediante el procesamiento bioinformático de los archivos resultantes de dicha secuenciación y su posterior análisis genómico e interpretación funcional a través de R.

El grupo de enfermedades en el que se centra este trabajo son las neoplasias mieloproliferativas cromosoma Filadelfia negativas. Este grupo de cánceres hematológicos está integrado por la policitemia vera (i.e., cáncer de eritrocitos), la trombocitemia esencial (i.e., cáncer de plaquetas), y la mielofibrosis (i.e., cáncer de precursores hematopoyéticos y médula ósea). El estudio se centra en ocho familias distintas; tres de las cuales corresponden a tres parejas de gemelos idénticos, todas ellas con un gemelo afectado por enfermedad y el otro sano, y cada una de ellas correspondiente a cada una de las tres enfermedades antes mencionadas. Las otras cinco familias permiten la identificación de variantes para discernir entre cada una de las tres enfermedades además de leucemia mieloide crónica y anemia idiopática; así como un caso de trombocitemia esencial triple-negativa.

En esta comunicación, se presenta el procesamiento bioinformático desde los archivos FASTQ hasta los archivos de variantes genómicas (i.e., VCF o variant call format files) así como el posterior análisis genómico y el filtraje computacional mediante el uso de R para el aislamiento y la obtención de variantes genéticas relevantes desde un punto de vista funcional y biológico.

Keywords: Genómica, Computacional, Bioinformática, Cáncer, Variantes, Genética, Leucemia, Neoplasia

G03 - 343 - O

Aplicaciones de la Inteligencia Artificial (AI) en investigación del cáncer: búsqueda de marcadores pronóstico y de respuesta

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La clasificación molecular consenso (CMS) del cáncer colorrectal (CCR) es un sistema de estratificación de pacientes que tiene el potencial de poder aplicarse a las decisiones terapéuticas. Hakai (CBLL1) es una E3 ubiquitina-ligasa que induce la ubiquitinación y degradación de E-cadherina, induciendo la transición epitelio-mesénquima (EMT), la progresión tumoral y la metástasis. Mediante métodos bioinformáticos, hemos analizado la expresión de CBLL1 en una gran cohorte integrada de muestras de tumores primarios de pacientes con CCR. La cohorte incluía datos de supervivencia y se dividió en subtipos moleculares consensuados. Se utilizaron tumoresferas de cáncer de colon para analizar la expresión de marcadores de células madre cancerosas mediante RT-PCR y Western blotting. Demostramos que la expresión del gen CBLL1 se asocia específicamente con el subtipo canónico CMS2, asociado con la ruta de Wnt, Myc y EFGR. Los genes diana de WNT LGR5 y c-MYC muestran una asociación con CMS2 similar a la de CBLL1. Estos niveles de ARNm están altamente regulados en las tumoresferas cancerosas, mientras que el silenciamiento de CBLL1 muestra una clara reducción del tamaño de la tumoresfera y de los biomarcadores de células madre. Es importante destacar que los pacientes de CMS2 con alta expresión de CBLL1 mostraron una peor supervivencia global (SG), que es similar a la asociada con los tumores de CMS4. Nuestros hallazgos revelan que CBLL1 es un biomarcador específico para CMS2 y el potencial de utilizar CMS2 con alta expresión de CBLL1 para estratificar pacientes con mala supervivencia global.

Keywords: AI, Búsqueda, Cáncer

G04: Biología sintética y biotecnología molecular

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Alkaline pH-based *Komagataella phaffii* platforms for efficient expression of enzymes of industrial interest

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The methylotrophic yeast *Komagataella phaffii* (formerly *Pichia pastoris*) is widely employed for heterologous expression of enzymes of industrial and therapeutic interest. Production is often driven by the powerful *AOX1* promoter, which is induced when the cells are grown on methanol as C source. However, this requires large volumes of methanol, a toxic and explosive material, involving important safety issues, thus stirring the search for methanol-free alternative promoters. Many yeasts, including *K. phaffii,* respond to moderate alkalinization of the medium with a robust transcriptional response. Transcriptomic [1] and proteomic (unpublished) profiling of *K. phaffii* to alkalinization led us to identify promoters offering a vigorous response to a shift to pH 8.0. We showed that three of these promoters (*PHO89*, *HSP12* and *TSA1*) potently drive, without negative effects on growth, the expression of a secreted *E. coli* phytase. Alkali-induced expression from the *PHO89* promoter was potentiated by mild phosphate limitation in the medium, thus matching the expression levels using the *AOX1* promoter [2]. We currently focus in bringing at the bioreactor level the production of phytase and that of a CRL1 lipase from *Candida rugosa*, as well as to identify possible bottlenecks at the translation level by ribo-seq techniques. Therefore, our data firmly supports the notion that *K. phaffii* alkaline pH-driven platforms can favorably compete with current *AOX1*-based systems, thus emerging as a convenient and inexpensive methanol-free alternative.

Supported by grants PID2020-113319RB-I00 (MCIN/AEI/10.13039/501100011033) and 2023 PROD 00006 (G. de Catalunya).

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Keywords: Heterologous Expression, Methanol-Free Promoters, Yeast, Pichia Pastoris

G04 - 27 - P

Autofluorescent biosensor for protein retro-translocation

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In eukaryotic cells, up to one-third of the newly synthesized proteins move from the cytosol to the endoplasmic reticulum (ER) lumen in a process known as trasnlocation. Once in the ER lumen, they undergo a post-translational folding process, assisted by a wide range of proteins of the chaperon family. However, due to genetic mistakes, cellular stress or simply stochastic events, some proteins do not reach the proper folding state. These misfolded proteins are subjected to a quality control mechanism in order to avoid their accumulation in the ER lumen, and its possible disastrous consequences. Thus, they are retro-translocated to the cytosol to be targeted for ER-associated degradation (ERAD) and subsequently annihilated by the proteasome.

This retro-translocation process is very complex and poorly understood, especially in mammalians. For this reason, the main methods for the detection of retro-translocated substrates presents clear disadvantages, such us the low-limited detection efficiency. To address the difficulties in the detection of potential substrates, our group has developed an auto-fluorescent biosensor to observe in vitro retro-translocation. This sensor is based on the reassembly of GFP complementary fragments using our developed technology, the IC-Tagging system. As a model, we are studying the retro-translocation process of the the non-secretory Ig *k* light chain NS1 (NS1), the null Hong Kong mutant of α1-antitrypsin (NHK-α1AT). Besides that, we observe the effects caused by the inhibition of the proteasome.

Keywords: Retro-Translocation, ERAD, Protein Detection, Fluorescence

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Construction of phage-displayed single-chain antibody (scFv) libraries using Gibson assembly cloning

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Bacteriophages, also known as phages, are bacteria-specific viruses that have been of interest for molecular biology since its discovery in the early twentieth century. In 1985, George P. Smith pioneered genetic engineering by fusing peptides to phage coat proteins, leading years later to the display of single-chain human antibody fragments (scFv) on phage surfaces and their subsequent development for phage-display of antibody libraries, which earned him and Gregory P. Winter the Nobel Prize in 2018.

Phage display, used additionally for antibody affinity maturation, entails inserting a DNA library into phagemids, displaying a range of antibody fragments, which are then selectively screened against the target of interest through successive rounds of biopanning. This study proposes optimizing phage-display library construction by integrating Gibson assembly, an *in vitro* recombination system that would overcome the laborious and time-consuming steps associated with traditional restriction enzyme-based cloning. Gibson single isothermal reaction enables seamless assembly and repair of overlapping DNA molecules, simplifying and accelerating the construction of large DNA libraries. Our work demonstrates Gibson cloning efficiency to build scFv phage-displayed libraries by improving a recently humanized antibody against CDH17, which has been proven to be implicated in CRC development and metastasis.

Keywords: Phage-Display, Gibson Assembly, Antibody Engineering, In Vitro Affinity Maturation, ScFv Library

G04 - 44 - P

MiST-IC: A novel nanobiotechnological platform with multiple biomedical and industrial applications

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A few years ago, a nanobiotechnological tool called IC-Tagging system was developed. This methodology is a protein molecular labeling platform based on the muNS-Mi protein derived from an avian reovirus. This system allows the production of micro- or nanospheres loaded with any protein of interest that carries a small protein tag (called IC) in any expression system. Proteins remain correctly folded and functional in the spheres, where quaternary interactions occur and complex enzymatic reactions are allowed. This system allows for single-step production of encapsulated proteins: cells do all the work. Furthermore, these particles are easily purified by mechanical methods making this technology very cost-effective.

This system has already been used successfully in the production of vaccines without adjuvants, and also in the stabilization/immobilization of industrial or therapeutic enzymes. However, we recently generated an important improvement in this technology, already versatile, to allow the binding of any molecule of interest to the surface of our particles in a simple, controlled, covalent and oriented way through a specific reaction catalyzed by the enzyme Sortase A. This new platform was called MiST-IC and allows us to take advantage of the intrinsic properties of the IC-Tagging system, to design and produce vaccines and stabilized enzymes more efficiently. In addition, MiST-IC offers new applications such as the development of biosensors, diagnostic systems, bioimaging or targeted therapies.

We have experimentally demonstrated that MiST-IC allows us to functionalize our protein-loaded particles with different molecules such as fluorescent probes, peptides (CPPs), proteins (GFP, RFP) or affibodies and nanobodies (anti-HER2) in an efficient and oriented way.

Keywords: MiST-IC, Nanobiotechnology, Nanoparticles

G04 - 46 - P

Pushing the limits of lateral flow immunoassay by Digital SERS for the ultralow detection of SARS-CoV-2 virus

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Lateral flow immunoassays (LFIA) are widespread easy-to-use antigen tests that provide a colorimetric readout that can be visualized with the naked eye. However, despite their usefulness for self-testing, such analytical devices have relatively low sensitivity and produce qualitative or semiquantitative results, which greatly limit their diagnostic applications. Here we address these challenges by implementing a digital surface-enhanced Raman spectroscopy (SERS)-based LFIA for the accurate and ultrasensitive quantitative detection of SARS-CoV-2 nucleocapsid (N) protein. As compared with average SERS intensity measurements, the digital approach allowed to overcome fluctuations in Raman scattering signals, thereby significantly increasing the analytical sensitivity of the assay. Our method exhibited a quantification range of the viral protein in nasal swabs from 0.001 pg/mL to 10 pg/mL, and a limit of detection down to 1.9 aM (0.9 fg/mL). Importantly, our digital approach showed an analytical sensitivity of 0.03 TCID50/mL, which is far greater than that reported by other types of immunoassays. Whereas colorimetric tests can only detect the viral N protein after symptom appearance, the high sensitivity shown by the proposed method could enable an early detection of the virus, lowering the probability of further spreading an infection and contributing to improving the surveillance of the disease.

In conclusion, we successfully demonstrated the robust detection and quantification of SARS-CoV-2 N protein in nasal swabs at ultralow concentrations. We believe that the substantial improvement in the sensitivity of LFIA by digital SERS may pave the way to translate this technology into the diagnostic arena for the ultrasensitive detection of microbes and disease biomarkers.

Keywords: SERS Tags, SARS-CoV-2, Lateral Flow, Digital SERS

G04 - 73 - P

Determination of prebiotic capacity of wheat straw xylooligosaccharides

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Xylooligosaccharides (XOS) are prebiotic compounds derived from xylan in plant fiber composed by xylose residues. XOS reach the colon without being digested, serving as carbon source for beneficial bacteria in the gut, promoting their growth. Depending on the source, degree of polymerization and substituents, XOS can be assimilated by different species of probiotic bacteria. Their selectivity as prebiotics help to rebalance the gut microbiota, improving digestion and immune function, making XOS valuable for functional foods, dietary supplements and pharmaceutical formulations.

Research on wheat straw XOS has been made as wheat straw is an abundant and renewable residue from agriculture which will allow potential large-scale production.

To be used as nutraceuticals, wheat straw XOS must not be cytotoxic to mammal cells. Cytotoxicity was tested in the human monocytes (THP1-Blue NF-kB) and murine macrophages (RAW264.7) and not significant cytotoxicity after 24 hours was observed. Also, digestibility of wheat straw XOS in the intestine has been studied *in vitro*, incubating rat small intestine extract (with lactase and maltase activity) with the XOS hydrolysate for two hours at 37ºC and analysing the results by HPLC. Most XOS remained intact after 2 hours of digestion, being xylobiose the most digested one (19,4%).

To test prebiotic capacity of XOS, the *in vitro* growth of various probiotic strains of lactobacilli and bifidobacteria has been studied by measuring absorbance in time. XOS were only assimilated by some strains of bifidobacteria, which could make them appropriate for obese patients, where bifidobacteria are often scarce in the gut microbiota.

Keywords: Prebiotic, Xylooligosaccharides, Microbiota, Nutraceutical, Bifidobacteria

INDICE

G04 - 80 - O

PETzyme: Building enzymatic nanoreactors for biodegradation of plastic waste

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The accumulation of plastic garbage in the environment has become one of the biggest challenges facing the world in the 21st century, with imminent consequences not only for wildlife but also for human well-being. Polyethylene terephthalate (PET) is the most abundant polyester plastic in the world, with an annual production of 70 million tons. However, less than 20% of PET is recycled, with most of the volume being released into landfills and oceans. Although mechanical and chemical recycling methods have been explored, their negative impact on the biosphere and the requirement for extreme conditions have limited their potential benefits. In response, enzyme-based plastic biodegradation has emerged as an eco-friendly and cost-effective strategy for managing PET waste. In this work, we propose the use of our own technology, the IC-Tagging system, as a platform for the immobilization of PET-degrading enzymes in order to overcome some of their constraints, such as reusability or thermal stability. IC-Tagging allows us to load any enzyme of interest into protein nanospheres, maintaining its catalytic activity. Our results demonstrate the capability of this method for the stabilization of active *LCC-ICCG* and *duraPETase*, two of the most promising enzymes for PET degradation. Immobilized enzymes are resistant to temperature and pH, and can be reuse up to 10 cycles without losing activity. Furthermore, both enzymes have proved to depolymerize PET beads to different monomers; particularly, *LCC-ICCG* lead to a weight loss of ~30% in 48 hours. These results, in combination with the successful scale-up of the technology, lay the foundations for the use of IC-Tagging for recycling or upcycling PET residues to value-added products, contributing towards a circular plastic economy.

Keywords: Polyethylene Terephthalate (PET), IC-Tagging, Enzymatic Nanoreactors

G04 - 90 - O

Correction of pathogenic mutations associated with ALS by using PAM-less synthetic enzymes

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by the progressive loss of motor neurons and it has no cure. ALS has a strong genetic background and many key genes have been identified to be involved in the development of the disease such as SOD1. Mutations in this gene are mostly missense point-mutations and cause up to 20% of the ALS familial cases.

CRISPR-Cas systems, originally derived from bacterial immune mechanisms, offer precise, programmable, and efficient means to edit specific genomic loci, providing a powerful tool for correcting pathogenic mutations associated with ALS. However, CRISPR-Cas9 systems have significant limitations that hinder their application. In order to overcome these limitations and reach their full potential, we have generated synthetic Cas enzymes by Ancestral Sequence Reconstruction (ASR) that exhibit unique properties like the absence of a specific PAM requirement, known as PAMless activity. These engineered enzymes expand the targeting range of CRISPR-based therapies by allowing edits at previously inaccessible sites.

We have applied synthetic enzymes to target different ALS-associated mutations in SOD1. We have shown that these synthetic enzymes can efficiently target and edit mutations that SpCas9 cannot due to the lack of a NGG PAM nearby in patient-derived fibroblasts. These results demonstrate the PAM-less activity of synthetic enzymes ex-vivo in patient derived cells, allowing the correction of any pathogenic mutation regardless of their genetic context.. In conclusion, synthetic Cas enzymes can significantly enhance genome-editing and hold promise for providing a genetic cure for ALS.

Keywords: Crispr, ALS, Genome Editing, Synthetic Biology

G04 - 115 - P

Development of an in vitro system for mycobacterial ribosome reconstitution

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Stablishing an *in vitro* system for cell-free synthesis and assembly of translationally competent ribosomes is a powerful technique for understanding molecular translation and ribosome mutability. *Mycobacterium tuberculosis* is the causative agent of human tuberculosis (TB), which remains a threat to the health of people worldwide. More than a quarter of global deaths due to antimicrobial resistance are due to drug-resistant *M. tuberculosis*, with many antibiotics commonly used targeting the ribosome. Traditionally, both the function and structure of the ribosome have been considered highly conserved. Nevertheless, specific characteristics of mycobacterial ribosomes, which could confer antibiotic resistance and adaptability to hostile environments, have been recently acknowledged. In our work, we are setting up an *in vitro* system for the reconstitution of mycobacterial ribosomes that can later be used to *in vitro* evolve the ribosome under different conditions. Using the non-pathogenic *Mycobacterium smegmatis*, we have successfully optimised protocols for the isolation of all the components necessary for the molecular assembly of active ribosomes. Using these components in *in vitro* reactions, we have been able to detect super-folder GFP (sfGFP) production. We have further developed the system for ribosome display by incorporating a stalling sequence in the mRNA template and obtained a significant reduction in sfGFP production. Future work will be centred in the optimisation of the reaction buffer to maximise near physiological conditions and to improve yields for *in vitro* protein synthesis. Later, we will use this system to screen a library of *M. tuberculosis* rRNA mutants under antibiotic pressure to better understand its mechanisms of ribosomal resistance and translation.

Keywords: In Vitro Translation, Mycobacterium, Ribosome Reconstitution

G04 - 122 - P

Development of Multifunctional Polyethyleneimine Nanoparticles for Active Targeting to Epidermal Growth Factor Receptor

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Nanotechnology has recently garnered extensive attention in the biomedical field, particularly for its potential in early cancer diagnosis and treatment1. The development of multifunctional nanoparticles (NPs) for cancer theranostics represents a key trend in nanomedicine. Polyethyleneimine (PEI) polymeric NPs offer a versatile platform as non-viral vectors for biomedical applications due to their high transfection efficiency and possibility of conjugation with chemotherapeutics and nucleic acids as cargo2.

This study aims to functionalize PEI NPs with recombinant antibodies, termed Nanobodies (Nb) to achieve specific binding to cell surface biomarkers3. We report an approach for the bioconjugation of maltose modified PEI NPs with a Nb that specifically recognizes the epidermal growth factor receptor (Nb_{EGFR}). This strategy is based on the affinity between maltose and the maltose binding protein (MBP) of *Escherichia coli* genetically fused to Nb_{EGFR} (MBP-Nb_{EGFR}). To monitor the adhesion to cultured cells *in vitro* by fluorescence microscopy, the NPs were conjugated with fluorescein-5-isothiocyanate. Our results demonstrated the synthesis and successful bioconjugation of the polymeric NPs with the MBP-Nb_{EGFR} fusion protein. Studies are underway to evaluate the specific adhesion of the NPs to various cell lines. If the specific binding to the EGFR cell surface protein is demonstrated, we envisage that the reported approach could be a valuable method for targeted therapy based on PEI nanoparticles and a robust platform for specific targeting of cell surface biomarkers in cancer theragnostic.

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3. Jin BK, et al. Int J Mol Sci. 2023.24:5994.

Keywords: Nanotechnology, Nanobodies, Cancer Theragnostic

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G04 - 125 - P

Enhancing Bioethanol Production with Synthetic Cellulases

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Lignocellulosic biomass is the most abundant renewable source on the planet. This biomass can be used to produce bioethanol, an alternative fuel to those derived from fossil fuels. Second-generation bioethanol, unlike first-generation bioethanol which is obtained from food crops, is produced from agricultural residues and other non-food sources. This makes it more sustainable and avoids competition with food production. However, a major challenge to making this process economically viable is the difficulty of breaking down cellulose into glucose through enzymatic hydrolysis, due to the natural resistance of the biomass cell wall. This cell wall contains lignin, a complex and resistant polymer that hinders access to cellulose and, therefore, complicates the extraction of glucose necessary for ethanol fermentation. In recent years, the application of enzymes for the decomposition of lignocellulosic material has attracted industrial interest in bioethanol production, but this process faces a significant limitation as in many processes, cellulases must withstand the harsh conditions of the industrial bioconversion process, such as high temperatures and extreme pH levels. In this work, to enhance the tolerance of cellulases to extreme conditions, we have employed computational methods to produce synthetic enzymes. By combining these enzymes, we have designed an enzymatic cocktail that, in preliminary results, shows hydrolysis performance comparable to that of commercial cocktails widely used in the industry. In addition, our enzymatic cocktail also shows significant tolerance to high temperatures (over 50 ºC) and low pH (bellow 5), distinguishing it significantly from commercially available cocktails.

Keywords: Cellulases, Bioethanol, Synthetic Enzimes

G04 - 131 - P

Physiological characterization of a D-malate oxidase mutant in *Pseudomonas oleovorans* CECT5344

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Hoy día, muchos ecosistemas naturales están en riesgo debido al aumento de contaminantes producidos por la actividad humanas. Estas sustancias terminan en diferentes ecosistemas, alcanzando concentraciones nocivas para la vida y provocando un deterioro significativo en la capacidad de los ecosistemas para mantener su equilibrio. El cianuro es uno de estos contaminantes, y la biotecnología ambiental trabaja con microorganismos que son capaces de eliminar este compuesto del ambiente de forma respetuosa y sostenible. *Pseudomonas oleovorans* CECT 5344 tiene la capacidad de asimilar este cianuro, utilizándolo como fuente de nitrógeno. Relacionada con la producción de energía, esta cepa carece de una malato deshidrogenasa para convertir el L-malato en oxalacetato. En su lugar utiliza una malato-quinona oxidorreductasa (MQO). Por otro lado, la asimilación del isómero D-malato es llevada a cabo por la enzima D-Malato Oxidasa (DMO) transfiriéndose los electrones al menos al Citocromo C en la cadena de transporte electrónico. La actividad de esta enzima no depende de cofactores nicotínicos sino de otros como la PQQ o FAD. En este trabajo se muestra la estrategia para la obtención de un mutante D-Malato Oxidasa mediante mutagénesis dirigida, y su comportamiento en la asimilación de D-malato en *Pseudomonas oleovorans* CECT 5344. Para la caracterización de este mutante se han utilizado dos métodos propios, uno espectrofotométrico y otro colorimétrico en geles de poliacrilamida, en la determinación de la actividad D-Malato Oxidasa.

Agradecimientos: *Consejería de Economía, Ciencia y Agenda Digital de la Junta de Extremadura (IB20148 y GR21016), Unión Europea (Next Generation EU), Plan de Recuperación Transformación y Resiliencia), Una Manera de Hacer Europa. Universidad de Extremadura*

Keywords: D-Malato Oxidasa, Biorremediación, Pseudomonas

G04 - 139 - P

Development of new engineered Synthetic CRISPR-Cas systems compatible with AAV delivery platforms

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The search for gene editing tools that allow programmable and targeted manipulation has gained increasing popularity in recent years due to their wide range of applications in both industry and biomedicine. One of the most widely used tools is the CRISPR/Cas gene editing system. Despite having displaced techniques such as zinc finger nucleases (ZFN) and TALENs, this methodology has certain limitations that reduce its effectiveness and versatility. These restrictions include the need for a compatible Protospacer Adjacent Motif (PAM) sequence near the area to be edited, non-specific activity (off-target), and the acquired immune response against Cas enzymes in some individuals.

The development of delivery techniques and advances in biomedicine have allowed CRISPR/Cas gene editing systems to be administered in vivo using adeno-associated viral vectors (AAVs). However, these vectors have a significant limitation that negatively affects their applicability to CRISPR/Cas: the amount of genetic material they can package. For these reasons, CRISPR/Cas systems have been subject to improvements through rational design and residue mutagenesis. These efforts seek to optimize the qualities of these systems as gene editing tools.

With the assistance of bioinformatics tools and other advanced techniques, the objective of this project is to overcome the aforementioned limitations by designing new synthetic CRISPR/Cas systems with improved functionalities, reduced size, and higher activity and specificity.

Keywords: CRISPR, AAV, Synthetic Biology

G04 - 152 - P

Rationale design of a localised drug delivery system for the specific locoregional treatment of advanced ovarian cancer

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Peritoneal carcinomatosis (PC) is a common outcome of most abdominal malignancies, such as advanced ovarian cancer (AOC). Typically, PC is treated by cytoreductive surgery (CRS) combined with conventional chemotherapy, although these treatments present several limitations that must be overcome. New efforts in the treatment of PC intraperitoneally have focused their attention on drug delivery systems (DDS). These devices allow a locally specific treatment, avoiding most of the drawbacks generated by systemic chemotherapy. In addition, they also enable a sustained release of the drug over time, preventing rapid drug clearance.

This study aimed to explore the properties of a chemically modified hyaluronic acid (HA) hydrogel (HAH) for its use as a DDS in the administration of Olaparib (OLA), a novel and specific anticancer drug targeting poly ADP ribose polymerase (PARP). Furthermore, to achieve a more specific loco-regional effect, the HA hydrogel was loaded into a haemostatic patch (HP) commonly used to control bleeding after CRS.

Our in vitro results have shown how the entrapped HAH loaded with OLA exhibited a sustained release of the drug over six days, allowing for higher drug loading than when administered intraperitoneally alone. Moreover, OLA maintained its cytotoxic effects upon release, being effective on ovarian cancer cell lines.

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In conclusion, we have demonstrated that OLA can be formulated into an efficient HAH DDS for the locoregional treatment of AOC. These cutting-edge devices offer huge potential in the clinical management of peritoneal malignancies, and their use warrants further investigation. Thus, our preliminary results support the use of DDS for precisely delivering chemotherapeutic agents to any residual microscopic disease in PC following CRS.

Keywords: Drug Delivery, Ovarian Cancer, Olaparib, Localised Intraperitoneal Chemotherapy

G04 - 160 - P

New enzymes and microorganisms in the degradation of polyethylene terephthalate

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In the constant search for solutions to plastic waste management, polyethylene terephthalate (PET) stands out as one of the most challenging materials due to its remarkable resistance to degradation. Although the discovery of *Piscinibacter sakaiensis* offers a potential solution to the problem, being able to break down PET into its basic components, ethylene glycol (EG) and terephthalate (TPA) which are metabolized, its industrial application is restricted due to the limited efficiency of its enzymes.

In this context, this study focuses on exploring various microbial strains, both from scientific collections and environmental isolates from different habitats, in search of those that can facilitate PET degradation at the industrial level. The main focus is to identify enzymatic activities related to the degradation of PET or its oligomers, especially bis-(2-hydroxyethyl) terephthalate (BHET), as a key indicator of biotransformation. This analysis has led to the identification of promising microorganisms, such as *Rhodococcus* sp. HE24-12 and *Gordonia* sp. HE24-4J, capable of degrading BHET to its main constituents (EG and TPA).

Detailed analysis of the secretome of these microorganisms has revealed the presence of potentially useful enzymes, such as esterases, involved in the biotransformation of BHET. These results suggest the existence of enzymes with the potential to degrade both BHET and possibly PET, which may be more efficient than those of *P. sakaiensis*. Moreover, these enzymes could, potentially, be improved by protein engineering techniques, thus broadening the prospects for sustainable plastic waste management.

Funding: The project ABERPLAS (TED2021-132593B-I00) is funded by MCIN/AEI/ 10.13039/501100011033 and "Unión Europea NextGenerationEU/PRTR".

Keywords: Polyethylen Terephthalate, Enzyme, Terphthalic Acid

G04 - 167 - P

Study of HMF and furfural degradation pathway in *Pseudomonas*

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La producción de bioetanol de segunda generación, usado como combustible o producto químico, es una buena alternativa a los combustibles fósiles. Éste se obtiene a partir de fuentes renovables como compuestos lignocelulósicos derivados de deshechos vegetales. Sin embargo, el proceso presenta algunos inconvenientes que disminuyen el rendimiento como, por ejemplo, la formación secundaria de 5-hidroximetilfurfural (HMF) y furfural (2-furaldehído). Estos compuestos son inhibidores del proceso por su toxicidad, afectando a la levadura que produce el etanol por fermentación de los azúcares liberados. Aunque son compuestos muy tóxicos, existen algunas especies de bacterias capaces de crecer en presencia de ellos e incluso de utilizarlos como única fuente de carbono y energía. Estas bacterias pueden usarse como herramienta para la eliminación de los inhibidores, mejorando el rendimiento en la producción de bioetanol. Estudios previos sobre las rutas de oxidación de HMF y furfural en otras bacterias, demuestran que la ruta de HMF y furfural convergen en ácido furoico. En este trabajo se estudia la capacidad de asimilación de HMF, furfural y posibles intermediarios entre HMF y ácido furoico en una bacteria que asimila muy bien el furfural (*Pseudomonas oleovorans* CECT 5344) y otra que asimila muy bien el HMF (*Pseudomonas sp.* 4A). El objetivo final es conocer las rutas metabólicas en estas dos especies de bacterias, con el fin de formular cultivos sinérgicos que permitan la eliminación total de inhibidores presentes en los hidrolizados y mejorar el proceso de obtención de bioetanol.

Agradecimientos: Universidad de Extremadura. Junta de Extremadura. Consejería de Economía, Ciencia y Agenda Digital (IB20148). Fondo Europeo de Desarrollo regional (FEDER). Una Manera de Hacer Europa.

Keywords: HMF, Furfural, Pseudomonas, Bioetanol

G04 - 168 - P

Polyethylene terepthale degradation using genetically modified bacterial chassis: a synthetic biology approach

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The high production of PET, its rapid disposal after use, and inherent resistance to degradation have caused a massive accumulation of waste based on these polymers in the environment. Due to its novelty in nature, only a few bacteria and fungi have shown the ability to partially degrade PET into its oligomers [bis-(2-hydroxyethyl) terephthalate], its monomeric units [mono-(2 -hydroxyethyl) terephthalate] and, finally, in its basic constituents ethylene glycol (EG) and terephthalate (TPA). *Piscinibacter sakaiensis*, can depolymerize PET to EG and TPA, using these as energy and carbon sources. In this bacterium, PET mineralization is initiated by a PETase and a MHETase. Their gene expression in biotechnological chassis could enable the treatment, disposal, and even revaluation of PET waste introducing them into a circular economy system.

Versions of the *P. sakaiensis* genes have been synthesized, adapting the codon usage for *Pseudomonas putida* U, *Rhodococcus* sp. HE24-12 and *R. jostii* RHA1. These genes have been cloned in replicative plasmids for each bacterium, and the design of promoters for the expression of these genes and efficient secretion peptides is in development.

In parallel, a bottom-up analysis of the capabilities of the host strains is being carried out for their potential application in the degradation of PET, determining its ability to metabolize BHET, TPA, and EG. The microorganisms studied have shown distinctive metabolic differences, providing different genetic and metabolic backgrounds for expressing optimized genetic systems.

Acknowledgments: Grant ABERPLAS (*Biotechnological alternatives for the elimination of petrochemical plastic waste*, Ref. TED2021-132593B-I00) funded by MCIN/AEI/ 10.13039/501100011033 and the "European Union NextGenerationEU/PRTR".

Keywords: PET, BHET, MHET, Terephthalate, Pseudomonas, Rhodococcus, Biodegradation

G04 - 169 - P

Innovative use of *Bacillus* sp. in biodegradable mulch films for a sustainable agriculture and an environmental footprint reduction

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The extensive use in agriculture of mulch film offers significant advantages for weed and pathogen control, as well as soil moisture and temperature conservation. However, the high environmental impact of these materials due to the seasonal removal and recycling technical problems and costs, along with the generation of secondary microplastics when the environmental degradation is the selected option, poses serious challenges. The demand for biodegradable mulch plastics is increasing, as they offer agricultural benefits without the environmental impact of conventional plastics. The adoption of these bioplastics represents a promising solution and aligns with the United Nations Sustainable Development Goals.

In this context, the BioPAC project (Ref. TED2021-131864B-C21) focuses on evaluating the amylolytic activity of various *Bacillus* sp. species for use in the degradation of starch-based thermoplastics with properties similar to that of traditional mulch films. Starting from a collection of *Bacillus* sp. strains, both amylolytic activity and antagonistic skills against phytopathogens were evaluated. The strain with the best performance in these aspects was a *Bacillus amyloliquefaciens* strain, for which a sporulation induction method was also optimized. The resistance of its spores to differ-

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ent physical and mechanical processes was demonstrated, opening commercial possibilities for developing a spore formulation that can be used in the field as an agent for the biodegradation of starch-based thermoplastic mulches, while also providing plant protection and growth stimulation.

Funding: This work has been developed within the BioPAC Project (Ref. TED2021-131864B-C21) funded by MCIN / AEI / 10.13039/501100011033 by the European Union NextGenerationEU / PRTR.

Keywords: Bacillus, Mulch Film, Biodegradable Plastic

G04 - 171 - O

Enhancement of Bioethanol Production Through the Use of *Pseudomonas*

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To produce bioethanol, a hydrothermal and thermo-mechanical pre-treatment of pruning waste is conducted. This is followed by the hydrolysis of carbohydrates and the alcoholic fermentation of sugars by yeasts. The hydrolysis of polymers releases sugars essential for yeast-driven alcoholic fermentation, but it also generates fermentation inhibitors such as acetic acid, furfural, and 5-hydroxymethylfurfural (HMF). This study aims to eliminate those inhibitors from elm hydrolysate (*Ulmus pumila*, an attractive biomass for developing various value-added chains), while preserving sugars. To achieve this, the ability of two *Pseudomonas* strains to utilize these compounds has been investigated. *Pseudomonas oleovorans* CECT 5344, can assimilate furfural and acetic acid but not HMF, although it is converted into a less toxic intermediate. An *edd* defective mutant strain, unable to assimilate sugars has been generated. A similar strategy is being employed with *Pseudomonas* sp. 4A, which assimilates HMF very efficiently but has difficulties with furfural. It has been verified that *P. oleovorans edd-* can utilize the hydrolysate, eliminating furfural or HMF and reducing acetic acid, while leaving the initial sugars intact. The process has been optimized by performing different dilutions of the hydrolysate, and currently, the intermediates are being characterized. A biodetoxification strategy enhance the yield in bioethanol production, adding value to waste and promoting a sustainable bioeconomy.

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Keywords: Bioethanol, Elm Hydrolyzate, Pseudomonas, Biodetoxification, Furfurals

G04 - 175 - P

Engineering of Escherichia coli biosensor strains with optimized expression of Raman Reporters

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Herein we report the development of *Escherichia coli* biosensor strains bearing a new class of genetically encoded optical reporters, termed Raman reporters (RaRs), detectable by surface-enhanced Raman scattering (SERS) spectroscopy. SERS is a highly sensitive analytical technique with “fingerprint” chemical information provided by Raman scattering. Another major advantage of SERS for biodetection is that, as compared to conventional light and fluorescence spectroscopies, the characteristic narrow bandwidths of Raman spectra facilitate the detection of multiple distinct molecules simultaneously as the Raman spectrum is typically 50 times narrower than that of fluorescence. The proposed RaRs are characterized by: (i) high SERS activity; (ii) amenable to heterologous expression in E. coli, and; (iii) unique spectral fingerprints for unambiguous identification by SERS. As a source of RaRs we investigated violacein and its derivatives from Chromobacterium violaceum. In this study we report the optimization of the violacein biosynthesis pathway expression by Ribosome Binding Site (RBS) engineering, which was leveraged for the generation of *E. coli* strains with highly stable and robust chromosomal expression of RaRs. Our results pave the way for the generation of a new class of biosensors with genetically-encoded optical reporters based on SERS with improved sensitivity and multiplexing capabilities.

Keywords: Bacterial Biosensor, Engeneered Bacteria, RBS Optimation, SERS

G04 - 198 - P

Construction of a mutant of biotechnological interest in the glucose assimilation pathway in *Pseudomonas* 4A strain

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Las bacterias del género *Pseudomonas* son capaces de utilizar un amplio rango de fuentes de carbono, una de ellas es la glucosa. Esta se asimila por la ruta de Entner-Douroff, ya que carece de la enzima 6-fosfofructoquinasa, y por lo tanto no puede metabolizarla vía glucolisis. En esta ruta, la glucosa se metaboliza vía 6-fosfogluconato hasta gliceraldehído-3-fosfato y piruvato. La gluconato-6-fosfato deshidratasa, codificada por el gen *edd*, es esencial para que la bacteria pueda asimilar este azúcar. *Pseudomonas* sp. 4A es una bacteria aislada en nuestro laboratorio por su capacidad de asimilar eficientemente Hidroximetil Furfural (HMF). Este compuesto se forma en el pretratamiento ácido de residuos lignocelulósicos por deshidratación de hexosas, inhibiendo posteriormente el proceso de fermentación alcohólica de las levaduras que finaliza con la producción de bioetanol de segunda generación.

Con el objetivo de obtener una cepa que elimine el HMF pero deje intactos los azúcares del hidrolizado, se ha construido un mutante en el gen *edd* de la cepa *Pseudomonas* sp. 4A. Dicho mutante se ha construido por doble recombinación homóloga sustituyendo la región central del gen por un casete de resistencia al antibiótico gentamicina. El mutante no asimila glucosa, pero mantiene intacta su capacidad de utilizar HMF, por lo que tiene un enorme potencial biotecnológico.

Agradecimientos: Universidad de Extremadura. Junta de Extremadura. Consejería de Economía, Ciencia y Agenda Digital (IB20148)., Fondo Europeo de Desarrollo regional (FEDER). Una Manera de Hacer Europa.

Keywords: Edd, Glucosa, HMF, Pseudomonas

G04 - 216 - P

Unraveling bacterial cyanide metabolism

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A pesar de que el cianuro es una molécula simple (HCN), su metabolismo parece ser bastante complicado. Por una parte, su presencia disminuye la biodisponibilidad de metales, especialmente de hierro, e inhibe algunas metaloproteínas como la citocromo *c* oxidasa, la fumarasa o la aconitasa. Por otra parte, las bacterias que asimilan cianuro deben poseer una ruta que conduzca el cianuro hasta rutas centrales del metabolismo. *Pseudomonas oleovorans* CECT 5344 es una bacteria capaz de asimilar cianuro y responde al cianuro induciendo una oxidasa terminal insensible al mismo, lo cual le permite una respiración aeróbica. A la vez, la presencia de cianuro modifica el patrón de inducción de algunas isoenzimas del ciclo de Krebs, como la fumarasa, la aconitasa y la L-malato:quinona oxidorreductasa. Las isoenzimas difieren unas de otras en su resistencia a estrés oxidativo y al cianuro, y también a su necesidad de hierro. En su conjunto, los mecanismos de resistencia proveen las enzimas necesarias para que funcione tanto el ciclo de los ácidos tricarboxílicos como la respiración. Por otra parte, para la asimilación de cianuro se han propuesto diferentes rutas que podrían conducir a la producción de amonio o aminoácidos. La inclusión de los datos bioquímicos disponibles en un modelo metabólico a escala de genoma permite integrar el metabolismo del cianuro en las rutas metabólicas centrales de *P. oleovorans* y predecir la biomasa que se puede obtener con cianuro como fuente de nitrógeno.

Agradecimientos: Este trabajo ha sido financiado por la Consejería de Economía, Ciencia y Agenda Digital de la Junta de Extremadura (IB20148 y GR21016), Universidad de Extremadura y Fondo Europeo de Desarrollo regional (FEDER). Una Manera de Hacer Europa.

Keywords: Cianuro, Aconitasa, Fumarasa, Respiración, Ciclo De Krebs

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G04 - 227 - P

From Chitin to High-Tech Bioink: Enzymatic Production and Enhancement of Crystalline Nanochitin

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Polysaccharides such as cellulose and chitin are the most abundant biomolecules on Earth. Consequently, extensive efforts have been made to harness these resources, converting them into high-value materials with biotechnological applications. Among these polysaccharides, chitin offers significant advantages over cellulose due to the presence of N-acetyl groups, which provide greater fracture resistance and improved adhesion to plastic or crystalline materials.

One of the potential uses of chitin is the production of crystalline nanochitin (CNCh). CNCh is a high-value material with significant applications in profitable biotechnological processes. One notable application involves combining CNCh with graphene oxide to create a conductive bioink with strong adhesion to plastic materials. Traditionally, the production of nanocrystals from biopolymers has involved acidic treatment. To avoid the use of acids, which are environmentally hazardous and toxic, enzymatic processing has been successfully employed to produce CNCh. Specifically, lytic polysaccharide monooxygenase (LPMO) enzymes have been characterized for their role in forming CNCh. However, enzymatic methods often struggle to generate nanocrystals as small as those produced by acidic treatment. In this work, we investigated both native and engineered proteins to enhance CNCh production by reducing the size of the nanocrystals.

Our findings demonstrate the potential of enzymatic processing to produce advanced materials with unique properties. By reducing the size of the nanocrystals, we have enabled the generation of bioink with potentially improved properties, avoiding the use of environmentally hazardous acids in the process. This emphasizes the value of CNCh in biotechnological innovations.

Keywords: High-Value Biomaterials, Crystalline Nanochitin, Conductive Bioink, Environmental Impact, Protein Engineering

G04 - 235 - P

Isolation and characterization of a *Pseudomonas putida* strain as a sustainable manner of epoxy thermoplastics recycling

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Epoxy thermoplastics are notoriously difficult to degrade using conventional methods, creating an urgent need for sustainable recycling techniques. Hence, the ESTELLA project—a pioneering initiative funded by the EU—aims to revolutionize waste management practices. By employing cutting-edge biotechnological methods, the project seeks to transform the handling of thermoplastic waste and promote a more sustainable circular economy. One such way, the biotechnological method involves the use of microorganisms capable of degrading epoxy resins. Thus, a strain of *Pseudomonas putida* isolated from an environmental sample has shown the remarkable ability to survive for extended periods by utilizing epoxy resins as its sole carbon source. The draft genome of this strain has been sequenced, revealing a single circular chromosome with a G+C content of 61.87%, encompassing 6,181 annotated open reading frames and an approximate size of 6.5 Mbp. Current research is focused on bioinformatic analysis of the genome to identify specific genes or enzymes linked to the microorganism's survival capabilities. Additionally, efforts are underway to optimize culture conditions and employ proteomics approaches to elucidate the mechanisms behind its ability to degrade epoxy resins. These studies aim to identify key enzymes, such as epoxide hydrolases, and secondary metabolites that may enhance the strain's adaptability and efficiency in breaking down complex chemical structures. The discovery of this strain offers new biotechnological opportunities to revolutionize plastic waste management by merging laboratory discoveries with industrial strategies.

Funding: ESTELLA project (no: 101058371), EU, Horizon Europe Program, call: HORIZON-CL4-2021-RESILIENCE-01-11 (https://estellaproject.eu/)

Keywords: Pseudomonas Putida, Epoxy, Thermoplastics, Resines, Enzymes

G04 - 247 - P

Nanoparticle-mediated delivery optimization of CRISPR/Cas RNP for gene therapy

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CRISPR/Cas systems are one of the most versatile genomes editing tools. However, its therapeutic use must face several challenges, including the delivery into cells due to many extra- and intracellular barriers. For this delivery, the use of RNPs (pre-formed protein-crRNA complexes) is the most straight forward strategy with a faster effect and fewer off-target cuts, but have some limitations including cell entrance, lysosomal degradation and intracellular localization. Our previous studies confirmed that the combination of Cas9 and Cas12a RNP with magnetic nanoparticles (MNPs) favoured intracellular delivery and allowed their CRISPR-Cas activity in cells. In this work we aimed to optimize the CRISPR intracellular delivery and their use in therapy. Thus, we produced recombinant Cas proteins containing a 5 amino acid peptide, endosomal escape domain (EED), that can disrupt endosomal membranes and induce endosomal escape. Considering these properties we though it could improve magnetic nanoparticle functionalization and even allow the use MNPs for Cas13b delivery. During this study, we were able to effectively express and purify the modified proteins and characterize its activity ´in vitro´ and ´in cellullo´, as well as determining their interaction and edition rate with MNPs.

Keywords: Nanoparticle, Crispr RNP, Gene Therapy, Endosomal Escape Domain

G04 - 275 - P

Recombinant Production of Bovine Serum Albumin (BSA) in Yeast: Optimization and Enhanced Blocking Capacity in Immunoassays

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At Levprot Bioscience we have developed an approach to the production of Bovine Serum Albumin (BSA) using recombinant methods in yeast, eliminating the need for animal-based sources. By optimizing the expression strain, production and downstream processes, we have achieved significant advancements both in yield and functionality of recombinant BSA (rBSA).

Traditionally sourced from animal sera, BSA is a critical component in various biological assays and experiments. However, concerns regarding animal welfare and batch-to-batch variability have prompted the exploration of alternative production methods. Through genetic engineering and precision fermentation techniques, we have successfully developed a sustainable and scalable method for rBSA production in yeast.

Our research demonstrates not only the feasibility of recombinant BSA production but also its superior performance compared to conventionally sourced BSA. The optimized production process yields high quantities of pure BSA, ensuring consistency and reliability in experimental outcomes. Furthermore, our recombinant BSA exhibits enhanced blocking capacity in immunoassays, improving the accuracy and sensitivity of detection methods.

By presenting our findings, we aim to advocate for the adoption of recombinant BSA as a viable alternative to animal-derived sources. This innovative approach not only aligns with ethical considerations but also offers practical advantages in terms of scalability, reproducibility, and performance in immunoassays. We believe that embracing recombinant production methods represents a significant step forward in the field of protein production and biotechnology.

Keywords: Yeast, Recombinant BSA, Animal-Free, Blocking Capacity

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G04 - 280 - O

Designing RNA-binding repeat proteins for splicing modulation in SMA

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Transcriptome editing has opened a variety of possibilities for new therapeutic strategies, including the modulation of mRNA splicing using antisense oligonucleotides (ASOs), a treatment already approved for spinal muscular atrophy (SMA) [1]. However, to overcome some of the disadvantages of the use of ASOs, is important to explore alternative strategies, such as engineered RNA-binding repeat proteins. These proteins, such as the pentatricopeptide (PPR) and the Pumilio and FBF (PUF) families, are modular proteins that can selectively bind specific RNA sequences based on an amino acid code, with each repeat recognizing a single base [2,3]. This allows for the design of proteins programmed to bind a target sequence with elevated affinity and specificity.

In this project, we design and characterize a PPR and a PUF protein that specifically bind a sequence in *SMN2* mRNA to induce exon 7 inclusion as a therapeutic strategy to correct splicing in SMA [4].

To obtain stable protein scaffolds as backbone, we use a consensus PPR sequence, and design a workflow to generate a *de novo* PUF sequence based on natural modules. These newly engineered proteins efficiently modulate *SMN2* splicing and are promising tools for transcriptome editing in other diseases.

Keywords: Splicing, RNA, PPR, PUF

G04 - 319 - P

Regulation of the biosynthesis of fungal secondary metabolites by reactive oxygen species (ROS)

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Our group discovered that lovastatin (hypocholesterolemic) is positively regulated, at transcriptional level, by reactive oxygen species (ROS) generated during idiophase. We also showed that ROS similarly regulate the biosynthesis of other industrially important secondary metabolites like penicillin and cephalosporin.

To investigate the mechanism by which ROS induce biosynthetic genes in *Aspergillus terreus* we studied the role of oxidative-stress defense transcription factors: Yap1 and AtfB, by generating knocked down (silenced) mutants by RNAi, and determined its effect on lovastatin production, and other parameters.

Yap1 transformants (Siyap1) showed higher sensitivity to oxidative stress. Interestingly, knockdown mutant showed an important increase in lovastatin production in submerged and solid-state fermentations: 60 and 70% increase respectively, but contained higher ROS levels in idiophase. Furthermore, sporulation also increased by 600%. By normalizing ROS levels, it was shown that AtYap1 does not regulate lovastatin biosynthetic genes, and that production increase observed in the knockdown strains was an indirect effect caused by ROS increase.

Surprisingly, AtfB transformants showed decreased lovastatin production in solid-state fermentation, while increased production in submerged fermentation, also displaying decreased ROS levels in both fermentation systems. Nevertheless, in this case differences in production were conserved when ROS levels were normalized.

It is concluded that Yap1's effect was indirect (by increasing ROS levels), while AtfB regulates lovastatin biosynthesis in *A. terreus*, but has different functions in submerged and solid-state fermentation.

Keywords: Lovastatin ROS Regulation, Transcription Factors, Yap1 And AtfB

G04 - 345 - O

Writing genomes and metagenomes, and the advent of synthetic evolution

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Our research is focussed in developing new principles and technologies to engineer biological systems. First, I will present our vision on how we are levereging bioprospecting, AI and evolution to create new biological functions. Then, I will focus on 1) how we developed a gene writer for human cells and its deployment ex vivo (T cells) and in vivo (liver targeting). 2) how we use Cutibacterium acnes as a chassis to engineer human skin properties.

Keywords: Synthetic Biology, Molecular Design, CRISPR, Gene Editing

G05: Biomembranas

G05 - 39 - P

Wnt trafficking in the early secretory pathway

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The secretion of Wnt signaling proteins is essential to control cell proliferation, cell polarity and cell fate determination during embryonic development and tissue homeostasis. Wnt secretion also contributes significantly to the pathogenesis of human diseases such as cancer, diabetes and congenital malformations. Although much focus has been placed on understanding intracellular Wnt signal transduction in signalreceiving cells, the molecular mechanisms that control the secretion of Wnt proteins in signal producing cells are still unclear. Lipidation of newly synthesized Wnt proteins in the endoplasmic reticulum (ER) confers a unique mode of membrane association within the lumen of secretory organelles that may lead to specialized trafficking through the secretory pathway. Here, we used the RUSH (Retention Using Selective Hook) system and superresolution microscopy to investigate whether the association of lipidated Wnt proteins with specific membrane lipids regulates their trafficking from the ER to the Golgi apparatus. In the results obtained, we found that depletion of the sphingolipid ceramide accelerates the exit of Wnt-RUSH from ER exit sites and its arrival at the Golgi. Since ceramide depletion increases membrane fluidity, our results suggest that ceramide may control ER export of lipidated Wnt proteins by forming compact and rigid lipid membrane domains.

Keywords: Wnt, Early Secretory Pathway, RUSH System, Ceramides

G05 - 42 - P

Unraveling the mechanisms of lipid - mediated protein sorting at the ER

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Protein sorting in the secretory pathway is crucial to maintain cellular compartmentalization and homeostasis. While coatmediated sorting is well understood, the involvement of membrane lipids in driving protein sorting during secretory transport remains a longstanding and unresolved question. To address this question, we have investigated in the yeast Saccharomyces cerevisiae how a special type of lipidlinked cell surface proteins, the GPIanchored proteins, are differentially exported from the endoplasmic reticulum (ER). Our findings reveal that ceramide plays a pivotal role in clustering and sorting GPIanchored proteins into specialized ER exit sites. Here we provide a better comprehension of the potential mechanism for this ceramidebased sorting process.

Keywords: GPI-APs, Ceramide, Lipids, ERES, TMD, Sorting, Membrane Trafficking, Secretory Pathway

G05 - 56 - O

Characterizing the biogenesis of SARS-CoV-2 Membrane protein in live cells

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The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Membrane (M) protein organizes the assembly and structure of new virions and is essential for virus formation (Yu et al., 2021; Finkel et al., 2021). It induces curvature in the membrane (Zhang et al., 2024), a process that could allow the assembly of viral particles through M oligomerization (Neuman et al., 2011). Cryo-EM structures of M protein confirm the presence of three transmembrane domains (TMDs) and a homo-dimeric conformation (Zhang et al., 2022; Dolan et al., 2022). As the link between its function and structure is still enigmatic, we studied M protein membrane insertion and folding using several biochemical approaches. First, we used an arrest peptide to thoroughly study the pulling forces (Nicolaus et al., 2021) dominating its co-translational folding. We observed that TMDs are inserted into the membrane in a sequential manner. Secondly, we worked with cell lines to analyze M biogenesis in the endoplasmic reticulum (ER). Using a glycosylation reporter system, we demonstrated that TM1 is sufficient to target the protein to the ER, also confirming co-translational insertion of the TMs. Then, we fused TurboID biotin ligase (Branon et al., 2018) to M truncated and full-length versions, revealing a detailed landscape of proximal insertase complexes and chaperones that potentially assist M insertion and folding in the ER membrane. Lastly, complementation (Kerppola, 2006) assays in cell culture reported that the expression of TM1 and 2 is sufficient to reconstitute M oligomers. In summary, we (1) demonstrate that M protein TMDs constitute the major driving force in terms of its folding and oligomerization, and (2) reveal potential host factors involved in the biogenesis and maturation processes.

Keywords: Membrane Protein, SARS-CoV-2, Arrest Peptides, Proximity Labeling, Viral Protein Biogenesis

G05 - 82 - O

Lipid-mediated control of collagen secretion

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Collagen is the main structural protein found in the extracellular matrix (ECM) and its defective synthesis, secretion and crosslinking is related to a number of diseases and conditions such as collagenopathies, cancer fibrosis, ageing and senescence. Procollagen is a large cargo that, after being synthesized by cells requires not only a specialized export machinery, but also very specific physical requirements at the membrane level. However, even if lipids are the major components of membranes and they largely control membrane biophysical properties, little is known about the role of lipids in the export of big cargoes such as ECM components. Previous data from our group shows how the knockdown of genes involved in collagen export from the endoplasmic reticulum renders cells hypersensitive to sphingolipid depletion, which showcases a role for lipids in collagen secretion, but the mechanism behind this phenotype has not been explored yet. We have analysed the lipid composition of fibroblasts that stably express collagen VII (RDEB/FB/C7) that either secrete or retain this collagen intracellularly, in order to find new modulators of collagen export. Cells that accumulate intracellular collagen aggregates show significant changes in their ceramide profile, supporting a role for these lipid species in collagen trafficking within cells. Future experiments will analyse how sphingolipid metabolism is orchestrated to support collagen homeostasis which could also lead to the discovery of new targets to treat collagenopaties and skin diseases.

Keywords: Secretion, Collagen VII, Sphingolipids

G05 - 85 - P

Cytokines inhibit store-operated calcium entry in U251 astroglioma cells without altering cytosolic calcium levels

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Astrocytes are the most abundant brain cells and are involved in many structural and physiological brain functions. They are required for neuronal survival and the maintenance of blood–brain barrier integrity. Besides, they make contacts with adjacent neurons, and maintain the extracellular ion homeostasis, and synapsis. A key feature of astrocytes is their capacity to respond to pathological conditions, which results in their functional and morphological transformation in toxic A1-like reactive astrocytes, that induce neuroinflammation, potentiating neuronal death. Also, aberrant Ca2+ signals are considered as a hallmark of reactive astrocyte in neurodegenerative diseases. In this work, we have induced A1-like reactive astrocytes by treating U251 human astroglioma cells with a cocktail of cytokines TNF-α, IL1-α and C1q. These reactive astrocytes show an increased production of the complement protein C3, a biomarker of A1 reactive astrocytes. Measurements of cytosolic Ca2+ show a 75±10% decrease of cytosolic Ca2+ in reactive astrocytes with respect to untreated cells, after depletion of intracellular Ca2+ stores by thapsigargin, and in the presence of EGTA. The further addition of Ca2+ did not produce an additional increase of cytosolic Ca2+, compared to the large Ca2+ increase observed in untreated cells. These results reveal a potential upregulation of a system responsible for the extrusion of Ca2+ out of the cell, such as the plasma membrane Ca2+-ATPase, and the inhibition of SOCE, a major mechanism of extracellular calcium entry, in A1-like reactive astrocytes, induced by cytokines.

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Keywords: Cytokines, Astrocytes, Calcium

G05 - 89 - P

Assembly of the *Escherichia coli* porin OmpF into nanodiscs as a tool for the characterization of its interaction with amyloidogenic peptides

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Nanodiscs are nanoscale model lipid bilayers consisting of phospholipids surrounded by an amphipathic helical protein (membrane scaffold protein, MSP). Nanodiscs can accommodate single molecules of membrane proteins, which thus stay in solution, offering advantages over liposomes or detergent micelles in terms of size and stability, and are suitable for single particle microscopy and spectroscopy assays (1).

The outer membrane (OM) of Gram-negative bacteria is an efficient permeation barrier central for antibiotic resistance and with a great biotechnological potential. OM is composed of segregated phospholipid (PL; inner leaflet) and lipopolysaccharide (LPS; outer leaflet) molecules embedding different proteins (OMPs) with a common -barrel fold (2). Porins are OMPs that carry out many functions relevant for cell survival (nutrient trafficking) and virulence (cell and bacteriophage adhesion). In this work, we present both wild-type and an engineered variant of OmpF, a porin from *Escherichia coli,* assembled in nanodiscs including both PLs and LPS. That engineered OmpF variant carries an insertion of an amyloidogenic sequence in its fifth extracellular loop (L5), which once expressed in *E. coli*, enables bacteria to

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scavenge on its surface the same amyloid as bait (3). To further characterize this homotypic interaction of OmpF, we have inserted the porin trimers into nanodiscs, and studied its functionality as ion channels, in the way to explore their interactions with amyloidogenic peptides of environmental and biomedical interest.

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Keywords: Nanodisc, Amyloidogenic

G05 - 100 - P

Comparative cellular study between the ATG8 proteins LC3B and LC3C

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Autophagy is a process through which superfluous or damaged material is degraded and recycled. This process is characterized by the formation of a double membrane structure called autophagosome (AP), which engulfs cytosolic material that is going to be degraded. ATG8 proteins are ubiquitin-like proteins that have been proposed to be involved in AP elongation by fusing vesicles to the growing phagophore. In mammals, there are 6 homologs divided into two subfamilies, LC3 and GABARAP. Among these, LC3B is the most thoroughly studied ATG8 protein. It is generally considered as an autophagosomal marker and a canonical representative of the LC3 subfamily. LC3C is less studied, but recent data have reported its implication in various processes, crucial to cellular homeostasis. Previous results from *in vitro* studies in our laboratory have shown that LC3C induces a higher fusion of liposomes than LC3B. The aim of this work was to test whether the differences observed in previous *in vitro* studies between LC3C and LC3B correlate with different behaviour in cells. To evaluate these differences, HeLa HexaKO cells (knockout of six ATG8 homologs) with stable transfection of LC3C or LC3B bound to GFP or RFP were used and their behaviour was analysed by confocal microscopy. Our results showed that LC3C formed larger GFP puncta than LC3B. In addition, preliminary results of co-transfection of RFP-LC3B and GFP-LC3C showed that LC3B and LC3C do not co-localize to the same vesicles. These results suggest that LC3C and LC3B could localize to different autophagosomes and that ATG8-induced fusion is involved in the regulation of AP size, suggesting a role in phagophore expansion.

Keywords: Autophagy, LC3B, LC3C, Phagophore Expansion

G05 - 208 - P

Unlocking Advanced Imaging Capabilities: Introducing Spain's Euro-BioImaging Nodes to the Molecular Biology Community

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Spain's recent inclusion in Euro-BioImaging marks a significant advancement in accessible imaging capabilities. This presentation introduces the five new Spanish nodes—four focused on bioimaging and one on medical imaging—equipped with cutting-edge technologies for molecular and medical biology research.

The bioimage nodes are strategically located at prestigious institutions, Institute for Research in Biomedicine (IRB), Institute of Photonic Sciences (ICFO), Centre for Genomic Regulation (CRG), University of Barcelona, Achucarro Basque Center for Neuroscience, and Instituto Biofisika, offer functional imaging, advanced live-cell imaging, and super-resolution microscopy, along with comprehensive data analysis support.

The medical imaging node in Valencia specializes in computational solutions, radiomic methods, and AI algorithms for clinical decision support, focusing on oncology and neurodegenerative diseases. It also manages an extensive population bank for translational research and personalized medicine studies.

Learn about the specific services, access procedures, and collaboration opportunities available at each node. By integrating these resources into the broader Euro-BioImaging network, Spain aims to foster innovation, facilitate high-impact research, and promote international collaborations across both molecular and medical biology. Highlighting key projects utilizing these facilities demonstrates their potential to advance our understanding of complex biological systems and improve healthcare outcomes.

Keywords: Euro-BioImaging, Advanced Imaging Technologies, Transnational

G05 - 269 - P

Identification of a crosstalk between ClC-1 C terminal CBS domains and the TM region

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CLC channels and transporters have large C-terminal regions which contain two cystathionine b-synthetase (CBS) domains. It has been hypothesized that conformational changes in these domains upon nucleotide binding modulate the process of common gating of the CLC dimer. It is not clear how rearrangements that occur in the CBS domains are transmitted to the ion pathway, since CBS domains interact with the rest of the channel at multiple locations and some of these sites are not visible in recent solved cryo-EM structures. Starting with a described ClC-1 mutation (H835R), located in the second alpha helix of the CBS2 domain, that changes the voltage dependence of gating, we identified several residues located in the disorganized loop after helix R (R-linker) that revert the phenotype of this mutation. We additionally proved that the R linker was functionally connected to the CBS2 domains by mutant cycle analysis. Furthermore, crosslinking studies using split-Cysless ClC-1 channels containing specific cysteine mutants in the R-linker and the CBS2 domain indicate that these two regions are in close contact. Considering these new results, we propose that conformational changes occurring in the CBS domains could be transmitted to the CLC intracellular chloride binding site by means of its interaction with the R-linker.

Keywords: Chloride Channel, CBS Domains, Intramolecular Interactions

G05 - 315 - O

Design of nanoreactors for microplastic depolymerization based on pore-forming toxins

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Petroleum-based plastics are durable and accumulate in all ecological niches. Approximately 80% of waste objects are made of macroplastics and most of them end up being dumped into the oceans. PET (polyethylene terephthalate) is the main component in many synthetic fibers and water bottles, and it comprises at least 5% of the total identified plastic particles, accounting for 14.4% of total plastic waste. Our work focuses on improving the biotechnological processes of PET degradation by designing more efficient enzymes. For this purpose, biocatalytic nanopores are designed based on the homo-octamer of a sea anemone toxin that forms pores in biological membranes. This pore is assembled into nanodiscs, forming individual and water-soluble particles. A computational modeling approach based on protein structure is used, followed by a specific assembly method in the form of the aforementioned nanopores. At only 40 °C temperature and neutral pH, this biocatalytic nanoreactor efficiently hydrolyzes bis(2-hydroxyethyl)-terephthalate (BHET). It is also capable of degrading PET nanoparticles from a plastic bottle and other commercial versions of PET used to manufacture plastic products. The products of these reactions are soluble BHET dimer, BHET itself and mono-(2-hydroxyethyl)-terephthalic acid (MHET). The results obtained so far already constitute a proof-of-concept for building biodegradable pore-based catalytic nanoreactors that could drive new developments in nanobiotechnology, here exemplified by efficient systems to decompose PET at levels of the best-performing known engineering PETases and, moreover, at relatively low temperatures.

Keywords: Microplastics, Pore-Forming Proteins, Enzymes

ÍNDICE

G05 - 328 - O

Sorting of secretory proteins at the trans-Golgi network

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The trans-Golgi network (TGN) is the central sorting station of the secretory pathway, where cargo proteins are packaged into different transport carriers for export to their respective destination. Despite its fundamental importance in maintaining cellular homeostasis and function, how secretory cargoes are sorted and specifically packaged into nascent transport carriers at the TGN still remain poorly understood. Here, we focus on explaining our recent results on the sorting of cargoes into a class of sphingomyelin-rich, Rab6 and protein kinase D (PKD)-dependent TGN-to-plasma membrane transport carriers, named CARTS. We present our results showing that the single-pass type I transmembrane (TM) protein TGN46 (a protein contained in CARTS) plays a key role in the sorting of its clients into nascent carriers. These data reveal that the topological determinants that describe the proper intracellular and intra-Golgi localization of TGN46, as well as its own incorporation in CARTS, are mainly contained in its lumenal domain. Notably, we present evidence showing that the lumenal domain of TGN46 is both necessary and sufficient for the export of cargo proteins into CARTS and also that this domain mediates the cargo sorting function of TGN46. Interestingly, this domain has the capacity to form liquid droplets in vitro, a mechanism that may assist in TGN46-mediated cargo sorting. Altogether, our data suggests an essential role for TGN46 in the sorting of cargo proteins into transport carriers at the TGN.

Keywords: Secretory Proteins, Trans-Golgi, Network

G05 - 329 - O

Understanding structural details of membrane proteins – Current state and trends

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Membrane proteins (MPs) mediate intercellular communication, transport across membranes as well as to provide unique physicochemical properties to the cell surface. Furthermore, MPs are the target for 60% of clinically approved drugs. Understanding MP functional mechanisms is key understand physiology as well as to design new and improved therapeutics. Structural information and in vitro studies are key players in this endeavour however, this is currently dependent on our ability to extract and maintain functional MPs outside the membrane environment. In this talk I will aim to summarize the current state in MP structural characterization, covering the basics of MP protein production, membrane mimetics as well as structural determination by cryo-electron microscopy, providing examples from our lab on G protein-coupled receptors. I will then share my personal view on trends in technology that will further allow to understand membrane protein functional mechanisms, signalosome assembly and regulation by native lipid environments. The aim of this talk will be to both cover the current state and future trends in MP structural biology as well as to provide the experimental basics and requirements for newcomers.

Keywords: Membrane Proteins, Structural Details, Trends

G05 - 354 - O

Cryo-EM as a magnifying glass into the action mechanism of protein–nucleic acid complexes important for biomedicine and biotechnology

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The first half of the seminar will describe cryo-EM studies on the mechanism of action of eukaryotic translesion DNA synthesis polymerase ζ, a key enzyme to preserve genome integrity and a target for cancer therapy. The second half will focus on the structural basis for the expanded target access and substrate recognition for nucleic acid editing and detection of a resurrected ancestor of Cas12.

Keywords: Replication, Genome Integrity, Gene Editing, Structural Biology, Cryo-Electron Microscopy

G06: Educación

G06 - 66 - P

Aprendizaje activo en la materia de bioquímica mediante clases invertidas

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En las clases invertidas el alumnado trabaja el material proporcionado por el profesorado previamente a la clase presencial, habiéndose evidenciado un mayor grado de comprensión conceptual. Además, permite visualizar el material explicativo las veces que sea necesario.

Hemos usado clases invertidas para fomentar el trabajo autónomo y mejorar la adquisición de competencias, en los Grados en Bioquímica (Laboratorio General de Bioquímica -LGB) y Enfermería (Bioquímica y Nutrición -BQN) de la Universitat de les Illes Balears.

En LGB, se proporcionó un vídeo explicativo e interactivo del protocolo de la práctica a realizar. Mediante la herramienta H5P se incrustaron preguntas sobre los conceptos clave a lo largo de la explicación. Estas preguntas son de obligada cumplimentación para proseguir la visualización. En BQN, se proporcionó un vídeo obtenido del recurso JoVE en el que se explican conceptos clave, e iban asociados a un cuestionario en el Aula Digital. La resolución de las preguntas se vinculó a un 10% de la evaluación.

Los resultados se evaluaron: (1) realizando un cuestionario anónimo de escala tipo Likert en una escala del 1 al 5 para evaluar el grado de satisfacción del alumnado con la metodología utilizada. (2) comparando las calificaciones obtenidas en la resolución de las preguntas de los vídeos con la calificación final de la asignatura.

En conclusión, la metodología del aula invertida ha sido bien aceptada y valorada por el alumnado y se ha observado la existencia de una correlación significativa entre los resultados obtenidos en la resolución de las preguntas de los vídeos y la calificación final de las asignaturas.

Agradecimientos: Actividad realizada en el marco del proyecto de innovación concedido por el IRIE (UIB) nº PID222424.

Keywords: Clases Invertidas

ÍNDICE

G06 - 347 - P

Transforming experimental practices in the laboratory. A pharmacological approach for biotechnology students

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As university instructors, it should be our priority to make the contents of the laboratory practices attractive to our students. We consider that this adaptation is crucial to achieve the learning outcomes we expect. In order to do so, one of the key points is that students should manage to integrate the theoretical knowledge from different courses and apply them to the same experimental approach in the lab, achieving a multidisplinary and holistic knowledge of their future profession.

In this context, during the last three years students in the 3rd year of the Biotechnology degree at Universidad Europea de Madrid we have carried out a PBL (project-based learning) activity involving 7 different subjects: Experimental Biotechnology, Molecular Pathology, Bioinformatics, Molecular Genetics Engineering, Advanced Instrumental Techniques, Chemistry and Engineering of Proteins, and Functional Genomics and Transcriptomics. Now, we have implemented new experimental practices in the Pharmacology course using the same drug and cultured cells which they also work with in other courses of the same academic year. The experimental work consisted of assessing IC50 of methotrexate drug in Jurkat cells (ATCC TIB-152) by incubating different concentrations tested for 24 and 48 hours and assessing cell viability by MTT.

A total of 40 students performed the new methodology in the 2023-2024 school year and satisfaction surveys were conducted online after attending the laboratory. Results show that the perception of the new practices has been very positive, and the students were more deeply involved in their tasks because they knew that their outcome would be linked and integrated in the contents of the rest of the courses. We encourage you all to implement these initiatives in your subjects!.

Keywords: Best Practices, Biotechnology, Satisfaction, PBL, Education, Innovation

G06 - 348 - P

Adopting Flipped Classroom Methodology to Enhance Student Learning Experience: A Case Study in Biology Education

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Students' educational needs are evolving, demonstrating that new generations learn differently than they did a few years ago. Traditional lecture-based teaching often results in students playing a passive, unmotivated role. Additionally, this approach is not well-suited for practical subjects. In recent years, the second-year Biology subject 'Integrated Practices of Molecular and Cellular Biology' has experienced a notable rise in failures and complaints, along with a clear decline in student motivation.

For this reason, we adopted the Flipped Classroom methodology, creating interactive videos with embedded questions for students to watch before lab practice (10% of the final grade). Additionally, students conducted a group seminar (5% of the final grade) and a trophy system was used to motivate participation (5% of the final grade).

As a result, we observed a significant improvement in both academic performance and students' attitudes towards the subject. Notably, in previous academic years, the average failure rate ranged between 60-70% of the student body. In contrast, in the current academic term, we observed a failure rate of 10% in the first partial exam and a 15% in the second, which is typical for this type of subject. Surprisingly we achieved a 100% pass rate during the recovery period.

In surveys regarding the new methodology, 97% of students highlighted the usefulness of the videos for studying, though they acknowledged it requires additional work at home. Despite this, most students would like to continue with this approach in the future, as it effectively addresses exam-related doubts (83%). However, they identified areas for improvement, particularly in the implementation of the trophy system and seminar sessions, which are already under consideration.

Keywords: Classroom, Students, Biology Education

G06 - 349 - P

El compromiso social trabajado en el aula mediante el aprendizaje basado en investigación

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La competencia transversal "Compromiso social" es una de las menos desarrolladas en los grados de Biociencias. El proyecto de innovación educativa "BioGela" de la UPV/EHU busca formar al alumnado en los Objetivos de Desarrollo Sostenible (ODS), para fomentar esta competencia e integrar el contenido curricular de dos asignaturas compartidas en grados de Biociencias

Mediante el Aprendizaje Basado en Investigación (ABI), el estudiantado diseña un proyecto para la sobreexpresión y purificación de una proteína cuyo uso responda a un reto social. Este proyecto debe estar alineado con uno o varios ODS, lo que permite al alumnado adquirir conocimientos propios de dos asignaturas, a la vez que se forma en aspectos relativos a la Agenda 2030. Un grupo control realiza el mismo trabajo sin recibir formación en desarrollo sostenible, pero debiendo justificar el interés de la proteína seleccionada

Los resultados del estudio muestran que el grupo experimental adquiere un mayor conocimiento y concienciación sobre desarrollo sostenible, y es capaz de integrarlo en el contenido curricular de su grado. Además, se observa un aumento significativo en la percepción del grupo experimental con respecto al control en varios aspectos de la tarea realizada. Por ejemplo, percibe que el proyecto le ha ayudado a familiarizarse con los ODS, a adquirir las competencias específicas y a aumentar su motivación por las materias cursadas. Además, la satisfacción global con la tarea es significativamente mayor en el grupo experimental

Estos datos sugieren que la sinergia entre asignaturas de un grado y el abordaje de los ODS mediante un ABI son herramientas eficaces para el desarrollo de la competencia transversal "Compromiso social" en Biociencias, así como las competencias específicas de las asignaturas.

Keywords: Compromiso Social, Objetivos De Desarrollo Sostenible (ODS), Innovación Docente, Aprendizaje Basado En Investigación, Purificación Proteica, Tecnología Del DNA Recombinante

G06 - 350 - P

Molecular parasitology for dummies: connecting art and science as the key to engage with the general audience

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A public engagement activity to explain general aspects of the molecular biology of the parasite Toxoplasma gondii was designed to be included in a Science Fair organised by the Society of the Spanish Researchers in the UK (SRUK), in the context of their annual symposium, in Glasgow, in 2018.

The main objective of the activity was to explain T. gondii lifestyle and adaptations to parasitism by showing the main organelles and functions that compose a T. gondii cell. To relate to the audience and facilitate comprehension, the explanation was humanised by comparing the cell with a house, more specifically a mooving house, a van. Besides, an analogy was stablished between the organelles of the cell and the components of the house, to explain their functions. As an example, mitochondrion was compared to the engine of the van and plasma membrane, to the door.

To engage with the public in a way that they could actively participate, regardless of their artistic habilities or age, the activity was designed to paint their own parasite by using preformed stamps of the organelles as well as other tools such as rollers or sponges; creating their artistic piece by using printing techniques.

As a complementary part of the activity, for advanced public, in order to explain adaptations of T. gondii related to parasite lifestyle success, the general concept of the immune system was introduced by creating and analogy with traffic signs, which can stop ot impede the movement of the cell, the moovement of the van. Moreover, some parasite specific organelles as rhoptries (drill), or parasitophorlus vacuole (garage) were also included here.

Keywords: Public Engagement, Parasite, Art

ÍNDICE

G07: Emprendimiento e innovación

G07 - 301 - O

Innovative strategy for osteoarthritis treatment using peptide-based therapeutic

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Osteoarthritis (OA) is characterized by the chronic breakdown of articular cartilage and the degeneration of synovial joints. Our group has identified Connexin 43 (Cx43) as a significant factor in the progression of OA, due to its association with aging and inflammation. In this study, we designed two small peptides derived from the C-terminal domain of Cx43 to interfere with its specific activities. Our results demonstrate that these peptides effectively reduce the accumulation of senescent cells and decrease the expression of pro-inflammatory molecules associated with senescence-associated secretory phenotype (SASP), which contribute to cartilage degradation and disease progression. The peptides were shown to disrupt the channel activity of Cx43, resulting in a decrease in gap junction intercellular communication (GJIC) and hemichannel (HC) activity, evidenced by reduced ATP release and lower production and secretion of SASP factors. Furthermore, we observed that Cx43 peptides promote the redifferentiation of chondrocytes and the formation of extracellular matrix in 3D *in vitro* models, *ex vivo* studies, and in an osteoarthritis mouse model, leading to decreased inflammation. These peptides not only decrease senescence but also promote extracellular matrix formation and tissue regeneration as shown by an increase in the levels of key extracellular matrix components such as collagen and aggrecan. Our findings indicate that Cx43 peptides are able to reduce the deleterious effects of Cx43 activity, thereby presenting a promising avenue for the development of new strategies to treat OA. Additionally, these compounds may have potential applications in addressing other age-related diseases characterized by the accumulation of senescent cells and the upregulation of Cx43.

Keywords: Conexin43, Peptides, New Therapy

G07 - 334 - O

Unraveling Ageing through RNA Methylation: Age-Related Changes in m5C on mRNA

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Ageing is an unavoidable and complex process that constitutes the highest risk factor for most human diseases, becoming a challenge in the health, social and economic policies of modern societies. It is characterized by a myriad of molecular alterations, with modifications in DNA methylation at the 5 position of cytosines (5mC) well established as a hallmark of the ageing process. RNA chemical modifications, collectively known as the epitranscriptome, play pivotal roles in regulating several biological processes, influencing RNA structure, localization, and function. While the role of cytosine methylation in DNA has been extensively described in ageing, the impact of 5-methylcytosine at the RNA level (m5C) remains to be fully elucidated. In this study, we investigated the potential role of m5C modifications in RNA in the context of ageing through bisulfite sequencing (BS-seq) using primary fibroblasts derived from 2- and 28-month-old mice, to elucidate the relationship between m5C modification and ageing. By RNA-seq analysis, we show that our ex-vivo model transcriptionally recapitulates ageing features. Most importantly, we have unraveled for the first time the dynamics of m5C modifications with age in mRNAs, revealing a significant age-dependent increase in global m5C levels in coding RNAs. Our analysis show that our identified methylated sites are enriched in NSUN2- and NSUN6-like binding motifs, the two main known mRNA methyltransferases. We validated these sites using amplification methods of bisulfite-converted RNA followed by sequencing. Our work uncovers for the first time a correlation between ageing and m5C modification in several RNA species, highlighting the importance of investigating ageing from an epitranscriptomic perspective.

Keywords: Epitranscriptomics, Ageing, Methylation, Bisulfite-Sequencing

G07 - 351 - O

NBusCeliac rapid test: towards non-invasive diagnosis of celiac disease

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Celiac disease (CD) is a systemic immune-mediated disorder induced by gluten present in cereals such as wheat, rye, and barley, affecting genetically susceptible individuals. Celiac disease is one of the most common food-related illnesses worldwide, with an estimated prevalence of around 1% of the total population in Western countries. However, despite advances in diagnostic methods, it is estimated that up to 70% of celiac patients remain undiagnosed due to the heterogeneity of their clinical manifestations.

Currently, there is no serological test that provides 100% sensitivity and specificity. Definitive diagnosis requires an intestinal biopsy, an invasive and costly method, highlighting the need for alternative and non-invasive diagnostic tests. In response to this issue, the NBusCeliac rapid test emerges as an entrepreneurial project aiming to develop new diagnostic and monitoring solutions that are quick, simple, and cost-effective for celiac disease. NBusCeliac rapid test consists of a Point-Of-Care Test (POCT) based on a lateral flow assay, for the simultaneous detection of serological biomarkers and a characteristic intestinal damage biomarker, combined with microfluidic quantification of the latter. The idea behind this approach is to achieve a rapid, simple, and effective diagnosis without the need for invasive tests or laboratory equipment, making it suitable for routine use in medical consultations or pharmacies. Currently, this device is in its initial stages, but has the support of the Universidade de Santiago de Compostela (USC) or the Federation of Associations of Coeliacs of Spain (FACE), as well as the scientific-technical endorsement of specialists in the sector.

Keywords: NBusCeliac Rapid Test, Celiac Disease, Diagnosis

G07 - 359 - O

Bringing basic science into clinical applications: the Batea Oncology experience

JORGE BARBAZAN

Batea Oncology/Health Research Institute of Santiago de Compostela, Santiago De Compostela, La Coruña, España

Research in life sciences, and particularly in cancer, is an incredibly dynamic field, and fundamental research is at the basis of every advancement of the modern age. However, bridging the gap from academic research can be challenging, yet essential. In this talk I will talk about the (still short) journey of Batea Oncology, a newly created startup focused in the development of therapeutic solutions for hard to treat cancers, particularly through the use of biomaterials and medical devices in the field of glioblastoma. I will emphasize about the science that it's being done at Batea, explaining how a intimate relationship between academia and industry is essential for the proper advancement of technology to reach the patients as products. As well, I will navigate the, sometimes tricky, scenarios that an academic researcher has to face when deciding to enter the entrepreneurship world. This talk aims to inspire, or at least serve as a support group, for the youngest generations that are curious enough about the startup world and would like to make a direct impact on patients, transforming their scientific discoveries into potential solutions.

Keywords: Entrepreneurship, Cancer Therapy, Glioblastoma, From Bench To Bedside. MedTech

G07 - 367 - O

Entrepreneurship as an instrument of health innovation: opportunities from EIT Health

ANTONIO RODRÍGUEZ

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It represents a challenge for scientific environments that the innovation developed by them can be transferred to society.

This gap is especially complex in the healthcare environment due to barriers specific to the sector. Aspects such as regulation or the multitude of actors are barriers to the generation and adoption of health innovation and adoption of healthcare innovation. Notwithstanding the above, entrepreneurship represents an alternative to classic technology transfer (patent licensing) and is an and is an agile instrument that can accelerate the path “from idea to market”, also having a greater economic and social impact in the region.

EIT Health is an alliance co-funded by the European Commission that brings together the main players in healthcare innovation (hospitals, universities, startups, corporations, etc) and whose mission is not only to foster collaboration between them, but also to support healthcare innovators and entrepreneurs through a series of specially designed programs. EIT Health is aware of the challenges of healthcare startups (need for specific knowledge, access to funding, need for clinical validation, etc) and therefore offers different programs that meet the needs of healthcare startups (public and private funding, acceleration, scaling, training, etc.) during the different stages of maturation.

Whether you are a researcher considering starting out in entrepreneurship or an established entrepreneur, EIT Health represents a platform where a team of people specialized in the field will meet with you to identify needs and offer you those programs and contacts that can add value to your development plan.

Keywords: Entrepreneurship, Innovation, EIT Health

G08: Estructura y función de proteínas

G08 - 4 - P

Flexible structural arrangement and DNA-binding properties of protein p6 from *Bacillus subtillis* phage φ29

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The genome-organizing protein p6 of *Bacillus subtilis* bacteriophage φ29 plays an essential role in viral development by activating the initiation of DNA replication and participating in the early-to-late transcriptional switch. These activities require the formation of a nucleoprotein complex in which the DNA adopts a right-handed superhelix wrapping around a multimeric p6 scaffold, restraining positive supercoiling and compacting the viral genome. Due to the absence of homologous structures, prior attempts to unveil p6’s structural architecture failed. Here, we employed AlphaFold2 to engineer rational p6 constructs yielding crystals for three-dimensional structure determination. Our findings reveal a novel fold adopted by p6 that sheds light on its self-association mechanism and its interaction with DNA. By means of protein–DNA docking and molecular dynamic simulations, we have generated a comprehensive structural model for the nucleoprotein complex that consistently aligns with its established biochemical and thermodynamic parameters. Besides, through analytical ultracentrifugation, we have confirmed the hydrodynamic properties of the nucleocomplex, further validating in solution our proposed model. Importantly, the disclosed structure not only provides a highly accurate explanation for previously experimental data accumulated over decades, but also enhances our holistic understanding of the structural and functional attributes of protein p6 during φ29 infection.

Keywords: B. Subtilis Phage, Molecular Dynamics, DNA–protein Interaction, Protein–protein Interaction, Protein Structure; Protein Oligomer, Protein Fiber, Protein P6, AlphaFold, DNA Supercoiling, Nucleocomplex

G08 - 7 - P

Androgen receptor post-translational modifications and their implications for pathology

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A major mechanism to modulate the biological activities of the androgen receptor (AR) involves a growing number of post-translational modifications (PTMs). In this review we summarize current knowledge on the structural and functional impact of PTMs that affect the different domains of AR. Next, we will discuss the crosstalk between these different PTMs and the presence of clusters of modified residues in the AR protein. Finally, we will discuss the implications of these covalent modifications for the aetiology of diseases such as spinal and bulbar muscular atrophy (SBMA, Kennedy’s disease) and prostate cancer, and the perspectives for pharmacological intervention.

Keywords: Androgen Receptor, Post-Translational Modifications, Pathology

G08 - 9 - P

Structural basis for glucocorticoid receptor multimerization

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The glucocorticoid receptor (GR) is a ubiquitously expressed ligand-regulated transcription factor essential for life and one of the most targeted proteins in drug discovery due to its powerful anti-inflammatory actions. The functional oligomeric state of the full-length receptor, which is essential for its transcriptional activity in cells, remains disputed. Here we present a new crystal structure of agonist-bound ancient GR-LBD in a large cell, along with a thorough analysis of previous structural work. The building block of the current structure is a homodimer we previously identified in GR-LBD crystals and its biological relevance has been verified by studying a battery of GR point mutants including crosslinking assays in solution and quantitative fluorescence microscopy in live cells. Several mutually exclusive multimeric assemblies of this dimer in the crystal highlight the versatility of GR-LBD for self-association and reveal implications for the conformation of the active full-length receptor. Our results underscore the relevance of non-canonical dimerization modes for GR-LBD, especially of contacts made by key residues such as Tyr545, Pro637 and Asp641. Of note, a non-conservative mutation of the latter, p.Asp641Val, causes Chrousos syndrome in humans. Understanding relevant quaternary assemblies of the GR is pivotal not only to understand and predict the therapeutic outcome of major blockbuster drugs but also to lessen their deleterious side effects and open new avenues for drug design.

Keywords: Glucocorticoid Receptor, Oligomerization, Chrousos Syndrome

ÍNDICE

G08 - 11 - O

The *Pseudomonas aeruginosa* effector Tse5 forms membrane pores disrupting the membrane potential of intoxicated bacteria

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Bacterial competition is a significant driver of toxin polymorphism, which allows continual compensatory evolution between the toxin and the resistance developed to overcome it. Bacterial Rearrangement hot spot (Rhs) toxins represent a widespread example of toxin polymorphism. Rhs toxins are organised into three domains, each having a specific function. The N-terminal domain targets the toxin to a secretory pathway, while the toxicity is localised in the C-terminal domain. Recently, we combine structural, biophysical and *in vivo* techniques to investigate the molecular function of the *Pseudomonas aeruginosa* type VI secretion system (T6SS) exported effector Tse5. We will present recent results demonstrating that the C-terminal toxin (Tse5-CT) is a pore-forming toxin that can transport ions across the membrane, causing membrane depolarisation and bacterial death [1]. Remarkably, we will provide the first structural and functional insight into an Rhs protein that encapsulates and delivers a pore-forming toxin to the membrane of competing bacteria [2].

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Keywords: Pore-Forming Toxins, Type VI Secretion System, Cryo-EM, Biophysics

G08 - 17 - P

The N-terminal helix of MarA as a key element in the mechanism of DNA binding

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Efflux is one of the mechanisms employed by Gram-negative bacteria to become resistant to routinely used antibiotics. The inhibition of efflux by targeting their regulators is a promising strategy to re-sensitise bacterial pathogens to antibiotics. AcrAB-TolC is the main Resistance- Nodulation-Division efflux pump in Enterobacteriaceae. MarA is an AraC/XylS family global regulator that regulates more than 40 genes related to the antimicrobial resistance phenotype, including *acrAB*. The aim of this work was to understand the role of the N-terminal helix of MarA in the mechanism of DNA binding. An N-terminal deletion of MarA showed that the N-terminal helix has a role in the recognition of the functional marboxes. By engineering two double cysteine variants of MarA, and combining *in vitro* electrophoretic mobility assays and *in vivo* measurements of *acrAB* transcription with molecular dynamic simulations, it was shown that the immobilization of the N-terminal helix of MarA prevents binding to DNA. This new mechanism of inhibition seems to be universal for the monomeric members of the AraC/XylS family, as suggested by molecular dynamics simulations done with the two-domain protein Rob. These results point to the N-terminal helix of the AraC/XylS family monomeric regulators as a promising target for the development of inhibitors.

Keywords: RND-Efflux Pump Regulation, AraC/XylS Family, MarA, Transcription Factor Inhibition

G08 - 18 - P

Structural insights into *Streptococcus pneumoniae* NADPH oxidase

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NADPH oxidases (NOXs) represent a family of transmembrane proteins pivotal in the physiology of eukaryotic cells, orchestrating the generation of reactive oxygen species (ROS). Recent discoveries have unveiled evolutionarily distant counterparts in Bacteria, sharing the NOX catalytic core. Notably, the 46-kDa Streptococcus pneumoniae NOX (SpNOX) emerges as a promising model for NOX studies, with remarkable activity and stability within detergent micelles. Using high-resolution cryo-EM techniques, we've elucidated structures of substrate-free SpNOX, as well as SpNOX bound to stably reduced NADH and NADPH under turnover conditions, including a variant with a Phe397Ala mutation. These structures allowed for structure-guided mutagenesis and biochemical analyses, unraveling the basis of constitutive activity and the lack of substrate specificity towards NADPH. Moreover, the Phe397Ala mutation offered relevant insights into the catalytic regulation of the conserved C-terminal aromatic residue.

Keywords: NOX, CryoEM, MembraneProtein, Oxidases, NADPH

G08 - 19 - P

Microbial production and biochemical characterization of amidohydrolases to improve the bioavailability of soil N

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Soil N fertilisation is a common practice to increase crop yields. Unfortunately, these fertilisers are not efficiently used by plants and can cause long-term environmental damage such as greenhouse gas emissions and eutrophication. Amidohydolases play an important role in the mineralisation of organic N in soils and thus in the availability of N to plants. Coating seeds with these enzymes would lead to the development of natural fertilisers that minimise the use of chemical fertilisers.1 This work investigated the microbial production and biochemical characterisation of amidohydolases for subsequent use as seed coatings to improve N assimilation.

Bacillus subtilis (CECT 356) and Aspergillus niger (CECT 2805) were selected for a plate screening assay to detect amidohydrolase producing microorganisms. The conditions for amidohydrolase biosynthesis were optimised for both microorganisms: incubation time and temperature, nitrogen source (asparagine, proline, methionine, tyrosine and bactopectone) and carbon source (fructose, glucose, lactose and sucrose). The amidohydrolases synthesised from B. subtilis and A. niger showed typical Michaelis-Menten behaviour with Km values of 14.6 and 21.9 mM and Vmax of 0.57 U and 3.89 U, respectively. In addition, their pH activity profiles showed high activity for both enzymes over a wide range of pH values, with an optimum pH of 6 for the bacterial enzyme and pH 8 for the fungal enzyme.

On the other hand, both amidohydrolases showed their maximum activity at a temperature of 37 ºC.

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Acknowledgements: This work is part of the PID2022-139246OB-I00 grant, supported by the MCIN/AEI/10.13039/501100011033 and FSE+"

Keywords: Amidohydrolases, Microbial Production, Biochemical Characterization

ÍNDICE

G08 - 21 - O

Cryo-EM structure of the gamma-Tubulin Ring Complex reveals the mechanism for *de novo* microtubule

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Microtubules (MT) are essential cytoskeletal elements that provide structural support to cells and play critical roles in processes such as cell division, motility, and intracellular transport. While the dynamic instability of the MT has been extensively characterized, the mechanism of MT nucleation, which relies on the gamma-tubulin ring complex (gTuRC), remains a fundamental question in cell biology.

Previous structural studies revealed an asymmetric, and seemingly inactive conformation of this complex1,2,3,4, challenging the previously suggested role of gTuRC as a MT template. We have solved the cryo-EM structure of the human gTuRC during the process of MT nucleation5. This sheds light on how this complex becomes active and adopts its functional conformation.

Image processing of the cryo-EM images of gTuRC while nucleating MTs was complex, and the image processing workflow designed to address the challenges encountered will be discussed. Sample heterogeneity consisting of gTuRC-nucleated MTs at several stages during the initiation of MT formation represented one of the major challenges during image processing. This heterogeneity has been addressed by employing deep learning algorithms, enabling the reconstruction of gTuRC at several stages during MT nucleation. This approach revealed the mechanism for gTuRC closure, shaping it into a perfect template for 13-protofilament MTs.

This work establishes a structural framework for understanding the regulation of gTuRC activity in cells, paving the way for future research on the mechanisms governing MT cytoskeleton organization and its implication in diseases.

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Keywords: Microtubules, GTuRC, Tubulin, Nucleation, Cryo-EM, Single Particle

G08 - 24 - P

Structural insights into RcsB binding to DNA and metal ion

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RcsB belongs to the Rcs phosphorelay system. It is conserved in the Enterobacteriaceae family and regulates gene transcription upon different stresses on the outer membrane. Once it is phosphorylated homodimerizes by the receiver domain (REC) and by the DNA binding domain (DBD) to act as a transcriptional factor. Our group has obtained a canonical structure of S. *Typhimurium* RcsB activated. However, an alternative dimeric structure has been obtained in which the REC domains have a crossed conformation that can be stabilized by a disulfide bridge in the S207C mutant.

In the canonical RcsB dimer, the K180 of each monomer recognizes the binding box. However, in the crossed dimer, the α6 helix of the linker between the REC and DBD domain in one monomer seems to be involved in binding to the major groove of the DNA through R136 and K140. In this work, single and double Ala mutants of R136, K140 and K180 on S2017C have been produced to evaluate the role of DNA binding in the crossed conformation. Electrophoretic mobility shift assays were performed to evaluate the binding of wild-type and mutant RcsB to DNA. Our studies have shown that RcsB in the crossed conformation binds DNA differentially compared to the canonical form, and that K180 plays a crucial role supported by K140 and R136. Furthermore, phosphorylation disrupts such differential binding.

The crossed conformation has revealed a binding site for divalent metal ion. Therefore, we have evaluated the binding of RcsB to different metals using thermofluor and observed binding to magnesium and zinc. Magnesium is required for the phosphorylation process, but zinc does not compete with it. Through anomalous diffraction, the zinc binding site has been identified in the structure of the RcsB mutant S207C K180A.

Keywords: Rcs System, RcsB Response Regulator, DNA Binding, Phosphorylation

G08 - 35 - P

The Vip3Aa Insecticidal Protein: Functional Role of Protoxin and Activated-Toxin Structural Conformations in its Mode of Action

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The Vip3Aa insecticidal protein, produced by *Bacillus thuringiensis*, has been effectively used to manage lepidopteran pests. Upon ingestion by larvae, the protoxin is processed by midgut proteases into the activated toxin and binds specifically to its receptors in the midgut, leading to pore formation and subsequent insect mortality.

Cryo-EM resolution of the trypsin-processed Vip3Aa unveiled a structural remodelling at the N-terminal region during the transition from protoxin to the activated toxin. To better understand the relevance of this major conformational shift for the insecticidal activity of the protein, two structural mutants that lock the protein in either its protoxin or activated conformations were obtained and characterized for their toxicity against the insect pest *Spodoptera exigua*. The results show that the structural remodelling is essential for toxicity, as well as the integrity of Domain I, which is involved in membrane insertion.

Additionally, to further characterize the relevance of the structural remodelling for the protein's mode of action, we investigated the ability of both structural conformations to bind to midgut receptors. We conducted *in vitro* binding assays with radiolabelled proteins on Brush Border Membrane Vesicles (BBMV) extracted from *S. exigua* larvae, and *in vivo* competition assays. Our findings indicate that both structural conformations share receptors in the insect's midgut epithelium, and that Vip3Aa is able to bind to functional receptors both as protoxin and activated toxin.

Hence, i*n vivo*, either spontaneous structural shift upon protease cleavage or receptor-mediated remodelling could be occurring, showing the complex interplay between proteolytic processing, protein structure, receptor interactions and toxicity.

Keywords: Conformational Shift, Protein-Receptor Interaction, Biopesticide, Lepidopteran

G08 - 36 - P

The importance of saccharides in GAS1 function

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GAS1 (Growth Arrest-Specific protein 1) is a monomeric soluble protein, anchored to the plasma membrane, which carries out different functions, highlighting its role as coreceptor of Sonic Hedgehog ligand (SHH) (Wierbowski, et al. 2020). In animal cells, this signaling pathway plays an essential role in embryogenesis and stem cells homeostasis in adult tissues. The alteration or absence of GAS1 has been related to the appearance and severity of a disease called holoprosencephaly, characterized by an incomplete division of the forebrain (Seppala et al. 2007). One of its characteristics is the presence of a glycosylation at the residue Asn117. This modification has been revealed to be very important for GAS1 function, but, quite surprisingly, its role has not been studied in detail yet. So, the main purpose of our investigation is to characterize the glycosylation pattern of this protein and examine how different glycans introduced by host expression systems can affect its function. With this purpose, we have cloned and produced the soluble domain of GAS1 in the yeast recombinant expression system *Pichia pastoris* and in human cells. The proteins were structurally characterized, and functional experiments were performed to evaluate how the different glycosylation patterns affect its function. We tested its ability to bind cholesterol, which is key for its function (Huang et al 2022); and their ability to extract the ligand SHH from HEK293 membranes. We were able to determine that some of the differences observed between both recombinant proteins were very probably being caused by the different glycans added by the two host expression systems employed. More experiments are still required to confirm and quantify these already preliminary, though very promising, results.

Keywords: GAS1, Hedgehog, Structure, Glycosylation

ÍNDICE

G08 - 49 - P

Repurposing non-immunosuppressive cyclosporin analogs for targeting *Toxoplasma gondii* cyclophilin

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Toxoplasmosis persists as a prevalent disease, facing challenges from parasite resistance and treatment side effects. Consequently, identifying new drugs by exploring novel protein targets is essential for effective intervention. Cyclosporin A (CsA) possesses antiparasitic activity against *T. gondii*, with cyclophilins identified as possible targets. However, CsA immunosuppressive nature hinders its use as an anti-toxoplasmosis agent. Here, we report a drug repurposing approach targeting TgCyp23, a previously characterized toxoplasma cyclophilin, using three CsA derivatives devoid of immunosuppressive activity: NIM811, Alisporivir, and dihydrocyclosporin A. We determined the X-ray crystal structures of TgCyp23 in complex with the three analogs and elucidated their binding and inhibitory properties. The high resolution of the structures revealed the precise positioning of ligands within the TgCyp23 binding site and the details of protein-ligands interactions. Comparison with established ternary structure involving calcineurin indicates that position 4 in CsA derivatives is critical for their immunosuppressive activity, abolishing calcineurin binding.

Keywords: Toxoplasmosis, Cyclosporin

G08 - 52 - P

Reconstitution of the *Mycobacterium tuberculosis* translation system to understand translation initiation control

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis, one of humankind's deadliest diseases. During its infection cycle, this pathogen can persist for prolonged periods in a non-replicating state, adapting to harsh environments. Recent studies have revealed differences in translational control between Mtb and other organisms, which may contribute to its ability to adapt during infection. We have characterized that over 50% of its genes lack the canonical signals for translation initiation, and they are robustly translated during exponential growth and under conditions that mimic persistence inside the host, suggesting the existence of alternative mechanisms for translation initiation. However, the molecular basis and the role of non-canonical translation during stress adaptation remain unknown. For this reason, we are interested in characterizing translation initiation in Mtb. To achieve this, we have acquired Mtb genes encoding translation factors to be recombinantly expressed and purified in *Escherichia coli*. We have purified all initiation factors (IF), including the full-length IF2 and a truncated IF2 version that lacks the N-terminal unstructured extension of the protein. We have also isolated ribosomal subunits of *Mycobacterium smegmatis* and obtained fMet-tRNAfMet from *E. coli*. Together with the remaining components, we will establish an *in vitro* translation system to characterize variations in initiation mechanisms. Additionally, we aim to obtain high-resolution structures of canonical and non-canonical initiation complexes using cryo-electron microscopy. We expect that these structures, together with our *in vitro* translation experiments, will help us identify crucial elements involved in translational control in Mtb.

Keywords: Tuberculosis, Translation Initiation, Ribosome, Cryo-EM

G08 - 58 - P

Effect of a soluble variant of Apoptin on ovarian cancer cells

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Cancer is one of the most common causes of death in the world and, amongst them, ovarian cancer has one of the worst mortality rates. Currently, treatment is based on classical chemotherapy or surgery, decreasing the quality of life of the affected patients. In addition, the high heterogeneity that ovarian cancer presents often drives tumor treatment failure. This shows the need of developing new treatments to overcome this problem. In this study, we have assessed the potential cytotoxic activity of Apoptin, a protein from leukemia chicken virus that induces apoptosis in a selective way in different types of cancer cells but that its use is hampered by its low solubility. We have previously developed a truncated soluble Apoptin variant that is as effective as Apoptin against different cancer cell lines. Here, we have tested its effectiveness against ovarian cancer cells. Protein production was carried out by *Escherichia coli* Rosetta (DE3) previously transformed with pET28a ApopΔLeu. Apoptin variant was purified with a methodology consisting of cell lysis, solubilization of inclusion bodies, affinity chromatography, protein refolding and size exclusion chromatography. The cytotoxicity against different ovarian cancer cell lines was measured by cell viability assays where half maximal inhibitory concentration (IC50) was determined. These analyses showed that ApopΔLeu is cytotoxic against different ovarian tumor cell lines cultured in both 2D and 3D. Our results show that the truncated Apoptin variant can be an interesting candidate for the treatment of ovarian cancer.

Keywords: Apoptin, Antitumor Drug, Ovarian Cancer, Recombinant Protein Production

G08 - 60 - O

Restoring Susceptibility to β-Lactam Antibiotics in Methicillin-Resistant *Staphylococcus aureus*

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Antibiotic Resistance (AR) constitutes a global health concern. Resistance to β-lactams in Methicillin-resistant *Staphylococcus aureus* (MRSA) and in the Methicillin-sensitive *Staphylococcus aureus* (MSSA) is inducible, governed by the *bla* and *mec* operons respectively, which regulate the expression of BlaZ β-lactamase or PBP2a. The *bla* system is more extensively studied and holds mechanistic importance. The central component of this mechanism is the BlaR1 protein, an integral membrane protein with an extracellular domain sensor of β-lactams, a membrane domain and a cytoplasmic domain possessing a metallopeptidase activity. Interrupting this inducible system would constitute a potent strategy to combat both MSSA and MRSA strains. Based on this strategy, we have identified effective inhibitors targeting the BlaR1 soluble domain and crystallographic structures have elucidated the mechanism underlying this inhibition. The combination of one of these compounds with β-lactams showed a potentiation activity *in vivo*.

Keywords: Antibiotic Resistance, Meticillin-Resistant Staphylococcus Aureus, Chemical Inhibitors

G08 - 62 - P

Peptide editing in class II antigen presentation and CD4+ T cell responses: towards scoring the impact of natural variants of HLA-DM on disease predisposition

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T cell epitopes, triggering CD4+ T cell function, consist of antigenic peptides loaded onto classical major histocompatibility complex class II (MHCII) molecules. MHCII fold in the ER with a self-peptide (CLIP) chaperoning their binding groove. T cell epitopes only result when CLIP is replaced by peptides from extracellular proteins degraded in late endosomal compartments. The non-classical MHCII molecule HLA-DM (DM) is responsible for catalyzing peptide exchange and selecting high kinetically stable peptide-MHC complexes. Whether and to what extent peptides are exchanged, hence positive or detrimental responses occur, ultimately depends on DM editing function. Genetic variants of DM had been considerably neglected until we proved the distinct editing function of a particular allotype present in 2-4% of the individuals of the 1000 Genomes Project. Here, we elaborate on our previous work to define a comprehensive catalogue of natural variants in coding regions of DM genes on the same dataset. We determined the presence of more than 15 new protein coding sequences that assemble into 36 haplotypes. Functional characterization of up to 20 of these variants at the molecular level allow us to conclude that larger differences than previously observed exist in terms of thermal stability and catalytic activity. Cellular experiments for the subset of the most abundant variants confirm altered functions of these molecules and a positive correlation between expression levels and thermal stability. On-going efforts aim at validating the impact of altered functions of these variants on peptidome editing and T cell responses related to relevant clinical conditions.

Keywords: MHC Class II, HLA-DM, Peptide Editing, CD4+ T Cell Responses, Immunopeptidome

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G08 - 64 - P

Intracellular Antibody and Nanobody Delivery via Nanotechnology: Targeting High-Value Oncoproteins KRAS and STAT3

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Introduction

Antibody-based therapies have represented a therapeutic milestone. However, their full potential is hindered by their inability to cross cell membranes and reach intracellular targets. Nanotechnology has the potential to make intracellular oncoproteins druggable. Here, we introduce novel nanocarriers (NCs) designed for the intracellular delivery of therapeutic monoclonal antibodies (mAbs) or nanobodies (VHHs) in diseased tissues.

Results

We have developed functionalized NCs for efficient encapsulation of anti-KRAS-G12V mAb or anti-STAT3-VHHs. The reproducibility, stability, and non-toxic profile of all prototypes were confirmed.

Cellular uptake studies revealed the presence of intracellular signals related to the mAb/VHHs when associated with the NCs, but not for free biologicals. Target engagement assays confirmed the ability of the encapsulated mAbs/VHHs to reach their targets inside cells. As functional readouts, anti-STAT3-VHH loaded NCs impaired IL6-dependent STAT3 nuclear translocation in HeLa-STAT3 expressing cells. For anti-KRAS mAb-loaded NCs, a significant reduction in colony formation and cell proliferation in mutated KRAS cells (CMT167) was observed.

The capacity of NCs to deliver VHH intracellularly was confirmed in CMT167 subcutaneous tumors through multiplex immunofluorescence. Furthermore, anti-KRAS mAb-loaded NCs significantly impaired pancreatic tumor growth as compared to blank prototypes following intravenous administration.

Conclusion

We have developed functionalized NCs that enable the intracellular delivery of therapeutic mAbs or VHHs. In vitro and in vivo studies confirmed the efficacy of our nanotechnology, which is expected to contribute to the expansion of novel antibody/nanobody-based therapies toward intracellular targets.

Keywords: Antibodies, Nanobodies, Nanocarriers, Intracellular Targets, KRAS, STAT3, Cancer

G08 - 84 - P

Structural basis of PHOX2B DNA binding and its impact in irreversible phase transitions

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Abnormal trinucleotide repeat expansions alter protein conformation producing dysfunction and contribute to a significant number of incurable human diseases. PHOX2B is a transcription factor with a 20 polyalanine tract whose elongation is frequently observed in patients suffering from Congenital Central Hypoventilation Syndrome (CCHS), a rare disease affecting children. The length of the polyaA tract is correlated with the development and severity of symptoms related to CCHS (1). We have described the solution structure and dynamics of the C-terminal fragment of human PHOX2B containing a wild type alanine tract (20A), a subpathogenic construct (23A) and a pathogenic expansion (26A). The structure of the different variants is highly similar, at least in its major conformation. Surprisingly, only the mutant protein displayed structural transitions towards nascent disordered conformations that favored its irreversible liquid-liquid phase separation (LLPS). This observations of emerging polymorphs in expanded PHOX2B postulates unbalanced phase transitions as pathophysiological mechanism in homorepeat expansion diseases. Next, we aim to understand the impact of DNA binding on PHOX2B irreversible aggregation (2). We have assigned the NMR moieties of PHOX2B homeodomain and mapped the interaction sites of canonical DNA target sequences. We have also studied the role of DNA binding in the LLPS of wild type PHOX2B and showed that DNA binding does not enhance LLPS. We aim to understand the effect of DNA binding in the LLPS of expanded PHOX2B, as well as the role of additional domains present in the protein. These studies are key to provide fundamental understanding into the pathogenic mechanisms of CCHS.

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Keywords: PHOX2B, Polyalanine Tract, Homeobox Domain, LLPS, CCHS

G08 - 96 - P

Antimicrobial Resistance Era: Dissecting the Molecular Basis of Bacterial Defense Systems

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Antimicrobial resistance (AMR) stands as one of the most pressing global health challenges, compromising the effectiveness of the treatment of infections worldwide. In 2022, the inaugural comprehensive assessment of the global health impact of AMR estimated that 4.95 million deaths in 2019 were linked to AMR, with 1.2 million directly attributable. Despite confirming that AMR´s impact on morbidity, mortality, and disability rivals that of HIV and malaria, the incidence of AMR has escalated notably during the COVID-19 pandemic. Consequently, the growing AMR crisis and the relevance of bacterial biofilm formation have reinforced exploration into antimicrobial alternatives, with bacteriophage therapy emerging as a particularly promising avenue.

Bacteria and their phage adversaries are engaged in an ongoing arms race, resulting in the development of a broad antiphage arsenal and corresponding viral countermeasures. In recent years, the identification and utilization of bacterial CRISPR–Cas systems have driven a renewed interest in discovering and characterizing antiphage mechanisms, revealing a richer diversity than initially anticipated. Currently, these defense systems can be categorized based on the bacteria's strategy associated with the infection cycle stage. Thus, bacterial defense systems can (i) degrade the invading genetic material, (ii) trigger an abortive infection, or (iii) inhibit genome replication. Understanding the molecular mechanisms of processes related to bacterial immunity holds great potential for phage-based therapies and the development of new biotechnological tools.

Here, we present a picture of the current knowledge about bacterial defense systems focusing on the most recent discoveries.

Keywords: Antimicrobial Resistance, Bacterial Defense Systems, Phage Therapy, CRISPR–Cas, Abortive Infection Systems, Bacterial Defense Islands

G08 - 134 - P

The arbitrium system: more than a microbial communication system

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Bacterial viruses (phages) parasitize their host's machinery to replicate and produce progeny, resulting in the lysis of the cell. Temperate phages have an alternative stage to the lytic cycle called lysogeny, where the viral DNA integrates into the host chromosome and becomes inactive. Recently, it has been discovered that some *Bacillus*-infecting phages encode a peptide-based communication system, called arbitrium, which allows the virus to communicate with its kin and coordinate the lysis-lysogeny decision. In the phage phi3T, the arbitrium system consists of three main components, all encoded in the phage genome. One component is AimP, a signaling peptide secreted into the medium after phage infection, which, once processed, is internalized by surrounding bacteria. This signaling peptide binds to AimR, a transcription factor that, in the absence of AimP, promotes the expression of *aimX*, which exerts a negative regulatory effect on lysogeny through an unknown mechanism.

In an effort to understand the molecular mechanism be-

hind the arbitrium system, we have demonstrated that it is not only a communication system entailing three phage genes, but a complex process that controls a host bacterial defense mechanism, such as the toxin-antitoxin system MazE-MazF, to regulate the phage life cycle. Our data reveal the evolutionary strategy used by phages with the arbitrium system to control lysis-lysogeny by domesticating a bacterial immunity mechanism.

Keywords: Quorum Sensing, Microbial Communication, Phages, Bacterial Immunity

G08 - 136 - P

Unraveling *the role of* $EccC_5$DUF domain in the ESX-5 secretion as potential antimicrobial application

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M. tuberculosis (*Mtb*) is the agent responsible of Tuberculosis (TB), causative of more than 1 million deaths every year (1). However, current treatments to combat the pathogen remains ineffective and present high toxicity. The ability of *Mtb* for the secretion of a wide range of virulence factor is essential in the infection, containing up to five ESX/Type VII secretion systems (ESX-1 to ESX-5). ESX-5 system is mainly presents in slow-growing pathogenic mycobacteria, where it mediates the secretion of substrates involved in nutrient uptake, intracellular colonization and modulation of immune system response (2).

$EccC_5$ is a member of the ESX-5 system, which is an ATPase belonging to the Ftsk/SpoIII family and the central component of the pore complex. That protein is made by two transmembrane (TM) helices in the N-terminal region, connected to four cytosolic domains in tandem. Two additional helices connect the TM region to the first cytosolic domain, referred as domain of unknown function ($EccC_5$DUF), and which is followed by three ATPase domains ($EccC_5$ATPase 1-2-3) (3).

Our research is focused in the structural and functional characterization of $EccC_5$DUF domain in order to unravel its role in the protein translocation. If so, that domain would be a possible target in drug discovery for abolishing the secretion. Here, we report the absence of ATPase activity showed by $EccC_5$DUF domain in biophysical and calorimetric assay. We also provide a high resolution crystallographic structure showing a degeneration of the essential motifs for the ATP hydrolysis supporting our previous results.

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Keywords: ESX System, T7SS, ATPase Activity, Mycobacteria

G08 - 140 - P

Structural determination of Metallo-β-lactamase CAU-1 from *Caulobacter crescentus.* Exploring new inhibitors by a Fragment Screening Strategy

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Increasing resistance of some bacterial pathogens to β-lactam antibiotics is a major threat to public health. Resistance to β-lactams can be due to various reasons, the most common being the presence of β-lactamases. These enzymes catalyze the hydrolysis of the β-lactam ring, generating products that can no longer perform their function. There are 4 classes of β-lactamases (A, B, C and D), all except class B have a nucleophilic serine in the active site, responsible for the cleavage of the antibiotic. Members of class B are zinc-dependent metalloenzymes (MBLs) that, unlike the previous ones, do not bind covalently to the antibiotic.

The study of MBLs is especially interesting because they are normally chromosomally encoded enzymes, they do not respond to inhibitors developed for serine-β-lactamases and they are resistant to last generation antibiotics. It is important to increase the information of B3 family of MBLs, less studied than B1 and B2 families.

Herein, we have carried out the production, crystallization and structure determination of the B3 MBL CAU-1 from the bacterium *Caulobacter crescentus***,** whose three-dimensional structure was not known until now. The final objective is to perform a complete structure-function analysis in this protein and use it as a model to understand B3 MBLs in more depth.

For that purpose, we have also performed Fragment Screening experiments in Diamond Synchrotron in Oxford, UK. Which have allow us to increase our knowledge about the catalytic mechanism and has started to lay the foundations for the development of new inhibitors.

Keywords: Metallo-ß-Lactamase, Antibiotic Resistance, Protein Structure, Protein Function, Fragment Screening

G08 - 156 - P

Structural Characterization of PdaA, an N-deacetylase from *Clostridioides difficile*

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Clostridioides difficile is a Gram-positive bacteria capable of forming spores. Spores are multilayered structures containing a peptidoglycan layer (cortex) that differs from the peptidoglycan of the bacterial wall.

Among the main differences between the cortex and the bacterial wall of C. difficile are structures called muramic-δ-lactams. The formation of these structures requires the involvement of an amidase (CwlD), which cleaves the peptide attached to muramic acid Subsequently, N-deacetylation of muramic acid is facilitated by an N-deacetylase named PdaA.

PdaA, classified as an N-deacetylase within the family 4 carbohydrate esterases, diverges from the typical metalloenzyme configuration. While most proteins in this family exhibit a metal-binding triad (Asp-His-His), PdaA substitutes the aspartic acid residue with an alanine residue.

In order to deepen the molecular basis of this enzyme, Extensive crystallization experiments were performed to solve the 3D structure of PdaA. Anomalous data processing and biophysical techniques allowed the identification of a zinc atom in the active site of the protein coordinated by the two conserved Histidines of the triad.

Details on the structure of PdaA, the structure of an inactive mutant and the structure with an analogous substrate will be shown in detail in the presentation.

Keywords: Spores; N-Deacetylase; Bacteria; Structural Biology

G08 - 176 - P

XAIRA: Advancing macromolecular crystallography with low background diffraction and high-precision helium environments

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The new microfocus beamline XAIRA at ALBA will host the first macromolecular crystallography (MX) experiments in fall 2024 with the aim to offer an optimal solution for some major factors limiting the quality of MX data, namely the crystal size, the radiation damage and the background of diffraction patterns.

Micron-sized crystals will be optimally illuminated by the 3×1 μm² beam at focal position. The beam spot will be enlarged and tailored to match crystal dimensions by either defocusing the optics or moving the sample along the beam axis. Radiation damage will be minimized in rotation experiments using helical collection strategies enabled by a custom-made sub-100nm run-out goniometer, as well as in serial experiments, using a fixed target SSX setup compatible with standard and custom supports.

Nevertheless, the landmark feature of XAIRA aims to be the exceptionally low background in diffraction images, achieved by enclosing the entire end-station in He atmosphere, including the sample environment, diffractometer, cryostream and detector, while remaining compatible with automated sample mounting, standard cryocrystallography sample formats and operation in air.

The high flux, above 1013 ph/s at 12keV, and the EIGER2 X 9M HE fast detector will enable fast oscillation experiments (~1 s), raster and helical scans. X-ray-based raster scans and AI algorithms are being developed to locate the micron-sized crystals.

The two MX beamlines at ALBA, XAIRA and XALOC, will share resources to tailor project needs through a joint proposal submission system. A new dewar shipment system and a new data portal to provide a single access point, automated processing and a catalog identification for all data acquired will be in service soon to improve user experience.

Keywords: Macromolecular Crystallography, Serial Synchrotron Crystallography, Helium Atmosphere, Microfocus Beamline, X-Ray Diffraction

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Scientific opportunities in macromolecular structural biology at ALBA

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ALBA synchrotron is currently building a strategy to provide integrated solutions for structural macromolecular biology based on a multi-scale, integrative and multidisciplinary vision. Here we present the existing and planned instruments and assets:

BL13-XALOC is a versatile MX beamline (5–22 keV), with a photon flux of 2.5x1012 ph/s and adjustable beam size (50×7 µm2). Recent upgrades include a Dectris Pilatus3 X detector with acquisition times ~10 ms; an ISARA2 sample changer with expanded sample capacity; an unattended data collection protocol, which is being commissioned for fragment screening experiments; and a high viscosity extruder for SSX and time-resolved MX.

BL06-XAIRA is a microfocus beamline (3×1 µm2 at 1 Å, 4–14 keV, >3x1013 ph/s), set to launch experiments by fall 2024. The end station enables experiments in cryogenic and room temperatures, both in air or helium atmosphere to minimize image background. XAIRA will support oscillation and time-resolved SSX experiments in the millisecond range (Dectris Eiger2 detector).

In addition, ALBA offers a **CryoEM facility** (JEMCA) with a 200kV Glacios TEM with autoloader and energy correction. The microscope is used for grid screening in sample preparation optimisation as well as for high resolution data collection.

Besides, the **Biological Laboratory** offers the user community several services for structural biological studies. These include 1) recombinant protein production facility; 2) crystallization platform (Mosquito, Dragonfly and Formulatrix imager); 3) support for fragment screening studies (RO3 library, crystal shifter); 4) CryoEM sample preparation and 5) offline SSX setup for sample preparation/optimization.

A new BioSAXS beamline (**CALIMA**) is planned in the frame of the major upgrade, the ALBA-II project.

Keywords: Synchrotron, Structural Biology, Macromolecular Crystalography, Microfocus Beamline, CryoEM, Serial Crystallography, SSX, Time-Resolved MX

G08 - 196 - O

Identification of a novel fold in the H_2S-producing cystathionine β-synthase enzyme from multidrug-resistant *Pseudomonas aeruginosa*

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Hydrogen sulfide (H_2S) is an emerging gasotransmitter involved in various roles in cell signaling. In bacteria, H_2S is considered a crucial component of defense mechanisms against the host immune system. Its enzymatic production is mediated by two key enzymes: cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE).

Pseudomonas aeruginosa (*Pa*), notorious for its antibiotic resistance, is a common cause of hospital acquired-infections, highlighting the need for new therapies. Selective inhibition of CBS and CSE in pathogens has recently been highlighted as a promising therapeutic strategy to combat multidrug resistance. In this study, we characterized recombinant CBS from *Pa* (PaCBS) using crystallographic and biochemical techniques. Unlike most bacterial CBSs, which usually consist only of the catalytic core, PaCBS contains the Bateman module, making it an interesting region for structure-based drug design analysis. Typically, the Bateman module is present in highly complex organisms: in humans it changes its conformation upon binding S-adenosylmethionine (SAM) and increases CBS activity threefold.

Our results show that PaCBS produces cystathionine from O-acetylserine and H2S from cysteine. Notably, PaCBS has a high affinity for SAM, crucial for a 30-fold increase in H2S production, an unprecedented level of regulation in CBSs. More importantly, the elucidation of the PaCBS crystal structure, both alone and in the presence of substrates and SAM, has provided further insight by revealing a novel fold that had never been noticed before in CBS enzymes. Such evolutionary divergence observed in the allosteric regulation of PaCBS, as well as its unique structural features, provide valuable insights for the development of selective inhibitors to combat *Pa* infection.

Keywords: Cystathionine ß-Synthase, Hydrogen Sulfide, Pseudomonas Aeruginosa, Crystal Structure

G08 - 204 - P

EM01-Cryo-TEM: A new Cryo-Electron Microscopy Platform at ALBA

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The recent development of transmission electron microscopy has opened the door to data acquisition at atomic resolution and, consequently, to a new dimension in the research of high resolution molecular structures, both from biological and materials samples. While other regions in Spain have made a strong commitment to equip themselves with state-of-the- art equipment, in Catalonia we are far behind with these kind of infrastructures. This situation has finally changed thanks to the installation of a Glacios 200kV TEM located in the JEMCA (Joint Electron Microscopy Center at Alba), in the ALBA synchrotron. This new infrastructure, devoted to high-end transmission electron microscopy analyses, emerged thanks to the collaboration between several local and national institutions.

The IBMB-CSIC Cryo-electron Microscope Platform possess a specialized cryo-electron microscope for structural biology applications. The platform will give access to state-of-the-art cryo-EM equipment for structure determination projects using the latest technology and methods. Glacios 200kV transmission electron microscope equipped with a cryogenic sample manipulator robot and with the last generation of direct electron detector, a Falcon 4 that can take up to 400 movies per hour. Its high level of automation and user guidance of experimental settings enable scientists to efficiently unravel protein structures in 3D, as well as understand their functional context in the biological cell.

Keywords: Glacios, Falcon 4, EER, Platform

G08 - 212 - O

Inhibition of Parkinson's Disease-related LRRK2 by type-I and type-II kinase inhibitors: activity and structures

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Leucine-rich repeat Kinase 2 (LRRK2) is a large multi-domain protein harboring a GTPase and a kinase as well as several protein interaction domains. The implication of LRRK2 in both familiar and sporadic Parkinson's disease (PD) has motivated efforts to understand the role of LRRK2 in cell signaling in both normal and disease states. Increased kinase activity has been shown in both familial and sporadic PD patients, making LRRK2 kinase inhibitors a major focus of drug development efforts, and several type-I inhibitors have been developed. MLi-2, a Type-I ligand with a high affinity and selectivity profile, has been regarded as the gold standard LRRK2 inhibitor and has been used in numerous studies. Although several type-II inhibitors with a broad kinase target spectrum, such as GZD-824, have been shown to bind to LRRK2 kinase with high affinity, no LRRK2-specific type-II inhibitors exist in the public domain. A major stumbling block to the design of new inhibitors is the absence of structures of LRRK2:inhibitor complexes. In this work, we solved cryo-EM structures of LRRK2, both wild-type and PD-linked mutants, bound to the LRRK2-specific type-I inhibitor MLi-2 and the broad-spectrum type-II inhibitor GZD-824. Our structures revealed LRRK2's kinase in the active-like state, stabilized by type-I inhibitor interactions, and an inactive DYG-out type-II inhibitor complex. Our structural analysis also showed how inhibitor-induced conformational changes in LRRK2 are affected by its autoinhibitory N-terminal repeats. The structural models provide a template for the rational development of LRRK2 kinase inhibitors covering both canonical inhibitor binding modes.

Keywords: Cryo-EM, Parkinson'S Disease, LRRK2

ÍNDICE

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Structural basis of near cognate codon discrimination by the yeast translation initiation complex

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Eukaryotic translation initiation is a multistep process that starts when a dozen of initiation factors (eIFs) and methionyl initiator tRNA (Met-tRNAi) associate with the 40S ribosomal subunit to form a 43S pre-initiation complex (PIC). Then this 43S PIC attaches to the capped 5' end of the mRNA and scans the 5´ untranslated region in the 5′ -to-3′ direction for an AUG nucleotide triplet start codon using complementarity with the anticodon of Met-tRNAi in the 40S P site. Recognition of a suitable start codon (usually AUG in a good Kozak context) is followed by a major conformational change in the PIC to a scanning arrested closed (P_{IN}) complex in which Met-tRNAi is more tightly bound, eIF1 dissociates and is replaced by the N-terminal domain of eIF5 and the codon:anticodon duplex is stabilized by the N-terminal tail (NTT) of eIF1A.

We have used cryoelectron microscopy (cryoEM) to understand, at a structural level, how the various eIFs, structural elements of tRNAi and rRNA and protein components of the 40S contribute to the stringency of initiation codon discrimination. We have produced all eIFs, 40S ribosomes, a near-cognate (AUC) capped mRNA and Met-tRNAi from *Saccharomyces cerevisiae*, and then *in vitro* reconstituted the 48S PIC and placed it in cryoEM grids. After large extensive 3D classifications of a large cryoEM dataset, we isolated several distinct and well defined maps of yeast 48S PICs in P_{IN}-like conformations at high resolutions (from 3.35 to 4.7Å-resolution). These complexes show in detail the geometry of a near-cognate codon:anticodon duplex with a mismatch in the third position of the codon and the role of eIF1, eIF5 and the eIF1A NTT in the discrimination of that third nucleotide of the mRNA initiation codon.

Keywords: 40S Ribosome, CryoEM, Codon

G08 - 222 - P

Bcl-2 family protein Harakiri's putative transmembrane domain and its role in pro-apoptotic function.

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Harakiri (Hrk) is a pro-apoptotic BH3-only protein from the Blc-2 family. These proteins regulate the intrinsic pathway of apoptosis. By interfering with apoptosis-repressor proteins Bcl-XL and Bcl-2, Harakiri blocks their function and causes cell death. Like other Bcl-2 family members, Hrk has a putative transmembrane domain (TMD) corresponding to a highly hydrophobic C-terminal domain that could be responsible for its cellular localization in intracellular membranes. In this work, we have tested Harakiri's membrane insertion capacity via glycosylation assays both in vitro using microsomal membranes and in vivo in eukaryotic cells (CLTM and lep' systems). Having confirmed the insertion capacity of the hydrophobic region of Harakiri, we went on to study the importance of this TMD in protein function. We first focused on testing whether the transmembrane domains of Hrk, Bcl-XL, and Bcl-2 could interact on their own without requiring the presence of the soluble part of the full-length protein. Interaction assays were performed in *Escherichia coli* (BLaTM) and eukaryotic cells (Bimolecular Fluorescence Complementation). We were able to corroborate the interaction between Hrk TMD and the other two transmembrane domains, suggesting that Harakiri's TMD is involved in the Bcl-XL and Bcl-2 blockade responsible for the pro-apoptotic effect of Harakiri. To verify this, we performed cell survival assays in eukaryotic cells and our preliminary results confirmed that the expression of Harakiri's TMD alone is enough to reduce the survival rate of the cells, indicating that Hrk TMD may play an important role in the functions performed by the full-length protein.

Keywords: Harakiri, Bcl-2, Transmembrane, Apoptosis

G08 - 228 - P

An allosteric switch between the activation loop and a c-terminal palindromic phospho-motif controls c-Src function

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Autophosphorylation controls the transition between discrete functional and conformational states in protein kinases, yet the structural and molecular determinants underlying this fundamental process remain unclear. Here we show that c-terminal Tyr 530 is a de facto c-Src autophosphorylation site with slow time-resolution kinetics and a strong intermolecular component. On the contrary, activation-loop Tyr 419 undergoes faster kinetics and a cis-to-trans phosphorylation switch that controls c-terminal Tyr 530 autophosphorylation, enzyme specificity, and strikingly, c-Src non-catalytic function as a substrate. In line with this, we visualize by X-ray crystallography a snapshot of Tyr 530 intermolecular autophosphorylation. In an asymmetric arrangement of both catalytic domains, a c-terminal palindromic phospho-motif flanking Tyr 530 on the substrate molecule engages the G-loop of the active kinase adopting a position ready for entry into the catalytic cleft. Perturbation of the phospho-motif accounts for c-Src dysfunction as indicated by viral and colorectal cancer (CRC)-associated c-terminal deleted variants. We show that c-terminal residues 531 to 536 are required for c-Src Tyr 530 autophosphorylation, and such a detrimental effect is caused by the substrate molecule inhibiting allosterically the active kinase. Our work reveals a crosstalk between the activation and c-terminal segments that control the allosteric interplay between substrate- and enzyme-acting kinases during autophosphorylation.

Keywords: Src, Allosteric, Protein, Structure,

G08 - 238 - P

Shedding light into bacterial communication by Rap systems

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Quorum sensing (QS) is a mechanism by which bacterial communities interact and coordinate several processes. In Firmicutes, RRNPPA family predominantly governs the QS system through pheromone-receptor signaling, controlling key processes such as sporulation, competence or biofilm formation. This involves the synthesis and release of an inactive propeptide into the environment, which external proteases then mature into a functional pheromone (Phr). This mature pheromone is reimported into the cell, where it binds to cytoplasmic RRNPPA receptors leading to the activation or repression of RRNPPA proteins. Pheromones are usually encoded in the propeptide sequence of the receptor, showing highly specific binding. Recently, the presence of multiple active pheromones within a single propeptide sequence has been described. These can include multiple copies of the same pheromone or putative pheromones with sequence variations, similar to other signaling systems. Our previous research has demonstrated that different pheromones encoded within the same propeptide can bind to receptors with varying affinities and kinetics, suggesting that this diversity may enable different biological functions within the bacterial community. In this work, we further investigate this hypothesis by analyzing the affinity interactions between putative pheromones in propeptides from Rap proteins, specially with Phi3T and Phi105 bacteriophages. Using a range of biophysical methods, we analyzed these interactions and structurally characterized the recognition mechanisms of these putative pheromones. Our goal is to elucidate the functionality and biological role of this 'partial selectivity' observed in Phr-Rap interactions, contributing to a deeper understanding of QS dynamics in bacterial communities.

Keywords: Quorum Sensing, Rap Proteins, Bacteriophage Phi3T

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Cargo and Membrane Recognition Act Synergistically to Promote SNX17 Activation and Retriever Recruitment

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The fate of internalized membrane proteins, known as cargoes, is determined in the endosomes. Endosomes serve as cellular sorting hubs that can direct cargoes towards lysosomal degradation or promote their recycling to various destinations. This recycling pathway plays a key role in regulating protein distribution within the plasma membrane, which is essential for maintaining cellular homeostasis. The recycling of cargoes depends on their recognition by the adapter protein SNX17, whose activation promotes Retriever recruitment to endosomal membranes. We have reconstituted the recruitment of Retriever to membranes *in vitro* using recombinant proteins and liposomes. Through biophysical assays and site-directed mutagenesis guided by AlphaFold2 modelling, we have shown that the interaction of Retriever with SNX17 is prevented by an intramolecular autoinhibitory interaction of the C- terminal tail of SNX17 with its cargo binding pocket. The interaction of SNX17 with selective cargo or its association with membranes containing phosphatidylinositol-3-phosphate allows the release of the C-terminal tail overcoming this autoinhibited state. The released C-terminal tail of SNX17 interacts with the VPS35L-VPS26C interface. Our proposed model illustrates two complementary activation mechanisms that facilitate the interaction between SNX17 and Retriever at endosomes for cargo recycling.

Keywords: Intracellular Trafficking, Endosomal Recycling Pathways

G08 - 251 - P

Cryo-EM structures of functional and pathological amyloid ribonucleoprotein assemblies

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Amyloids are implicated in neurodegenerative and systemic diseases, yet they serve important functional roles in numerous organisms. Heterogeneous nuclear ribonucleoproteins (hnRNPs) represent a large family of RNA-binding proteins (RBPs) that control central events of RNA biogenesis in normal and diseased cellular conditions. Many of these proteins contain prion-like sequences of low complexity, which not only assemble into functional fibrils in response to cellular cues but can also lead to disease when missense mutations arise in their sequences. Recent advances in cryo-electron microscopy (cryo-EM) have provided unprecedented high-resolution structural insights into diverse amyloid assemblies formed by hnRNPs and structurally related RBPs, including TAR DNA-binding protein 43 (TDP-43), Fused in Sarcoma (FUS), Orb2, hnRNPA1, hnRNPA2, and hnRNPDL-2.

Keywords: Amyloid Fibrils, Functional Amyloids, RNA-Binding Proteins (RBPs), Low-Complexity Domains (LCDs), Neurodegenerative Diseases

G08 - 259 - P

When AlphaFold3 models fall short: structural insights on a novel transpeptidase to fight superbacteria

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LD-transpeptidases play a critical role in bacterial cell wall remodeling, making them essential targets in the fight against antibiotic resistance. Our study focuses on a novel transpeptidase whose structure, as predicted by AlphaFold, was significantly different from the experimentally determined structure. This disparity provides new insights into the enzyme's functional mechanisms and potential vulnerabilities.

Using advanced crystallographic techniques, we resolved the protein's structure, revealing unique conformational features not anticipated by computational models. These findings challenge current predictive models and highlight the importance of empirical validation in structural biology. Furthermore, the unexpected structural elements identified may inform the development of more effective antibacterial agents. By comparing predicted and real-life structures, this work underscores the complexities of protein dynamics and the necessity for continued refinement of predictive algorithms. Our results pave the way for novel therapeutic strategies targeting bacterial cell wall synthesis and combating antibiotic resistance.

Keywords: Bacterial Resistance, Cell Wall, Structure Prediction

G08 - 261 - P

Evidence for an Epistatic Mechanism Expanding the Evolvability of a Pathway of Folding into the Ubiquitin-like Fold

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Biophysical models have increased our understanding of protein evolution by rationalizing the relationship between protein thermodynamic stability and fitness. However, the impact of mutations arising during evolution on the pathways and mechanisms of protein folding has remained mostly unexplored.

We show that the remarkable difference in folding and unfolding rates between the main human SUMO domains results from their transit along different folding pathways. More importantly, whether the SUMO domain follows one or another pathway appears to be dictated mainly by the amino acid identity at a single position—and, by exchanging amino acids at this position, we can largely switch the folding kinetics of SUMO domains. This allowed us to achieve what, to the best of our knwoledge, represents the first switch between folding pathways by means of a single amino acid subtitution reported to date.

Our results suggest that the epistatic emergence of an interaction before or early during Metazoan divergence resulted in an alternative folding mechanism enabling a different pathway into the SUMO Ubiquitin-like native state.

We hypothesize single point mutations yielding drastic changes in amino acid physicochemical properties can be a source of protein innovation by expanding folding evolvability.

Keywords: Protein Folding, Folding Pathway Evolution, Ubiquitin-Like Proteins

G08 - 270 - P

Proteins, RNA and Medicinal Chemistry: from In Silico Approaches to Biomedical Applications

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Advancements in computational biology and molecular dynamics simulations have unlocked new frontiers in understanding the structure and function of proteins and RNA, propelling us closer to resolving critical health issues. The current work highlights the transformative potential of these technologies in accelerating scientific discoveries and translating them into tangible health benefits.

In collaboration with medical professionals, we apply cutting-edge in silico methodologies to tackle the intricate challenge of predicting the phenotypic effects of difficult-to-anticipate mutations. Our research focuses on enhancing the accuracy of genetic disease diagnosis and the development of personalized medicine strategies. Through the integration of molecular dynamics simulations and structural analysis on protein systems, we classify the impacts of mutations, facilitating more precise and individualized treatment plans.

Furthermore, our recent studies extend beyond protein targets to explore RNA and RNA-protein complexes as thera-

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peutic targets. We demonstrate –through a case study– the utility of RNA-centric strategies combined with state-of-the-art computational approaches in expediting the identification of new therapeutic agents.

This presentation encapsulates the synergy between computational biology and clinical applications, emphasizing the necessity of bridging scientific innovation with practical healthcare solutions.

Keywords: Computational Biology, Molecular Dynamics Simulations, Genetic Disease Diagnosis, Personalized Medicine, Drug Discovery, RNA Therapeutics

G08 - 320 - P

Characterization of B albumin fraction from four Costa Rican varieties of Phaseolus vulgaris (Fabaceae) Beans: arcelins, phytohematoglutinin (PHA) and α-amylase inhibitors.

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Proteins in beans (Phaseolus vulgaris, Fabaceae), play pivotal roles in their quality, taste, and pest resistance. Phaseolins, constituting up to 50% of bean proteins, influence texture and water absorption during cooking. While arcelins bolster crop resistance to pests by being toxic to insects, their presence raises food safety concerns, necessitating proper management of arcelin-containing varieties. Globulins, another essential protein group, contribute to beans nutritional and functional properties, playing roles in seed germination and environmental responses. In this study we assessed the B albumin fraction composition of four bean varieties in Costa Rica by analyzing their content of arcelins, phytohemagglutinin (PHA), and α-amylase inhibitors, due to their potential relation to natural resistance against insect attacks. The soluble protein fraction was extracted via sequential steps over fine bean flour using 10 mM NaCl and 50 mM glycine buffer at pH 2.40. Following concentration and desalination using 30 kDa and 10 kDa Amicon membrane tubes, arcelins were isolated from albumins through exclusion molecular chromatography using Sephadex G-75, monitored by UV absorption at 280 nm. Subsequently, samples were analyzed via SDS-PAGE. High-performance thin-layer chromatography (HPTLC) coupled with fluorescamine derivatization enabled quantitative analysis of soluble proteins, indicating minimal arcelin content but abundant PHA and other albumins. Superior results in protein extraction and separation were achieved using 10 kDa dialysis systems. Notably, Costa Rican bean varieties exhibited a prevalence of PHA and other albumins over arcelins. Optimal separation of soluble proteins via HPTLC was attained using silica gel in BuOH/H2O/HOAc (28:8:2) at 366 nm.

Keywords: Arcelinas, Fitohemaglutininas, Faseolinas,

G08 - 333 - O

Arbitrium communication shows a complex and novel phage-host interaction to coordinate lysis-lysogeny decision

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Arbitrium is a new quorum sensing mechanism used by bacterio(phages) and other mobile genetic elements of the phylum Bacillota to communicate via peptides. In phages of the SPbeta family, where arbitrium was initially described, this system is used to coordinate the decision between lysis and lysogeny. The implementation of this communication system in their life cycle has involved the development of a novel repression mechanism that differentiates SPbeta phages from the prototypical one present in lambda phages. Our structural and functional studies show that this new repression mechanism is based on a six-gene operon that we have named “SPbeta phages repressor operon’’ (*sro*) and that has not one but two master repressors for the establishment and maintenance of lysogeny. Interestingly, our structural data show that one of the master repressors has a typical recombinase folding, differing from the typical cI-cro phage repressors. The three initial proteins of *sro* operon, which are more variable in sequence, are required to transduce the information provided by the arbitrium system to the activity of the repressions. In this process, the *sro* transducer proteins regulate systems form the host, involving the bacteria in the decision between lytic and lysogenic phage cycles. In the presentation will show the intricate and specialised repression system employed by phages of the SPbeta family necessary for their life cycle decision as a function of phage and host population density.

Keywords: Quorum Sensing, Bacterial Communication, Mobile Genetic Elements, Phage Cycle, Estructural Biology

G08 - 344 - O

Complementary approaches for the regulation of the TGF-beta signaling pathway in disease

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SMAD proteins are a family of highly conserved transcription factors that act as downstream effectors of Transforming Growth Factor Beta (TGFβ) signaling. Because this cascade has key roles in embryonic development, tissue homeostasis, immune system maintenance and neuroprotection, among other functions, the misregulation of SMADs has severe pathogenic implications.

While major therapeutic strategies to tackle TGF-β pathway are focusing on modulating the membrane receptor function or inhibiting the hormone activation, no therapeutic strategies have been tested targeting SMAD proteins. Targeting SMAD4 might be of special interest since it is the most mutated element in the SMAD driven TGF-β pathway in primary tumors, especially in pancreatic and gastrointestinal tract cancers, and plays key roles in advanced cancer stages, fibrosis and rare diseases. Individuals with Juvenile Polyposis Syndrome (JPS) or Hereditary Hemorrhagic Telangiectasia (HHT) patients usually suffer from alterations in the proper function of epithelial tissue in various organs. The SMAD4 variants associated with these epithelial disorders, which accumulate mainly in the MH2 domain of the protein, cause inhibition of SMAD complex formation. Individuals with Myhre syndrome (MyS) have specific SMAD4 point variants associated with stabilization of SMAD proteins.

Driven by the urgent societal need to find new treatments for cancer patients as well as individuals with Myhre syndrome and other rare diseases, we have analyzed the transcription factor SMAD4 as a target for drug discovery. In this talk, I will discuss the results we have obtained and how we plan to proceed with the project to obtain molecules with pharmacological applications.

Keywords: SMAD Proteins, Structures, TGF Beta, Pharmacological Applications

G09: Mitocondria, comunicación celular y estrés oxidativo

G09 - 26 - P

Effects of GLP-1 AR on atherosclerosis, mitochondrial function and inflammation in type 2 diabetes

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Objective: Type 2 diabetes (T2D) is linked to metabolic, mitochondrial and inflammatory alterations, atherosclerosis development and cardiovascular diseases (CVDs). The aim was to investigate the potential therapeutic benefits of GLP-1 receptor agonists (GLP-1 RA) on oxidative stress,

mitochondrial function, leukocyte-endothelial interactions, inflammation and carotid intima-media thickness (CIMT) in T2D patients.

Research design and methods: Type 2 diabetic patients (255) and control subjects (175) were recruited, and separated into two groups: without GLP-1 RA treatment (196) and treated with GLP-1 RA (59). Peripheral blood polymorphonuclear leukocytes (PMNs) were isolated to measure reactive oxygen species (ROS) production by flow cytometry and oxygen consumption with a Clark electrode. PMNs were also used to assess leukocyte-endothelial interactions. Circulating levels of adhesion molecules and inflammatory markers were quantified by Luminex's technology, and CIMT was measured as surrogate marker of atherosclerosis.

Results: Treatment with GLP-1 RA reduced ROS production and recovered mitochondrial membrane potential, oxygen consumption and MPO levels. The velocity of leukocytes rolling over endothelial cells increased in PMNs from GLP-1 RA-treated patients, whereas rolling and adhesion were diminished. ICAM-1, VCAM-1, IL-6, TNFα and IL-12 protein levels also decreased in the GLP-1 RA-treated group, while IL-10 increased. CIMT was lower in GLP-1 RA-treated T2D patients than in T2D patients without GLP-1 RA treatment.

Conclusions: GLP-1 RA treatment improves the redox state and mitochondrial respiration, and reduces leukocyte-endothelial interactions, inflammation and CIMT in T2D patients, thereby potentially diminishing the risk of atherosclerosis and CVDs.

Keywords: Atherogenesis/Atherosclerosis; GLP-1 RA; Leukocytes; Mitochondrial Dysfunction; Oxidative Stress; Type 2 Diabetes

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Neural mitochondrial transfer as a signalling of asteocytes metabolism reprogramming

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In the brain, the maintenance of a proper mitochondrial function and metabolic balance is crucial to define cell viability and fate (1). Metabolic states between neural cells are known to be coordinated by an active exchange of metabolites. An overlooked mechanism driving a major form of metabolic reconfiguration, involve sharing of whole, functional mitochondria. Exogenous mitochondria reconfigure the cells that incorporate the organelles to their native network. Transfer has proven critical to define cell survival (2-3). However, since mitochondrial content and function are key in metabolic regulation, it is expected that this process is regulated, (3-4). This is more relevant because metabolism ultimately controls neural cell fate (3). Despite the growing structural and functional characterization of mitochondrial transfer, its impact on metabolism and neural fate remains largely unknown. Here, we show that neurons and astrocytes can acquire whole mitochondria from each other neural partner in culture. The acquisition of whole mitochondria remodels the native mitochondrial network, as well as the morphology of the recipient cells. Functionally, transfer results in a reconfiguration of the mitochondrial content, respiratory metabolism and in a metabolic fluxes reprogramming, as assessed by respirometric and radiometric analysis of major metabolic paths such as glycolysis, ketogenesis or TCA cycle. Altogether, our data suggest that the incorporation of exogenous neural mitochondria rewires brain metabolism.

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Keywords: Mitochondrial Transfer, Astrocytes, Neurons, Metabolic Reprogramming

G09 - 87 - P

The GFAP R239C mutation causing Alexander disease disrupts lysosomes and increases their vulnerability to stress in an astrocytoma cell model

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Glial Fibrillary Acidic Protein (GFAP) is an intermediate filament protein characteristic of astrocytes that is vital for their function and for brain homeostasis. Various GFAP point mutations are associated with Alexander disease (AxD), a severe leukodystrophy. At the cellular level, GFAP mutants form aggregates, disrupt the morphology and function of cell organellesand provoke a proteostasis defect. These alterations can be recapitulated in a model of U-87 MG astrocytoma cells by transfection of AxD GFAP mutants [1]. Here, we have used this cellular model to explore the impact of the GFAP R239C mutation on lysosomal function. We have observed that expression of GFP-GFAP R239C disrupts lysosomal position and impairs lysosomal acidification and activity with respect to cells expressing GFP-GFAP wt. In addition, cells expressing the AxD mutant suffer a more intense lysosomal damage upon incubation with H_2O_2 or irradiation with UV light. This is evidenced by increased lysosomal permeabilization, with recruitment of galectin-1 to the inner lysosomal membrane, and by a greater decrease in the total number of lysosomes. Interestingly, the recovery from H_2O_2-induced lysosomal damage is hampered in cells expressing GFP-GFAP R239C, suggesting that lysosomal repair mechanisms could be impaired. Taken together, these observations show that expression of a GFAP mutant causing AxD exerts deleterious effects on the cell degradation machinery in this cell model. Moreover, they suggest that lysosomal impairment could contribute to altered astrocyte homeostasis in AxD.

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Keywords: GFAP, Lysosomes, Stress

G09 - 98 - P

NOX2 and glutamine metabolism in acute myeloid leukaemia cells

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The NADPH oxidases (NOX) are enzymes involved in the production of reactive oxygen species (ROS), which are crucial as second messengers in hematopoiesis. NOX are related to increased ROS levels in tumor processes, specifically in acute myeloid leukemia (AML). AML is a heterogeneous hematological cancer characterized by the uncontrolled proliferation of myeloblasts in the bone marrow, which suppresses the normal development of blood cells. Compelling evidence supports the involvement of NOX2 in the control of AML metabolism. Our group has reported that NOX2 and metabolism can be prognostic factors in AML, and that the deletion of NOX2 hampers energetic metabolism in AML cells.

Moreover, several studies have highlighted the relevance of glutamine in the metabolism of AML. Both metabolic reprogramming and redox imbalance are interesting areas of study that could lead to potential AML therapies. To explore these questions, various experiments were conducted using the THP-1 cell line (an AML model) and its NOX2 knockout version (THP-1NOX2/KO). Both were subjected to treatment with glutaminase inhibitors (DON, Telaglenastat) and glutamine depletion. Cell proliferation was assessed in response to these conditions using the MTT assay, while cell viability was measured by flow cytometry using Annexin/7AAD staining and cell counting.

Additionally, since glutamine is a precursor of glutathione, a fundamental antioxidant, total glutathione levels (GSH + GSSG), reduced glutathione (GSH), and oxidized glutathione (GSSG) were determined in both cell lines. Finally, protein signaling alterations were analyzed using Western Blot, focusing on mTOR, P70, and Nrf2 proteins. All of this has provided a deeper understanding of the involvement of NOX2 and glutamine metabolism in AML cells.

Keywords: Acute Myeloid Leukemia (AML), Reactive Oxygen Species (ROS), NADPH Oxidases (NOX), Glutamine Metabolism,

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NOX2 correlation with lipid metabolism, prognosis and survival in acute myeloid leukaemia

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NADPH oxidases (NOX) are a family of enzymes with a key role in redox signaling. Recent evidence highlights the significance of NADPH oxidases in regulating metabolism, with NOX2 playing a prominent role in the metabolic control of acute myeloid leukemia (AML). Previous work in our laboratory identified the 29G gene panel, which contains 28 metabolic genes related to *CYBB* (the gene encoding NOX2) and shows a high prognostic value in AML. Deletion of NOX2 in the AML model cell line THP-1 (THP-1NOX2/KO) slowdowns basal metabolism but increases glycolytic and mitochondrial capacity under high energy demand. Transcriptomic studies showed that these cells exhibit 725 differentially expressed genes compared to control cells, revealing a significant number of genes related to lipid metabolism. Given these findings, the objectives of this work are to analyse whether the metabolic genes altered by NOX2 deletion have prognostic and survival value, and to explore the role of NOX2 in the context of lipid metabolism in AML.

Among the 725 DEGs found in THP-1NOX2/KO cells, we identified a panel of 30 metabolic genes with high prognostic and survival value, strongly supporting the NOX2-metabolism relationship not only *in vitro* but also in the clinical context. Additionally, Seahorse technology revealed that NOX2 deletion enables activation of lipid metabolism more efficiently. Finally, MTT assays suggested that the combination of several NOX inhibitors with etomoxir, an inhibitor of CPT1a (a key transporter on fatty acid catabolism), significantly reduces proliferation compared to individual treatments. All these results allow for a detailed characterization of the role of NOX2 in lipid metabolism and highlight its importance in the prognosis and survival of AML patients.

Keywords: Acute Myeloid Leukemia (AML), Reactive Oxygen Species (ROS), NADPH Oxidases (NOX), NOX2, CYBB, Lipid Metabolism

G09 - 132 - P

Lipid composition and traffic are altered in the absence of PRDX6 sensitizing cells to ferroptosis

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Peroxiredoxin 6 (PRDX6) is a multifunctional enzyme provided with peroxidase, phospholipase A2 and lysophosphatidilcoline acyltransferase activities, being involved, among other processes, in phospholipid peroxide repair and metabolism. In this work we report a comparative study between SNU475 cell lines with (WT) or without (KO) PRDX6 at the level of global lipid composition and lipid-related cellular processes.

Human hepatocarcinoma SNU475 cells lacking PRDX6 have shown modified lipid

composition and lipid-related cellular processes presenting a general decrease in all kinds of lipids. Among these modifications, the formation of lipid droplets accumulating various polyunsaturated fatty acids (PUFA) and PUFA-containing triacylglycerols has been described, indicating an altered fatty acid flux in absence of PRDX6. An increment in arachidonic acid (AA) containing phosphatidylcholines was also observed, suggesting a preference of the PLA2 activity of this enzyme for these AA-storing glycerophospholipids.

SNU475-KO cells also showed increased total lipid hydroperoxide levels, which reverted to the levels of SNU475-WT cells after transfection with PRDX6. Moreover, lack of PRDX6 enhanced sensitivity to erastin-induced ferroptosis, leading to changes in morphology and survival of SNU475-KO cells, which could be explained by an alteration in plasmalogen homeostasis.

The results presented here show that all three enzymatic activities of PRDX6 contribute to the role of this enzyme in a variety of cellular processes, from membrane phospholipid remodelling and functional diversity of glycerophospholipids to the fate of lipid peroxides and modulation of AA levels. These contributions explain the complexity of the changes that loss of PRDX6 exerts on cellular functionality.

Keywords: Peroxiredoxin 6, Oxidative Stress, Lipid Peroxidation, Lipid Traffic, Ferroptosis

G09 - 141 - P

Effect of PRDX6 deletion in the human colon cancer cell line HCT116 on mitochondrial function and biogenesis

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PRDX6 is a unique member within the peroxiredoxin family that presents not only peroxidase but also phospholipase A2 (iPLA2) and lysophosphatidylcholine acyl transferase (LPCAT) activity, acting on phospholipid hydroperoxides. It is also the only peroxiredoxin member that translocates to damaged mitochondrial membranes to regulate mitophagy by suppressing ROS. PRDX6 has been considered a tumor promoter, associated with the proliferation and invasive capacity of different tumor cell lines including colorectal cancer cells, although the effect of its complete deletion in these cells has not been studied. Here, using CRISPR/Cas9 technology, we constructed a colorectal cancer cell line HCT116 knockout for PRDX6 to study the effect of its removal in mitochondrial function and whether it differs from that observed in other tumor cells. The lack of PRDX6 in HCT116 cells induced oxidative stress as indicated by the increase in ROS and lipid peroxidation and a decrease in the antioxidant response regulator NRF2, sensitizing cells to ferroptosis. Furthermore, there is also a severe impairment of mitochondrial function and biogenesis when PRDX6 is deleted as showed by the alteration in mitochondrial morphology and the decrease of mitochondrial complexes I/III activity and respiratory capacity. Therefore, PRDX6, through its membrane turnover functions, can provide a deeper ROS tolerance, avoiding lipid peroxidation, which is essential to maintaining the proper organelle functionality and preventing ferroptosis in the presence of large ROS. This means that PRDX6 downregulation could be considered a good therapeutic strategy together with a ferroptosis inducer because of its capacity to sensitize colorectal cancer cells to ferroptosis cell death.

Keywords: Peroxiredoxin 6, Mitochondria, Lipid Peroxidation, Ferroptosis, Oxidative Stress

G09 - 155 - P

A new view on the role of mito-ribosomal fidelity on human disease: structural analysis of deafness-related mtDNA variants mapping to mitochondrial rRNA genes

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Studies directly tackling the phenotypic effects of altered mito-ribosomal fidelity have been performed in the last few years. In an upcoming paper, we have reviewed the issue of mito-ribosomal fidelity during mitochondrial protein synthesis and placed it in the context of the emerging high-resolution structures of the mito-ribosome. Much needs to be done to arrive to a clear picture of how defects at the level of mito-ribosomal translation eventually result in the complex patterns of disease observed in patients. However, all available evidence shows that altered mito-ribosome function, even at very low levels, as is often the case with fidelity mutations, may become highly pathogenic. This shifts the theoretical framework for the evaluation of the pathogenic potential of mitochondrial (mt-) rRNA variants. Under this new light, essentially, any base change capable of inducing a fidelity phenotype may be considered non-silent. We have used this new framework to study the potential pathogenicity of 93 mt-rRNA variants, reported by others as presumably associated to deafness. After inspection of these variants in the mito-ribosomal structure with the highest resolution currently available (2.2Å), 49 such variants were deemed as potentially non-silent. These results drastically update our view on the implication of the primary sequence of mt-rRNA in the etiology of deafness and mitochondrial disease in general. In particular, our data shed much needed light on how mt-rRNA variants, specially those located at non-conserved positions, may lead to mitochondrial disease. Our data also provides clues on the predicted effect of haplotype context in the manifestation of some mt-rRNA variants.

Keywords: Mito-Ribosome, Mitochondrial RRNA Variants, Translational Fidelity, MtDNA Disease, Deafness (Hearing Loss)

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G09 - 177 - P

Acute hypoxia and hypoglycemia in arterial chemoreceptors: Role of ATP

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Carotid body (CB) glomus cells are the main arterial chemoreceptors responsible for the cardiorespiratory reflexes induced by acute hypoxia. These cells contain specialized mitochondria in which the activity of mitochondrial (MC) IV is modulated during physiological levels of O_2 tension. In hypoxia, MCIV activity is slowed down, causing a backlog of electrons along the electron transport chain with production of NADH and H_2O_2, which are the signaling molecules that inhibit membrane K+ channels to elicit transmitter release. This, in turn, activates the respiratory centers in the brainstem. **A relevant unsolved mechanistic question is whether or not hypoxia causes a decrease in cytosolic ATP levels in glomus cells and whether this can work as a hypoxic signaling molecule.** Glomus cells are highly sensitive to mitochondrial poisons and are also activated by hypoglycemia. Seahorse analysis of the bioenergetic status of glomus cells showed that OXPHOS is their main source of ATP. Measurement of glomus cell secretory activity showed that rotenone, an MCI inhibitor, activates CB cells and occludes any further effect of hypoxia. In contrast, rotenone does not abolish responsiveness of the cells to hypoglycemia. In the presence of rotenone, cyanide (a MCIV inhibitor) produced a strong activation of glomus cells. Direct measurement of cytosolic ATP/ADP ratio in glomus cells expressing the fluorescent ATP sensor PercevalHR revealed that cytosolic levels of ATP remain unaltered during acute hypoxia. Contrarily, the mitochondrial inhibitor CN and hypoglycemia elicited a significant decrease in ATP/ADP ratio. These data suggest that cytosolic ATP does not participate in the response of glomus cells to acute hypoxia, although it could be involved in their response to hypoglycemia.

Keywords: Mitochondria, Oxygen, ATP, Glucose, Carotid Body

G09 - 180 - P

Effect of peroxiredoxin 6 deletion in the colon cancer cell line, HCT-116, on cell proliferation, migration and invasion

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PRDX6, an atypical member of the peroxiredoxin family, exhibits two additional enzymatic activities, phospholipase A2 and lysophosphatidylcholine acyl transferase, apart from its canonical peroxidase activity, and has been found to act repairing peroxidized cell membranes. PRDX6, through its activities, plays a role in cellular proliferation, migration and invasiveness as has been demonstrated in different tumoral cell lines, including colorectal cancer cells. However, its deprivation in these cells has never been studied. Here, we constructed a PRDX6 knockout colon cancer cell line HCT116 using CRISPR/Cas9 technology to examine whether the role of this enzyme on proliferation, migration and invasiveness observed in other cell lines also applies in these tumor cells. The PRDX6 knockout cell line showed decreased proliferation rates and lower metabolic status but no changes in survival. The decreased proliferation is consistent with the altered cell cycle in PRDX6-deficient cells, indicating cell cycle arrest in S and G2/M phases. Furthermore, reduced migration and invasiveness capacities were detected in PRDX6 knockout HCT116 cells, consistent with lower expression of N-cadherin and reduced activity of pro-invasive enzymes like metalloproteinases. The mechanisms underlying the effects of PRDX6 elimination may vary between different cell lines, as the results obtained in HCT116 differ slightly from those previously obtained in hepatocarcinoma HepG2 cells. This underlines the importance of assessing the role of PRDX6 in the development of different tumors. Considering all the above-mentioned, our results point to this protein as a promising therapeutic target also for colorectal cancer.

Keywords: Peroxiredoxin 6, Colorectal Cancer, Cell Cycle, Migration, Invasiveness

G09 - 186 - O

Loss of mitochondrial plasticity negatively impacts hepatocellular carcinoma development in the context of MAFLD

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Liver steatosis is a highly prevalent condition associated with the metabolic syndrome that can progress to metabolic associated fatty liver disease (MAFLD), characterized by abnormal mitochondrial metabolism and oxidative stress, represented by a reduction in the levels of PGC-1α, the master regulator of oxidative metabolism. This induces inflammatory and pro-fibrotic processes that can, in turn, lead to the development of cirrhosis and Hepatocellular Carcinoma (HCC).

To evaluate the contribution of mitochondrial dysfunction to the progression of fatty liver (steatosis), the development of HCC in the context of fatty liver is analyzed in wild-type (WT) mice and PGC-1α deficient mice. WT and PGC-1 KO mice were used and treated under four different conditions: normal or high-fat diet, with or without teratogens. At the end of the treatment, the liver was analyzed through histological and biochemical analyses. We observed that the high-fat diet, teratogens, and the absence of PGC-1α increase the tumor burden and generate an altered fibrotic profile. Additionally, KO mice seem to have a different fat distribution compared to their controls.

These results support the hypothesis that mitochondrial dysfunction plays a significant role in the development of HCC apart from fatty liver and indicate that PGC-1α could be a potential therapeutic target.

Keywords: Mitochondria, Metabolic Plasticity, Oxidative Stress, Hepatocellular Carcinoma, PGC-1a, Metabolic Associated Fatty Liver Disease (MFLD)

G09 - 193 - P

Characterization of mitochondrial metabolism in cells afected by Bardet-Biedl and Alström syndromes

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Ciliopathies consist of rare diseases caused by the malfunction of cilia. Bardet-Biedl Syndrome (BBS) and Alström Syndrome are two conditions that fall under this group. The former is caused by mutations in a large number of genes related to the BBSome (a heterooctameric protein complex that performs many functions), while the latter is caused by alterations in a single gene (ALMS1). The phenotypes of both diseases are very similar, with patients presenting blindness, obesity, and renal problems (among others), and they seem to be related to mitochondrial function. This study focused on characterizing mitochondrial metabolism in both wild-type phenotype cells and cells affected by the two syndromes. For this purpose, four markers were used: two related to mitochondrial fission (DRP1, MFF), one related to mitochondrial fusion (OPA1), and another involved in Ca+2 transport (MCU), in addition to three different cell models (two for Bardet-Biedl and one for Alström).

Keywords: Ciliopathies, Bardet-Biedl, BBSome, Alström, Mitochondria

G09 - 200 - P

Ciliary dysfunction compromises mitochondrial homeostasis

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Ciliopathies are a group of genetic disorders arising from defects in cilia, which play essential roles in cellular signalling, motility, and balance. Two notable ciliopathies, Bardet-Biedl Syndrome (BBS) and Alström Syndrome (AS),

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share symptoms like obesity and retinal degeneration but are caused by distinct molecular mechanisms. BBS is associated with mutations in 26 cilia-related genes (BBS26) and manifests with obesity, retinal degeneration, polydactyly, renal insufficiency, and cognitive challenges. In contrast, AS is caused by mutations in the ALMS1 gene and presents with retinal degeneration, hearing loss, obesity, type 2 diabetes, heart and kidney problems, liver fibrosis, and pulmonary complications. However, the molecular mechanisms underlying this variability is poorly understood. To further investigate these syndromes, we molecularly analysed retina cells lacking BBS1 and BBS4 by large-scale proteomics. We saw an enrichment of the mitochondrial serine/threonine-protein phosphatase PGAM5 which has been related with changes in mitochondrial fission through the phosphorylation of the dynamin-1-like protein is a GTPase (DRP1). In keeping with these findings, we observed altered DRP1 activation as well as mitochondrial dynamics changes by either electron microscopy and/or confocal using the membrane marker TOM20 in both cellular models which was also accompanied with an increase of mitochondrial biogenesis and function. Although further studies are necessary to understand the role of mitochondrial function and dynamics in the formation of the cilia and its associated disorders, our data points to an important role of the mitochondria in these pathologies.

Keywords: Mtochondria, Bardet-Biedl Syndrome, Ciliopathies

G09 - 205 - P

Effects of Thioredoxin-Interacting Protein ablation in adipose tissue homeostasis

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Obesity is a well-known risk factor for multiple types of cancer. Consequently, adipocytes have been proposed as key players in the tumour microenvironment, linked to their signalling and metabolite fuelling capabilities. Moreover, although they are fully differentiated cells, adipocytes from white adipose tissue (WAT) maintain some cell plasticity that allows them to respond to different environments, including transdifferentiating to brown adipose tissue (BAT). BAT poses as a novel therapeutic target in cancer, but its effects and mechanisms need to be further dilucidated. Therefore, our goal was to study the role of redox signalling as a potential driver of adipose tissue plasticity within the tumour microenvironment.

To this aim we employed a Thioredoxin interacting protein (TXNIP)-knockout model in a prostate cancer transgenic (TRAMP) background previously developed in the lab. Androgen deprivation in this model showed distinct features in the general adiposity, depending on TXNIP status, although browning within the tumour microenvironment was not drastically impacted. As adipose tissue is widely considered to influence immune cell population, we also studied tumour-associated macrophages upon TXNIP ablation by immunofluorescence. Lastly, we performed a metabolomic assay in visceral WAT and BAT tissue from these mice, in order to determine which metabolic pathways are primarily affected in our models.

To sum up, TXNIP seems to have a significant role in adipose tissue homeostasis although further studies are needed to stablish the precise mechanisms of action.

This work was supported by MCI-PID2019-111418RB-100. BGS is supported by FPU20/04045. DPC and SAR are supported by the Severo Ochoa program.

Keywords: TXNIP, Adipose Tissue, Tumor Microenviroment

G09 - 230 - P

Mitochondrial oxidative stress as a key determinant for cardiovascular disease in type 2 diabetes

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Type 2 diabetes (T2D) is characterized by a loss of metabolic plasticity and an impairment of mitochondrial function. We hypothesis that this mitochondrial damage, accentuated by conventional therapies, is responsible for the development of CV disease (CVD) in diabetic patients. Currently, there are no biomarkers that allow predicting the development of CVDs and we aimed to evaluate if non-invasive blood tests could be used to determine mitochondrial activity and be used for CVD risk assessment in T2D patients. Since oxidative stress is generally linked to an altered immune response several cytokines were monitored. We isolated PBMCs from T2D patients and found that subjects with abnormal IM thickening (IMT) of the carotid artery had an increased correlation between inflammatory factors such as TNFa, IL-6 or TGFb and mitochondrial genes as Prx3 and Tfam. Futhermore, we found an increase in iNOS expression levels and a significant increase in the levels of oxidized gDNA present in plasma samples in abnormal IMT patients; despite having equivalent or higher levels of antioxidants when compared to normal IMT patients, which could indicate an insufficient compensatory capacity and greater production of ROS. These results suggest a connection between oxidative stress and the alteration in the immune response. Next, we observed that mitochondrial DNA copy was significantly lower in patients with abnormal IMT, consistent with a possible loss of mitochondrial function and mitochondrial genomic instability, supporting that reduced mitochondrial oxidative capacity could be associated to CVD development. We conclude that T2D patients with CVD show signs of mitochondrial dysfunction, oxidative stress and altered immune profile.

Keywords: Mitochondria, Oxidative Stress, T2D, CVD

G09 - 234 - P

Understanding the impact of mitochondrial function on lung carcinoma spheroid formation. A 3D model of personalized medicine

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Mitochondrial metabolism acts as a driver in cancer. Its functions are organized in two genomes: nuclear and mitochondrial (mtDNA). The mtDNA is more likely to suffer somatic mutations associated with different prognosis, but it remains controversial whether those mutations act as a driving force or have a functional impact. mtDNA evolved accumulating adaptative modifications, creating phylogenetic related population groups known as mitochondrial haplogroups. Haplogroups are related with different risk of developing several kinds of cancer, but the molecular mechanisms underlying their effect on oncogenesis remain yet unknown. Recent data from our laboratory showed that mtDNA background regulates key pathways in the metabolic regulation such as mTORC1 (regulator of protein synthesis) or HIF1a (hypoxia inducible factor) which could explain variability in the anticancer response. Thus, to analyse how mtDNA variation regulates oncogenesis, here, we modulated growth, viability, cell compactness and number in a 3D model of lung adenocarcinoma (LUAD) carrying different mtDNA backgrounds as well as the frequent tRNA heteroplasmic mutation m3243A>G. We saw that mtDNA variation drives 3D growth and compactness depending on the mtDNA background independently of the cell number and viability. Given the importance of the mitochondria in drug and hypoxia responses, then, we studied the impact of hypoxia (1% of O_2) as well as several drugs in the formation of spheroids. We observed that mtDNA backgrounds modified the response to O_2 as well as many treatments used in LUAD. Thus, pointing to a crucial clinical relevance of mtDNA variation in cancer treatments. Moreover, we believe that this 3D model is a helpful tool to model drugs used in cancer and other age associated disorders.

Keywords: MtDNA, Haplogroup, Lung Cancer, Spheroid Model, Hypoxia

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G09 - 237 - P

mtDNA regulates epithelial-mesenchymal transition processes in lung adenocarcinoma

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The epithelial-mesenchymal transition (EMT) is an important process for tumour cells migration and metastasis. Mitochondrial DNA (mtDNA) variants have been shown to play an essential role in cancer; potentially altering the risk of developing various cancer types. However, the mechanisms by which mtDNA variation affects cancer penetrance and molecular markers of oncogenesis are not fully understood. In this study, using a cellular model of lung adenocarcinoma with different mtDNAs, we demonstrate that mtDNA variation differentially regulates mitochondrial function, influencing levels of EMT-associated proteins such as Slug, Snail, cadherins, and β-catenin.

By either depleting mtDNA or exposing cells to 1% O2 hypoxia to shut down mitochondrial function, here, we show that EMT activation by mtDNA variants is dependent on the mtDNA molecule regulates 3D growth and cell morphology and is independent of mitochondrial function. Although further research is needed to elucidate the complete molecular mechanisms, our findings underscore the significance of mtDNA variation and signaling in EMT processes.

Keywords: Mitochondrial DNA, Epithelial-Mesenchymal Transition

G09 - 243 - P

Bioavailability and antioxidants properties of blueberry by-products against endothelial oxidative damage

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Hyperglycemia-induced endothelial cell dysfunction is a key event in the onset and progression of CVD, it promotes the overproduction of reactive oxygen species, leading to increased oxidative stress, inflammation, and cellular death. Blueberries by-products (BP) are considered an important source of antioxidants, can be a strategy for hyperglycemia management, collaborating to modulate endothelial dysfunction through antioxidant and anti-inflammatory actions. The aim of this study was to evaluate the bioavailability of the BP and the protective effect against hyperglycemia-induced oxidative damage in endothelial cells. The study of bioavailability was carried out in a transwell epithelial culture system with Caco-2 cells as intestinal layer, and the bioactivity was evaluated by assessing oxidative stress biomarkers and the gene expression under normoglycemic or hyperglycemic conditions. The bioavailable polyphenolic constituents of the BP showed a regulatory effect on the integrity of the intestinal barrier. Hyperglycemia reduced cell viability, which was re-stored to normoglycemic levels by bioaccessible fractions of BP. These fractions were able to counteract hyperglycemia induced oxidative stress as evidenced by significant decreases in carbonyl groups and MDA level. Furthermore, the bioaccessible fractions could exert a protective effect by modulating genes involved in endothelial cell ROS and NO balance (NOX4 and eNOS) and the antioxidant response (Nrf2 and NF-κB). These results confirm the promising healthy properties of bioaccessible fraction of BP in cardiovascular diseases. The authors thank the financial support of Ministry of Science and Innovation Spanish State Research Agency and European Regional Development Fund (Project PID2021-125400OB-I00).

Keywords: Bioavailability, Blueberry, Antioxidant, Endothelial Dysfunction

G09 - 276 - O

The Role of Mitofusin-2 in the Metabolic Adaptation of Skeletal Muscle

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Introduction: Metabolic flexibility is crucial for adapting to variations in metabolic and energy demands, and its loss is linked to insulin resistance (IR) and type 2 diabetes (T2D). Correct mitochondrial function is essential for proper nutritional adaptation and energy homeostasis. Mitochondrial dynamics have emerged as a key process in regulating mitochondrial function. Previously, our findings identified mitochondrial dynamics protein Mitofusin-2 (Mfn2) as a key regulator of mitochondrial function and glucose homeostasis, with reduced expression in insulin-resistant muscle. This study aims to elucidate the role of Mfn2 in mitochondrial plasticity and metabolic adaptation in skeletal muscle.

Results: The study showed a significant increase in Mfn2 protein expression during fasting in both in vivo and in vitro models, which was associated with increased mitochondrial elongation. Importantly, Mfn2 was essential for metabolic transitions from glucose to lipid oxidation in vivo and in vitro. The findings strongly suggest the critical role of Mfn2 in controlling skeletal muscle metabolic adaptation and whole body metabolic flexibility, providing insights into mechanisms influencing IR and T2D and emphasizing mitochondrial dynamics in skeletal muscle metabolism.

Conclusion: Mfn2 emerges as a key determinant of metabolic flexibility in skeletal muscle, influencing mitochondrial plasticity during metabolic transitions. Its reduced levels in obesity and T2D underscore its potential significance in IR. Understanding Mfn2's role in maintaining mitochondrial function in muscle provides a foundation for interventions. Further research into modulating Mfn2 expression may yield strategies to enhance metabolic flexibility and alleviate IR in T2D.

Keywords: Mitofusin-2, Metabolic Flexibility, Type 2 Diabetes

G09 - 293 - O

Neuroprotective Role of NDI1 expression in Parkinson's Disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder whose etiology is poorly understood. Mitochondrial dysfunction is now established to play a major role in the pathophysiology of PD. Indeed, a genetically modified mouse (MCI-Park model) with absence of mitochondrial complex I (MCI) in the dopamine neurons showed a progressive and dramatic nigrostriatal neurodegeneration associated with locomotor decline.

To search for possible therapeutic treatment to reestablish mitochondria functional activity, we generated a mouse model (MCI-Park/NDI1 model) with transgenic expression of the yeast NADH-quinone oxidoreductase (NDI1), an al-

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ternative NADH dehydrogenase, in dopamine neurons with MCI dysfunction. To determine whether the parkinsonian phenotype could be recovered in MCI-Park/NDI1 mice, we compared them to their pathological MCI-Park and wild-type littermates. Our study examined the fine motor skills and locomotor coordination, as well as the integrity of the dopamine nigrostriatal pathway at a molecular level.

The phenotypic analyses show that MCI-Park/NDI1 mice recover their behavior in full, just like wild-type mice. In addition, MCI-Park/NDI1 mice have the same number of dopamine neurons (stained with tyrosine hydroxylase) compared to wild-type mice. HPLC analysis show that MCI-Park/NDI1 animals have also a restored dopamine content in the striatum. These observations indicate that MCI-Park/NDI1 mice completely recovered from parkinsonism characteristic of MCI-Park mice. Furthermore, NDI1 expression in dopamine neurons prevents MPTP-induced degeneration, a classical model of PD. In summary, NDI1 expression can reverse nigrostriatal degeneration in MCI-Park mice. Improving MCI function could be beneficial for treating PD patients.

Keywords: Parkinson, Mitochondria, Degeneration, Metabolism

G09 - 294 - P

Explorando la funcionalidad mitocondrial y sus implicaciones en el desarrollo del cáncer colorrectal. Utilidad como herramienta en el diagnóstico y el pronóstico

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El cáncer colorrectal es el 2º más frecuente y la 2ª causa de muerte por cáncer dado que el 50% presenta estadios avanzados al diagnóstico.

La obesidad y la disfunción mitocondrial se consideran factores de riesgo. Decidimos determinar si los biomarcadores mitocondriales en sangre tienen valor diagnóstico al correlacionarse con las alteraciones tumorales. Se analizaron muestras de sangre y tejido de 60 pacientes clasificados por estadio tumoral. Se observó que, en PBMCs, la expresión de genes implicados en el metabolismo oxidativo aumentaba en estadios avanzados y que la correlación de TFAM con PFKB3 y PRX3 aumentaba también, sugiriendo la activación coordinada del metabolismo oxidativo y glucolítico. Además, los niveles de ADNmt en el tumor se correlacionaron con los de PBMCs, que sugiere una coregulación metabólica sistémica. Observamos mayor inestabilidad del ADNmt en el tumor, niveles más altos de ND1 en estadios avanzados, pero no de ND4 ni de ADNmt total. La correlación de ND1 con ADNmt fue mayor en estadios avanzados, relacionando el aumento de ND1 con la inestabilidad. También, se observaron niveles mayores de un ND1 mutado en tumores avanzados, pero no en PBMCs. Por otro lado, en plasma los cambios fueron consistentes: una reducción del cociente ND4/ND1 en estadios avanzados, similar al tejido y PBMCs, indicativo de inestabilidad sistémica en el ADNmt, detectable especialmente en plasma. Los tumores avanzados tenían mayores niveles de DNA genómico detectable en plasma, indicativo de mayores índices de muerte celular vinculada un cociente menor de ADNmt/ADNg. Esto sugiere que lahiperactivación metabólica sistémica relacionada con inestabilidad en el ADNmt son características de tumores avanzados detectables en sangre pudiendo ser de utilidad en el diagnóstico.

Keywords: Cáncer Colorrectal, Funcionalidad Mitocondrial, Estrés Oxidativo.

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Role of mitochondrial Na^+ import by the Na^+/Ca^{2+} exchanger NCLX in brain hypoxia and stro ke

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We have recently described a mechanism by which the mitochondrial Na+/Ca2+ exchanger NCLX takes part in the superoxide burst produced in response to acute hypoxia1. Mitochondrial complex I deactivation in hypoxia triggers calcium phosphate granules solubilization and NCLX activation. NCLX thus drives mitochondrial Na+ import, which diminishes coenzyme Q diffusion and regulates the electron transport chain, increasing superoxide production. NCLX inhibition abolishes the superoxide burst, altering acute responses to hypoxia, but also HIF-alpha stabilization and activation of the HIF pathway.

We have now studied the role of this mechanism in brain hypoxia. Using neuron cell models and *ex vivo* hippocampal slices, we show that NCLX activity is necessary for different responses to hypoxia, namely acute superoxide production and HIF-alpha stabilization. These results confirm our previous results in different cell types linking NCLX activity to ROS production and activation of the HIF pathway.

In animal models of stroke we show that this mechanism is operating, with complex I deactivation taking part independently of NCLX, while HIF-alpha stabilization depends on NCLX activity. Indeed, brain damage after stroke also depends on NCLX activity, which can be related to oxidative damage.

References:
1. Hernansanz-Agustín et al. Nature (2020) 586:287-291. DOI 10.1038/s41586-020-2551-y

Keywords: Mitochondrial Sodium/Calcium Exchanger NCLX, Brain Ischemia, Stroke, Hypoxia, Oxidative Stress

G09 - 303 - P

Culture of Bovine Aortic Endothelial Cells in galactose media enhances mitochondrial plasticity and changes redox sensing altering Nrf2 and FOXO3 levels

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Understanding complex biological processes of cells in culture, particularly those related to metabolism, can be biased by culture conditions, since the choice of energy substrate impacts all main metabolic pathways. When glucose is replaced by galactose cells decrease their glycolytic flux, working as an *in vitro* model of limited nutrient availability. However, the effect of these changes on related physiological processes such as REDOX control is not well documented, particularly in endothelial cells where mitochondrial oxidation is considered to be low. We evaluated the differences in mitochondrial dynamics and function in endothelial cells exposed to galactose or glucose culture medium. We observed that cells maintained in galactose-containing medium show higher mitochondrial oxidative capacity, more fused mitochondrial network, and higher intercellular coupling. These factors are documented to impact the cellular response to oxidative stress. Therefore, we analyzed the levels of two main REDOX regulators and found that Bovine Aortic Endothelial Cells (BAEC) in galactose media had higher levels of FOXO3 and lower levels of Nrf2 than those with glucose in the media. Thus, culture of endothelial cells in galactose containing medium may provide a more suitable condition to study *in vitro* mitochondrial related processes than glucose media, and deeply influence REDOX signaling in these cells

Keywords: Glucose, Galactose, Mitochondria, Antioxidants, Oxidative Metabolism, REDOX, FOXO3, NRF2.

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G09 - 309 - P

Sex differences influence hepatic oxidative stress in a photoperiod-dependent manner in cafeteria-fed obese rats

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Obesity is associated with an increased risk of oxidative stress in mammals. Both sex differences and changes in photoperiod can also affect oxidative status. However, to our knowledge, it is still unknown how these factors affect oxidative stress and antioxidant enzyme activities in obesogenic conditions. The aim of this study was to determine whether hepatic oxidative stress is affected by sex and photoperiod in cafeteria diet-induced obese rats (60 males and 60 females) exposed to a 6-h light (L6) or 18-h light (L18) photoperiod for 9 weeks. A significant decrease in hepatic catalase activity was observed in females compared to males in both photoperiods. This loss of catalase activity in females correlated with a significant increase in hepatic biomarkers of oxidative stress, such as malondialdehyde (oxidised lipids) and thiols (oxidised proteins) concentrations. However, this increased oxidative stress in the liver of females was apparently attenuated by upregulation of several antioxidant genes including glutathione peroxidase (*Gpx*) and superoxide dismutase Mn (*Sod2*). Notably, this transcriptomic response was more significant in females exposed to L6 than to L12 photoperiod. In accordance with these results, plasmatic concentrations of melatonin significantly resulted higher in females than in males, being these differences more pronounced in L6 photoperiod. Altogether, this study evidences the complex relationship between sex differences, photoperiod and oxidative stress in obesogenic conditions. Nevertheless, further research is needed for a better understanding and thus validate the underlying mechanisms.

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Keywords: Antioxidant, Catalase, Liver, Melatonin, Obesity, Rhythms, ROS, Seasonality

G09 - 311 - P

Mitochondrial characterization of triple negative breast cancer: Understanding tissue specificity of Connexin43

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Breast cancer stands as the most prevalent cancer globally, posing a significant burden on cancer-related mortality worldwide. Among the various subtypes of breast cancer, triple-negative breast cancer (TNBC) emerges as the most aggressive and challenging to treat, representing a major cause of death of young women (<40 Years) due to its particularly bleak prognosis. TNBC presents limited therapeutic options and 10-20% of patients carry BRCA1/2 mutations that elevate cancer risk by disrupting crucial DNA repair mechanisms. Although targeted therapies based on PARP inhibitors are being used for the treatment of TNBC tumors, drugs resistance is the main cause of treatment failure in these patients. Metabolically, TNBC exhibits a strong reliance on glycolysis linked to pronounced aggressiveness and resistant TNBC cells tend to shift towards oxidative phosphorylation (OXPHOS). Unpublished results from our group have found how Connexin43 (Cx43) modulates mitochondrial function by increasing mitochondrial biogenesis, oxidative phosphorylation (OXPHOS) capacity and mitochondrial reactive oxygen species (ROS) in BRAF-mutated tumours. Here, we investigate the mitochondrial characterization of TNBC cells under Cx43 upregulation in order to understand its role and metabolic reprogramming in a drug-resistant context.

Keywords: Mitochondria, Breast Cancer, Connexins

G09 - 314 - P

Time- and sex-dependent changes in connexin-based channels during aging

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Intercellular communication is crucial for retinal homeostasis, facilitating the exchange of metabolites between neighboring cells. Gap junctions, formed by structural elements known as connexins, allow this exchange. Their degradation is mediated by a process called autophagy, responsible for eliminating molecules and subcellular elements through lysosome-mediated degradation. During aging, the autophagic process diminishes, impacting connexin biogenesis, degradation, and consequently, cell homeostasis and survival. New evidence suggests that disrupting connexin-mediated cellular communication is crucial in aged-related retinal degeneration. A comprehensive understanding of the retinal connectome throughout aging remains aspirational, with no published systematic analysis of the retinal connexins to date. Retinas from C57BL/6J mice at 1 month, 4 months, 12 months, and 24 months were collected and immunohistochemical analysis was performed for several Cxs isoforms: Cx43 (expressed in RPE and ganglion cells), Cx50 (expressed in Müller cells), Cx36 (expressed in cones, bipolar and amacrine cells) and Cx45 (expressed in neural retina and ganglion cells). Transcriptional levels of connexins’ genes were measured by qPCR using QuantumStudio5. Pictures were captured using the EVOS M5000 microscope and deconvolution plus 3D modeling was performed using Celleste 3D Deconvolution Module. The content of Cxs isoforms showed no significant alteration until 12 months. As mice aged, isoform-specific differences in quantity and localization were observed. Sex-dependent variations in localization and quantity were also observed. Our findings provide new evidence regarding the relationship between Cxs-based communication and the pathophysiological process associated with aging.

Keywords: Connexins, Gap Junction, Aging, Retina

G09 - 316 - P

Connexin43 modulates mitochondrial biogenesis and function through metabolic regulation in BRAF-mutated melanoma

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Mutations in the oncogenic protein kinase BRAF are involved in the onset and progression of several tumours, including more than 50% of melanoma cases and around 8% of all cancers worldwide. Currently, BRAF/MEK inhibitors (BRAF/MEKi) are the standard therapy for BRAF-mutated melanoma. However, development of drug resistance urgently calls for the discovery of new therapeutic targets. Our group recently found that the channel protein connexin43 (Cx43) has an anti-tumour effect in BRAF-mutated tumours enhancing BRAF/MEKi efficacy. Cx43 co-localises at ER as well as in the mitochondrial membranes and regulates mitochondrial membrane permeability in cardiomyocytes, suggesting its role as a metabolic regulator. We therefore investigated the impact of Cx43 on mitochondrial function and cellular responses in BRAF-mutant melanomas. We show that overexpression of Cx43 in BRAF-mutated melanoma models leads to increased mitochondrial biogenesis and oxidative phosphorylation (OXPHOS) capacity, thereby promoting metabolic plasticity and improved adaptability in glucose-free environments. Our results suggest that this increase in mitochondrial biogenesis may be due to either an ER-mediated upregulation of the unfolded protein response (UPR) and/or dysregulation of mTORC1 identifying Cx43 as a novel modulator of mitochondrial activity. Although further research is necessary to elucidate the role and mechanism of action of Cx43 as a modulator of mitochondrial, stress and metabolic dynamics in melanoma our data points that

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prevention of mitochondrial dysfunction in BRAF-mutated cancers might act as a good therapeutic co-adjuvant.

Keywords: Connexin43, BRAF-Mutant, Mitochondria

G09 - 323 - O

Mitochondrial strategies to extend healthspan: lessons from the oocyte

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Oocytes form before birth and remain viable for several decades before fertilization. Although poor oocyte quality accounts for most female fertility problems, little is known about how oocytes maintain cellular fitness, or why their quality eventually declines with age. We found that *Xenopus* and human dormant oocytes employ unique strategies to preserve their mitochondria and cytosol in pristine conditions for many years. Combining live-cell imaging, proteomics and biochemical assays, we discovered that dormant oocytes evade ROS by remodeling the mitochondrial electron transport chain through elimination of mitochondrial complex I, which was thought to be essential for any physiological animal cell. This is accompanied by a highly active mitochondrial proteostatic response, which may help preserve the fitness of the mitochondrial proteome for their many years of dormancy. Now, we are investigating the mechanisms of such adaptations, as they represent evolutionarily conserved strategies that allows longevity while maintaining mitochondrial biological activity in long-lived oocytes.

Keywords: Mitochondria, ROS, Dormant Oocytes, Complex I

G09 - 330 - O

Deciphering Differential Sensitivity to Mitochondrial Dysfunction Across Brain Cell Types

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Deficiencies in the electron transport chain (ETC) give rise to a diverse spectrum of genetically inherited disorders collectively known as mitochondrial diseases. Despite mutations being present throughout the entire organism, not every cell type or tissue is equally sensitive to ETC disruption, and the molecular mechanisms underlying this differential sensitivity are poorly understood. In this study, we have uncovered that, upon ETC inhibition, a non-canonical tricarboxylic acid (TCA) cycle is upregulated to maintain malate levels and concomitant production of NADPH. We found that Pyruvate carboxylase (PC) and Malic Enzyme 1 (ME1), the key mediators of this metabolic reprogramming, are selectively expressed in astrocytes compared to neurons, underlining their differential sensitivity to ETC inhibition. Morevover, our data demonstrate that the observed phenotype in these brain cells upon ETC inhibition is specifically attributed to a decline in NADPH levels, rather than ATP. Strikingly, augmenting ME1 levels in the brain suppresses neuroinflammation and corrects motor function and coordination in a preclinical mouse model of CI deficiency. These studies provide a cohesive explanation for the differential sensitivities of distinct types of brain cells to ETC inhibition, which might have profound implications in the management of mitochondrial diseases.

Keywords: Mitochondria, NADPH, Malic Enzyme 1, Mitochondrial Dysfunction, Astrocytes, Neurons

G09 - 358 - O

Finding the missing pieces in mitochondrial RNA processing

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The biogenesis of the oxidative phosphorylation (OXPHOS) system requires the correct expression of the genes encoded in the mitochondrial genome, a process aided by an array of nuclear encoded proteins, which are responsible for the transcription, processing and maturation of the mitochondrial RNAs. During canonical mitochondrial RNA processing, tRNAs are recognised and cleaved by nucleases to release the flanking transcripts. However, not all mitochondrial transcripts are punctuated by tRNAs, and their mode of processing has remained unsolved. Using Drosophila and mouse models, we demonstrated that processing non-canonical RNAs results in the formation of 3' phosphates, and that phosphatase activity by the CCR4 domain-containing family member ANGEL2 is required for their hydrolysis so that the mRNAs to proceed to polyadenylation. Our results have shed light into mitochondrial RNA processing and have identified a new essential player for the biogenesis of the OXPHOS system.

Keywords: Mitochondria, OXPHOS, RNA Processing

G10: Muerte Celular e Inflamación

G10 - 32 - P

Combined intravenous administration of poly(I:C)+resiquimod reprograms tumor associated macrophages in solid tumors and prevents metastasis

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Introduction

Tumor associated macrophages (TAMs) are key drivers of immunosuppression in solid tumors and support cancer progression, metastasis and recurrence after treatment. Poly(I:C) (pIC) and Resiquimod (R848) are agonists of toll-like receptors (TLR3, TLR7/TLR8, respectively). We have recently demonstrated the activation of STAT1 and TAM reprograming by the poly(I:C)+R848 combination. Now, we investigated the synergistic antitumoral activity of poly(I:C)+R848 intravenously administered in several preclinical models of solid tumors.

Results

The intravenous administration of the poly(I:C)+R848 combination significantly reduced the growth of primary tumors, evaluated in orthotopic models of lung cancer (CMT167), fibrosarcoma (MN/MCA), breast cancer (4T1) and glioma

(GL261). Treated mice presented an increase in circulating pro-inflammatory cytokines, such as TNF-alfa and IL-6, evaluated by Luminex. By multispectral immunophenotyping analysis, we observed that the tumor microenvironment of treated mice presented higher infiltration of macrophages showing their reprogramming towards an M1-antitumoral phenotype, characterized by increase in CD86 and decrease in Arginase1. TAM reprogramming was validated by PCR analysis of primary tumors. Furthermore, in the orthotopic lung cancer model, a significant reduction of CD206 was observed in the interstitial macrophages. Notably, lung metastasis (quantified by Bouin fixation or H&E staining) was prevented by i.v. poly(I:C)+R848 treatment both in the fibrosarcoma and in the breast cancer models.

Conclusions

Intravenous administration of poly(I:C)+R848 showed the capacity to reprogram TAMs to reduce tumor progression and prevent metastasis in a variety of pre-clinical murine cancer models.

Keywords: Cancer Immunotherapy, Toll Like Receptors, Tumor Associated Macrophages, Tumor Microenvironment

G10 - 33 - O

Combined inhibition of STAT3 and TGF-β reprograms tumor associated macrophages, unleashing effective antitumoral activity in lung and pancreatic cancer models

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Introduction

We evaluated the signalling pathways activated by the combination of Stattic, a STAT3 inhibitor, with Galunisertib, a TGF-β inhibitor, with a particular focus on their action on cancer cells and tumor-associated macrophages (TAMs), *in vitro* and *in vivo*.

Results

In vitro, using CMT167 lung cancer cells, Galunisertib inhibited the TGF-β-induced EMT process, by increase in epithelial markers and decrease of TGF-β-induced mesenchymal markers, while no effect was observed for Stattic. RAW264.7 or HMDMs treated with Stattic and/or Galunisertib reduced the expression of M2 markers (i.e. CCL2). BMDMs treated with Stattic presented increased cytotoxic activity towards CMT167 cells, while no effect was observed for Galunisertib. *In vivo*, the intratumoral synergistic combination of Stattic+Galunisertib decreased tumor growth in lung and pancreatic cancer models. Experiments performed in IFN-γ KO mice and immune deficient mice (NSG and Balb/c nude) revealed that a fully functional immune system is crucial for the antitumoral response. Analysis of the tumor microenvironment showed a decrease in STAT3 and SMAD2 phosphorylation, and increased activity of CD4 and CD8 T cells. Furthermore, combination of Stattic+Galunisertib with the immunostimulatory molecule Resiquimod (R848) enhanced the antitumoral efficacy. Nanoemulsions with a PEGylated surfactant, encapsulating Stattic+Galunisertib+Resiquimod showed faster antitumoral activity *versus* the free drugs, and lead to tumor eradication in some cases.

Conclusions

Our data reveal the antitumoral efficacy and reprogramming of the tumor microenvironment achieved by the combined use of Stattic and Galunisertib, which can be further enhanced by the addition of R848, and the use of nanoemulsions for the loading of these drugs.

Keywords: Tumor Associated Macrophages, Tumor Microenvironment, Immunosuppression, Combination Therapy

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Reprograming macrophages through autophagic modulators to reduce inflammatory phenotypes

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Chronic and excessive alcohol intake causes aberrant immune activation, which leads to diseases like alcoholic steatohepatitis, with a high mortality rate. Previous research indicates that alcoholic steatohepatitis is characterized by an abundance of classically activated macrophages (M1/pro-inflammatory) which show less autophagic activity and they accumulate lipid droplets (LDs). The less abundant alternative activated macrophages (M2/anti-inflammatory) show autophagic activation and few LDs. We hypothesize that boosting lipophagy in M1 macrophages will promote M2 activation, reducing alcoholic steatohepatitis symptoms. Our objectives are to elucidate the role of lipophagy in activated macrophages and the effect of enhanced lipophagy to improve the inflammatory phenotype. We used *in vitro* models to study macrophage polarization and boost lipophagy for reprograming macrophages. Then, we evaluated lipophagy inducers and global autophagy machinery using immunofluorescence and western blot. We observed a decreased colocalization between Lamp1 (lysosome) and the lipid droplet and an accumulation of LDs in M1 macrophages, suggesting impaired lipophagy. However, M2 presented elevated colocalization, indicating lipophagic activation. Additionally, western-blot and qPCR analysis showed M1 and M2 classical markers described in the literature. Then, we modulate autophagy and evaluate macrophages polarization. Finally, alcoholic steatohepatitis mice with increased lipophagy showed an improvement in their symptoms. Lipophagy is impaired in M1 macrophages respect to M2 ones and the autophagic modulation could switch macrophages activation. Thus, modulation of lipophagy seems a promising novel tool to ameliorate alcoholic steatohepatitis inflammation.

Keywords: Macrophages, Autophagy, Inflammation

G10 - 86 - P

Role of FSP1 in cancer cells ferroptosis resistance

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Ferroptosis is a type of cell death triggered by iron accumulation and lipid peroxidation. Many pathways by which the cell defends itself against ferroptosis have been identified by researchers. Among these is the system Xc^- which is an amino acid antiporter which usually mediates the exchange of extracellular cystine and intracellular glutamate across plasma membrane. Cystine enters the cell, it is reduced to cysteine and used to synthesize GSH. GPX4 can use tripeptide GSH as a cofactor to detoxify lipid hydroperoxides formed during oxidative stress. Ferroptosis suppressor protein 1 (FSP1) has been verified as a ferroptosis suppressor and catalyzes the regeneration of coenzyme Q10 (CoQ10) with an important antioxidant role, using NADPH. Inhibition of FSP1 strongly synergized with GPX4 inhibitors to trigger ferroptosis in various cancers. The FSP1-CoQ10 pathway cooperates with GPX4 and glutathione to suppress phospholipid peroxidation and ferroptosis. RAS-selective lethal 3 (*RSL3*) is a ferroptosis inducer that inhibits GPX4 enzyme. In this work, we investigated the role of FSP1 in ferroptotic ovarian cancer cells. First, we successfully induced ferroptosis in the SKVO3 ovarian cancer cell line using *RSL3*. Crystal violet staining technique was used to ensure our results. Moreover, Western Blot analysis was done for ferroptotic cells induced by *RSL3*, and results showed a significant increase in the level of FSP1 in these cells compared to untreated cells. Our findings demonstrate that FSP1 remains active even when GPX4 is inhibited, protecting cells from ferroptosis independently of glutathione levels and GPX4 activity. In conclusion, these results align with previous studies suggesting that FSP1 plays a crucial role in ferroptosis resistance in ovarian cancer cells.

Keywords: FSP1, Ovarian Cancer, Ferroptosis Resistance

G10 - 92 - P

p53: A key modulator of neuroinflammation induced by amyloid-beta

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive decline, whose pathological hallmarks are amyloid-β (Aβ) and Tau protein aggregates. The pathogenesis is not limited to neurons, being neuroinflammation an early event in AD. Here, microglia is the major component involved in this process, contributing to disease progression.

p53 has been postulated as a microglial modulator. Also, we described that p53 regulates Aβ-induced neurodegeneration *in vivo*. Here, we evaluate the effect of p53 in Aβ-induced neuroinflammation and its impact on neuronal and cognitive status.

$A\beta_{25-35}$ oligomers (9 nmol) was stereotaxically injected into the right ventricle of WT and p53KO mice. Some animals were intraperitoneally treated with the p53 transcriptional activity inhibitor, pifithrin-α (2 mg/kg). Also, primary neuronal cultures were incubated with conditioned medium from primary microglia previously treated with Aβ (10 μM). Neuroinflammation and neurodegeneration were assessed *in vivo* and *in vitro*. Cognitive status was tested 5 days post-injection.

Aβ caused early p53 accumulation in microglia. Thus, p53 mediated neuroinflammation, inducing an early proinflammatory profile that evolved into an antiinflammatory state, at day 5. This led to neurodegeneration and memory loss, which were prevented by p53 ablation. Besides, p53 controlled microglial function *in vitro*, which regulated neuronal susceptibility to Aβ through neuronal p53 stabilization.

Our findings highlight a key role for p53 in Aβ-induced neuroinflammation, contributing to neurodegeneration in AD.

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Keywords: Neuroinflammation, Alzheimer'S Disease, P53, Amyloid-Beta, Microglia

G10 - 146 - P

Forced weaning versus physiological weaning: implication in breast cancer

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Durante el embarazo, la glándula mamaria se prepara para la lactancia sufriendo una serie de cambios morfológicos y fisiológicos para transformarse en un órgano maduro y funcional. Una vez acabado el periodo de lactancia, la glándula mamaria involuciona para volver al punto de partida o punto de reposo no lactante. En la involución poslactancia, se produce la muerte de las células alveolares mamarias con el fin de permitir la regresión de la mama a través de una respuesta inflamatoria, donde aumenta el reclutamiento de macrófagos y la expresión de genes proinflamatorios. Se ha propuesto este ambiente inflamatorio como uno de los principales factores favorecedores del incremento a largo plazo del riesgo de cáncer de mama asociado al embarazo. Aunque se ha documentado el efecto protector de una lactancia prolongada frente al desarrollo de cáncer de mama, una lactancia corta y destete abrupto favorecerían la formación de un ambiente inflamatorio protumoral. OBJETIVO: Estudiar posibles diferencias morfológicas y moleculares de la glándula mamaria murina tras una lactancia corta por destete forzado o larga por destete espontáneo de las crías que pueda confirmar la hipótesis anterior. El análisis y caracterización exhaustiva de la mama mostró que aunque en ambos modelos se produce un aumento de la respuesta proinflamatoria, está era más moderada tras el destete espontáneo que tras el destete forzado de las crías. La posible relación entre el modelo de lactancia reducida de la sociedad occidental y el riesgo de cáncer de mama asociado al embarazo, pone de manifiesto la importancia de este estudio.

Keywords: Lactancia, Destete, Inflamación, Glándula Mamaria

G10 - 151 - P

Autophagy pathways in endothelial cells as modulators of the inflammatory response

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Endothelial cells (ECs) line the inner surface of all vascular walls and as such form a vital barrier between blood and interstitial tissues. In response to inflammatory insults (e.g. injury or infection), this barrier is reduced, allowing for the selective vascular crossing of bloodborne proteins and circulating immune cells. Key to this function is the ability of ECs to form cell-cell junctions. Our previous work has shown that the homeostatic and degradative pathway autophagy, in ECs, regulates EC barrier function and junctional morphology during acute inflammation *in vivo*. In particular, EC-intrinsic autophagy can modulate the inflammatory response, most notably the recruitment of leukocytes to inflamed tissues and vascular leakage. However, the associated molecular mechanisms are not fully understood and require further exploration. Interestingly, we unveil that, non-canonical forms of autophagy, which until now have been only described in immune and epithelial cells, operate in ECs *in vitro* and *in vivo*. Using Human Umbilical Vein Endothelial Cells (HUVECs), pharmacological inhibition, and genetic ablation of different autophagy components, we reveal that inflammatory stimuli such as IL-1β, TNFα, IFNγ or LPS impact EC autophagy pathways. Moreover, using HUVEC-immune cell co-cultures and autophagy reporter mice (expressing the autophagy marker LC3 fused to GFP; GFP-LC3-Tg), we explore whether the regulation of EC autophagy during inflammation depends on the crosstalk between immune and vascular cells.

Keywords: Inflammation, Autophagy, Endothelial Cells, HUVECs

G10 - 182 - P

Novel Titanocene Y derivative with albumin affinity exhibits improved anticancer activity against platinum resistant cells

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La actividad antitumoral de los compuestos derivados de titanio ha sido previamente demostrada en el tratamiento del cáncer, en particular en tumores resistentes al cisplatino. Sin embargo, su falta de estabilidad y solubilidad dificultan su uso en la práctica clínica. En este trabajo, hemos diseñado y sintetizado un nuevo complejo de titanio (Myr-TiY), que contiene un fragmento de titanoceno unido a un ligando dicarboxilato tridentado para mejorar su estabilidad en agua y, una cadena alifática que facilita la interacción no covalente del compuesto con albúmina. Mediante diferentes técnicas, demostramos una alta estabilidad del complejo Myr-TiY en tampón salino fosfato y, una fuerte interacción con la albúmina sérica humana gracias a la presencia de la cadena alquílica mirística. También se observó la interacción de Myr-TiY con el ADN a través de los oxígenos del grupo éter. En cuanto al perfil de citotoxicidad, Myr-TiY exhibió una alta actividad anticancerígena en líneas celulares de cáncer de ovario y pulmón resistentes a cisplatino, mostrando una IC50 más baja que el titanoceno Y. Además, el compuesto Myr-TiY demostró una potencial selectividad tumoral cuando se analizó en líneas celulares no tumorales. Finalmente, Myr-TiY mostró un efecto antiproliferativo sobre las células tumorales y promovió la apoptosis, lo que concuerda con su capacidad de unión al ADN. En conclusión, la estrategia de síntesis propuesta no solo aumenta el efecto antitumoral de los compuestos derivados de titanoceno, sino que contribuye a aumentar su vida media en el torrente sanguíneo y mejorar su llegada al microambiente del tumor. Todo esto convierte al nuevo compuesto Myr-TiY

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en un candidato firme para continuar con más estudios sobre su potencial terapéutico *in vitro* e *in vivo*.

Keywords: Cancer, Titanium Complex, Albumin, Chemotherapy

G10 - 187 - P

New titanocene-derived compounds with different albumin affinity as alternative therapy against cisplatin-resistant cancer cells

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Los compuestos derivados de titanio suponen una nueva estrategia terapéutica para el tratamiento de pacientes con cáncer, especialmente aquellos resistentes a cisplatino. Las limitaciones de algunos de estos compuestos en cuanto a estabilidad y solubilidad han frenado su desarrollo. En este trabajo, hemos sintetizado una familia de complejos derivados de titanoceno, caracterizados por un ligando dicarboxilato tridentado y una cadena alifática de longitud variable que facilita su interacción no covalente con albúmina, proteína importante en el transporte de compuestos poco solubles.

La estabilidad de todos los complejos sintetizados fue demostrada por RMN. Los estudios de afinidad por albúmina sérica humana han mostrado una relación directa entre la longitud de la cadena alifática y la afinidad por la proteína, siendo mayor para los complejos con mayor longitud de cadena (Ste-Ti y Ole-Ti). En paralelo, Ste-Ti y Ole-Ti demostraron una mayor actividad citotóxica tanto en células tumorales, incluídas aquellas resistentes a cisplatino, como no tumorales, evidenciando una relación entre dicha afinidad y su actividad antitumoral. Para ahondar en esta relación, hemos comparado los efectos celulares de los derivados de titanoceno Myr-Ti (14C) y Ole-Ti (18C), observándose un mayor efecto proapoptótico y antiproliferativo para el compuesto de mayor longitud de cadena. Asimismo, la capacidad de producir especies reactivas de oxígeno, mecanismo inductor de la apoptosis, fue significativa en el caso del complejo Ole-Ti, no siendo evidente a las mismas concentraciones en células tumorales tratadas con su homólogo de cadena alifática más corta Myr-Ti. Nuestros resultados convierten al complejo Ole-Ti en un buen candidato para profundizar en su potencial terapéutico *in vitro* e *in vivo*.

Keywords: Cancer, Titanocene-Derived, Albumin, Chemotherapy

G10 - 189 - P

Generation of an *in vitro* model of ferroptosis resistance in epithelial ovarian cancer cells

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The low survival rate and limited therapeutic options for epithelial ovarian cancer (EOC) necessitate the exploration of new treatments. Inducing ferroptosis, a type of non-apoptotic regulated cell death, is a promising approach due to EOC cell characteristics, such as the overexpression of genes related to ferroptosis regulation and abnormal iron metabolism. Despite its potential, ferroptosis is a recently discovered process that requires further study.

This research aimed to generate and characterize ferroptosis-resistant EOC cell lines. Initially, ferroptosis was induced to observe its impact on cell proliferation and to characterize various parameters (Fe2+, MDA, and ROS levels). To understand the behavior of ferroptosis-resistant cells, the study employed silencing techniques (siRNA and Crispr/Cas9) to target GPX4, a key ferroptosis regulator.

The findings revealed that unmodified EOC cells underwent ferroptosis upon treatment with the ferroptosis inducer RSL3, evidenced by decreased proliferation and increased ferroptosis markers (Fe2+, MDA, and ROS). However, siRNA-treated cells did not exhibit significant changes in proliferation or ferroptosis markers when exposed to RSL3. Additionally, Crispr/Cas9 failed to achieve complete *GPX4* knockout, underscoring the critical role of GPX4 in cell survival.

These results suggest that inducing ferroptosis could be a viable treatment strategy for EOC, potentially reducing tumor cell proliferation. Future research should focus on further elucidating the mechanisms of ferroptosis and exploring new treatments, including novel inducers and other regulatory targets.

Keywords: Ferroptosis, Epithelial Ovarian Cancer (EOC), Cell Death, GPX4, SiRNA, Knockdown, Knockout, Iron

G10 - 213 - O

Identification of a novel ASC-dependent inflammasome inhibitor

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Inflammasomes are cytosolic multiprotein complexes within the immune system which detect harmful stimuli and trigger inflammatory responses. These structures play a crucial role in host defence by activating caspase-1, which in turn processes pro-inflammatory cytokines such as interleukin (IL)-1β into its active forms, and Gasdermin-D thus leading pyroptotic cell death. Dysregulation of inflammasomes have been implicated not only in several autoimmune and inflammatory diseases, but has also extended its impact to cancer. In the current study we present a new multi-inflammasome inhibitor QM372, which prevented the secretion of IL-1β and pyroptosis in macrophages cell lines and in an *in vivo* mice model of peritonitis by affecting ASC oligomerization. Consequently, it provides a valuable tool for exploring the modulation of inflammasomes across various disease contexts.

Keywords: Inflammation, Inflammasome, Drug Discovery.

G10 - 219 - P

Bioavailable bread melanoidins: Neuroprotection Against Hypoxia in SH-SY5Y Cells

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Melanoidins, formed from the Maillard reaction during bread baking, have potential health benefits due to their antioxidant and anti-inflammatory properties. However, their effects on neuronal cells under oxidative stress such as hypoxia are not fully understood. Hypoxia can lead to cellular damage, especially in neuronal cells and is commonly associated with neurological disorders, including Alzheimer's and Parkinson's diseases. The ability to mitigate the detrimental effects of hypoxia could have profound therapeutic implications. Previous studies have indicated that melanoidins can exert protective effects against oxidative stress, a key factor in cellular damage during hypoxia. This study investigates the protective effect of bioavailable melanoidins from bread on differentiated SH-SY5Y neuronal cells exposed to hypoxia. Cells were treated with bioavailable melanoidins and exposed to hypoxia to simulate an oxidative stress environment. Measurement of oxidative stress, apoptosis-associated speck-like protein (ASC), caspase, cell viability and gene expression were performed. The results showed a significant protective effect of melanoidins on SH-SY5Y cells exposed to hypoxia. Specifically, ASC and caspase were reduced in cells treated with melanoidins. Furthermore, cell viability was notably higher and a positive modulation of protective genes was observed. These findings demonstrate that melanoidins from bread can significantly protect neuronal cells from hypoxia-induced damage by reducing oxidative stress, inhibiting apoptosis, and promoting cell survival. The findings suggest potential applications in developing functional foods or supplements that incorporate melanoidins to support brain health. The authors thank to MICIU and ERDF (TED2021-132195B-I00)

Keywords: SH-SY5Y, Melanoidins, Hypoxia

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G10 - 220 - P

Innovative lung cancer therapy: disrupting MCL1-BOK interaction with MBoIN179

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Lung cancer remains the leading cause of cancer-related deaths in men and the second in women worldwide. Despite treatment advances, survival rates for non-small cell lung cancer (NSCLC) are dismally low, highlighting the need for novel therapies. Our study focuses on the roles of the anti-apoptotic protein MCL1 and the pro-apoptotic protein BOK in lung cancer, aiming to exploit their interactions for therapeutic gain.

We analyzed the expression profiles of BOK and MCL1 across various lung cancer cell lines, revealing significant insights into their interplay. BOK's role in lung cancer is controversial, with studies suggesting both tumor-suppressive and tumor-promoting functions. Our research emphasizes BOK's potential as a therapeutic target. We introduced MBoIN179, a novel small molecule that disrupts the MCL1-BOK transmembrane interaction, freeing BOK to selectively induce cancer cell death.

MCL1 overexpression is common in lung cancer, contributing to metastasis and therapy resistance. Traditional MCL1 inhibitors target its cytosolic domain but are limited by cardiotoxicity due to BAK release. In contrast, MBoIN179 targets the transmembrane domain, minimizing cardiotoxicity by releasing BOK, which is low in cardiomyocytes.

This innovative approach targeting the MCL1-BOK interaction offers a promising new avenue for lung cancer therapy, addressing the urgent need for more effective treatments.

Keywords: Cell Death, BOK, Lung Cancer, Mitochondrial Pore

G10 - 221 - P

Elucidating BOK's Role in Mitochondrial Pore Formation: Implications for Cancer Therapy

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BCL2-related ovarian killer (BOK) emerges as a key player in cancer biology, exhibiting intricate roles in tumorigenesis and apoptosis regulation. Recent studies highlight its potential as a therapeutic target, particularly in lung cancer. Understanding the molecular mechanisms underlying BOK's action is crucial for devising effective cancer therapies. One notable aspect of BOK's function is its ability to form pores in the mitochondrial outer membrane, leading to cell death induction.

In our laboratory, we have recently characterized the interaction between MCL1 and BOK through the transmembrane domain. Additionally, we have discovered a first in class inhibitor of MCL1/BOK interaction, MBoIN179. This inhibitor has demonstrated the ability to induce cell death in tumor cells by releasing BOK, which subsequently initiates pore formation in the mitochondrial outer membrane.

In this work we show in liposome permeabilization assays, utilizing calcein-loaded large unilamellar vesicles (LUVs), that MBoIN179 does not affect LUV structure, validating the specificity of its action and we will study the role of BOK.

Moreover, we set up the conditions to purify the transmembrane protein BOK. By overexpressing BOK in Escherichia coli, we have the tool to elucidate its pore-forming capabilities in a controlled environment, laying the groundwork for understanding its mechanism of action.

We expect that our findings shed light on the dynamics of BOK-mediated pore formation and its modulation by MBoIN179, offering insights into cancer therapeutics. By elucidating the mechanisms underlying BOK's function, this research paves the way for the development of innovative cancer therapies targeting BCL-2 family proteins.

Keywords: Bok, Cell Death, Cancer, Mitochondrial Pore

G10 - 224 - O

Endoglin (CD105) overxpression drives immunosuppressive conditions in the tumor microenvironment of mice

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Tumors can be classified based on their microenvironment as hot or cold. Hot tumors contain a higher number of antitumor inflammatory cells and have a better prognosis. In contrast, cold tumors exhibit hypoxic conditions that favor the survival of aggressive malignant cells and contain more protumor inflammatory cells, resulting in a poorer response to therapies and a worse prognosis. Traditionally, tumors with high levels of endoglin have been associated with a worse prognosis, thought to be due to increased tumor growth driven by its proangiogenic properties. However, in a previous study we demonstrated that tumors developed in human endoglin-overexpressing mice (*ENG*+) do not grow larger or have more blood vessels, but that these do not mature correctly, leading to poor perfusion and a greater appearance of metastases.

Here, we demonstrate through flow cytometry and immunofluorescence that xenograft tumors in *ENG*+ mice exhibit greater hypoxia and higher recruitment of protumor cells, such as Treg lymphocytes, M2 macrophages, and myeloid-derived suppressor cells (MDSCs), compared to wild type (WT) mice. These tumors also express higher levels of protumor cytokines and chemokines, including IL-6, IL-12a, CXCL12, and Cox2. On the contrary, *ENG*+ tumors have fewer antitumor cells such as CD8+ cytotoxic lymphocytes. These findings were also confirmed in an orthotopic lung cancer model, although leukocyte infiltration in these tumors in both WT and *ENG*+ mice was much lower.

Therefore, we conclude that the worse prognosis of tumors with high endoglin levels could be due to the development of metastases as well as to the cold phenotype of their microenvironment that allows the most aggressive malignant cells to survive.

Keywords: Endoglin, Tumor Microenvironment, Cold Tumor, Hipoxia, Angiogenesis

G10 - 335 - P

Mining immunomodulatory myeloid programs in cancer

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Recent advances in tumor immunology have uncovered a tremendous complexity of myeloid cellular states. However, before trying to design therapeutic strategies targeting these pathogenic states, we need a deeper understanding of the biology of myeloid cells in solid tumor microenvironments by functional means.

As myeloid cells represent the most abundant and diverse compartment across all leukocytes, our goal is to identify and manipulate innate leukocytes as a first approach to harness anti-tumor immunity.

In this talk, I will cover the latest advances from my laboratory, in which we particularly focus: *(1)* on the temporal timing of myeloid-focused immunotherapies; *(2)* the identification of novel innate biomarkers in lung metastasis, and *(3)* how we envision cancer evolution, taking advantage of molecular tools to trace and map clonal co-evolution between tumors and the immune microenvironment at metastatic sites.

Keywords: Myeloid Cells, Solid Tumors, Heterogeneity

G10 - 337 - O

Mitochondria as inducer of inflammasome-mediated neuroinflammation in Alzheimer's disease

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Evidence indicates that the perpetuation of glial activation, resulting in sustained production of pro-inflammatory cytokines, plays a critical role in the pathogenesis of Alzheimer's disease (AD). However, the exact mechanisms leading to chronic neuroinflammation remain unrevealed. In the present study, we evaluated how dysfunctional mitochondria, an early event in AD, can regulate innate immune

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signaling. Using cell cultures and AD mouse models that combine enhanced amyloid beta synthesis with high intracellular cholesterol levels, we show mitochondria-mediated activation of the NLRP3 inflammasome, with different outcomes in microglia and neurons. In activated microglia, cholesterol enrichment promotes a neuroprotective phenotype, with increased phagocytic capacity and release of neurotrophic factors. By contrast, in cholesterol-enriched neurons, with enhanced mitochondrial oxidative stress, inflammasome assembly results in gasdermin D-mediated pyroptosis. Furthermore, conditioned media of pyroptotic neurons eliminates the increased phagocytic capacity of cholesterol-enriched microglia, thereby indicating that microglia-neuron communication can ultimately modify microglia behavior and perpetuate a vicious cycle that sustains neuroinflammation.

Keywords: Mitochondria, Cholesterol, Inflammasome, Alzheimer'S Disease

G10 - 355 - O

Breaking cancer's defense: overcoming drug resistance by targeting anti-apoptotic mechanisms

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Cell death evasion is a hallmark feature of cancer cells. The altered levels of anti-apoptotic proteins often found in cancer cells aid in escaping the apoptotic program, required for anticancer drugs to exert their cytotoxic effect, thus developing drug resistance. This makes anti-apoptotic proteins an attractive therapeutic target for the development of novel treatment strategies capable of overcoming cancer resistance.

Breast cancer remains the leading cause of cancer-related death in women. Triple-negative breast tumors (TNBC) is particularly aggressive and has the worst prognosis due to their marked heterogeneity, which contributes to the absence of targeted treatments. Additionally, patients often develop high resistance to chemotherapy, highlighting the need for targeted strategies to treat these tumors, especially refractory ones.

Combining computational prediction and Bcl-2 (B-cell lymphoma-2) expression profiling in TNBC patients before and after neoadjuvant treatment, we identified alterations in the expression pattern of Bcl2 and other family members in patient who did not respond to drug treatment. Experimental evaluation of the Bcl2 family members and assessment of sensitivity to BH3 mimetics in TNBC cell lines revealed a particular susceptibility to the pan-Bcl2 inhibitor Obatoclax, especially in chemotherapy-resistant cells. This compound also reduced migration, invasion capacity, and clonogenic potential, inducing apoptosis even in refractory cells. *In vivo*, Obatoclax reduced tumor growth in a xenograft model derived from a taxane-refractory patient and prevented tumor cells dissemination and metastasis. Our data demonstrate that Bcl2 pan inhibition could serve as a promising antitumor approach in chemotherapy-resistant TNBC.

Keywords: Anti-Apoptotic Proteins, Drug Resistance, Targeted-Therapy

G11: Neurobiología molecular

G11 - 37 - P

Calcium-dependent regulation of the neuronal glycine transporter GlyT2 by the hedgehog pathway.

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Hyperekplexia (OMIM 149400) is a syndrome of great perinatal clinical relevance. Neonates suffer energic and sustained startle responses to trivial stimuli, generally tactile or acoustic, which can cause sudden infant death due to apnea episodes. Some mutations in the neuronal glycine transporter GlyT2 gene (SLC6A5) are the second most common cause of human hyperekplexia, being the first one, mutations in the postsynaptic glycine receptor. Although adult patients still suffer disabling motor alterations and recurrent unprotected falls throughout their entire life, there is a partial remission of the hyperekplexia phenotype around the first year of life. This suggests that there is a nervous system developmental component that determines the intensity of the disease. Since hyperekplexia affecting GlyT2 produces more drastic phenotypes than those affecting the postsynaptic glycine receptor, we hypothesized a role for GlyT2 in the development of glycinergic neurotransmission. In this line, we previously demonstrated that the Hedgehog pathway, clearly involved in development, modulates GlyT2 in primary mouse spinal cord neurons causing a drop in GlyT2 expression and function by promoting transporter ubiquitination and degradation. Here, we report that the down regulation of GlyT2 by the smoothened receptor agonist purmorphamine requires calcium efflux from the endoplasmic reticulum as pharmacological inhibition of ryanodine receptors with dantrolene prevents the GlyT2 ubiquitination and degradation caused by the activation of the Hedgehog pathway. The biological meaning of this regulation is being investigated.

Keywords: GlyT2, Hyperekplexia, Neurotransmission, Hedgehog, Calcium

G11 - 57 - P

Zinc and Copper Ions Induce Aggregation of Human β-Crystallins. Implications for Cataracts

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Cataracts are defined as the clouding of the lens due to the formation of insoluble protein aggregates. Metal ions exposure has been recognized as a risk factor in the cataract formation process. The γ and β crystallins are members of a larger family and share several structural features. Several studies have shown that copper and zinc ions induce the formation of γ-crystallins aggregates. However, the interaction of metal ions with β-crystallins, some of the most abundant crystallins in the lens, has not been explored until now. Here, we evaluate the effect of Cu(II) and Zn(II) ions on the aggregation of HβA1, as a representative of the acidic form, and HβB2, as a representative of the basic β-crystallins. We used several biophysical techniques and computational methods to show that Cu(II) and Zn(II) induce aggregation following different pathways. Both metal ions destabilize the proteins and impact protein folding. Copper induced a small conformational change in HβA1, leading to high-molecular-weight light-scattering aggregates, while zinc is more aggressive towards HβB2 and induces a larger conformational change. Our work provides information on the mechanisms of metal-induced aggregation of β-crystallins.

Keywords: Cataracts; Copper; Crystallins; Human Beta Crystallins; Zinc.

G11 - 65 - P

The role of noradrenaline in the development of Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by neuronal damage in motor cortex, brainstem, and spinal cord. Higher levels of noradrenaline have been described in ALS patients, but its impact is certainly unknown. Alpha1-adrenoceptors (ADRA1s) mediate noradrenalines signaling on motoneurons and Ingenuity Pathway Analysis (IPA) software predicts that ADRA1A and ADRA1D participate in ALS. Cell death caused by noradrenaline was proposed in several tissues, but it was not investigated in ALS context. Besides, mitochondrial apoptosis was described in rat brain as a possible mechanism of neuronal death induced by high noradrenaline levels acting on ADRA1s. These findings support a plausible role of noradrenaline overstimulation on ADRA1s in ALS neurodegeneration. However, the effect of high noradrenaline levels acting on ADRA1s in ALS context has not been investigated before. Our unprecedented results showed altered gene expression of ADRA1s and corticosterone receptors *Nr3c1* and *Nr3c2* in SOD1G93A mice specially in brainstem, supporting the relevance of brainstem and the noradrenergic overstimulation in ALS. Besides, inhibition of phenyletanolamine N-methyltransferase (PNMT) enzyme, which catalyze noradrenaline to adrenaline conversion, was suggested to increase noradrenaline levels and cause neuronal dysfunction in mice. Our results indicate for the first time a significant decrease in *Pnmt* gene expression in mutant mice, pointing out PNMT as a new target in the study of ALS. Thus, modulation of noradrenaline signaling using doxazosin, an ADRA1s antagonist, and regulation of noradrenaline levels through overexpression of PNMT could lead to the development of potential therapies for the disease.

Keywords: Noradrenaline, Alpha1-Adrenoceptors, Phenyletanolamine N-Methyltransferase

G11 - 99 - P

PFKFB3 activity is essential for postnatal astrocyte differentiation

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The adult brain consumes 20% of the total glucose availability, while representing only about 2% of the total body weight. In the developing brain, glucose consumption is even higher, reaching around 40% in the early postnatal age, which correlates with a peak in glycolysis. Moreover, neural precursor cells (NPCs) have high glycolytic activity, facilitated by the key regulatory glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphastate-3 (PFKFB3), to meet the demands of rapid proliferation and differentiation. Considering that many neurodevelopmental diseases are associated with impaired glucose metabolism, here we focus on the role of PFKFB3 on brain development.

To this end, C57BL6/J Pfkfb3flx/flx mice were crossed with mice expressing Cre recombinase under the Nestin promoter control, to eliminate PFKFB3 in the nervous system at mid-late gestation (E14.5). We found that PFKFB3 loss impaired neural cell distribution in the brain, after birth. Thus, astrogliogenesis was decreased, but neuron and oligodendrocyte generation were not altered, in PFKFB3 KO mice. Interestingly, astrocyte depletion was maintained in the adult PFKFB3 KO mice, which was accompanied with dendrite disruption and microgliosis. Moreover, different behavioral tasks revealed that PFKFB3 loss caused cognitive decline.

In conclusion, PFKFB3 loss in NPCs impairs brain development and astrocyte number and distribution, which promotes cognitive decline. Our data highlights the key role of PFKFB3 in brain development after birth, which is essential to sustain memory and mice welfare.

Leticia Sancha Ortega is the recipient of a FPU contract from the MICIU.

Keywords: Neurodevelopment, Glucose Metabolism, PFKFB3

G11 - 110 - P

ECM deposition mediated by YAP in neural stem cells regulate quiescence maintenance.

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The formation of new neurons takes place in very specialized domains or niches in the adult mammalian brains, with the subependymal zone (SEZ) being the largest and most active germinal region. The era of transcriptomic and single-cell analyses has led to a well-accepted dogma: neural stem cells (NSCs) coexist in the SEZ in different states of activation: quiescent (q) and activated (a). Transitions between q/a states are dynamic and reversible and are regulated by a wide array of regulatory signals, some derived from the specialized microenvironment in which they reside. One of the niche elements whose role has been largely underestimated is the extracellular matrix (ECM), recently described as having a unique composition and properties compared to other brain regions.

In this work, we set out to elucidate the role of ECM in regulating NSC activation state. We confirmed that NSCs in specific states of activation differentially contribute to and interact with the ECM niche. Furthermore, we developed a bioassay (iQ assay) to study NSC adhesion to the self-generated ECM, demonstrating that adhesion to this unique matrix is sufficient to induce quiescence in active NSCs. In this scenario, we found that Yes-associated protein (YAP), a coactivator of TEAD transcription factors, is required both for matrix deposition and for the transcriptional switch required for quiescence induction in response to adhesion.

Keywords: Neural Stem Cells, Quiescence, Adhesion

G11 - 119 - P

Niche-specific microglia as key regulators of neural stem cell quiescence

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Within the adult mammalian brain, neural stem cells (NSCs), located in the subependymal zone (SEZ), generate millions of neurons, contributing to the olfactory bulb circuits. The SEZ, lining the lateral ventricle wall, forms a specialized microenvironment where NSCs receive signals from the niche, orchestrating transitions among three recently identified distinct states: quiescent (qNSC), primed (pNSC), and activated (aNSC), each characterized by unique molecular identities. While the role of various niche elements in regulating NSC activation has been explored, the contribution of microglia in this context remains obscure. Here,

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we show that SEZ microglia actively induce/sustain NSC quiescence. Additionally, this work reveals distinctive morphological and localization features that set SEZ microglia apart from those in other brain regions. We also unveil previously unrecognized transcriptomic heterogeneity in the adult SEZ microglia under homeostatic conditions. Specifically, our research identifies a microglial state, that we have named 'niche-associated microglia' or NAM, which shares similarities with diverse microglial types identified in different contexts, such as DAM or WAM. Notably, NAM closely interact with NSCs, emphasizing its crucial role in maintaining the quiescent state vital for NSC long-term viability. Using single-cell transcriptomic techniques, we have explored the molecular landscape of microglial signals that could be mediating the observed pro-quiescence effect. Experimental validation of several of the identified candidate signals suggests a complex scenario where microglia might exert multiple and finely tuned effects on NSCs with an overall result leading to the induction/maintenance of NSCs.

Keywords: Neural Stem Cells, Quiescence, Microglia, Neurogenesis

G11 - 127 - O

Metabolic control of adult neural stem cell quiescence

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Adult Neural Stem Cells (NSCs) exist in different states of activation that go from a quiescent non-proliferative (qNSCs) to an actively proliferating (aNSCs) state[1]. Knowing the factors responsible for NSCs activation could be a promising strategy to modulate neurogenesis. One of the factors modulated during NSCs activation is metabolism. The quiescence state relies on glycolysis and fatty acid oxidation, whilst aNSCs are more dependent of oxidative phosphorylation[2,3]. In this work, we studied the role of glycolysis for the induction and maintenance of quiescence in adult NSCs of the subependymal zone (SEZ). To approach this, we have used a conditional knock-out mouse to eliminate 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase-3 (PFKFB3$^{LoxP/LoxP}$), an enzyme that regulates the flux of glycolysis[4], in a cell-specific manner. AdV-mediated delivery of a Cre recombinase to primary cultures of neurospheres from adult PFKFB3$^{LoxP/LoxP}$ mice resulted in a downregulation of glycolysis that rendered NSCs more proliferative. To study the relevance of glycolysis in qNSCs **in vivo**, we electroporated early postnatal PFKFB3$^{LoxP/LoxP}$ mice with episomal non-integrative plasmids, only retained in qNSCs, carrying GFAP-Cre^{ERT2}. Recombination following tamoxifen injection at 2 months of age resulted in an increased number of aNSCs, neuroblasts, and proliferating cells in the SEZ of PFKFB3 knock-down animals. These results indicate that the glycolytic metabolism characteristic of qNSCs is necessary for the maintenance of the quiescent state.

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Keywords: Neural Stem Cells, Quiescence, Metabolism, Glycolysis

G11 - 170 - O

Variant specific mitochondrial responses regulate hypoxia rescue of tRNA mutants independently of HIF

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Mitochondrial diseases (MDs) are metabolic disorders characterized by the disruption of the oxidative phosphorylation (OXPHOS), which can be caused by mutations in both mitochondrial (mtDNA) or nuclear genomes (nDNA). mtDNA is polyploid and several alleles can coexist inside each mitochondrion and cell, in a state called heteroplasmy. Studies on MDs models have recently pointed to the hypoxia and other stress responses like ISR as therapeutic tools for these disorders. However, little is known about the molecular mechanisms responsible for these observations as well as their role in heteroplasmy regulation and OXPHOS biogenesis in humans. Here, by using heteroplasmic mtDNA variants causing mitochondrial translation deffects, we studied the role of these pathways in mitochondrial dysfunction as well as in heteroplasmy regulation. Our results confirmed high complexity in these responses being pathway as well as variant and complex specific. Although further analysis is needed to confirm these effects and describe the molecular mechanism, O_2 sensing pathways play a pivotal role in mtDNA heteroplasmy regulation with impact on mitochondrial proteostasis and metabolic rewiring.

Keywords: Mitochondrial DNA (MtDNA), Mitochondrial Diseases (MDs), Hypoxia

G11 - 209 - P

Discovery and profiling of new genes associated with ALS, first approaches

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Amyotrophic lateral sclerosis is a rare motor neuron disease with no cure or treatment, that significantly impacts the patient lives by severing the connection of these neurons with their innervated muscle, resulting in a complete paralysis of the patient. There have been several environmental and lifestyle-related risk factors that have been linked to the disease, such as exposure to cyanotoxins, smoking habits or even head traumas resulting from a lifetime of sports or sporadic accidents. It has been established that around 90% of the cases are sporadic with the rest being considered familiar cases. Different GWAS studies and bulk/single cell RNA-seq analysis have found thousands of genes associated and differentially expressed to this disease. Although these genes may appear to play individual roles in the pathology, a systems biology approach and the use of biological networks shows an intricate connection between all of them. In this work, by applying a network-based approach to "seed genes" already associated to ALS, new candidates' genes previously unrelated were detected. By testing the transcript levels 10 candidates' genes in the spinal cord and frontal cortex of an ALS mouse model, it was possible to determine transcriptional dysregulation at different stages of disease, with further validations by Western blots of a neuro muscular synaptic gene of promising interest. Future cellullar studies of this and other genes could provide new insights into the pathology, nevertheless this work provides new candidate genes that could play different roles in ALS, which could help in the fight against the disease by expanding our knowledge and allowing us to develop genetic markers, treatments, or preventive measures.

Keywords: ALS, Biological Network, Neurodegeneration, Candidates Genes

G11 - 246 - O

Peptides inhibiting isoforms phospholipase C beta show a potential anti-inflammatory activity

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Phospholipases C (PLC) are a family of signalling proteins which hydrolyze the membrane phospholipid phosphatidylinositol-4,5-biphosphate (PIP_2) into diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3), which trigger a wide range of intracellular events. In particular, isoforms PLCβ play an essential role regulating the activity of ion channels involved in pain transduction, such as the Transient Receptor Potential Vanilloid 1 (TRPV1). PLCβ activation by proinflammatory mediators induces TRPV1 sensitization and increases the excitability of peripheral sensory neurons. Thus, inhibition of PLCβ in inflammatory pain conditions could be an innovative approach to attenuate the activity of dysregulated nociceptors.

Using computational techniques of molecular modelling we have designed small peptides to modulate the activity of isoforms PLCβ3 and PLCβ4. The effect of PLCβ peptides has been validated analysing intracellular calcium fluxes triggered after PLCβ activation by acetylcholine or bradykinin. Our results show a partial inhibition of PLCβ (50%) in the presence of the most potent peptide. The effect of PLCβ inhibition over TRPV1 sensitization has been tested in a culture of neonatal rat dorsal root ganglion neurons with multielectrode arrays. Peptides significantly reduce the firing of action potentials triggered by bradykinin and there is a tendency to attenuate the sensitization of TRPV1.

Finally, the best PLCβ3 peptide has been tested in the CFA-induced inflammatory pain model in mice. Intradermal injection of the peptide in the hindpaw, before administering CFA, reduces the inflammation of the paw and the sensitivity to noxious heat and mechanical stimulation. Our peptides pave the way for the modulation of PLCβ isoforms considered undruggable targets.

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Keywords: Phospholipases C, TRP Channels, Inflammation, Pain, Therapeutic Peptides

G11 - 248 - P

Neuron Type-Specific Transcriptomic Alterations in the Human Parkinsonian Striatum

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by impaired movement and cognitive decline due to the degeneration of dopaminergic neurons in the substantia nigra and the formation of Lewy bodies. The irreversible loss of these dopaminergic neurons, which project to the striatum, leads to significant detriments in motor control and cognitive function. The striatum, considered the main input structure for the basal ganglia system, is crucial for these processes. Therefore, understanding the neuronal diversity in this region, as well as the primary alterations that occur in Parkinson's disease, is essential.

In our study, we employed single-nuclei RNA-seq to characterize the heterogeneity and abundance of the two main neuronal groups in the human striatum: projection neurons, or Medium Spiny Neurons (MSNs), and local neurons, also known as interneurons. Analyzing postmortem samples from the Caudate Nucleus and Putamen (dorsal striatum) in an extensive cohort of PD and control patients, we identified eight main classes of interneurons and six main classes of MSNs, further subdivided into subclasses.

Our investigation revealed that two subclasses of interneurons and three of MSNs were significantly affected by PD, each displaying specific transcriptomic patterns and regional differences. Furthermore, we elucidated the biological implications of these changes and the involved pathways. Subsequently, we validated some of our findings in tissue using the Xenium *in situ* platform, confirming the previously described neuronal alterations.

These results underscore the molecular variations within striatal neuronal populations, exhibiting both cell-type and brain-region specificity. Importantly, they open new avenues for cell-type targeted therapy in PD.

Keywords: Neuron, Parkinson'S Disease, Single-Cell Sequencing, Striatum

G11 - 273 - P

Stimulation of brain striatum GDNF by modulating parvalbumin interneurons intracellular pathways: effect on nigrostriatal dopamine neurodegeneration

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The glial cell line-derived neurotrophic factor (GDNF) is an endogenous protein with well-known neuroprotective properties on the dopamine-producing neurons, which are primarily affected in Parkinson's disease. In the striatum, GDNF is produced by a subset of parvalbumin (PV) gabaergic interneurons at low physiological level. Increasing endogenous GDNF production is a therapeutic target to provide trophic support and protection to dopamine neurons.

From previous data published by our group, we hypothesized that modulating intracellular pathways such as cyclic AMP and beta-catenin may stimulate GDNF synthesis by PV interneurons in the striatum. We tested this on mouse brain slices (*ex vivo*) and *in vivo* mice by targeting: 1/ various phosphodiesterases (PDE), responsible for the breakdown of cAMP, and 2/ glycogen synthase kinase 3 beta (GSK-3β), which is involved in β-catenin phosphorylation and its subsequent degradation. Specific phosphodiesterase inhibitors (blocking PDE2A and PDE4B activities) increase GDNF synthesis in *ex vivo* slices of striatum. However, these pharmacological compounds, supposedly brain penetrant, did not increase GDNF levels in live animals. A broader range PDE inhibitor (Ibudilast) showed robust increase of *Gdnf* gene expression *ex vivo* and *in vivo*. Pharmacological blockade of GSK-3β activity also resulted in striatal GDNF levels rise in both *ex vivo* and live mice. We finally tested Ibudilast on a mouse model of dopamine neurons degeneration. The results are still under analysis, but our data support the view that inhibition of PDEs and GSK-3β are two potential therapeutical approaches to up-regulate striatal GDNF synthesis to provide neuroprotection on dopamine nigrostriatal pathway. Anyhow, further testing of alternative compounds is essential.

Keywords: Phosphodiesterase, GDNF, Parvalbumin Interneuron, Beta-Catenin, Parkinson

G11 - 279 - O

Personalized medicine for ALS by using novel synthetic editing systems - SynCas

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that causes the loss of motor neurons and therefore the loss of voluntary muscle movement. Approximately 15% of ALS cases are associated with inherited mutations in genes such as SOD1, C9orf72, FUS, TARDBP and TBK1, although dozens of ALS-associated genes have been described. In addition, there is a relevant genetic component in non-familial ALS cases. The advent of CRISPR gene editing makes it possible to correct mutations. The need for recognition of a motif adjacent to the target (PAM sequence) or the immune response to natural CRISPR systems are limitations of the natural CRISPR system. The new revolutionary tool for designing CRIPSR Cas proteins. This system is based on the reconstruction of much more flexible and versatile ancestral forms of Cas proteins. We will use a new class of fully synthetic Cas enzymes, created in the CIC BioGUNE lab, which uses the ancestral variants as a base. We intend to generate variants capable of correcting all ALS-associated mutations, in addition to generating a lesser immune response and more efficient editing. We will design a molecular transport system based on messenger RNA and lipid nanoparticles to edit cells from ALS patients. Finally, we will study the genetic bases of non-hereditary ALS to determine the possibility of using our designed CRISPR systems in these cases, later, we will apply our finding to a suitable animal model. The main goal of our Project, to probe an extremely innovative science concept – "gene Therapy through genetic editing" in human patients.

Keywords: ELA, Neurobiología, CRISPR/Cas, Terapia Génica, Neurodegeneración

G11 - 290 - P

Role of Aldehyde Dehydrogenase 1A1 in Parkinson's Disease

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A defining feature of Parkinson's disease (PD) is the impaired function of mitochondrial complex I (MCI) in dopaminergic (DA) neurons located in the substantia nigra pars compacta (SNpc). MCI-Park, a novel mouse model of PD, has yielded significant insights into the metabolic alterations that contribute to the degeneration of the nigrostriatal pathway. This model shows that MCI dysfunction alone is sufficient to induce progressive, human-like parkinsonism, where the reduction in DA release from the SNpc is pivotal in the manifestation of motor dysfunction. These SNpc DA neurons possess a pacemaker feature and a large axonal arbor, suggesting that they need a higher metabolic sustain than other neurons. Aldehyde dehydrogenase 1A1 (ALDH1A1), a protein expressed by DA neurons in the ventral SNpc, has been described as a vulnerability marker of PD, linked to the detoxification of DA metabolites in physiological conditions. In MCI-Park mice, DA neurons expressing ALDH1A1 within the ventral SNpc are more vulnerable than other subset of DA neurons present in the lateral and dorsal areas. Moreover, we observed a puzzling ectopic expression of ALDH1A1 in brain regions of the MCI-Park mouse such as the midbrain and striatum, where SNpc DA neurons project their axon. This expression does not match the DA neuron expression specifically stained with tyrosine hydroxylase (a specific marker for DA neurons). This discrepancy suggests that other cell types, which remain to be determined, could produce ALDH1A1 expression in the degenerative brain. These results highlight the need to decipher the relationship between ALDH1A1 and the vulnerability of DA neurons after mitochondrial dysfunction, as a path to better understand the pathogenesis of PD.

Keywords: Aldehyde Dehydrogenase 1A1, Parkinson'S Disease, Mitochondrial Complex I, Vulnerability

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Deciphering the functional interplay between transcription and replication stress in the cellular hierarchies of Glioblastoma

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Glioblastoma (GBM) represents the most prevalent and lethal primary intrinsic brain tumour. Although radiotherapy is the most effective non-surgical GBM therapy, recurrence is universal, in part due to highly tumorigenic Glioblastoma Stem Cells (GSCs, CD133 + cells) population. Targeting GSCs remains a challenging task because of their unique biology and dependency on vital survival pathways, which have equal importance for the maintenance of normal stem cells and progenitors. Given the high degree of genomic instability, and well-defined cellular hierarchies with therapeutically resistance GSCs at the apex, GBM represents an ideal model for identification and pre-clinical evaluation of putatively druggable targets. Our studies showed concurrently high levels of replication stress and global transcription rates in human primary GSCs population, suggesting that Replication-Transcription Conflicts (RTCs) might be increased, triggering a heightened DNA Damage Response leading to the expansion of aggressive GSC clones underpinning the therapeutic resistance. Hence, players involved in solving RTCs will become targets to sensitize GSCs to therapeutic intervention. In this work, we pointed to Homologous Recombination RAD52 protein as one of the key factors in solving RTCs because it is involved in pathways related to Replication-Transcription Conflicts, such as 1) BIR mediating repair and restarting collapsed DNA replication forks, and 2) Transcription-Associated HR Response (TA-HRR) sensing R-loops and repairing precisely transcriptional active regions. Therefore, our hypothesis points to RAD52 as a critical factor for restarting replication forks and promoting the resolution of persistent R-loops in these RTCs, avoiding GSCs apoptosis and promoting therapeutic resistance.

Keywords: Glioblastoma, Cancer Stem Cells, Transcription, Replication Stress

G11 - 307 - P

The Neuronal Calcium Sensor 1 as a drug target for nervous system pathology: development of protein-protein interaction modulators.

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The Neuronal Calcium Sensor (NCS-1) is a calcium signaling protein implicated in a wide range of functions in the central nervous system. It is involved in learning, memory, neuroprotection and axonal regeneration processes, as well as in pathological conditions, thus constituting an interesting pharmacological target. NCS-1 is an integrator of Ca2+ and G signaling pathways since it recognizes in a selective manner proteins such as the molecular chaperone and guanine exchange factor Ric-8A and the dopamine D2 receptor, both related with neurodevelopmental disorders and neurodegeneration. In the past years we have demonstrated that NCS-1 is a druggable target and it is possible to use small molecules to regulate the interaction with Ric-8A in a therapeutic manner (1, 2, 3). Furthermore, we have recently unveiled the molecular mechanism by which NCS-1 controls Ric-8A activity at atomic level (4). Our lab is also focused in understanding how NCS-1 modulates dopamine D2 receptor (D2R) activity at the molecular level. For this purpose, we have discovered protein-protein interaction modulators of the NCS-1/D2R complex using a drug-repurposing approach and an FDA-approved drug library. The crystal structure of the NCS-1/FDA-drug complexes show the molecular mechanism of action. In addition, we have demonstrated the activity of the compounds in cellular assays. We are currently studying the effect of NCS-1 in dopamine D2 receptor signaling and exploring the therapeutic potential of these compounds.

Keywords: Calcium Sensor, Dopamine Receptor, GPCR Signalling, Drug Development

G11 - 313 - P

Análisis del fluido lagrimal para la determinación de biomarcadores en esclerosis múltiple

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La esclerosis múltiple (EM) es una enfermedad inflamatoria y neurodegenerativa del sistema nervioso central. La búsqueda de biomarcadores no invasivos para la EM es esencial para mejorar el diagnóstico temprano y el seguimiento clínico de la patología. La película lagrimal contiene una variedad de biomoléculas que pueden reflejar cambios patológicos en el cuerpo, además es un fluido biológico fácilmente accesible y no agresivo. Este estudio investigó las diferencias en los perfiles lipídicos y proteicos en las muestras de lágrimas de 14 pacientes con EM y 15 controles sanos. Las lágrimas de los participantes se extrajeron a través de las tiras de Schirmer. Se utilizaron técnicas de cromatografía de líquidos de alto rendimiento (HPLC) y de espectrometría de masas (MALDI) para analizar las muestras. Se llevó a cabo el correspondiente análisis estadístico de los ácidos grasos (AG) C18:1, C18:2 y C20:4; y se examinó la relación que había entre cada uno de ellos con el AG C17:0. También se analizaron los perfiles electroforéticos obtenidos del análisis proteico. Se identificaron diferencias significativas en la composición lipídica y proteica de las lágrimas entre ambos grupos de estudio. Estos hallazgos revelan el potencial de las lágrimas como herramienta de análisis no invasiva para la detección y seguimiento de la EM

Keywords: Esclerosis Múltiple (EM), Biomarcadores Lagrimales, Lipidómica, Proteómica, ácido Araquidónico

G11 - 332 - O

Searching for the origin of the adult neural stem cells

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Neural stem cells (NSCs) in the dentate gyrus (DG) enter quiescence during early postnatal development, before the adult hippocampal neurogenic niche is fully established. However, the mechanisms controlling NSC first quiescence entry and the correct level of quiescence are largely unknown. Using conditional mutant mouse during embryonic or postnatal stages, we have determined that transcription factor Sox5 is required to restrict first entry in quiescence. Moreover, we have found a critical window during the second postnatal week when NSCs build up a shallow or primed quiescent state. Loss of Sox5 leads to an excess of primed NSCs prone to activate, leading to a neurogenic burst in the adult DG and precocious depletion of the NSC pool. Mechanistically, Sox5 prevent an excess of BMP/Smad1/5/9/Id4 activation, which is associated to the primed state in NSCs. In conclusion, our results demonstrate that Sox5 is required to control the correct balance between primed and deep quiescence during the first postnatal weeks of DG development, a balance which is essential for establishing long-lasting adult neurogenesis.

Keywords: Neurogenesis, Quiescence, Neural Stem Cells, Hippocampus, Transcription Factors

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G11 - 362 - O

The Pthlh Interneuron Population: A Critical Cell Type in Parkinson's Disease

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PD is the second most common and fastest-growing neurodegenerative disease, with no cure yet available, and its underlying cellular and molecular mechanisms remain incompletely understood.

Our research aims to unravel the cellular diversity of the striatum, one of the key structures in PD, and elucidate cell type-specific molecular mechanisms. Recently, we identified a highly abundant population of GABAergic interneurons in the striatum that express Pthlh and varying levels of Pvalb that correlate with intrinsic electrophysiological properties (Muñoz-Manchado et al., Cell Reports, 2018). Notably, we have also showed that this interneuron population is among the most abundant in the human caudate (CN) and putamen (Pu), (Garma et al., Nat Com 2024, accepted).

Using snRNA-seq and spatial transcriptomics on postmortem human CN and Pu samples from PD patients and control subjects, we identified the Pthlh population as one of the critical interneuron cell types in PD. We mapped the transcriptomic pathways involved and located them within the tissue.

To further understand the role of this novel interneuron population and its involvement in PD, we combined our recent developed mouse line Pthlhcre::R26R-tdTomato mouse line with parkinsonian mouse models as the classic toxic unilateral 6-OHDA model.

Keywords: Interneuron, Striatum, ScRNA-Seq, Parkinson'S Disease, Basal Ganglia

G12: Parasitología molecular e infecciones emergentes

G12 - 5 - O

West Nile, SARS-CoV-2 and HCV infection and antiviral treatments for emerging virus monitored by multimodal imaging

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Recent advancements in virology have revealed a common feature among positive-strand viruses:the alteration of cellular membranes to form replication complexes. Despite variations in origin and structure,these membranous compartments, classified as double-membrane vesicles (DMVs) or invaginated vesicles (IVs),are observed across diverse viruses, including hepatitis C virus (HCV) and SARS coronavirus. Notably, West Nile virus (WNV) generates IVs, suggesting a conserved strategy across distant viruses

In this study we have performed infrared microscopy, confocal immunofluorescence and correlative cryogenic light-soft X-ray tomography (CLXT) in the water window photon energy range to investigate in whole, unstained cells, the morphology of the membranous rearrangements induced by WN, HCV and SARS-CoV-2 infection and after antiviral treatments in near-native conditions.

These infection alterations could be reverted by combination of different antiviral treatment and monitored the healing process by multimodal imaging techniques. In addition to providing structural insight into cellular aspects of viral pathogenesis, our study illustrates how cryo-SXT is a powerful three-dimensional wide-field imaging tool for the assessment and understanding of complex cellular processes in a setting of near native whole hydrated cells. Our results also constitute a proof of concept for the use of cryo-SXT at ALBA synchrotron and at lab-scale soft X-ray microscope (SXM) as a platform that enables determining the potential impact of candidate compounds on the ultrastructure of the cell that may assist drug development at a preclinical level.

This study was funded by ALBA Synchrotron proposals 2022065884, 202102489920, 23087667; and for the CID EU project and by CLEXM, (EU MSCA-DN),

Keywords: West Nile, SARS-CoV-2, HCV

G12 - 81 - P

Tenofovir attenuates cytokine storm and bronchiolar damage in a mouse model of bleomycin-induced acute lung injury

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Background: SARS-CoV-2 pandemic has converged with the HIV epidemic. Although immunocompromised patients show an elevated risk of death due to COVID-19 compared to HIV infection, the impact remains contradictory. One reason could be the use of antiretroviral therapy (ARV). Patients with HIV receiving ARV, such as tenofovir (TDF), have fewer symptoms of COVID-19. In mice, bleomycin (BLM) induces acute lung injury, resulting in an acute inflammatory response similar to the cytokine storm and lung damage observed in COVID-19. This study aimed to evaluate the preventive role of TDF administration prior to BLM administration.

Methods: Four experimental groups of mice of both sexes (n=8), were used: i) oropharyngeal aspiration of saline (SAL), ii) SAL-TDF in drinking water 7 d before BLM until euthanasia (TDF); iii) oropharyngeal aspiration of BLM (BLM); and iv) BLM-TDF (TDF-BLM). Animals were euthanized 3 d after BLM administration, and serum and lungs were collected for analyses.

Results: BLM-TDF lungs showed significantly reduced bronchioalveolar lavage fluid protein content and total cell counts, specifically reduced macrophages and neutrophils counts, but an increased presence of minority lymphocytes. TDF downregulated BLM-induced levels of *Il1β*, *Il6*, *TNFa*, and *Tgfβ* mRNA. Preventive treatment with TDF counteracted BLM-induced alveolar and bronchiolar cell damage. Male mice showed more severe symptoms after BLM administration for most parameters, and the preventive action of TDF was similar in both sexes.

Conclusions: Preventive TDF administration counteracted BLM-mediated acute lung damage. These data support the epidemiological observations suggesting the potential benefits of TDF as a preventive therapy against COVID-19.

Keywords: Tenofovir (TDF), Bleomycin, ALI, Mouse Model, Cytokine Storm, COVID-19

G12 - 203 - O

Targeting Key Drivers of Pseudomonas aeruginosa Pathogenesis in Cystic Fibrosis Bronchial Epithelia

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In Cystic Fibrosis (CF), CFTR defect leads to abnormal airway surface liquid composition and impaired muco-ciliary clearance in the lungs, promoting the colonization of the respiratory epithelia by *Pseudomonas aeruginosa* (PA). Previous pathogen-host proteomic studies in our laboratory have highlighted the significance of rapid lipid metabolism turnover, aminopeptidase production and biofilm regulation during PA early acute infection stages in CF.

To assess the phenotypic impact of these processes during pathogenesis, we selected 18 bacterial proteins highly upregulated during CF bacterial infection and performed knockout assays *in vivo* using NuLi-1 (healthy bronchial epithelium), CuFi-1 (CF bronchial epithelium), and A549 (non-differentiated alveolar epithelium) cell lines. We evaluated biomass production *in vitro* against the wild-type PAO1 strain, viable bacterial counts *in vivo*, and pathogenic phenotype variations via immunohistochemistry and SEM imaging.

Our results indicate that two lipid-related determinants significantly influence biomass expansion in the fibrotic cell line. Additionally, three porins appear to modulate microcolony formation and alter bacterial distribution during biofilm stages. Notably, one specific protease seems essential for CF infection in mucus environments, presenting a potential novel target for antimicrobial development.

These findings offer insights into PA pathogenicity modulation during CF infection and lay the groundwork for developing new therapeutic options for CF-related lung infections.

Keywords: Cystic Fibrosis, Pseudomonas Aeruginosa, Pathogen - Host, Proteomics, Immunohistochemistry,

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Pathogenic Phenotype, SEM Imaging

G12 - 210 - P

Molecular characterization of 207 bacteraemia-causing Escherichia coli strains from a Spanish hospital isolated between 2020 and 2022

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Antecedentes: En los últimos años se ha observado un aumento de bacteriemias causadas por E. coli patógenos extraintestinales (ExPEC), E. coli uropatogénicos (UPEC) y E. coli patógenos aviares (APEC), junto con un incremento en la resistencia a los antimicrobianos. Nuestro objetivo es caracterizar las cepas pertenecientes a complejos clonales emergentes, buscando entender las razones de su éxito. Determinar las características de estas cepas es vital para diseñar vacunas adaptadas a la epidemiología actual de nuestra área sanitaria.

Materiales y métodos: Utilizamos métodos fenotípicos (serotipado, determinación de CMIs) y moleculares para estudiar 207 cepas de E. coli causantes de bacteriemias en un hospital lucense (HULA) durante los años 2020 a 2022. Determinamos serotipos, grupos filogenéticos y genes de virulencia para establecer los estatus ExPEC, UPEC y APEC.

Resultados: La colección incluye 20 cepas O1, 33 O2, 12 O4, 24 O6, 29 O8, 10 O9, 14 O15, 7 O18, 34 O25, 10 O75 y 14 O101, de las cuales 63 son multirresistentes (MDR). La caracterización molecular muestra que el grupo filogenético más abundante es el B2 (126 cepas), seguido de B1 (21), C (20), A (14) y E (12). En base a los genes de virulencia, 148 cepas son ExPEC, 136 UPEC y 86 APEC. Se detectaron 27 cepas del clon de alto riesgo ST131, 24 ST95 y 8 del nuevo clon emergente ST1193.

Conclusiones: Los resultados confirman que una variedad de serogrupos causan bacteriemias en la población lucense, con un porcentaje significativo de cepas positivas para ExPEC, UPEC y APEC. El 30% son MDR y se describe la presencia del nuevo clon de alto riesgo ST1193.

Keywords: Bacteriemia, E. Coli, Multirresistentes, Serogrupos, Virulencia, ExPEC, UPEC, APEC.

G12 - 217 - O

Inhibition of bacterial conjugation applying cationic Solid Lipid Nanoparticles

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Antimicrobial resistance threatens global health by making existing antibiotics ineffective against resistant pathogens. The primary mechanism for spreading antibiotic resistance genes among bacteria is plasmid-mediated conjugation. Inhibiting conjugative proteins is a viable strategy to mitigate this spread. Solid lipid nanoparticles (SLNs) offer an attractive option to enhance the efficacy and delivery of conjugation inhibitors. This study focused on encapsulating an inhibitory compound within cationic SLNs. Four types of nanoparticles were developed: blank SLNs, SLNs with the compound, SLNs with linoleic acid but without the compound, and SLNs with both linoleic acid and the compound. Linoleic acid was included in some formulations for its known inhibitory effect on bacterial conjugation. Characterization of these nanoparticles involved measuring size, polydispersity, and zeta potential via Dynamic Light Scattering (DLS) and determining encapsulation efficiency through spectrophotometry. Toxicity and conjugation studies assessed the impact of SLNs on bacterial cells using a high-throughput method. The results indicated that the SLNs remained stable for up to two months at 4ºC. SLNs containing linoleic acid, as well as those with both linoleic acid and the compound, significantly reduced plasmid transmission, indicating a notable inhibition of conjugation. This work highlights the potential of SLNs as a promising strategy to combat antibiotic resistance. Further research could explore optimizing these formulations in various bacterial strains and environments.

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Keywords: Cationic Solid Lipid Nanoparticles, SLN, Antimicrobial Resistance, Antibiotics, Bacterial Conjugation, Inhibition.

G12 - 257 - P

Mechanism and dynamics of 3-methylcrotonyl-CoA carboxylase, an essential complex for *Trypanosoma brucei* metabolism

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3-methylcrotonyl-CoA carboxylase (MCC) catalyzes the two-step, biotin-dependent production of 3-methylglutaconyl-CoA, an essential intermediate in leucine catabolism. Given its critical metabolic role, deficiencies in this enzyme associate with organic aciduria, while its overexpression is linked to tumor development. MCC is a dodecameric enzyme composed of six copies of each α- and β-subunit. We present the cryo-EM structure of the endogenous MCC holoenzyme from *Trypanosoma brucei* in a non-filamentous state at 2.4 Å resolution. Biotin is covalently bound to the BCCP domain of α-subunits and positioned in a non-canonical binding pocket near the active site of a neighboring β-subunit dimer. Moreover, flexibility of key residues at α-subunit interfaces and loops enables pivoting of α-subunit trimers to partly reduce the distance between α- and β-subunit active sites, required for MCC catalysis. Our results provide a structural framework to understand the enzymatic mechanism of eukaryotic MCCs and to assist drug discovery against trypanosome infections.

Keywords: 3-Methylcrotonyl-CoA, Trypanosome, Mitochondria, Cryo-EM, Catalytic Mechanism, Biotin, Multimeric Enzyme, Conformational Changes

G12 - 287 - P

Development and characterization of cationic solid lipid nanoparticles for antifungal applications

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Candidiasis is the most common opportunistic mycosis. The WHO has classified some *Candida* species as priority fungal pathogens because they are resistant to the main antifungal agents and cause invasive candidiasis infections with high mortality rates. The drugs used to treat invasive candidiasis have drawbacks such as toxicity and bioavailability, limiting their use. Furthermore, their extensive clinical use and long-term treatments can lead to resistance.

Drug delivery systems offer a therapeutic alternative by serving as drug administration vehicles that enhance drug efficacy, reduce the necessary therapeutic dose and minimize toxicity. Specifically, solid lipid nanoparticles (SLN) stand out for as a highly attractive option due to their numerous advantages, including non-toxic and targeted drug delivery. SLNs loaded with antifungal drugs could be an effective alternative against resistant *Candida* strains.

Citral, a monoterpene composed of neral and geranial, exhibits a range of biological activities, including antibacterial, antifungal, antibiofilm, and antiparasitic properties. However, it presents low water solubility difficulting its systemic administration.

This work focuses on encapsulating different citral amounts within cationic SLNs and characterizing the obtained suspensions. Nanoparticles were characterized by measuring size, polydispersity, zeta potential, encapsulation efficiency, and toxicity. Our results indicate that the obtained SLN suspensions present good characteristics as citral-delivery systems. Overall, this work highlights the potential of citral-SLNs as a promising strategy to combat antimicrobial resistance.

This work was supported by MCIN/AEI/10.13039/501100011033 (PID2020-116495RB-I00) and the Basque Government (IT1578-22 and IT1607-22).

Keywords: Drug Delivery, Solid Lipid Nanoparticles, Candidiasis, Antifungal, Citral

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G12 - 292 - O

Design of a DNA vaccine against *Plasmodium falciparum* malaria with chimeric antigen genes containing IgM epitopes

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Plasmodium falciparum is the most lethal species of malaria parasites. This species causes severe malaria as cerebral malaria, mostly in children, with more than 600,000 annual deaths. Developing countries in Africa account for 95% of the malaria cases and 96% of deaths, of which 80% are in children (WHO). RTS/S is the only licensed vaccine. Other updated formulations are in the pipeline, such as R21/MatrixM, to protect against severe disease in children. These vaccines are partially protective (up to 66% efficacy that waned in 1-5 years) and complex to administer requiring four inoculations in a 18-month period.

A custom immunoproteomics study using high-density overlapping peptide microarrays revealed IgM against immunodominant epitopes recognizing parasite's surface proteins in sera from asymptomatic patients from Ghana and D.R. Congo. After that, chimeric antigens enriched in such protective epitopes were devised by computer-aided structural design. Then, DNA sequences encoding them were cloned in the antibiotic resistance-free plasmid vector pPAL to obtain DNA vaccines. This vector was used for Neoleish®, a DNA vaccine authorized by the European Medicines Agency (EMA/CVMP/858971/2022) to prevent canine leishmaniasis. DNA vaccines are suitable for easy distribution and administration. Hence, it is ideal for malaria vaccines in developing countries. Specific profiles of T and B cell responses, including memory cells, the humoral immune response, and protection against challenge are going to be evaluated. Then, those DNA immunogens inducing better immune response activation and protection are going to be mixed to obtain a multigenic DNA vaccine to be tested in mice and in Macacus rhesus. The ultimate goal is to prevent severe malaria and decrease mortality.

Keywords: Plasmodium Falciparum, DNA Vaccine, IgM

G12 - 327 - O

Multiomics characterization of the translational machinery of Trypanosoma cruzi during metacyclogenesis and cell cycle

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Trypanosoma cruzi (T. cruzi), the etiological agent of Chagas disease, regulates its gene expression mainly by post-transcriptional mechanisms. Our group observed that translational regulation is an important mechanism during metacyclogenesis and G1/S cell cycle transition. Recently, it has been shown that the composition of the ribosomes may be variable at the protein level, leading to regulatory changes. Thus, we set out to perform a detailed characterization of the translational machinery of T. cruzi in the epimastigote and metacyclic trypomastigote life cycle stages, as well as in G1 and S cell cycle phases. First, we reviewed and polished the current annotation of ribosomal proteins (RP), analyzing copy number, location in ribosome, protein extensions at terminal ends, expression values and putative extraribosoma functions. Addressing this characterization in an experimental way, we observed through Ribo-Seq that there is a global repression of RP mRNAs translation in the metacyclic trypomastigote stage, but there is also individual variation, finding some RP mRNAs that resist that repression. We also performed a multiomics approach (RNA-seq, Ribo-Seq and proteomics experiments) in parasites synchronized on G1 and S cell cycle phases. We observed individual variation in the translational efficiency changes of RP mRNAs and also in RP stationary level. To explore this further we performed quantitative proteomics on ribosomes enriched fractions in parasites synchronized in G1 and S cell cycle phases observating interesting variations of ribosome components. These observations obtained so far could be in line with the hypothesis that translational machinery composition may be variable during these transitions.

G12 - 363 - O

Multi-omic data analyses and modelling: developing computational tools to systematise the modelling and optimisation of dynamic systems

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Microbial metabolism plays a crucial role in various aspects of life, from sustaining ecological balance to contributing to industrial processes. Understanding and harnessing microbial metabolism is vital for biotechnology or environmental conservation applications, offering new opportunities for improving human health, advancing bioprocess optimization, and promoting sustainable industrial practices.

In this presentation, we will delve into the potential of computational models to integrate multi-omics data to understand and predict the dynamics of microbial metabolism. We will discuss dynamic knowledge-based and artificial intelligence models, highlighting their pros and cons while emphasizing the importance of understanding their limitations. Furthermore, we will explore how to enhance the resolution of our models by integrating genomics, metabolomics, and transcriptomics data into dynamic genome-scale models. These models, with their ability to provide comprehensive insights into the metabolic pathways leading to different observed phenotypes, have the potential to guide us in optimizing industrial bioprocesses.

To illustrate these concepts, we will present the different modeling approaches with real-life examples related to yeast physiology and metabolism during batch fermentation, a crucial process in industrial biotechnology. For instance, we will show how different yeast species use different pathways to achieve redox balance or how we embed models into digital twins that enable re

Keywords: Dynamic Genome Scale Models, Artificial Intelligence, Yeast Physiology And Metabolism; Systems Biotechnology

G13: Química biológica

G13 - 20 - O

Disrupting a non-canonical G-protein interaction that promotes metastasis. A molecular recognition study

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The α subunits of heterotrimeric guanine nucleotide-binding proteins (G-proteins) participate in signal transduction and in intracellular signaling pathways. When the Gα subunit binds GTP it dissociates from Gβγ and interacts with other proteins downstream the pathway. After hydrolysis, the inactive GDP-bound Gα can reassociate with Gβγ. G-protein-coupled receptors (GPCRs) activate Gα subunits in response to extracellular stimuli by accelerating the exchange of GDP for GTP. Gα subunits can also be activated by intracellular proteins like GIV, of which elevated levels correlate with increased cell migration and cancer metastasis. The small molecule IGGi-11 disrupts the interaction with GIV and inhibits pro-invasive traits of metastatic breast cancer cells. IGGi-11 binds to the same site on Gαi3, as GIV does, but with a 10-fold lower affinity. A high-resolution structure of the complex would help designing derivatives of IGGi-11 with higher affinity. Crystallization of human Gαi3 has only been achieved bound to other proteins, presumably due to its high flexibility. The relative flexibility of the Gαi3 main chain has been measured by NMR and the most flexible residues at the chain ends have been removed to facilitate its crystallization. Mixtures with IGGi-11 yielded crystals that showed the protein and the nucleotide but not IGGi-11. Computational docking filtered by NMR observations suggests that the binding of IGGi-11 is heterogeneous due to the elongated shape of the cavity and the symmetry of IGGi-11 and that chemical modifications breaking its molecular symmetry might yield derivatives with higher affinity.

Keywords: Molecular Recognition, Protein Interaction, G-Protein, Cancer

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G13 - 22 - O

Control of biological processes using metal complexes

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Nucleic acids play a key role in the storage and transmission of genetic information and alterations in their activity are at the origin of many diseases, including cancer. Therefore, the development of synthetic systems capable of interacting with specific DNA sequences with high affinity and selectivity is of critical relevance. We have relied on transition metal chemistry to develop new tools for the *controlled*, *specific recognition of DNA*. We have demonstrated the viability of using zinc fingers for the construction of synthetic peptide chimeras that display excellent DNA recognition properties in terms of affinity, selectivity and cell transport.[1] We have also demonstrated the potential of using nickel[2] complexes as tethering coordination systems to promote the DNA recognition. Moreover, we have demonstrated the use of a Pd-mediated self-immolative cleavage to modulate the DNA-binding of a dimeric peptide.[3]

We have also developed ruthenium complexes for the selective metalation of parallel G-quadruplexes, specifically [Ru(tpy)(bpy)X]n+ (X = Cl, RSR', Met). This ruthenation of the DNA promoted an enhancement of the *c-MYC* gene expression in cells by disrupting the GQ structure present in its gene promoter site. [4]

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Keywords: DNA, Gene Expression, Artificial Transcription Factors, Metallopeptides, Metal Complexes

G13 - 106 - O

The anti-tumor peptide TAT-Cx43$_{266-283}$ binds to albumin enhancing its plasma stability

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Our group designed a cell-penetrating peptide, TAT-Cx43$_{266-283}$, with important anti-tumor effects in several preclinical models of glioblastoma, the most malignant and frequent primary brain tumor. Peptides are very promising therapeutic molecules due to their high specificity and low toxicity, as reported for TAT-Cx43$_{266-283}$. However, they usually present unfavorable pharmacokinetic properties, mainly due to their rapid degradation and elimination. Therefore, our aim was to study the plasma stability of TAT-Cx43$_{266-283}$.

The analysis of TAT-Cx43$_{266-283}$ by MALDI-TOF mass spectrometry showed a great reduction of TAT-Cx43$_{266-283}$ signal when diluted in mouse plasma compared to saline. Interestingly, TAT-Cx43$_{266-283}$ diluted in saline plus albumin also showed this signal reduction, suggesting that TAT-Cx43$_{266-283}$ binds to albumin, the most abundant plasma protein with high capacity to bind ligands. Indeed, when these experiments were performed under reducing and denaturing conditions TAT-Cx43$_{266-283}$ signal was recovered. To confirm these results, biotinylated-TAT-Cx43$_{266-283}$ was intraperitoneally administered to glioma-bearing mice and blood samples were taken at different time points. Western blots showed that the peptide appeared bound to albumin in native conditions while it appeared free under reducing and denaturing conditions. In addition, we found that TAT-Cx43$_{266-283}$ remained detectable in plasma ex vivo for at least 6 days, with a half-life of 90 min. Finally, the binding mechanism was investigated by using peptides with different amino acid modifications.

In conclusion, TAT-Cx43$_{266-283}$ binds to albumin, which may strongly improve its pharmacokinetic properties, enhancing its therapeutic potential against glioblastoma.

Keywords: Peptide-Based Drug, Albumin, Glioblastoma

G13 - 117 - O

Multiscale molecular simulations: from chemical biology to biomedical applications.

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Quantum Mechanics/Molecular Mechanics (QM/MM) is a multiscale approach widely used to simulate biomolecular systems, from enzymes to membrane proteins. This talk will showcase two QM/MM applications in which proton transfer is key for protein function.

The first example involves the unknown human gut bacterium mannoside phosphorylase (UhgbMP), an enzyme investigated for its biomedical and biotechnological potential. Our QM/MM simulations [1] showed that the phosphorolysis reaction follows a novel substrate-assisted mechanism, in which the 3' hydroxyl group of the mannosyl unit of the substrate acts as a proton relay between the catalytic aspartate and the glycosidic oxygen atom. Moreover, our simulations unraveled the conformational itinerary followed by the substrate along the reaction; such information can be used to design enzyme inhibitors.

The second example corresponds to a proton-coupled fluoride transporter (CLCF), which, unlike other members of the CLC family of chloride channels and transporters, can transport fluoride (F–) in exchange for one proton. Our QM/MM simulations [2] showed how Nature capitalized on the singular chemical properties of F– (and the weak acid HF) to make fluoride transport efficient. In particular, the interplay between two glutamate residues is essential to enable the intracellular release of proton and fluoride simultaneously, as HF, thus providing a molecular explanation for contrasting mutagenesis data.

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Keywords: Quantum Mechanics/Molecular Mechanics, Enzymes, Transporters

G13 - 150 - O

Biocompatibility and antibacterial efficacy of xanthones: structural influence on in vitro biological activity in human gingival fibroblasts and *Porphyromonas gingivalis*

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Plants are valuable sources of antimicrobial agents due to their ability to produce secondary metabolites effective against pathogens, offering alternatives to synthetic chemicals like antibiotics and antiseptics. Garcinia mangostana Linn, known as mangosteen, is a widely cultivated fruit tree in Asia. Its pericarp is used in traditional medicine for treating diseases, attributed to the abundant xanthones, a class of polyphenolic compounds with many biological activities, including antibacterial.

The chemical structure of xanthones consists of 9-H-xanthene-9-one, characterized by a dibenzo-γ-pyrone system with various functional groups among which the most important are isoprenyl, methoxy, hydroxyl, and the different groups can be cyclised to form pyrane-type cycles. I. The biological activity of xanthones is predominantly influenced by the substituents on the benzene rings and their hydrophobic nature. However, the relationship between their molecular structure and in vitro biological activity needs to be elucidated.

Thus, this study aimed to determine the biocompatibility and antibacterial activity of 30 different xanthones with different substituents. Antibacterial efficacy was evaluated against Porphyromonas gingivalis by measuring growth rate and live/dead ratio. Biocompatibility was assessed by determining metabolic activity and lactate dehydrogenase activity release in human gingival fibroblasts.

The findings revealed structural relationships influencing both antibacterial efficacy and biocompatibility based on the functional groups present. In conclusion, the biological activity of xanthones is notably shaped by the functional groups they harbor, specially with hydroxyl and biprenyl groups, which notably enhance this activity.

Keywords: Natural Products, Antimicrobial, Xanthones

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G13 - 195 - P

Enhanced Biodegradation of Low Density Polyethylene and Polypropylene Films through Microbial Consortium

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In this study, unpretreated and pretreated films of low density polyethylene (LDPE) and polypropylene (PP) underwent in vitro biodegradation processes. Microorganisms isolated from water, sediment and plastics obtained in Sabón beach, Arteixo (A Coruña), were screened, using a minimal medium with plastics as the only carbon source. After a month, strains that had grown in this medium were identified via 16S rRNA (bacteria) or ITS rRNA region (fungi) sequencing. 12 species were identified. The most promising strains were selected and a consortium comprising Gordonia hongkongensis, Pseudomonas knackmussii, Stutzerimonas stutzeri, Pseudomonas sabulinigri and Rhodococcus fascians was cultured for three months in the presence of LDPE or PP in a minimal medium.

Various pretreatments including thermal exposure, enzymatic oxidation with commercial laccase, ultraviolet radiation, and Photo-Fenton were applied to the plastics over a 14-day period. Results indicated that unpretreated LDPE exhibited a weight reduction of 8.9%, while unpretreated PP did not show any weight loss. The biodegradation of LDPE and PP was also confirmed through Fourier Transformed Infrared spectroscopy (FTIR) and Scanning Field Microscopy (SEM). FTIR analysis revealed a slight increase in vinyl bond index and internal double bond index in LDPE, whereas unpretreated PP showed no significant changes. These findings align with the commonly observed higher resistance of PP to biological degradation.

This research highlights the potential of microbial consortia in enhancing the biodegradation of plastics,offering insights into the effectiveness of different pretreatment methods in facilitating the breakdown of LDPE and PP.

Keywords: Biodegradation, Polyethylene, Polypropylene, Microbial Consortia

G13 - 211 - P

Red Box: a novel cyclophane for specific G-Quadruplex binding

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G-quadruplexes (G4s) are non-canonical secondary structures formed in guanine-rich DNA sequences which are specifically located in regulatory regions such as gene promoters and telomeres to control replication, transcription, and telomere lengthening. Some oncogenes, such as c-MYC, c-KIT, and h-TERT, may be significantly regulated by these structures, making them potential targets for the development of anticancer drugs.

Our research group has successfully synthesized the RedBox, a hydrazone-based polycationic cyclophane, which contains multiple aromatic rings arranged in a planar surface, allowing for specific non-covalent interactions with G-quadruplex (G4) DNA structures. Notably, under physiological pH conditions, the charged groups of the cyclophane promote electrostatic interactions between the molecule and the phosphate groups of the DNA thereby increasing the stability of the DNA-RedBox complex.

Using variable temperature circular dichroism, isothermal titration calorimetry, and in silico molecular docking, we have characterized the supramolecular interaction of the RedBox with G4 DNA sequences of several oncogenes and model double-stranded DNA sequences. We have tested the biological effect by analyzing its cytotoxicity in different cell lines, as well as its impact on the expression of different genes containing G4 structures.

Keywords: Red Box, G-Quadruplex

G13 - 254 - P

Binding Analysis of Potential Inhibitors of Bacterial Conjugation to the Coupling Protein TrwB Using EMSA and Proteinase K Digestion Assays

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Despite the role of antibiotics in modern medicine, abuse and misuse has led to the emergence of resistant bacteria, being antibiotic resistance (AR) a global health challenge. Type IV coupling proteins (T4CPs) are essential for bacterial conjugation, the main mechanism driving AR spread. Therefore, to control AR spread targeting T4CP emerges as a promising strategy.

Here, by proteolysis and electrophoretic mobility shift assays (EMSAs) we investigate the interactions between previously identified potential inhibitors and TrwB, T4CP of R388 plasmid. The soluble version of this membrane protein, TrwBΔN70 was digested with proteinase K in the presence/absence of the compounds to analyse whether the ligands induce a conformation change, rendering different proteolytic patterns. Some compounds prevented or reduced the protein degradation, indicating that they could be conjugation inhibitors.

By EMSA we investigated if the compounds prevent TrwBΔN70 from binding DNA. TrwBΔN70 was incubated with the plasmid pUC18 in the presence the compounds, followed by electrophoresis to discern DNA-protein complexes from free DNA. Only one compound induced partial dissociation of the complexes.

These experiments are part of work to control the dissemination of AR by inhibiting specifically bacterial conjugation.

MCIN/AEI/10.13039/501100011033 (PID2020 -116495RB -I00) and Basque Government (IT1578-22). NS-F received an Ikasiker grant (Basque Government).

Keywords: Antibiotic Resistance, Bacterial Conjugation, Proteinase K Digestion, Electrophoretic Mobility Shift (EMSAs)

G13 - 295 - P

Distinctive metabolic colorectal cancer subtypes identified by multi-omics data integration

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Colorectal cancer (CRC) is among the most frequent cancers worldwide and the second leading cause of cancer-related deaths in Western countries. In the last few decades, significant progress in survival has occurred with the use of chemotherapy and target-agents. But despite of high initial responses, practically all CRC patients succumb due to disease progression and metastatic CRC (mCRC). Metabolic reprogramming is a sensitive target for cancer therapy, but the wide landscape of metabolic phenotypes among CRC patients requires a more precise understanding of individual patient tumor metabolic reprogramming to predict the best metabolic targets in the design of new combined therapies.

Here, we have used transcriptomics data from biopsies of CRC patients to generate their Genome-Scale Metabolic Models (GSMMs), and this analysis revealed that CRC patients grouped in two distinctive metabolic subtypes: broadly speaking, one more glycolytic, and the other with enhanced oxidative phosphorylation (OXPHOS). We also applied this approach to classify transcriptomics data from different CRC cell lines, and we selected eight different cell lines, 4 closer to the glycolytic phenotype and 4 closer to the OXPHOS phenotype. All cell lines were subjected to different metabolic and transcriptomics assays, and multi-omics integration of all data into GSMM was used to better define their metabolic traits.

Altogether, our results demonstrate the existence of differ-

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ent metabolic phenotypes in CRC, that allow to predict their different sensitivity to metabolic drugs, thus opening new avenues to new CRC treatments tailored to the patient-specific metabolic subtype.

Keywords: Metabolic Network Models, Colorectal Cancer, Tumor Metabotypes, Fluxomics

G13 - 308 - O

First-generation protacs for progerin as a new therapeutic opportunity for progeria

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Hutchinson-Gilford progeria syndrome (HGPS) or progeria is a rare genetic disease that affects around 1 in 7 million newborns causing their death at 14-15 years. This is due to a mutation in the lamin A gene, causing the synthesis of the altered variant progerin. Its accumulation in the nuclear membrane is responsible for the characteristic phenotype of the disease such as altered nuclear morphology, high DNA damage and impaired cell proliferation among others. Recent studies have shown that the reduction of progerin levels in the nuclear membrane improves the phenotype of progeria. However, the only drug approved for the treatment of this disease in human patients (Zokinvy®) has moderate efficacy. Therefore, the search for new and effective therapeutic strategies is necessary. Aiming at reducing progerin levels, our research group has recently developed the first series of PROteolysis TArgeting Chimeras (PROTACs), molecules able to selectively degrade this protein. In this communication we will describe the biological characterization and cellular efficacy of the first generation PROTACs. Among all synthesized compounds, UCM-18142 stands out. It induces progerin degradation in a specific, proteome-dependent manner, and with no significant toxic effects. This PROTAC shows a good pharmacokinetic profile and it can revert some of the features of the disease, including nuclear damage and senescence. Finally, UCM-18142 shows efficacy in cells from progeria patients, thus confirming PROTACs directed to progerin as a new promising therapeutic approach for ameliorating progeria. Acknowledgments: funding by MCIN/AEI/FEDER (grant PID2022-138797OB-I00), The Progeria Research Foundation (grant PRF 2022-84) and Comunidad de Madrid (fellowship PIPF-2022/SAL-GL-24817).

Keywords: PROTAC, Progerin, Progeria

G14: Regulación de la expresión génica y dinámica del genoma

G14 - 14 - O

A search for factors involved in the repair of broken forks provides evidence for a replication fork restart mechanism in yeast

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Genome stability is essential for cell fitness. A major source of instability arises during DNA replication as a consequence of the encounter of the replication forks with DNA lesions and obstacles that threat their integrity and stability and can eventually result in their breakage. Such a situation is doubly deleterious as it generates double-strand breaks (DSBs) and compromises replication completion. How cells deal with these problems is largely unknown because the lack of genetic systems to induce the breakage of the replication forks. Since the ssDNA binding complex RPA is preferentially bound to advancing replication forks in the absence of massive DNA damage, we decided to generate a chimera (Rfa1-MN) of the largest subunit of RPA and the micrococcal nuclease, whose nuclease activity is activated with Ca2+ ions. The absence of the homologous recombination (HR) protein Rad52 is lethal in cells expressing Rfa1-MN, suggesting that the intracellular concentration of Ca2+ is sufficient to induce the nucleolytic activity of Rfa1-MN at a rate that has no effect on cell growth unless HR is absent. Therefore, we performed a Synthetic Genetic Array (SGA) analysis to search for factors involved in the rescue of replication fork-associated DSBs. This study revealed functions involved in DSB and replication-associated HR, replication fork stability, cohesion dynamics, dNTPs induction and G1-length control, providing evidence for a replication fork restart mechanism that is not associated with checkpoint activation.

Keywords: Replication Forks, DNA Repair, Yeast, Replication Dynamics

G14 - 47 - O

AlphaFold-based structural model of the priming mechanism of human PrimPol

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PrimPol is the second primase discovered in eukaryotic cells, whose main role *in vivo* is to rescue stalled replication forks during nuclear and mitochondrial DNA replication via repriming. Our current work is focused on deciphering the key structural elements implicated in its unique DNA primase activity. Regarding the structure, PrimPol contains an AEP catalityc core domain followed by a Zn finger-containing domain (ZnFD). Since 2016, the crystal structure of the AEP core domain has been resolved. However, the experimental structure of the ZnFD, which is essential for primase activity, remains unresolved. Using AlphaFold, we are working on modelling the conformation that PrimPol may adopt at primer initiation, in which both ZnFD (predicted) and AEP core (crystallized) domains are in a ‘closed’ state and interact with the DNA template and with the first 2 substrate nucleotides. We are using the model for identification of new key residues of the ZnFD implicated in template interaction and nucleotide binding.

Keywords: PrimPol

G14 - 53 - P

Using CRISPR interference to study the role of ribosomal protein bS1 in translation initiation in mycobacteria

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Traditionally, the ribosome has been considered as a core element in mRNA translation with an established function. However, recent findings suggest that ribosomal heterogeneity might play a key role in the biological adaptation of organisms. In prokaryotes, alternative mechanisms for initiating translation have been described when the mRNAs are devoid of the canonical Shine-Dalgarno (SD) sequence that the ribosome recognises to start protein synthesis. These either require direct recognition of the ATG start codon by the 70S monosome in mRNAs where a 5’ untranslated region (UTR) is missing, or mediation by ribosomal protein bS1 when the 5’UTR has either a weak or a completely absent SD sequence. In *Mycobacterium tuberculosis*, the causative agent of human tuberculosis, half of its genes are devoid of a SD sequence. Our previous ribosome profiling studies have demonstrated that non-canonical genes have differential recruitment of ribosomes to start codons, suggesting alternative initiation mechanisms. The mycobacterial ribosomal protein bS1 has an uncharacterised C-terminal domain that could influence the specificity of translation initiation, but the role it plays in regulating translation initiation in mycobacteria is completely unknown. Here, we are using CRISPR interference to silence the bS1 gene in *Mycobacterium smegmatis*, a non-pathogenic and fast-growing mycobacteria frequently used as a surrogate model for *M. tuberculosis*. To gain further understanding on the regulation of translation initiation in mycobacteria, the wild-type and knockdown strains (with both total and partial bS1 silencing) are being used to assess translational efficiency of both canonical and non-canonical mRNA templates using an *in vitro* translation system.

Keywords: CRISPRi, Mycobacteria, Ribosome, BS1

G14 - 143 - P

Functional role of different lncRNAs in ovarian cancer

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Ovarian cancer (OC) is one of the leading causes of cancer-related deaths in woman worldwide, with 206956 deaths among the 324603 cases detected in 2022 (International Agency for Research on Cancer, 2022). The high mortality is due to a late diagnosis caused by the lack of specific symptoms and an effective diagnostic method. The identification of new biomarkers could lead to an efficient early diagnosis and improved overall survival rate in OC. Long non-coding RNAs (lncRNAs) are a class of non-cod-

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ing transcripts of more than 200 nucleotides that regulate gene expression at different levels and are related to cancer hallmarks. Many lncRNAs are differentially expressed between OC and normal tissues and can act as biomarkers (Wang et al., 2019; Xie et al., 2021). In a previous published meta-analysis based on transcriptomic studies we identified different lncRNAs deregulated in ovarian cancer tissues and associated to diagnosis, prognosis, metastasis formation or chemoresistance (Salamini-Montemurri et al., 2023). Based on this data, we selected several lncRNAs for their study. To analyse the possibility of its use in liquid biopsy its differential expression was confirmed in blood samples from ovarian cancer patients compared to healthy women by qPCR. Moreover, under silencing conditions of each lncRNA in two different epithelial ovarian cancer cell lines, SKOV3 and PEO1, we analyzed their relationship with different processes associated with cancer such as proliferation, migration or cell invasion.

Keywords: Ovarian Cancer, LncRNA, Proliferation, Migration, Invasion

G14 - 181 - O

Resveratrol targets G-quadruplexes to exert its pharmacological effects

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Resveratrol (RSV) is a polyphenol that was discovered nearly a century ago. Since then, many pharmacological effects targeting multiple tissues have been described for this molecule, such as vascular protective functions, antioxidant capacity, regulation of cell metabolism and antimicrobial and antiaging properties1. Current research has been focused on studying isolated targets of RSV1, although a common underlying mechanism driving its full pharmacological activity has not been discovered to date. G-quadruplexes (G4s) are non-canonical nucleic acid structures usually found in genomic regulatory regions. In promoters, G4s are involved in the regulation of gene transcription. This study proposes a novel G4-dependent mode of action for RSV, explaining its multi-target traits. In a cellular context, RSV was shown to trigger nucleolar disassembly, RNA polymerase I inhibition, DNA damage and cell cycle arrest, all common traits of G4s ligands. Double strand breaks (DSBs) produced in response to RSV tended to accumulate around G4 regions. RSV binding was demonstrated for G4s in the rDNA and in the promoters of differentially expressed genes, directly influencing gene expression. Deciphering RSV mode of action might be helpful to enhance its therapeutic potential in a wide variety of health scenarios.

Keywords: Resveratrol, G-Quadruplex, Gene Expresion

G14 - 183 - P

Connection between the regulation of the Yen1 resolvase and the integrity of DNA secondary structures

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The homologous recombination pathway involves the formation of a series of branched intermediates that may culminate in the appearance of Holliday junctions (HJs), four-way DNA structures that connect sister chromatids or homologous chromosomes, and therefore must be processed prior to cell division. One of the enzymes responsible for their severance is the conserved budding yeast Yen1 structure selective endonuclease (GEN1 in humans).

The activity of Yen1 is restrained until the onset of the anaphase by Cdk-dependent phosphorylation, that keeps it inactive and excluded from the nucleus. This observation was quite unexpected, given that HJs begin to accumulate during the S and G2 phases. This strict regulatory mechanism prompted the development of a mutant that remains active and in the nucleus throughout the whole cell cycle, Yen1-ON, to investigate the potential consequences of premature processing of recombination intermediates. Cells expressing Yen1-ON display increased sensitivity to genotoxic agents, suggesting that Yen1 late activation may prevent the unscheduled targeting of DNA secondary structures in replication or recombination intermediates arising during S or G2. Moreover, an additional regulatory layer has been recently described by which the minor fraction of Yen1 that persists bound to chromatin in the G1/S transition is marked for degradation by SUMOylation-dependent ubiquitination.

In order to gain a better understanding of the precise DNA structures and cellular processes that may be disrupted by early Yen1 activation, in this work we studied the effect of the overexpression of Yen1 variants with combinations of mutations that bypass its different regulatory layers on cell viability, cell cycle progression and DNA damage checkpoint activation.

Keywords: Homologous Recombination, Genome Stability, Saccharomyces Cerevisiae, Structure Selective Nucleases, DNA Damage Checkpoint

G14 - 245 - P

Validation of meta-analysis of long-non-coding RNAs in plasma samples from patients with ovarian cancer

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Ovarian cancer is a frequently diagnosed gynaecological cancer in women. Unfortunately, early-stage screening is not effective, often leading to diagnosis at an advanced stage and poor patient outcomes. Long non-coding RNAs (lncRNAs) play a role in tumour progression by regulating gene expression through various mechanisms, such as modulating chromatin remodelling, affecting transcription activation or alternative splicing, generating miRNAs, or producing short biologically active peptides. LncRNAs are tissue-specific and can be detected in biological fluids, making them potential biomarkers1.

LncRNAs' different expressions were identified using computational tools over microarray- and bulk RNA-Seq-based gene expression profiling studies of ovarian cancer identified in PubMed and the Gene Expression Omnibus2. The expression of several lncRNAs, such as UCA1, SPINT-AS1, MIR100HG or ZNF232-AS1, was validated by qRT-PCR in an external cohort of plasma samples of non-ovarian cancer and ovarian cancer patients from stage III (FIGO classification) obtained in the Hospital Materno Infantil from La Coruña.

Most lncRNAs display trends consistent with the meta-analyses, indicating their potential to enhance early diagnosis of ovarian cancer and contribute to better patient outcomes. Additional research on these lncRNAs could shed light on their specific roles in the pathophysiology of ovarian cancer.

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Keywords: Ovarian Cancer, LncRNA, Meta-Analysis, Plasma

G14 - 281 - P

Deciphering the relevance of HMGB proteins in the contribution of tumor-derived extracellular vesicles to promote the Epithelial-mesenchymal Transition and Metastasis of Epithelial Ovarian Cancer

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Ovarian cancer is one of the most lethal gynecological malignancies globally due to its tendency to be diagnosed at advanced stages, post-metastasis (1). Numerous studies have explored the association of high mobility group box (HMGB) proteins with cancer, given their critical roles and diverse functions both within and outside cells. HMGB1 and HMGB2 contribute to various cancer hallmarks such

as sustained proliferative signaling, resistance to cell death, replicative immortality, genomic instability, increased mutation rates, tumor-promoted inflammation, insensitivity to growth suppressors, deregulated cellular energetics, evasion of immune destruction, metastasis, and angiogenesis stimulation. Additionally, HMGB1 has been frequently proposed as a diagnostic and prognostic biomarker for human ovarian cancer (2). Extracellular vesicles (EVs), including exosomes, are abundantly present in body fluids such as blood, urine, saliva, and seminal plasma.

In this study, we investigate the influence of HMGB1 and HMGB2 on the composition of extracellular vesicles derived from ovarian cancer cells and their implications in the EMT and metastasis, as well as the molecular mechanisms associated. HMGB1 and HMGB2 knockouts in SKOV3 ovarian cancer cell line were obtained by using CRISPR/Cas9 technology. We utilized EVs isolated from SKOV3 cells or derivative knockout cells to analyze differences in proteomic composition using Trapped Ion Mobility Spectrometry time-of-flight (timsTOF) technology. Additionally, we assessed transcriptomic analysis, cytotoxic assay and migration assays, as well as intracellular ROS level measurements, on primary cultures of ovarian epithelial cells treated with these characterize EVs to study their implications in the EMT and metastasis.

Keywords: HMGB1, HMGB2, Epithelial Ovarian Cancer, Extracellular Vesicles

G14 - 306 - O

RNAPII-facilitated repair of ribosomal DNA breaks in nucleolar caps guards against genomic instability.

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The nucleolus, whose canonical function is ribosome biogenesis, is a cellular sensor for stress. Oversized nucleoli and enhanced ribosome biogenesis are hallmarks of cancer, reflecting tumour cells' aberrantly high demand for protein synthesis during tumorigenesis. The high-copy ribosomal DNA (rDNA) genes are highly transcribed by RNAPI and inherently unstable, often subject to DNA double-strand breaks (DSBs) caused by transcription-replication conflicts. Due to exist more than 200 copies of rDNA genes, repair by homologous recombination (HR) into the nucleolus might result in loss or amplification of rDNA sequences, increasing genome instability.

Upon rDNA DSBs, ATM promotes transcriptional inhibition and rDNA and DSBs relocation to the nucleolar periphery for repair by HR in newly formed nucleolar caps. However, molecular mechanisms underlying these processes are incompletely understood. Our previous work shown that RNA polymerase II (RNAPII) has an essential role for cell choice between HR and NHEJ. This work assesses the theory that rDNA DSBs are actively moved out to the nucleolar periphery to interact with RNAPII and promote the HR pathway.

Taking advantage of an U2OS-based cell model that expresses Cas9 and a GFP-tagged version of the DDR protein NBS1, we can induce DSBs specifically in the rDNA. Using this cell model we have observed that rDNA DSB translocation and nucleolar cap formation require presence and activity of RNAPII. Nascent RNA deficiency upon RNAPII inhibition in rDNA-damaged cells provokes rDNA instability and cell death. Finally, the synergistic cytotoxic effect of combined RNA transcription inhibition and rDNA damage is consistent with addiction of cancer to nucleolar function, increasing the vulnerability of cancer cells.

Keywords: DNA Damage, Ribosomal DNA, RNA Polimerase II, Cancer

G14 - 338 - O

Trapping Ubiquitin (-like) E3 enzymes specific substrates to understand genome biology

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Protein function is regulated by different Post Translational Modifications (PTMs). Among them, modification of proteins with ubiquitin and ubiquitin and ubiquitin-like modifiers is performed by an enzymatic cascade performed by E1, E2 and E3 enzymes. E3 enzymes are the ones responsible of providing the substrate specificity. Determining which E3 modifies which substrate is challenging. To tackle this challenge we develop the TULIP and SATT methodologies. Among others we used to identify the E3-specific SUMO proteome.

Moreover, we made use of our approaches to study substrates for the BRCA1/BARD1 ubiquitin E3 enzyme. Deficiencies in the BRCA1 tumor suppressor gene are the main cause of hereditary breast and ovarian cancer. BRCA1 is involved in the Homologous Recombination DNA repair pathway, and, together with BARD1, forms a heterodimer with ubiquitin E3 activity. The relevance of the BRCA1/BARD1 ubiquitin E3 activity for tumor suppression and DNA repair remains controversial. Here, we observe that the BRCA1/BARD1 ubiquitin E3 activity is not required for Homologous Recombination or resistance to Olaparib. Using TULIP2 methodology, which enables the direct identification of E3-specific ubiquitination substrates, we identify substrates for BRCA1/BARD1. We find that PCNA is ubiquitinated by BRCA1/BARD1 in unperturbed conditions independently of RAD18. PCNA ubiquitination by BRCA1/BARD1 avoids the formation of ssDNA gaps during DNA replication and promotes continuous DNA synthesis. These results provide additional insight about the importance of BRCA1/BARD1 E3 activity in Homologous Recombination.

Keywords: SUMO, Ubiquitin, Genome Biology, DNA Damage Response

G14 - 340 - O

Mechanistic studies on SMC complexes and their role in fungal pathogenesis

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In all domains of life, cells face the challenge of packaging enormous amounts of DNA into chromosomal structures that not only ensure genomic integrity but also provide a highly dynamic platform for orchestrating and tuning genomic functions. To achieve this, chromosomes are subject to active remodelling in close coordination with cellular activities. Structural maintenance of chromosomes (SMC) complexes are ubiquitous regulators of chromosome architecture, dynamics and function. Eukaryotes contain three distinct SMC complexes: cohesin, condensin and Smc5/6. They form multisubunit ring structures and use ATP hydrolysis to fuel chromatin manipulation. Using purified holocomplexes from **S. cerevisiae**, we applied biochemical and biophysical techniques to investigate the mechanisms behind SMC functions. The combination of optical tweezers and single-molecule fluorescence allowed us to measure and visualise their interactions with DNA, providing new insights into processes such as DNA tethering and compaction, and identifying novel functional implications of their structural properties. Based on the translational potential of SMC complexes, we plan to apply these approaches to the field of fungal pathogens. We will investigate the canonical and novel roles of these complexes in shaping the structure and plasticity of the unique fungal accessory genomic compartments essential for niche adaptation and pathogenicity.

Keywords: Chromatin, SMC Complex, Optical Tweezers, Genome Plasticity, Fungal Pathogenesis

G15: Regulación metabólica y nutrición

G15 - 45 - P

Impact of a Mediterranean Diet and Physical Activity on Reducing chronic kidney disease Risk in Adults with metabolic associated fatty liver disease: a 2-Year Study

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Non-alcoholic fatty liver disease (NAFLD), now more commonly referred to as metabolic associated fatty liver disease (MAFLD), is the most prevalent chronic liver condition seen globally in clinical settings. This disease has been independently linked to an elevated risk of chronic kidney disease (CKD). The aim was to investigate whether a 2-year intervention involving a Mediterranean diet and physical activity, focussed on reducing intrahepatic fat content (IFC), also correlates with a reduced risk of CKD. Forty adults (20 men and 20 women), aged between 48 and 60 years and diagnosed with MAFLD, were recruited. Participants were split into two groups based on their improvement in IFC, assessed via nuclear magnetic resonance, after the dietary intervention. Responders, who showed improvement, also exhibited enhancements in anthropometric and clinical measures such as reduced weight, BMI, and waist circum-

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ference. Additionally, only responders showed better lipid profiles and liver enzyme levels. Responders had lower plasma IL-18 levels, but higher erythrocyte malondialdehyde levels compared to non-responders, who displayed increased erythrocyte catalase and superoxide dismutase activity. After 2 years, non-responders had elevated serum creatinine, MDRD, and CKD-EPI levels, whereas responders showed decreases in these parameters as well as in uric acid and the urine albumin-to-creatinine ratio (UACR). Positive correlations were found between changes in IFC and kidney injury biomarkers, including MDRD and serum creatinine levels. In conclusion, adhering to a Mediterranean diet and lifestyle significantly improves cardiovascular, hepatic, and renal health parameters.

Keywords: Fatty Liver, Kidney Disease, Nutrition, Oxidative Stress

G15 - 51 - P

Effect of 6-year Nutritional and Lifestyle Intervention on Oxidative and Inflammatory Markers in High-Risk Individuals for Cardiovascular Disease

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Obesity and overweight pose significant health risks, contributing to the prevalence of type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD). This study aimed to assess the impact of a 6-year lifestyle intervention on oxidative and inflammatory markers in elderly Spaniards at high risk of CVD. Eighty people with metabolic syndrome (MetS) in Mallorca, Spain, received a nutritional intervention based on the Mediterranean diet (MedDiet) and encouragement to engage in physical activity. Before and after the intervention, several parameters, including anthropometric and clinical data and blood pressure (BP) levels, were measured. Oxidative and inflammatory biomarkers in plasma were analyzed.

After the 6-year intervention, participants who reduced their BMI had greater reductions in abdominal obesity, waist-to-height ratio (WHtR), diastolic BP, and glucose levels, and increased high-density lipoprotein cholesterol (HDL-c) compared to those who did not reduce BMI. This BMI reduction was related to reduced energy intake, increased adherence to the MedDiet, and increased physical activity (PA). Furthermore, improvements in oxidative stress and proinflammatory status were observed in those who reduced their BMI, evidenced by significant reductions in markers such as myeloperoxidase (MPO) activity, malondialdehyde (MDA) levels, and monocyte chemoattractant protein-1 (MCP1). Those who did not reduce BMI showed higher levels of MCP1 and tumor necrosis factor alpha (TNFa) and increased catalase (CAT) activity.

Current findings suggest that an effective way to reduce BMI is a hypocaloric MedDiet combined with tailored physical activity to improve oxidative stress and proinflammatory status, potentially reducing the risk of CVD.

Keywords: Cardiovascular Diseases, Mediterranean Diet, Oxidative Stress, Inflammation

G15 - 76 - P

Isocaloric Time-Restricted feeding to fight aged-associated behavioral anomalies by western diet

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Western diet is mainly composed of rich-in-fat food. This causes a huge worsening in health conditions, which becomes extremely worrying when it comes to senescence. As an alternative to Caloric-Restriction (CR), Isocaloric Time-Restricted (ICTR) feeding has been recently proposed as a promising dietary habit, able to enhance both metabolic and cognitive features, but displaying higher adherence. Unfortunately, potential benefits of ICTR feeding in brain metabolism and function during aging remain unknown.

This is a longitudinal preclinical study in which 3 groups of C57BL/6 mice (n=50/group) were divided into 4 experimental conditions resulting from crossing two different feeding regimes, Ad-Libitum (AL) or Isocaloric Time-Restricted (ICTR) under two types of diet, Standard (SD) or High-Fat Diet (HFD). At the age of 6 months, mice were switched to one of these conditions for 3 (mature adult), 6 (middle-age), and 12 (old) more months, respectively. Finally, mice were assessed for metabolite blood levels and cognitive abilities before tissues and plasma extraction used for subsequent *ex vivo* analysis.

Our preliminary results show different metabolic profiles in the 4 groups. Mice fed with an AL-HFD show glucose intolerance compared to the other conditions. Mice fed with an AL-SD display higher ketone bodies blood level after fasting, with this outcome reversed in both ICTR conditions. This observation is confirmed by higher amount of lipid droplets in livers of both HFD conditions. Behavioral tests show a more active and less anxious phenotype in both ICTR conditions.

These results suggest that ICTR feeding seems to be a promising method to actively promote both healthy metabolism and higher-level cognitive behaviors during aging in individuals under western-like diets.

Keywords: Metabolismo, Cogniciòn, Cerebro, Envejecimineto, Nutriciòn

G15 - 78 - P

Assessment of Adenosine, Inosine and Uric Acid in cow's milk produced in Galicia

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Este trabajo se enmarca en el campo de estudios metabolómicos orientados al análisis de alimentos buscando una alimentación saludable. Nuestro objetivo es caracterizar la leche producida en Galicia en cuanto a su contenido en metabolitos bioactivos. Nos centramos en concreto en el análisis del contenido en Adenosina e Inosina, dos nucleósidos con conocidos efectos neuroprotectores, cardioprotectores e inmunomoduladores, y la base nitrogenada Ácido Úrico, que es un potente antioxidante.

En primer lugar, se ha puesto a punto un método de valoración de estas moléculas por HPLC. Es un método de cromatografía en fase reversa con elución en gradiente y detección ultravioleta para lo que se emplea un detector *diode array*. Para validar el método, se han calculado los límites de detección y cuantificación, se ha analizado la reproducibilidad tanto intra como inter-día y la linealidad. El análisis de la absorbancia entre 220 y 320 nm que nos permite el detector de diodos se emplea como método para identificar de manera inequívoca las moléculas, y también para asegurar la pureza de los picos en el cromatograma. Se ha prestado especial atención al método de preparación de las muestras de leche analizando la recuperación de los metabolitos de interés.

Keywords: Adenosina, Inosina, Ácido Úrico, Leche, HPLC

G15 - 83 - P

The prebiotic potential of red algae (*Palmaria palmata*) xylo-oligosaccharides

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Gut microbiota is a community of microorganisms that inhabit the gastrointestinal tract. They are involved with to digestion, stimulation of healthy digestive and immune systems development and metabolic homeostasis maintenance. Deregulation of gut microbiota, also known as "dysbiosis", has been related to different conditions such as obesity, metabolic syndrome, inflammatory bowel disease and even some types of cancer. In the last decade, researchers have proposed different approaches to modulate gut microbiota, such as the use of prebiotics1. Xylo-oligosaccharides (XOS) are non-digestible carbohydrates with prebiotic potential that can be obtained from agricultural an algae waste exploitation. Algae are an under-exploited source of different functional compounds in the western industry.

For all these reasons, in this work we studied *Palmaria palmata*'s XOS effect on cell viability, their digestibility and their capability to promote the growth of different gut related bacteria strains (*Lactobacillus spp*. and *Bifidobacterium spp*.).

Results show XOS are not cytotoxic towards RAW264.7 cell line and their digestibility is very low, only affecting XOS of high degree of polymerization, which means that XOS with most prebiotic potential (X2 to X4) could reach gut microbiota intact. Bacterial growth assays reveal that XOS don't have the potential to stimulate the assayed *Lactobacillus* strains, which is a positive result given the fact that this genera counts are increased in obese patients.

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Keywords: Gut Microbiota, Prebiotics, Xylo-Oligosaccharides, Red Algae, Obesity

G15 - 91 - O

Extracellular vesicles from hypothalamic astrocytes modify transcription factors of the leptin signaling pathway in proopiomelanocortin (POMC) neurons

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The hypothalamus is the central regulator of homeostasis with proopiomelanocortin (POMC) neurons in the arcuate nucleus playing a fundamental role. These neurons release neuropeptides that promote energy expenditure and satiety and are a target for leptin, an anorexigenic hormone of adipose tissue origin. POMC neurons are reported to communicate with hypothalamic astrocytes via extracellular vesicles (EVs) that contain proteins, lipids, and nucleic acids, and relay information regarding metabolic status. Our hypothesis is that astrocytes affect leptin signaling in POMC neurons in a nutrition-dependent manner. Primary hypothalamic astrocyte cultures were treated with 0.5 mM palmitic (PA), oleic (OA) or vehicle for 24 hours (h). EVs purified from the media (EV-PA, EV-OA or EV-V, respectively) were applied to the mHypoA-POMC/GFP-2 neuronal cell line for 4 or 24 h. POMC expression increased at 4 h in response to leptin, EV-PA and EV-OA. However, co-treatment with leptin and EVs at 4 h did not increase POMC expression. After 24 hours, POMC expression increased only in response to leptin and EV-OA, with co-treatment of both factors inducing POMC expression to a much greater extent than either treatment alone. Thus, these EVs could affect leptin signaling pathways in POMC neurons. For example, FoxO1 is a repressor of POMC transcription, and forms a complex with PGC-1α to regulate some genes. When FoxO1 is phosphorylated, it cannot repress POMC transcription. Preliminary results show that EV-PA decreases PGC-1α, while EV-OA increases both p-Foxo1 and PGC-1α. Thus, EVs from hypothalamic astrocytes may modulate leptin´s stimulation of POMC transcription through their effects on transcription factors, including miRNAs that are also present in these EVs.

Keywords: Extracellular Vesicles; Astrocyte; Neurons; Leptin; POMC; Fatty Acids

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SIRT3 in AgRP neurons protects against glucose intolerance in diet-induced obesity

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Sirtuin 3 (SIRT3) is a well-known mitochondrial energy sensor that has been linked to energy balance and glucose metabolism. Although hypothalamic SIRT3 has recently been shown to control energy homeostasis, its implication in the regulation of glucose and insulin sensitivity is still unknown. Thus, we explored the effect of SIRT3 modulation in neurons of the mediobasal hypothalamus on glucose homeostasis and insulin sensitivity.

We performed studies of loss-of-function and gain-of-function mouse models of SIRT3 in agouti-related protein (AgRP) and steroidogenic factor 1 (SF1) neurons to determinate their metabolic phenotype in different diet regimes and sexes.

We found that the genetic inhibition of SIRT3 in AgRP neurons induced an impaired glucose tolerance and insulin sensitivity in both lean and diet-induced obese male mice. Next, we observed that the ablation of SIRT3 in SF1 neurons did not alter glucose or insulin sensitivity. Consistently, the overexpression of SIRT3 in AgRP neurons improved glucose clearance in male mice fed on high fat diet.

Collectively, our findings establish a protective role of SIRT3 in AgRP neurons on glucose intolerance in diet-induced obesity.

Keywords: SIRT3, AgRP Neurons, Glucose Homeostasis, Obesity.

G15 - 112 - P

Effects of high-fat diet in cognitive and motor performances in middle-age rats

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High-fat diets (HFD) are related to negative long-term health consequences, since they can promote cognitive decline by worsening memory, learning or mental flexibility. The exact mechanisms are still being investigated, but it is believed inflammation, insulin resistance, and oxidative stress could play a central role. This study aimed to determine the effect of a HFD on cognitive and motor skills in middle-aged rats, and its effect on triglycerides and creatinine. Three groups of rats (male and female, 5 months old, n=6) were fed a HFD 3 months, and one followed a standard diet (pellet, control). After, two of the interventional groups changed the diet to a standard (HFD-SD) or antioxidant-rich (HFD-Anti-ox) diet for 2 months. At 3 and 5 months, learning/memory (Barnes maze) and motor abilities (Rota-rod) were analyzed. Once sacrificed, triglycerides and creatinine in plasma were analyzed. Rats with a HFD showed after 3 months a clear worsening in motor performance but no significant effects on memory results; but after 5 months memory deterioration was observed only in female rats and worsening in cognitive flexibility in both sexes. The change to healthy diet induced a motor performance improvement in all rats; and memory improvement in females. No effect on cognitive flexibility was observed. Triglyceride levels were higher in the group maintaining the HFD. Creatinine showed variations between the groups depending on the diet. Male animals fed with HFD, and independently on the diet change, showed lower levels compared to control; but in females, creatinine levels increased after an antioxidant diet change. In conclusion, a high fat diet intervention showed a worsening of the cognitive and motor abilities of rats with alterations in the markers in plasma.

Keywords: Fatty Liver, Cognition, Motor Abilites, Plasma Markers

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G15 - 126 - P

Acute metabolic responses of mice to the injection of EVs in the arcuate nucleus

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Hypothalamic POMC and AgRP neurons play a pivotal role in the regulation of metabolism. Extracellular vesicles (EVs) are involved in the horizontal transfer of information between astrocytes and neurons, facilitating astrocyte-neuron communication. In pathological or aged conditions, astrocytes secrete EV with altered protein and microRNA content. Our hypothesis is that astrocyte-to-metabolic neuron communication via extracellular vesicles (EVs) regulates metabolic status. Primary hypothalamic astrocyte cultures were treated for 24 hours with palmitic acid (PA; 0.5 mM), oleic acid (OA; 0.5 mM) or vehicle. EVs purified from the media (E-PA, A-OA or E-V) were bilaterally injected into the arcuate nucleus of AgRP-cre+Cas9+ mice that were monitored in metabolic cages for 24 h. Food intake, water ingested, O_2 consumed and CO_2 production were monitored every 30 minutes. The total food intake, expressed as area under the curve (AUC) was reduced in all EV-treated groups. The total water intake (AUC) showed a reduction that was notably more pronounced in mice treated with EV-PA. There was a decrease in O_2 consumption and CO_2 production (AUC) in mice injected with EV-OA compared to those receiving EV-PA that might reflect a shift towards less aerobic metabolism, possibly indicating increased efficiency. The respiratory exchange ratio (AUC) was reduced across all EVs treated groups, indicating greater fat utilization and oxidation. These data might indicate an overall shift towards a fasting-like metabolic state induced by EV treatments. EVs, depending on their source might carry specific lipids, proteins or miRNAs that could interact with hypothalamic neurons to alter their function and signaling pathways involved in metabolism.

Keywords: Fatty Acids, Astrocytes, Neurons, Extracellular Vesicles

G15 - 128 - P

The lipopolysaccharide-TLR4 axis regulates hepatic glutaminase 1 expression promoting liver ammonia build-up as steatotic liver disease progresses to steatohepatitis

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Introduction: Ammonia is a pathogenic factor implicated in the progression of metabolic-associated steatotic liver disease (MASLD). The contribution of the liver glutaminase 1 (GLS) isoform to hepatic ammonia build-up and the mechanisms underlying its upregulation in steatohepatitis remain elusive.

Methods: Multiplex transcriptomics and targeted metabolomics analysis of liver biopsies in dietary animal models representative of the whole spectra of MASLD were carried out. In addition, the acute effects of liver-specific GLS inhibition in hepatic ammonia content were evaluated both in cultured hepatocytes and in in vivo mouse models of diet-induced MASLD. Also, the regulatory mechanisms of hepatic GLS overexpression related to the lipopolysaccharide (LPS)/Toll-like receptor 4 (TLR4) axis in the context of steatohepatitis were explored.

Results: In mouse models of diet-induced MASLD, augmented hepatic GLS expression is closely associated with ammonia build-up as the disease progresses from steatosis to steatohepatitis. The acute silencing/pharmacological inhibition of GLS diminishes the ammonia burden in cultured primary mouse hepatocytes undergoing dedifferentiation, in steatotic hepatocytes, and in a mouse model of diet-induced steatohepatitis, irrespective of changes in ureagenesis and gut permeability. Under these conditions, GLS upregulation in the liver correlates positively with the hepatic expression of TLR4, an LPS receptor. Finally, the TLR4 pharmacological inhibition reduces GLS and hepatic ammonia content in LPS-stimulated mouse hepatocytes and *animal models of endotoxemia.*

Conclusions: Overall, we suggest that the LPS-TLR4 axis regulates hepatic GLS expression promoting liver ammonia build-up as steatotic liver disease progresses to steatohepatitis.

Keywords: Ammonia, Glutaminase, Urea Cycle, Toll-Like Receptor 4, Lipopolysaccharide, Metabolic-Associated Steatotic Liver Disease.

G15 - 161 - P

Fecal metabolomics to understand the problematic of male dairy beef calves at arrival to the rearing farm

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Fecal biomarkers are an important analytical tool since feces are in direct contact with the inflamed intestinal area and site for the gut microbiome. Young male dairy calves are subjected to stressful conditions during management from birth to the arrival to the rearing farm, provoking the appearance of intestinal pathologies. The objective of the study was the identification of potential fecal biomarkers by means of 1H-NMR to evaluate the management effects. First, lactoferrin was measured in fecal extracts from male Holstein calves with different colostrum feeding and nutrition protocols in order to select the more adequate time and type of treatment for the metabolomic analysis. Groups were CTR (high colostrum, milk replacer, no transport), LCMR (low colostrum, milk replacer and transport) and LCRS (low colostrum, rehydrate solution and transport). After aqueous and lipophilic extraction, forty-one polar metabolites, mainly corresponding to amino acid metabolism and short chain fatty acids, and ten non-polar lipid types were identified. The comparison between treatment groups showed that differential polar metabolites were the amino acid proline, the organic acid formate, and creatine, increased in the LCRS group, and butyrate and uracil, decreased in the LCRS group. In the analysis of non-polar metabolites, sphingomyelin (SM), and arachidonic acid and eicosapentaenoic acid (ARA+EPA) were different between groups. Multivariate analysis indicate that most of the differences are found between the LCRS group and the other two (CTR and LCMR), suggesting that feed restriction has a more important effect than colostrum consumption at this age. These metabolites are candidates to become useful biomarkers for the optimization of management procedures in young calves.

Keywords: 1H-NMR, Biomarker, Dairy Beef Calves, Feces, Inflammation, Lactoferrin, Metabolomics

ÍNDICE

G15 - 163 - O

TRIB2 as a novel switch of glycolytic metabolism in melanoma cells

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TRIB2, a member of the tribbles family of serine/threonine pseudokinases, plays a crucial role in various tumor-related processes. While TRIB1 and TRIB3 have been associated with metabolism, recent research has linked TRIB2 to glycolytic metabolism in lung cancer. Tumor cells often exhibit anaerobic glycolysis even under normoxic conditions, leading to chemoresistance. Identifying therapeutic targets that can modify or impact this metabolic pathway is essential for treating different cancer types. In melanoma, TRIB2 has been shown to confer resistance to chemotherapy. For that reason, we investigated whether this effect is mediated by alterations in glycolytic metabolism. Using UACC62 melanoma cancer cells with high TRIB2 expression, we demonstrated that suppressing TRIB2 via CRISPR/Cas reduces glucose consumption and lactate production. Consequently, this significantly decreases proliferation in TRIB2-knockout cells by inhibiting oxidative pathways compared to wild-type cells. Conversely, promoting oxidative pathways with drugs increases proliferation in TRIB2-knockout cells compared to wild-type cells. In summary, our findings highlight TRIB2 as a metabolic switch in highly metastatic melanoma cells, underscoring its potential as a therapeutic target against tumor resistance.

Keywords: Tribbles, Melanoma, Cancer Metabolism, Glycolysis

G15 - 173 - O

The absence of *Sucnr1* in hepatocytes disrupts gluconeogenesis and the zonal organization of the liver

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The liver is vital for maintaining energy balance in fasting-feeding cycles. Research notes that succinate, a metabolic sensor, increases in the bloodstream after eating. However, the metabolic role of succinate via its receptor SUCNR1 in the liver, especially in hepatocytes, remains poorly understood, particularly during the transition from fasting to feeding.

First, we analyzed *Sucnr1* expression in murine livers and hepatic cell lines under different nutritional states. Besides, we generated hepatocyte-specific *Sucnr1* KO mice *(Alb-Cre Sucnr1fl/fl* mice*)* to investigate their phenotype. We also conducted mechanistic studies in hepatocytes.

Sucnr1 expression increased in the livers of WT mice fed *ad libitum* compared to those fasted for 24 hours or subjected to a 60% caloric restriction for 4 days. Correspondingly, glucose and glutamine treatments *in vitro* enhanced succinate secretion and *Sucnr1* expression in hepatocytes. *Alb-Cre Sucnr1fl/fl* mice exhibited increased glucose excursions in pyruvate and insulin tolerance tests and elevated fasting plasma glucose and amino acids. Accordingly, hepatocytes in culture showed increased glucose production in response to SUCNR1 antagonism or deletion and, mechanistically, SUCNR1 agonism reduced p-AMPKα, SIRT1, and FGF21 expression. In addition, *Sucnr1* deletion in hepatocytes blunted the refeeding-induced mTOR activation in the liver. Finally, immunohistochemistry of SUCNR1 showed a central expression pattern and hepatocyte RNA-seq revealed that its absence disrupted the zonation.

In essence, our research underscores the crucial involvement of SUCNR1 in regulating the liver's response to fasting and feeding. Deleting *Sucnr1* in hepatocytes leads to enhanced gluconeogenesis, a dampened refeeding response, and altered hepatic zonation.

Keywords: SUCNR1, Hepatocyte, Gluconeogenesis, Zonation

G15 - 178 - P

Effects of LEAP-2 in inflammation, ER stress and fibrosis in in vivo and in vitro models of obesity and MAFLD

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MAFLD (metabollic associated fatty liver disease) is one of the most common comorbilities of obesity and the most common cause of chronic hepatic pathology. It can progress to more advanced stages like MASH, fibrosis or hepatocelular carcinoma. Ghrelin is a relevant hormone that regulates food intake, adiposity and other aspects related to MAFLD, LEAP-2 was recently discovered as an antagonist of the growth hormone secretagogue receptor(GHSR) and it has been proven to have the opposite effect to ghrelin in some aspects of metabolism when administered central and chronically in animals. The effects of this peptide on inflammation, ER stress and fibrosis in animal models of MAFLD have yet to be described, as well as its effects when administered directly to cell culture.

The goal of this work is to analyze the effect of a central, chronic treatment with LEAP-2 on animals with diet-induced MAFLD or MASH, as well as the direct effects of LEAP-2 on primary mouse hepatocytes and human cell line hepatocytes.

The results prove that a central chronic treatment with LEAP-2 for seven days causes a decrease in hepatic inflammation markers, as well as fibrosis and ER stress markers in diet-induced animal models of MAFLD and MASH. On the other hand, it is proven that the treatment of primary mouse hepatocytes as well as the human hepatocyte cell line HEPG2 with LEAP-2 causes a decrease in the accumulation of lipids induced by oleic acid treatment.

Because of this, we can conclude that the effects of central LEAP-2 on liver affected by MAFLD are relevant against inflammation, as well as ER stress and the progression to fibrosis; and LEAP-2 has a direct effect on the lipid metabolism of hepatocytes. We aim to find out the mechanisms of action explaining these effects in the future.

Keywords: MAFLD, LEAP-2, Inflammation, ER Stress, Animal Models, Hepatocytes

G15 - 179 - P

Influence of p107 in the evolution of metabolic liver disease

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Metabolic-associated fatty liver disease and its progression to MASH is one of the most important hepatic manifestations of the metabolic syndrome. p107 is a member of the RB family, essential in cell cycle regulation and in the modulation of brown adipocyte thermogenic activity. Also, the absence of p107 leads to an improvement in hepatic metabolism by preventing lipid accumulation in high-fat diet models. However, p107 hepatic functional relevance and the potential role in progression to MASH remains unknown.

Objective: To study the mechanisms through which p107 exerts its metabolic effect on liver and how the absence of p107 affects the development of fibrosis in a mouse model.

Methods: The animal models used were the male mouse wild type and global and liver-specific knockout (using virogenetic techniques) for the Rbl1 gene. The experimental groups were subjected to three types of diet: standard diet,

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high-fat diet and methionine and choline deficient diet. In addition, a human hepatocyte cell line (THLE2) and human hepatic stellate cells line (LX-2) has been used. Histological analysis, expression and protein levels were evaluated.

Results: The specific inhibition of p107 in the liver recapitulates the effects of the global p107 genetic inhibition, with a decrease of hepatic lipid accumulation due to an inhibition of de novo lipogenesis. Also, p107 liver-specific KO mice presented less fibrosis in NASH model with a decreased of fibrogenic and ER stress markers.

Conclusions: These data indicate that p107 has an important relevance in the metabolic activity of liver preventing or delaying the progression of liver disease to more severe stages. Therefore, it could be a suitable target in the development of new therapies that improve MAFLD and MASH.

Keywords: Metabolism, Liver, MASH, Fibrosis, Hepatocytes, Hepatic Stellate Cells

G15 - 206 - P

Bioavailability and organ-specific impacts of polyethylene-adsorbed bisphenol A compared to free bisphenol A in rats

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Introduction: Microplastics (MP) are emerging contaminants ubiquitously present in the food chain. Increasing evidence indicates that MP can be absorbed and accumulated in organs. Plastic additives of MP raise as a major health concern.

Objective: To analyze the bioavailability and health impact of polyethylene (PE)-adsorbed bisphenol A (BPA-PE) and free BPA.

Experimental design: In the study I, rats were gavaged with 2 mg/kg free BPA, BPA-PE (0.67 g PE/kg), or their respective vehicles. In the study II, rats were gavaged with 0,67 mg/kg free BPA, BPA-PE (0.22 g PE/kg), pristine PE, or the combined vehicles. All rats were sacrificed 24 h after administration.

Results: Plasma glucuronidated BPA (gBPA) was detected in BPA-PE rats of both studies. In the study I, only free BPA administration induced (P<0.05) the activity of hepatic antioxidant proteins (GPx, SOD). Conversely, only BPA-PE administration altered (P<0.05) the activity of lung antioxidant enzymes (GRd, SOD). Moreover, only BPA-PE increased the expression of genes involved in oxidative stress (*Mn-Sod*, *iNos*) in the liver, adipose tissue (AT), and lung; in lung repair (*Tgfb*, *Col1a1*); and in AT lipogenesis (*Srepb1c*, *leptin*). In the study II, gBPA levels from BPA-PE group were not different from those found in the free BPA group. In the gut, both free BPA and BPA-PE increased (P<0.05) the activity of a prooxidant enzyme (MPO), a phase II detoxification enzyme (GST), and the expression of *Mrd1*, involved in the protection from toxics. Both pristine PE and BPA-PE induced (P<0.05) Tgfb mRNA levels in the lung.

Conclusions: The bioavailability of MP-adsorbed BPA is similar to that of free BPA. However, the body distribution of BPA-PE appears to differ from that of free BPA, affecting organs such as the lung.

Keywords: Bisphenol A, Microplastics, Bioavailability, Oxidative Stress, Tissue Repair

G15 - 218 - P

Structure and biological activity of crust bread melanoidins extracted with different proteolytic enzymes

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Melanoidins are abundant in our diet, and scientific interest in them has increased due to their nutritional and physiological implications. The positive health effects of melanoidins are attributed to their known physiological functions, mainly due to their ability to regulate cellular oxidative stress. The aim of this study was to determine biological activity melanoidins obtained by enzymatic hydrolysis from crust bread, using the proteolytic enzymes Pronase and MP (metalloenzyme protease). The structural properties of melanoidins were characterized using UV/Vis spectrophotometry FTIR, and the nutritional components, antioxidant capacity and prebiotic activity were evaluated. The melanoidins revealed structure characteristics of melanoidins products in both UV/Vis and FTIR analyses, showing no significant differences between Pronase and MP extracts. The bioaccesible extracts, obtained by *in vitro* gastrointestinal digestion, showed high antioxidant capacity (ABTS) and antiradical capacity evaluated by HRSA (hydroxyl radical scavenger activity), without significant difference in the IC50 levels between Pronase and MP extracts. Although melanoidins from crust bread have been shown not to be cytotoxic, some compounds produced during the Maillard reaction can induce detrimental effects, including cytotoxicity. However, the results indicate no significant cytotoxic effect in neuroblastoma cells when incubated with the bioavailable fraction of melanoidins. A prebiotic effect was observed for both melanoidins extracts. These results indicate the importance of further research into the biological functions of melanoidins and their potential health benefits. Financial support of Ministry of Science and Innovation and European Regional Development Fund (TED2021-132195B-I00)

Keywords: Melanoidins, Bioactivity, Prebiotic

G15 - 240 - O

TXNIP's role in inhibiting glycolysis: A key factor in human erythroid differentiation

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The differentiation of Hematopoietic Stem Cells (HSCs) into the erythroid lineage is a dynamic process regulated by nutrient uptake and metabolism. Mitochondrial metabolism is actively induced during the commitment stage, whereas glycolysis is crucial in late erythropoiesis and red blood cells. While mitochondrial activity is associated with some erythroid disorders, the impact of an imbalance between glycolysis and oxidative phosphorylation during differentiation has not been described. TXNIP, a protein activated under hyperglycemic conditions like diabetes, promotes oxidative stress by inhibiting thioredoxin's antioxidant potential and alters the metabolic environment by decreasing glycolysis and promoting mitochondrial respiration. This study explores TXNIP's role in metabolic regulation during erythropoiesis. Our findings show that during EPO-induced differentiation of human CD34+ progenitors, TXNIP protein levels increase with glucose concentration, peaking in proerythroblasts and early basophilic erythroblasts. SRI-37330, a pharmacological inhibitor of TXNIP, affects these populations, while TXNIP-IN.1, a TXNIP-thioredoxin complex inhibitor, does not significantly impact erythropoiesis. Blocking TXNIP shifts the metabolic programming of erythroblasts towards glycolysis while maintaining high mitochondrial respiration. Additionally, inhibiting Lactate Dehydrogenase A (LDHA), which converts pyruvate to lactate, accelerates early terminal erythropoiesis under control conditions and restores differentiation in TXNIP-downregulated progenitors. In conclusion, promoting glycolysis without affecting mitochondrial metabolism alters early erythroid differentiation, emphasizing TXNIP's crucial role in maintaining low lactate production.

Keywords: Erythropoiesis, Glucose Metabolism, Glycolysis, Hematopoietic Stem Cell, TXNIP

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G15 - 253 - P

Disruption of Selenium Biochemistry in the Human Body via Dietary Mercury

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Mercury mainly interacts with selenium via 3 types of molecular targets: Selenoenzymes, Reactive Selenium metabolites and plasma proteins.

Mercury compounds inhibit the activity of selenoenzymes such as thioredoxin reductase (TrxR), glutathione peroxidase (GPx) [1], Selenoprotein P, K, and T. These selenoproteins are critical for various cellular functions, and their inhibition by mercury leads to disrupted redox balance and increased oxidative stress. Reactive selenium metabolites include Hydrogen Selenide (HSe-) and Glutathione Selenopersulfide (GS-SeH). These metabolites form stable complexes with mercury that reduce the bioavailability of selenium and disrupt its biological functions [2]. Mercury and selenium bind to specific plasma proteins at an equimolar ratio, forming high-molecular-weight inert substances, reducing mercury's toxicity. However, these also reduce the bioavailability of selenium [3].

These interactions have significant implications for mercury toxicity and selenium's protective effects. Understanding these mechanisms provides insights into mitigating mercury toxicity through selenium supplementation and other therapeutic strategies.

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Keywords: Mercury, Selenium, Interaction, Selenoenzymes, Thioredoxin Reductase (TrxR), Glutathione Peroxidase (GPx), Selenoproteins, Oxidative Stress, Selenium Transport, Reactive Selenium Metabolites

G15 - 258 - P

Target Molecules of Algae Phlorotannins in the Human Body

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Algae phlorotannins are a specific class of polyphenolic compounds predominantly found in marine brown algae. These compounds are known for their high molecular weight and complex structure, which include multiple phenolic rings. Phlorotannins are synthesized by algae as secondary metabolites and play a significant role in protecting these organisms against various environmental stressors, such as UV radiation and herbivory. Algae phlorotannins exhibit a range of biological activities, making them valuable for health applications. We focus on how algae phlorotannins influence key enzymes and proteins in the human body, contributing to their therapeutic potential. Phlorotannins inhibit protein tyrosine phosphatase 1B (PTP1B) and α-glucosidase, enzymes crucial for insulin signaling and carbohydrate digestion, offering strategies for managing type 2 diabetes. Additionally, they modulate thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD), essential in cancer therapy, enhancing the efficacy of chemotherapeutic agents like 5-fluorouracil. Novel fluorinated analogues of rhodol fluorophores target the endoplasmic reticulum (ER) in living cells, delivering cytotoxic agents specifically to the ER and inhibiting protein processing pathways. Phlorotannins also strongly inhibit tyrosinase activity by binding to the enzyme's active site, reducing melanin synthesis. Besides human health applications, this activity is also applied in fruits and fungi as the synthesis of melanin is one of the processes that occurs in the browning of these food products. These interactions highlight the multifaceted potential of algae phlorotannins. Continued research into their mechanisms of action and potential uses will further enhance their applicability in health and wellness.

Keywords: Marine Brown Algae, Polyphenolic Compounds, Phlorotannins, Enzyme Inhibition, Therapeutic Potential

G15 - 264 - P

Inhibition of the DNA damage response reduces the E2F2-induced hepatocyte metabolic dysregulation in MASLD

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DNA damage (DD) is a hallmark in metabolic dysfunction associated steatotic liver disease (MASLD). Our preliminary results show a correlation between hepatic levels of the transcription factor E2F2 and the DD in metabolic dysfunction associated steatohepatitis (MASH) patients. Thus, the aims were 1) to identify if the E2F2-induced DD in a subpopulation of patients with MASH promotes a worse metabolic phenotype; 2) if DDR inhibition in hepatocytes is able to prevent the metabolic dysregulation mediated by E2F2-induced DD.

Hepatic DNA damage (pH2AX), DDR activation (P-p53-Ser15) and E2F2 levels, the lipidome, mitochondrial-complexes activity and serum parameters were analyzed in patients categorised as MASH/no-MASH. DD was induced in primary mouse hepatocytes and overexpression of *E2f2* was achieved with adenovirus. For DDR inhibition ceralasertib was used.

Results showed increased hepatic lipid accumulation, increased mitochondrial activity and an inefficient fatty acid oxidation (FAO) in a subpopulation of MASH patients with increased DD. Among then, those with E2F2 levels above the media were a decade younger, exhibited a more marked metabolic impairment with insulin resistance, a higher hepatic lipid overload and worse atherogenic scores suggesting faster MASH progression than those with E2F2 below the media. Overexpression of *E2f2* in hepatocytes induced DD, increased P-p53-Ser15 levels and promoted lipid accumulation when exposed to PA or UV, while *E2f2* deficiency reduced lipid depot. Ceralasertib reduced lipid levels by an increased FAO in *E2f2* overexpressing hepatocytes.

In conclusion, E2F2 mediates the increased metabolic dysregulation induced by the DDR in MASH patients promoting progression so that inhibition of the DDR brings beneficial metabolic effects.

Keywords: MASH, Metabolic Dysregulation, DNA Damage, E2F2 Trasncription Factor

G15 - 265 - P

The activation of the PPARα/γ-CREB axis protects from APAP-induced hepatotoxicity

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Dysregulation of PPAR transcription factors have been related to metabolic disorders. In acetaminophen (APAP)-induced hepatotoxicity altered PPAR-α activation, fatty acid oxidation (FAO) and related lipophagy have been found. E2F transcription factors regulate metabolism and cell cycle, both processes involved in drug-induced liver injury (DILI). Preliminary results from our laboratory showed that *E2f1-/-* mice do not survive after APAP-induced liver injury, whereas *E2f2-/-* animals have a higher survival rate with increased FAO when compared to wild-type (WT) and *E2f1-/- mice.* This study **aimed** to 1) determine the involvement of E2F transcription factors in the regulation of PPARs in DILI; 2) establish its relationship with APAP toxicity. *For this, E2f1-/-*, *E2f2-/-* and WT mice were used. Hepatotoxicity was induced by IP injection of 360 mg/kg APAP. Mice were evaluated 6 and 48h post APAP-treatment. Survival, necrosis and protein analysis were performed. Additionally, PPAR-γ agonist (Rosiglitazone) and PPAR-α/γ agonist (Saroglitazar) were administered before and after APAP dose.

E2f1-/- mice exhibited reduced levels of PPAR-α and CREB activation, reduced FAO and levels of autophagy markers. However, *E2f2-/-* mice showed elevated PPAR-α/γ levels and CREB activation when compared to *E2f1-/-* mice. Pre- and post- APAP, rosiglitazone treatment improved survival to 100% and normalized transaminase levels in *E2f1-/-* and WT mice, reduced necrosis and restored autophagy markers and CREB activation at 6 and 48h post-APAP, providing near complete protection against toxicity. Saroglitazar treatment did not improve rosiglitazone´s effect but induced similar results. In conclusion, activation of the PPAR-CREB axis confers protection against toxicity and could be a potential treatment.

Keywords: APAP, Hepatotoxicity, Protection, PPARa/?, CREB

G15 - 331 - O

Skin glucocorticoid signaling impacts the whole-body metabolism

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Glucocorticoid (GC) excess contributes to the development of metabolic syndrome, defined by visceral obesity, abnormal glucose tolerance, and dyslipidemia. While it is accepted that loss of metabolic control is causative of cutaneous diseases, the systemic effects of epidermal dysfunction have received limited attention. Importantly, independent of GC blood levels, skin synthesis of these hormones can provide tissue-specific variations that may affect global homeostasis. We aimed to assess whether the epidermal-specific loss of the GC receptor (GR) had an impact on the specialized fat depot dermal white adipose tissue (dWAT) as well as on whole body homeostasis. GR epidermal KO (GREKO) and control female mice were treated with O corticosterone (CORT) for 4 weeks to induce metabolic dysfunction. Despite similar blood GC levels, GREKO mice were highly resistant to CORT-induced systemic metabolic anomalies including body weight gain, visceral and hepatic fat, hyperglycemia, and insulinemia. GREKO mice featured constitutively enhanced levels of cutaneous GCs relative to controls due to keratinocyte-specific increased expression of the key steroidogenic enzyme Cyp11b1. The higher ratio of skin-secreted protective *vs* inflammatory adipokines in GREKO *vs* controls correlated with higher capacity of adipogenic conversion in experiments using conditioned media from tissue explants. CORT- treated GREKO mice featured reduced dWAT hyperplasia and adipocyte hypertrophy, with increased Adipoq and decreased Lipocalin 2 expression in purified dermal adipocytes. Overall, epidermal GR loss results in paracrine actions on dermal adipocytes and endocrine actions on key metabolic tissues that significantly improve the whole body metabolism in a mouse model of metabolic dysfunction.

Keywords: Glucocorticoid Signaling, Skin, Metabolic Dysfunction, Inter-Organ Crosstalk

G15 - 336 - O

Deficiency of the nutrient sensor CPT1c in SF1 neurons disrupts the endocannabinoid system resulting in compromised satiety and fuel selection upon fat uptake

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The SF1 neurons of the ventromedial hypothalamus (VMH) are pivotal in governing body weight and adiposity, particularly in response to a high-fat diet (HFD). Previous studies have shown that the activation of SF1 neurons induces satiety, increases energy expenditure, and promotes the preferential use of fats as energy substrate. Furthermore, SF1 neurons are necessary for recovering from insulin-induced hypoglycemia. Here we demonstrate the essential role of the nutritional sensor CPT1c in the activation of SF1 neurons by dietary fats. Mice deficient in CPT1C in SF1 neurons (SF1-*CPT1c*-KO) are unable to adjust their caloric intake during the initial exposure to a HFD. This is associated with an impaired metabolic transition in the liver, muscle, and adipose tissue, despite a normal response to a glucose or insulin challenge. During chronic HFD exposure, SF1-*CPT1c*-KO mice are more prone to obesity and glucose intolerance than controls. CPT1c deficiency in SF1 neurons also leads to alterations in hypothalamic endocannabinoid levels and their metabolism. Our findings posit CPT1C in SF1 neurons as a sensor for dietary fats, regulating satiety responses and nutrient partitioning likely through the modulation of the endocannabinoid system.

Keywords: Ventromedial Hypothalamus, Diet-Induced Obesity, Endocannabinoids, CPT1

G16: Senescencia celular

G16 - 13 - P

Role of Connexin43 and purinergic signaling in modulating the senescent phenotype

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Cellular senescence is characterized by a stable cell-cycle arrest associated with macromolecular alterations and accompanied by a proinflammatory secretory phenotype (SASP). Interestingly, senescent cells exhibit several hallmarks of ageing, and preclinical studies have highlighted how senescent cells therapeutic targeting can alleviate age-related diseases (ARD), including osteoarthritis (OA). We have previously shown that in OA, as it happens in many other wound healing disorders, there is a chronic increase in connexin43 (Cx43), which is involved in disease progression limiting cartilage regeneration. The aim of this study was to investigate the role of Cx43 in senescence. For doing so, we have studied Cx43 gene and protein expression after DNA damage-induced senescence. Senescence was evaluated by cell cycle arrest, SA- β-Gal activity and RNA/protein analysis of several SASP factors. GJIC and hemichannel activity were evaluated by flow cytometry or ATP release, respectively. RNA interference experiments were used to further comprehend the involvement of Cx43 in the senescent phenotype. Our results showed that human fibroblasts display Cx43 overexpression after senescence induction; Cx43 was preferentially located in the membrane of senescent cells, but GJIC activity was impaired when compared to normal cells. However, Cx43

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seemed to be involved in an augmented hemichannel activity, promoting ATP release and triggering purinergic signaling, which exacerbates the NF-kB-dependent SASP. Finally, we found that inhibition of purinergic signaling receptors affects NF-kB activation and reduces the SASP. These results indicate that Cx43 may be involved in the inflammatory loop in senescent cells, hence participating in the development of ARD.

Keywords: Senescence, Cx43, SASP

G16 - 63 - P

Understanding the Role of CD13 in Cellular Senescence: A Novel Approach to Treat Age-Related Diseases

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In biomedical sciences, a key challenge is to unravel the molecular mechanisms of aging. One of the main features of aging is the disruption of cell proliferation, which defines the senescent state, linked to a proinflammatory secretome known as SASP. This secretome can propagate senescence to neighboring cells. The accumulation of senescent cells as we age creates an inflammatory environment that leads to a variety of age-related diseases.

Purine metabolism plays a crucial role in the synthesis of *de novo* purines, which are essential components of ribonucleic acids. CD13 is a metalloprotease involved in this pathway, which processes peptides into amino acids, thereby initiating the reaction cascade for purine formation. Prior to cell division, DNA replication incorporates these purines, making this pathway vital for cellular proliferation. When this pathway is disrupted, it can lead to reduced cell division and senescence.

The aim of this project was to determine the role of CD13 in the senescence process. To investigate this, we treated mesenchymal stem cells from umbilical cord stroma, chondrocytes (TC28a2 line), and ovarian cancer cells (SK-VO-3 line) with the CD13 inhibitor bestatine. This treatment induces senescence by hindering purine formation and subsequent DNA incorporation. We evaluated senescence by conducting proliferation assays and measuring the presence of senescence (p21) and SASP (IL-6, IL-8) biomarkers. Our results indicated a statistically significant decrease in proliferation and an increase in these biomarkers compared to the control.

This study highlights the dysregulation of purine metabolism as a potential mechanism for inducing senescence, positioning CD13 as a promising target for developing senodrug treatments to combat age-related diseases.

Keywords: Senescence, CD13, Purine, Metabolism

G16 - 95 - P

Understanding CDKi-induced senescence as anticancer therapy

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Cellular senescence is an effective anti-tumor response that needs to be overcome by cancer cells on their way to malignant conversion. Reactivating this response during cancer treatment might be an effective way to restrict tumor growth. Cyclin-dependent kinases (CDKs) are essential regulators of the cell cycle and drugs that mimic the activity of natural CDK inhibitors (CDKis) have been successfully developed as anticancer drugs. One of the best known CDKis is p16INK4a, who acts as a tumor suppressor by inhibiting the phosphorylation of the retinoblastoma protein (pRb) by CDK4 and CDK6, causing cell cycle arrest and senescence.

In this work, we have inducibly expressed high levels of p16INK4a to study whether its expression is sufficient to cause senescence in tumor cells, and whether this senescence is reversible by switching off p16INK4a. Robust overexpression of p16INK4a, although reducing proliferation, was not enough to induce cell cycle arrest in MCF7 or A549 cells, and pRb remained hyperphosphorylated. To test the possibility of inactivating mutations downstream of p16INK4a that could prevent cell cycle arrest, we treated cells with Palbociclib, a chemical CDK4/6i similar to p16INK4a, and found that the drug was capable of inducing cell cycle arrest and senescence. We performed co-immunoprecipitation assays to test if p16INK4a was bound to its target CDK4. Although immunoprecipitation of p16INK4a showed the co-immunoprecipitation of CDK4, p16INK4a was not detected when CDK4 was immunoprecipitated. These results may indicate that the activity of CDK4 in these cancer cells is much higher than the amount of overexpressed p16INK4a, and that Palbociclib treatment is a more powerful strategy to block CDK activity in cancer cells.

Keywords: Senescence, Cell Cycle, Cancer, CDKi, P16INK4a, Palbociclib

G16 - 114 - P

Best niosome formulations to target umbilical cord stroma mesenchymal stem cells with a senescent phenotype

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Age-related diseases are highly correlated with the accumulation of senescent cells. Hence, gene therapy constitutes a suitable tool to target this population and therefore, aging. Niosomes are nanocarriers with appealing properties but low transfection efficiencies. Minicircle (MC) DNA technology constitutes a solution for these. The aim of this work was to elucidate which niosome formulation is the best to genetically modify senescent umbilical cord stroma MSCs (UC-MSCs) with parental (PP) and MC-GFP plasmids.

Niosomes were formed using DOTMA (cationic lipid), polysorbate 20 (P20) or 80 (P80) (non-ionic surfactants) and chloroquine (P20CQ; ratio 1/2/1 and 1/2/2) or cholesterol (P80CH; ratio 1/2/2 and 1/2/4) (helper lipids). Characterization in terms of size, charge, complexation ability and DNase protection capacity were carried out. Nioplexes were prepared by mixing these niosomes with PP-GFP or MC-GFP plasmids at DOTMA/DNA ratios of 5/1, 10/1 and 15/1. Both transfection efficiency and viability were evaluated by flow cytometry.

All niosomes and nioplexes reached size and electropositivity values around 175 nm and 25 mV, except those based in the P80CH 1/2/4 formulation, that achieved negative charge values. This concurs with the results obtained in the complexation and DNase protection assays. Of note, the highest levels of transfection for the senescent UC-MSCs were achieved with CH, being always higher than the Lipofectamine (LPF) ones. Cytotoxicity was lower in cells with niosomes (~94%) than with LPF (~36%). Ongoing experiments are evaluating the most efficient formulation to transfect UC-MSCs with MC-GFP.

Concluding, niosomes constitute suitable nanocarriers to genetically modify senescent cells and consequently interesting tools to treat age-related diseases.

Keywords: Senescence, MSCs, Nanocarriers

G16 - 120 - P

Modulation of senescence and extracellular matrix homeostasis using RNA-loaded lipid nanoparticles

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Cellular senescence is a determining factor in tissue homeostasis that modifies the composition of the extracellular matrix and the phenotypic characteristics of cells. This physiological process is controlled by a multitude of molecular pathways involving different transcription factors, which can vary depending on the cell type. The COVID-19 outbreak has brought to light nucleic-acid-based therapeutics as a potential tool to control different physiological processes such as aging or oxidative stress, increasing the interest in these innovative approaches by biotech industries. However, the effectiveness of these strategies is critically limited by poor targeting ability, short circulation time and off-target effects of naked oligonucleotides-based agents.

To overcome these barriers, lipid nanoparticles have been extensively studied as delivery vectors to protect nucleic acids from the external environment, thereby reducing their degradation and enhancing their circulation time and targeted accumulation. In the present work, we studied the overexpression of the transcription factor *HES1* in senescent dermal fibroblasts using different strategies. First, we evaluated the temporal expression of *HES1* in HEK293T and U2OS cells using plasmid vectors. Subsequently, we used lipid-based vectors to corroborate the effect of *HES1* overexpression in senescent dermal fibroblasts. Finally, we analysed the impact of the cargo in these cells, comparing the efficiency our systems containing other anti-senescence cosmetic ingredients.

Thus, we conclude that *HES1* overexpression is linked to a partial reversion of senescence. The potential anti-senescence effect of *HES1* overexpression could be applied as a dermal rejuvenation strategy.

Keywords: Dermal Fibroblasts, Anti-Senescence Actives, HES1, Lipid Nanoparticles, Liposomes, Senescence.

G16 - 121 - O

Brain endothelial senescence and its impact on adult neurogenesis

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Aging involves a gradual accumulation of senescent cells in tissues. Driven by diverse intrinsic and extrinsic stresses, senescent cells undergo irreversible proliferation arrest and significant biological alterations, marked by increased β-galactosidase activity and a robust secretory phenotype (SASP) rich in proinflammatory factors that detrimentally affect tissue function. This environment depletes resident stem cells necessary for tissue repair, linking senescence to a variety of age-related diseases. As the global population ages, neurodegenerative diseases are becoming increasingly prevalent, presenting significant socio-health challenges. Neural stem cells (NSCs), responsible for neuron generation, interact with other cells within neurogenic regions that influence their behavior. Notably, brain endothelial cells (BECs) of blood vessels play a crucial role in modulating NSC quiescence-activation transitions through angiocrine factors and adhesion cues. Aging-related changes in BECs may contribute to the decline in neurogenesis observed with age. However, the potential senescent fate of these cells and its impact on the neurogenic process remain poorly explored. In our study, we delve into the effects of endothelial senescence on NSC dynamics and neurogenesis, providing new insights into brain aging and the associated neurodegeneration.

Keywords: BECs, NSCs, Neurogenesis, Cellular Senescence

G16 - 123 - P

Characterizing PAK1 and PAK2 as potential senolytic targets

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Cellular senescence is a state of stable cell cycle arrest. Senescent cells tend to accumulate over time and promote an inflammatory state implicated in age-related alterations. Recently, senolytic therapy has gained interest as a novel therapeutic strategy consisting of taking advantage of the specific characteristics of senescent cells for the treatment of diseases. These compounds can selectively kill senescent cells leaving normal healthy ones intact. In our laboratory, a compound, G-5555, with potential senolytic activity has been identified on a high-throughput compound screening. The proposed mechanism of action of this compound is the inhibition of the PAK family of serine/threonine kinases, which are known to be involved in aging and senescence, and whose silencing has been related with an increase in life expectancy in animal models. In this work, the activity of the compound G-5555 and the effect of the genetic knockdown of PAK1 and PAK2 genes using shRNAs have been studied.

In vitro cytotoxicity studies were performed with proliferating and senescent A549 cells. The results showed that the compound G-5555 did not present senolytic activity at low concentrations, while at high concentrations it killed both cell types, so its cytotoxic activity is not specific to the senescent state. Individual generation of two A549 cell lines with stable silenced PAK1 and PAK2 genes was achieved by lentiviral transduction, and correct silencing was verified by quantitative PCR (qPCR). Subsequent induction of senescence in these cell lines did not result in widespread cell death, nor were notable differences observed between proliferating and senescent cells and their corresponding non-silenced controls in SA-β-GAL staining assays or clonogenicity assays.

Keywords: Cellular Senescence, Senolytics, G-5555, PAK

G16 - 164 - O

Palbociclib increases the occurrence of the cellular cannibalistic programme entosis in breast cancer

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Entosis is the homotypic invasion of one cell into another cell, leading to the formation of a cell-in-cell (CIC) structure. This non-phagocytic process is observed in human tumors and has been correlated to cancer progression. Internalized cells may either escape or engage in cell division. However, the majority of inner cells undergo a non-canonical cell death pathway, known as entotic cell death (ECD).

Palbociclib is a selective inhibitor of the cyclin-dependent kinases CDK4 and CDK6, used for the treatment of metastatic or locally advanced ER+/HER- breast cancer. One well-characterized effect of this inhibitor is the induction of senescence. In this context, it has been found that chemotherapy-induced senescent cells engulf other cancer cells at higher rates. Therefore, we aimed to investigate whether Palbociclib treatment can induce entosis in cancer cells.

Methodology: MCF7, T-47D and MCF-10A cells were treated for a week with either vehicle, 5μM palbociclib and a combination of palbociclib and 20μM of Y-27632 (a well-known entosis inhibitor). We quantified the number of CIC structures by using both live cell imaging, immunofluorescence and correlative light-electron microscopy (CLEM) assay.

Results: We observed that treatment with Palbociclib induces an increase in CIC structures. Treatment with Palbociclib and Y-27632 significantly decreased the number of CIC structures, confirming indeed the induction of entosis, by Palbociclib treatment alone. Additionally, we highlighted that the majority of internalized cells undergo ECD and that non-treated cells invade at higher rates Palbociclib treated cells.

Finally, we here provide preliminary results that point to a possible mechanism of survival following Palbociclib treatment, based on the induction of entosis.

Keywords: Entosis, Cell-In-Cell, Entotic Cell Death, Breast Cancer

G16 - 197 - O

Role of Glutaminase isoform1 (GLS1) in Liver Senescence via Ammonium and Iron Modulation

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Metabolic alterations are key drivers of cellular senescence. Johmura et al. have recently identified glutaminase isoform 1 (GLS1), which converts glutamine to glutamate

and ammonium in mitochondria, as crucial for senescent cell survival. In this work we aimed to investigate whether disrupted ammonium homeostasis can be a metabolic trigger for liver senescence and if metabolite chelators can reverse senescence markers.

A hepatocyte-specific Gls1 overexpressing mouse model was generated by administering an AVV-CMV-FLEX-GLS1 adenovirus to transgenic Alb-Cre mice. *In vitro*, the effects of the ammonium chelator ornithine phenylacetate (OP) and the iron chelator deferiprone (Dfp) were studied.

We observed a positive correlation between age and serum glutamine/glutamate levels, a readout of GLS1 activity, liver iron content, and iron-storage protein levels among healthy individuals. *In vivo*, GLS1 overexpression led to elevated hepatic ammonium and iron levels, inducing a senescent phenotype with increased SA-b-Gal staining, p21 protein levels, oxidative stress and mitochondrial dysfunction in Cre+ mice. We hypothesized that pH changes from ammonium accumulation affect lysosomal activity and alter iron homeostasis. *In vitro*, following *Gls1* overexpression, transmission electron microscopy showed altered mitochondrial dynamics and iron aggregates in both mitochondria and lysosomes. Treatment with OP and Dfp reversed the senescent phenotype and restored both mitochondrial and lysosomal functions. Interestingly, OP and Dfp treatment were also effective in aged hepatocytes from 2-year-old mice.

Overall, the accumulation of toxic ammonium and iron can be considered metabolic stressors for senescence progression, suggesting that chelators may offer potential therapeutic approaches.

Keywords: Senescence, Ammonium, Iron, Mitochondria, Lysosome

G16 - 229 - O

Small extracellular vesicles (sEV): key players in paracrine senescence

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Secreted molecules as cytokines, chemokines, grow factors and the release of small extracellular vesicles (sEV) modulate the cellular microenvironment. Senescent cells main characteristics are the inhibition of the cell cycle, an increased β-galactosidase activity and a specific secretome of the previous compounds known as Senescence Associated secretory phenotype (SASP). SASP leads the microenvironment to a more pro-inflammatory state and induces senescence in the neighbouring cells through a paracrine and autocrine manner. As time goes by, the accumulation of senescent cells drives into age-related diseases. In this study, we focused on paracrine senescence transmission mediated by sEV. Our objective is to identify targets involved in the transmission of senescence.

For that, we knocked-down the expression of *RAB27A* and *RELA* in mesenchymal stem cells from human umbilical cord stroma. These proteins are implicated in sEV biogenesis and in every paracrine senescence compound respectively. The paracrine senescence transmission was reduced in the knock-down cells after the addition of sEV from senescent cells, validated by β-galactosidase and proliferation assays. Next, we performed a shotgun proteomic study of the recipient cells, identifying several proteins statistically significant dysregulated. These proteins are related to lysosomes, Golgi and calcium channels displaying a possible participation of the endomembrane system. In conclusion, the silencing of RAB27A prevents the paracrine senescence transmission and endomembrane system could be a possible target for the development of drugs against SASP, also known as senomorphics.

Keywords: Senescence, Extracellular Vesicles, SASP

G16 - 341 - O

Exploiting DNA damage response and senescence in cancer therapy and resistance

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Drug resistance is a major challenge in modern cancer therapy, despite the significant advances made with targeted therapies and immunotherapies for various cancers, specially advanced metastatic tumors. Our group has identified a new target that plays a critical role in cell-to-cell communication and enhances the effectiveness of targeted therapies involving senescence and DNA damage, such as BRAF or PARP inhibitors. By recruiting DNA repair complexes to lamina-associated domains and promoting senescence and persistent DNA damage, this target contributes to genome instability and synthetic lethality. The development of an innovative drug combination by using nanovesicles to deliver the protein and mRNA of the identified target, we were able to induce cell death and enhance the anti-tumor immunity. Our findings highlight a new player in DNA repair and drug response in the tumor context, with significant potential to improve treatment outcomes for patients with advanced tumors by exploiting key tumor vulnerabilities to overcome the limitations of current therapies.

Keywords: Senescencia

G16 - 342 - O

Discovery of new senomorphic targets

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Senescence, a state of permanent cell cycle arrest, is implicated in aging and age-related diseases. Senomorphic therapies, which modulate the senescence-associated secretory phenotype (SASP) or eliminate senescent cells, are a burgeoning field in biogerontology. Small extracellular vesicles (sEVs), including exosomes, have emerged as critical mediators of intercellular communication, influencing senescence and its associated phenotypes.

Recent research has uncovered novel senomorphic targets through the study of sEVs. These vesicles carry a diverse cargo of proteins, lipids, and RNAs, which can modulate senescence in recipient cells. Key findings highlight that sEVs derived from senescent cells contain unique biomarkers and bioactive molecules that contribute to the SASP and propagate senescence.

Identifying and characterizing these sEV-associated molecules have revealed several promising targets. For instance, sEVs enriched with specific microRNAs (such as miR-21 and miR-146a) and proteins (like p16^INK4a and p53) can modulate pathways involved in inflammation, DDR, and metabolic regulation. Targeting these sEV components offers a novel approach to alter the senescent cell microenvironment and reduce SASP-related damage.

This innovative focus on sEVs as senomorphic targets opens new avenues for therapeutic interventions. By manipulating sEV content or inhibiting their secretion, it is possible to mitigate the deleterious effects of cellular senescence, thereby promoting healthy aging and counteracting age-related pathologies.

Keywords: Cellular Senescence, SASP, SEV, Senomorphics

G17: Señalización celular

G17 - 28 - P

DEP Domain-Containing mTOR Interacting Protein (DEPTOR) governs cardiac fibroblast resistance to cell death triggered by calcium overload via regulation of BCL2 protein abundance

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Introduction: Cardiac fibroblasts (CF) survive to conditions that normally trigger cell death, such as heightened intracellular calcium levels related to hypertrophy. CF play a crucial role in extracellular matrix (ECM) secretion and scar formation. ECM deposition leads to fibrosis, which contributes to heart failure, a leading cause of mortality worldwide. Hence, targeting CF survival and function could offer significant therapeutic benefits. We previously identified BCL2 as a pivotal factor in CF survival, with its expression relying on the constitutive activation of the JAK2/STAT3 pathway in these cells. Hypothesis: We postulated that additional signaling pathways are involved in CF resistance to cell stress and aimed to further elucidate them. Methods and results: Gene set enrichment analysis (GSEA) from human fibroblast microarray data and rat fibroblast proteomics concur-

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rently identified upregulation of DEPTOR in CF compared to pulmonary and dermal fibroblasts. DEPTOR interacts with and can inhibit mTOR complexes 1 and 2, which are key players in regulating survival and proliferation in response to environmental cues. Silencing DEPTOR expression in cultured primary rat CF revealed that CF rely on DEPTOR for survival and proliferation under normal conditions, and that it is essential for CF survival under conditions of cytosolic calcium overload induced by Thapsigargin. Mechanistically, our findings demonstrate that elevated levels of DEPTOR are critical for sustaining increased BCL2 expression in CF under both basal and stress conditions through post-transcriptional processes. Conclusion: Our results identify DEPTOR as a crucial promoter of CF survival under conditions of calcium overload primarily through its role in maintaining high BCL2 abundance.

Keywords: Cardiac Fibroblast, Hearth Failure, Endoplasmic Reticulum Stress

G17 - 41 - P

Targeting the neuronostatin system: A new endocrine therapeutic avenue in chronic liver disease

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The somatostatin (SST) system is a pleiotropic endocrine system that inhibits hormone and enzyme secretion and suppresses cell growth in multiple tumours. In 2008, neuronostatin (NST) was discovered as a new ligand of this system and, in 2012, its receptor (GPR107) was identified. We previously reported that the NST-GPR107 system is a potential biomarker and treatment option in prostate cancer. Therefore, we aimed to explore the role of this system in chronic liver disease (CLD), mainly, in metabolic dysfunction-associated steatotic liver disease (MASLD) and hepatocellular carcinoma (HCC).

For this purpose, GPR107 expression was assessed in 2 CLD-HCC internal cohorts, 13 external cohorts (6 HCC, 7 MASLD) and 4 human liver cell lines (THLE-2, HepG2, Hep3B, SNU-387). In the cell lines, we evaluated the impact of NST treatment and GPR107 expression modulation using functional assays.

GPR107 was overexpressed in MASLD and HCC samples and associated with key liver cancer aggressiveness features (microvascular invasion and proliferative biomarkers). Functional assays revealed that NST exerts antiproliferative effects in all liver cancer cell lines and antitumorigenic effects (colony and hepatosphere formation) on Hep3B, which showed the highest GPR107 expression. Remarkably, GPR107 silencing blunted, while its overexpression enhanced, tumoral parameters in HCC cell lines. The protumorigenic effects of GPR107 overexpression were counteracted by NST co-administration.

Altogether, we unveil the NST-GPR107 system as a novel vulnerability in CLD that could be exploited as a putative biomarker and therapeutic target in these diseases.

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Keywords: Somatostatin System, Neuronostatin, GPR107, Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), Hepatocellular Carcinoma (HCC)

G17 - 54 - O

Identification of substrates of Cullin-RING E3 ligases (CRLs) by BioE3

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The specificity of the ubiquitination process is mediated by the E3 ligases. Discriminating specific substrates of E3s from interacting proteins is one of the major challenges in the field. For that aim, we previously developed BioE3, a biotin-based approach to identify specific substrates of E3 ligases. The use of BirA-E3 fusions together with ubiquitin fused to a low-affinity AviTag (bioGEFUb) allows a site-specific and proximity-dependent biotinylation of the target proteins. We proved the suitability of BioE3 to identify targets of RING and HECT-type E3 ligases. Here we adapt the BioE3 system to the multi-protein complex Cullin-RING E3s, choosing as proof of principle the substrate receptor Cereblon (CRBN). We show that BioE3 is able to identify both endogenous substrates and neosubstrates upon pomalidomide treatment. Furthermore, we observe that the molecular glue induces a decrease in the ubiquitination of the endogenous substrates, suggesting a competition by the neosubstrates. The ability of our system to detect changes in the specificity of the E3s in response to drugs may be of interest in the development of targeted protein degradation strategies.

Keywords: Ubiquitin, E3 Ligases, Cereblon, Targeted Protein Degradation

G17 - 74 - P

Understanding the Secretion Mechanism of IL1β in Breast Cancer-Associated Fibroblasts to Block Metastasis

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The tumor microenvironment (TME) also influences cancer progression, and it is defined by multiple cell types. Moreover, the TME contains soluble factors and extracellular matrix components (ECM) that shape the physical and chemical properties of tissues. Fibroblasts are the most abundant cell type in the tumor-associated stroma, also known as cancer-associated fibroblasts (CAFs). CAFs can contribute to the malignant phenotype of tumor cells through a variety of mechanisms.

The GTPase Rac1 is a molecule implicated in cancer progression and the poor prognosis of various tumor types. Rac1 has been shown to control the shape, mechanical and adhesive properties of fibroblasts, as well as their capacity to organize the ECM and myofibroblast formation. Because Rac1 is involved in malignant transformation, influencing tumorigenesis, we wondered if Rac1 activity could participate in CAF signaling to promote tumor development.

We demonstrated that targeting Rac1 activity enhances the pro-tumorigenic behavior of CAFs by altering the production of pro-inflammatory cytokines such as IL-1β. This leads to an increase in the aggressiveness of CAFs, affecting their communication with breast cancer cells.

To identify the mechanism involved in the regulation of IL-1β secretion, we conducted mass spectrometry analysis in combination with stable isotope labeling with amino acids in cell culture (SILAC). This allowed us to quantitatively track the protein changes in CAFs treated with Rac1 inhibitor. The identified proteins were then validated using different methods to evaluate their role in regulating IL-1β secretion.

We proposed that blocking of target protein involved in the secretion of IL-1β could be considered an interesting therapeutic option for breast cancer treatment.

Keywords: IL1ß, CAFs, Breast Cancer

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G17 - 88 - P

Pitpnm3, a new target of HGF/Met pathway in hepatic progenitor cells.

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Hepatic progenitor cells (HPC) are bipotential cells involved in liver regeneration after chronic damage. Their fate and role during the development and progression of chronic liver disease (CLD) relies on their intrinsic plasticity and the diversity of signals from their niche, having been described as having both pro-fibrogenic and pro-regenerative actions. Hepatocyte growth factor (HGF) is among the key signals involved in liver regeneration and, as a component of the HPC niche, modulates HPC behavior. However, the molecular mechanisms responsible for HGF-mediated regulation of HPC are only partially understood.

In order to address this question, a transcriptomic study using MACE (Massive Analysis of cDNA ends= 3'mRNA-Seq) technology has been performed to identify target genes of the HGF/Met pathway in HPC and, consequently, potential targets for liver regeneration in CLD. Among these genes is Pitpnm3, a member of a family of membrane-associated phosphatidylinositol transfer proteins.

Our results demonstrate that HGF induces an increase in both mRNA and protein levels of Pitpnm3 in HPC *in vitro*. More importantly, siRNA-based approaches show that both pro-proliferative and pro-migratory/invasive actions of HGF in HPC are abolished upon Pitpnm3 silencing. Pitpnm3 silencing also alters HGF-triggered signaling pathways in these cells. Altogether, results point to Pitpnm3 as a critical mediator of HGF-triggered signaling and biological activity in HPC. As an additional tool, HPC with stable Pitpnm3 silencing have been generated by using the CRISPR-Cas9 technology. Pitpnm3-edited HPC will be used to decipher the molecular mechanisms behind HGF/Met and Pitpnm3 functional interaction and its pathophysiological implications in CLD.

Keywords: HGF, Met, Pitpnm3, Hepatic Progenitor Cell, Chronic Liver Disease

G17 - 94 - P

TASR5 and TAS2R38 are bitter taste receptors whose colonic expressions could play important roles in age-associated processes

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Ageing disrupts how our bodies process nutrients, which leads to the deregulation of nutrient-sensing and increases inflammation. Dietary interventions such as calorie restriction can promote healthy ageing, which demonstrates the importance of both metabolism and the gastrointestinal tract for our health. Bitter taste receptors (TAS2R) present in the intestine are key members of metabolic regulation. These receptors are involved in controlling enterohormonal secretion, detect phenolic compounds in our diet, and potentially have a great impact on the ageing process.We aimed to analyse the potential role of the intestinal bitter taste receptors on the ageing process and establish potential impact of these receptors on the biomarkers.

Healthy subjects were divided into two age cohorts: young and aged. TAS2R expression was analysed in the colon mucosa. Metabolomic analysis and analysis of phenolic markers were performed in plasma samples. Best discriminatory parameters were obtained using three machine-learning methods. Finally, Spearman's rank correlation was also performed.

The best separators of the age cohorts were docosahexaenoic acid and multiple lipoprotein fractions; and TAS2R5 and TAS2R38. TAS2R5 correlated with multiple lipoprotein-derived fractions and inflammatory marker IL-6. A correlation with β-hydroxybutyrate was also observed, as were some connections with polyunsaturated fatty acids. TAS2R38 was much more selective, correlating with just membrane lipid sphingomyelin, acetone, and omega acids. TAS2R38 also correlated with β-hydroxybutyrate.

The parameters that correlated with TAS2R have known effects on the ageing process. This suggests that TAS2R5 and TASR38 are the bitter receptors most likely to play a role in the development and progress of ageing.

Keywords: Bitter Taste Receptors, TAS2R5 And TAS2R38, Ageing, Metabolomics, Phenolic Metabolites, Gastrointestinal Tract

G17 - 97 - P

Effect of germline *PTPN13* mutations in the interaction between PTPN13 and beta-catenin in the context of lymphoid differentiation

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We have previously shown that the phosphatase PTPN13 interacts with β-catenin in hematopoietic cells, and the levels of both proteins must be strictly regulated to control the quiescence of hematopoietic stem cells (HSCs). At The Hospital for Sick Children (Toronto, Canada), three different mutations in the *PTPN13* gene have been described in four patients from two unrelated families, who presented with a hereditary bone marrow failure syndrome (IBMFS) and acute lymphoblastic leukemia (ALL). To determine if these mutations are pathogenic, we have analyzed their effect on the function of PTPN13. Our results show that the mutations decrease the stability of PTPN13 in hematopoietic cells, inducing a drop in β-catenin levels. In addition, we have characterized the interaction between PTPN13 and β-catenin, and found that PTPN13 mutations alters this interaction. Furthermore, we observed that silencing of PTPN13 and β-catenin affects lymphoid differentiation. These results suggest that PTPN13 and β-catenin are important for regulating lymphoid differentiation. The reduction in the expression of effector proteins associated with the BCR signaling pathway, specifically PLCγ2 and Btk, seems to be linked to the silencing of PTPN13, where PTPN13 would regulate phospho Btk and stabilize β-catenin. All these findings in the present work support the idea that mutations in the PTPN13 gene could have a pathogenic role, especially in relation to the development of hematological disorders. Additionally, they suggest that the reduction in β-catenin levels due to these mutations could be related to the IBMFS seen in patients. This work highlights and involves, for the first time, germline mutations in PTPN13 associated with ALL and the development of IBMFS in patients.

Keywords: PTPN13, Beta-Catenin, Hereditary Bone Marrow Failure Syndrome (IBMFS), Acute Lymphoblastic Leukemia (ALL), Lymphoid Differentiation

G17 - 138 - P

Generation and characterization of a cisplatin-resistant ovarian cancer cell line

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Introduction: Ovarian cancer (OC) is the most lethal gynecological malignancy worldwide with a 5-year survival of <50%. High-grade serous ovarian carcinomas (HGSOC) represent 70% of all ovarian tumours and more than 70% of women are diagnosed with advanced stage disease. Interleukin-6 (IL6) is one of the key immunoregulatory cytokines and its levels correlate with poor prognosis and chemoresistance in OC. Currently, de-bulking surgery followed by chemotherapy (p.e. cisplatin (CDDP)) are the pillars of the treatment. Nevertheless, patients develop resistance to CDDP, making necessary the search of new therapeutic options.

Objectives: Generation and characterization of a CDDP-resistant *in vitro* model that precisely mimics patient's pathophysiology to later study alternative therapies, based on anti-IL6 immunotherapy, effectiveness.

Material and methods: The likely HGSOC Caov3 cell line was selected to develop the model due to its histological subtype and genomic characteristics. Cell line was generated by a "pulse method" and characterized by using MTT, ELISA and WB techniques, among others.

Results: The CDDP-resistant cell line, Caov3-cisR, was successfully generated and results demonstrated that it is 4 times more resistant (IC_{50}=10.1 vs 2.73 μM) and have a higher growth rate than Caov3. Finally, IL6 autocrine loop and signaling, including JAK-STAT signaling pathway, are upregulated in Caov3-cisR, probably due to the acquired drug resistance and aggressiveness.

Conclusions: HGSOC cell lines are frequently hypermutated and not suitable as disease models. Here, we have created a CDDP-resistant cell line, based in a likely HGSOC (featuring the major genomic characteristics of HGSOC), as a suitable model of OC with aberrantly hyperactivated IL6/JAK/STAT signaling axis.

Keywords: Ovarian Cancer, Interleukin-6, Cisplatin Resistance.

ÍNDICE

G17 - 158 - O

Small RNA cargo of circulating extracellular vesicles, deciphering the hallmarks of aging

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The biological process of aging evolves along human existence leading to a decline in cellular functionality and efficiency. Deciphering the hallmarks of aging is crucial, and extracellular vesicles (EV) have emerged as a potential of study. These nanometric lipid particles originate from all cell types and participate in cellular communication by transporting a diversity of biomolecules. microRNA (miRNA) presents a unique profile according to physiological and/or pathological conditions and its regulation and function has been associated to different age-related processes. Hence, the aim of the present study was to analyze circulating EV, as well as the smallRNA (sRNA) content profile, in patients of different age ranges, contributing to elucidate the molecular mechanisms of aging. Plasma samples were obtained from a total of 39 individuals stratified into young (20-39 years; n=12), middle-age (40-59 years; n=13) and old (> 60 years, n=14) groups. EV were isolated by size exclusion chromatography and characterized by nanoparticle tracking analysis (NTA) and flow cytometry. Total RNA extracted from EV was sequenced using the Illumina platform and a bioinformatics study of the sRNA profile was performed. EV characterization results demonstrate changes in surface proteins among experimental groups and a tendency of the number of circulating EV to increase in aging, although no significant differences were found in terms of concentration or size of EV. The sRNA profile shows a differential profile between groups, particularly evident in miRNA expression between the older and younger groups. In conclusion, the pattern associated with surface proteins, sRNA and miRNA evidences the potential applications of EVs as biomarkers and diagnostic tools.

Keywords: Extracellular Vesicles, SRNA, Aging, MiRNA

G17 - 159 - P

The process is the product: a multiomic characterization of Platelet-derived Extracellular Vesicles (pEV) isolated from different platelet concentrate sources.

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Platelet-derived Extracellular Vesicles (pEV), have shown significant therapeutic potential in regenerative medicine. The source of platelet concentrate and downstream processing to obtain pEV may determine their characteristics, functionality and molecular cargo. Here, we compare pEV isolated from platelet lysates (PL-EV), and from aged platelet concentrates (aP-EV). pEV were isolated by size exclusion chromatography (SEC) and characterized by transmission electron microscopy (TEM), Western blotting (WB), and nanoparticle tracking analysis (NTA). Functionality was assessed by wound healing assays, metabolic activity and cytotoxicity assays. Protein and miRNA profiling was conducted with LC-MS/MS and the GeneChip miRNA 4.0 Array respectively, followed by a bioinformatic analysis. The highest yield and purity was obtained when PL was used as pEV source, as well as improved in vitro wound healing. On the one hand, proteomic analysis showed enrichment of immune response and wound healing functions in PL-EV. On the other hand, we have identified hsa-miR-320a, hsa-miR-320b, and hsa-miR-210-3p as highly expressed on PL-EV and thus as potential molecules underlying the molecular mechanisms responsible for mediating wound healing. In conclusion, our results show that the method of preparation of the platelet concentrate can also determine the molecular cargo of the pEV, and, in turn, their functionality as we show in the in vitro studies.

Keywords: Platelet-Derived Extracellular Vesicles, Platelet Concentrates, Multi-Omics Analysis, Wound Healing, Molecular Cargo.

G17 - 231 - O

Variation in mtDNA modulates LUAD formation through differential activation of HIF

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The hypoxia-inducible factor (HIF) plays a crucial role in cellular adaptation to the tumor microenvironment. On the other hand, the oxidative phosphorylation system (OXPHOS), which regulates mitochondrial metabolism and is controlled by two genomes, the mitochondrial (mtDNA) and the nuclear (nDNA), both acts as a cancer driver by governing critical aspects of metabolism, retrograde response, epigenome and cell signaling; pathways that depend on nutrients and oxygen.

Inherited variants of mitochondrial DNA (mtDNA) are associated with increased risk of cancer, yet how they regulate oncogenesis remains unclear. Given that lung adenocarcinoma (LUAD) represents a significant global mortality rate, it is imperative to develop more effective therapeutic strategies for this type of cancer. To address this issue, here, we are exploring the molecular interface between HIF signaling and mtDNA variation in LUAD by combining in vivo (xenografts) and in vitro (spheroids and cell cultures) analyses in A549 LUAD cells carrying distinct mtDNA background. Our data shows that subtle differences in the mtDNA background modulate HIF signaling responses (in tumours and cell lines) as well as mitochondrial biogenesis and function, spheroid compactness, ROS levels and MTORC1-autophagy signals modulating tumor size and sensitivity to therapy. These findings highlight the importance of considering mtDNA in the diagnosis and treatment of LUAD, suggesting its potential as a tool for personalized cancer medicine.

Keywords: MtDNA, HIF, Haplogroups, Cancer

ÍNDICE

G17 - 236 - P

GRK2 enables aberrant proliferation of mammary epithelial cells by breaking mitogenic control over cell division

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Normal cell proliferation relies on continuous exposure to growth factors (GFs) to surpass the G1 phase restriction point and enable cell division. This mitogenic exposure can be mimicked by two short, appropriately spaced pulses of GF-receptor activation, which prime and enable the crossing of the restriction point.

Malignant cells can bypass the need for such robust two-pulse GF receptor activation by employing strategies like displaying mutated or over-expressed growth factor-receptor proteins or sensitizing non-genetically altered signaling cascades to respond to low levels of GFs. Regulatory molecular nodes, in this regard, are particularly suitable as non-oncogenic contributors to cellular growth under sub-optimal conditions.

We have recently identified that the G protein-coupled receptor kinase (GRK2), a signaling hub involved in various transduction cascades of GPCR and tyrosine kinase receptors, is part of an intrinsic regulatory pathway that ensures cell cycle progression and proper centrosome dynamics. In breast cancer, our group have shown that upregulation of GRK2 enhances cell proliferation and survival, promoting growth under adverse environmental stresses.

Our results show that cell cycle-dependent modulation of GRK2 activity during the G1 phase is crucial for the two-pulse mitogenic mechanism. GRK2 is involved in the commitment of normal mammary epithelial cells to the S phase by modulating the AKT/Mdm2/p53 pathway and p27Kip1 protein levels. Interestingly, overexpression of wild-type GRK2 increases cell proliferation even in the absence of GFs by overcoming starvation-induced E2F repression, thereby facilitating GF-independent proliferation that may contribute to breast transformation.

Keywords: Cell Proliferation, Mammary Epithelial Cell, Mitogenic Control, S-Phase Commitment, Cell Cycle, GRK2

G17 - 252 - P

Hypoxia-induced Bhlhe40 is a key regulator of proliferation and angiogenesis in mouse embryoid bodies

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Deciphering how cells respond to unbalanced oxygen levels is fundamental to understanding health and disease. Hypoxia-inducible transcription factors (HIFs) are the masterminds that alter gene expression to adapt to suboptimal oxygen conditions. Our study focused on the intriguing role of Bhlhe40, a transcriptional repressor that we recently identified in a meta-analysis as one of the most hypoxia-overexpressed genes in different cell types. Using a gene-editing strategy in mouse embryonic stem cells (mESCs) that we differentiated into embryoid bodies (EBs), we investigated the role of Bhlhe40 in controlling proliferation and angiogenesis. In mESCs, Bhlhe40 does not inhibit the rapid intrinsic proliferation of these cells. In EBs, we also found that a minority population of HOXD9+ mesodermal progenitors were not arrested by hypoxia, and their proliferation was independent of Bhlhe40. However, we uncovered that the deletion of Bhlhe40 reduced the cell cycle arrest in most progenitor cells and endothelial cells in the EBs under hypoxia. These findings implicate Bhlhe40 as a cell fate dependent regulator of proliferation under hypoxic conditions. On the other hand, deletion of Bhlhe40 increased the basal vascularization of EBs in normoxia and exacerbated hypoxia-induced vascularization. These results support a novel role for Bhlhe40 as a negative regulator of angiogenesis. Taken together, our findings implicate Bhlhe40 in the control of key functional adaptive responses to hypoxia such as proliferation arrest and angiogenesis.

Keywords: Hypoxia, Bhlhe40, Proliferation, Angiogenesis, Embryoid Bodies

G17 - 286 - O

Targeting oncogenic TRIB2 with small molecules in melanoma

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Tribbles homolog 2 (TRIB2) is one of three members of the Tribbles family of pseudo serine/threonine kinases. It acts as an oncogene whose expression is correlated with tumor stage and therapy response in melanoma. While metastatic melanoma, the most aggressive form of skin cancer now has two effective treatment options, namely MAPK pathway and immune checkpoint inhibitors, the majority of patients primarily fail to respond or acquire secondary resistance. Hence, innovative approaches to identify disease-relevant, druggable targets to remove the roadblock of therapy resistance are urgently needed. TRIB2 has been implicated in conferring resistance to various anti-cancer therapies suggesting TRIB2 as a therapeutic target for resistant tumors. This study explores the pharmacological targeting of TRIB2, revealing several independent routes of pharmacological manipulations. TRIB2 is a short-lived protein stabilized by inhibition of the PI3K/AKT pathway. Conversely, inhibitors of BRAF, MEK and ERK significantly decrease TRIB2 expression by a mechanism that involves transcription. Strikingly, increasing concentrations of the kinase inhibitor PIK75 effectively eliminate TRIB2 in melanoma cells surpassing its PI3K inhibitory activity. Additionally, Polo-Like Kinases (PLKs) inhibitors significantly reduce TRIB2 protein expression and stability. We demonstrate that inhibition or silencing of PLK2 leads to a decrease in TRIB2 levels. Overall, we identify three distinct classes of compounds that efficiently eliminate the oncogenic TRIB2 protein from melanoma cells based on different molecular mechanisms and exhibiting 40 to 200 times greater potency than the previously reported afatinib.

Keywords: Chemical Biology, Cancer, TRIB2, Signaling Pathways, Drug Discovery, Anti-Cancer Drug Resistance

G17 - 300 - P

Connexin43 influences cell cycle dynamics in ER+/HER2- breast cancer

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Cancer cells usually exhibit dysfunctional intercellular gap junction communication due to the lack of expression of connexin genes or the aberrant localization of connexin proteins. Connexins (Cxs) are channel-forming proteins involved in cell-to-cell communication that modulate the tumour microenvironment via hemichannels, gap junctions (GJ), exosomes or via channel-independent functions that regulate different cellular signalling cascades. Particularly, connexin43 (Cx43) has been found to be downregulated in breast cancer (BC) however, different reports suggest that Cxs act as tumour suppressors as their re-expression in tumour cells decrease their tumorigenicity.

Based or our previous data where we found that Cx43 reduces the colony formation capacity and proliferation of ER+/HER2- BC cells increasing the efficacy of CDK4/6 inhibitor, we wanted to further explore this fact and understand the underlying mechanism. To asses that, the cell cycle profile of BC cells expressing the vector control or Cx43 was analysed. Our results demonstrated that the cell cycle distribution changes upon Cx43 overexpression showing an increase in S-phase length in the presence of Cx43. Furthermore, the pull-down of Cx43 nuclear extracts revealed that Cx43 interacts with key cell cycle regulators. These results were confirmed using a proteomic analysis where we found that Cx43 recruits proteins implicated in cell cycle, inflammation and angiogenesis that could be participating in tumour microenvironment remodeling to avoid a metastatic

stage. In conclusion, our data identifies Cx43 as a novel cell cycle regulator in BC cells, highlighting its therapeutic potential to overcome current therapy limitations and improve treatment outcomes for patients with metastatic ER+/HER2- breast cancer.

Keywords: Conexin43, Breast Cancer, CDK4/6 Inhibitors

G17 - 339 - O

MAP kinase ERK5 modulates cancer cell sensitivity to extrinsic apoptosis induced by death-receptor agonists and Natural Killer cells

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Death receptor ligand TRAIL is a promising cancer therapy due to its ability to selectively trigger extrinsic apoptosis in cancer cells. However, TRAIL-based therapies in humans have shown limitations, mainly due inherent or acquired resistance of tumor cells. To address this issue, current efforts are focussed on dissecting the intracellular signaling pathways involved in resistance to TRAIL, to identify strategies that sensitize cancer cells to TRAIL-induced cytotoxicity. In this work, we describe the oncogenic MEK5-ERK5 pathway as a critical regulator of cancer cell resistance to the apoptosis induced by death receptor ligands. Using 2D and 3D cell cultures and transcriptomic analyses, we show that ERK5 controls the proteostasis of TP53INP2, a protein necessary for full activation of caspase-8 in response to TNFα, FasL or TRAIL. Mechanistically, ERK5 phosphorylates and induces ubiquitylation and proteasomal degradation of TP53INP2, resulting in cancer cell resistance to TRAIL. Concordantly, ERK5 inhibition or genetic deletion, by stabilizing TP53INP2, sensitizes cancer cells to the apoptosis induced by recombinant TRAIL and TRAIL/FasL expressed by Natural Killer cells. The MEK5-ERK5 pathway regulates cancer cell proliferation and survival, and ERK5 inhibitors have shown anticancer activity in preclinical models of solid tumors. Using endometrial cancer patient-derived xenograft organoids, we propose ERK5 inhibition as an effective strategy to sensitize cancer cells to TRAIL-based therapies.

Keywords: Extrinsic Apoptosis, TRAIL, FasL, TNFa, Cancer, ERK5, TP53INP2

G17 - 352 - O

RAS-ERK signaling regulation by RNA

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The RAS-ERK pathway is deeply involved in the promotion and progression of human malignancies, with about 40% of human cancers harbouring mutations in components of this signalling pathway, in particular RAS and RAF. A tight regulation of RAS-ERK pathway is crucial for the proper maintenance of cellular homeostasis, mostly undertaken by post-translational modifications and by protein-protein interactions, as well as the recently discovered regulation by ncRNA. At this respect, the mechanisms by which ncRNA could influence RAS-ERK signals have not been fully addressed and characterised. In order to unveil whether the kinases populating the pathway could be regulated by ncRNA binding, we have generated kinases-bound RNA libraries by CLIP assays. From that, we have determined MEK-binding RNA partners finding a new lncRNA that specifically binds MEK in vivo. The expression of this new ncRNA impacts on MEK kinase activity, since its modulation entails changes on MEK and ERK phosphorylation in vitro. Besides this biochemical effect, we have found changes on cell morphology due to cytoskeleton disorganization, and this could be linked with our observations on processes such as cellular migration and invasion in melanoma cell lines.

In conclusion, MEK activity is regulated by its binding to ncRNA, having an impact on RAS-ERK signaling pathway and some related biological readouts. Therefore, the characterization of the binding between MEK and ncRNA, as well as, its implication in tumorigenesis and metastasis could be crucial for developing new therapeutic strategies.

Keywords: RAS-ERK Signals, Kinase, LncRNA, Melanoma

G17 - 368 - P

VTRNA-1-2 downregulation in prostatic carcinogenesis contributes to the reduction of tumor cell immunity

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Vault RNAs (vtRNAs) are ≈100 nt long eukaryotic noncoding RNAs transcribed by RNA polymerase III, named after the vault particle. Among the four human vtRNAs, the eutherian-born vtRNA2-1/nc886 is the youngest paralog.

We found that the epigenetic repression of vtRNA2-1/nc886 correlates with the prostate cancer cell cycle progression gene signature in prostate tissue of the TCGA-PRAD, consistent with a tumor suppressor role in prostate cancer revealed by functional studies. To uncover the molecular pathways regulated by vtRNA1-2/nc886, transcriptomic and proteomic changes elicited by prostate cancer cell lines gain and loss of function transfectants. We found that vtRNA2-1/nc886 modulates native immune system pathways and confirmed interferon regulation by reporter gene assays. Concordantly, unbiased gene set enrichment analysis of vtRNA2-1/nc886 promoter methylation in PRAD-TCGA RNA-seq identifies native immune responses and inflammation at the top of the ranking. In addition, extracellular fractionation of DU145 conditioned medium and metanalysis of extracellular RNAs in RNA-seq studies, suggest that vtRNA2-1/nc886 is enriched in the extracellular space, possibly at the exosomal fraction. Concordantly, the incubation of mice BMDCs obtained from LNCaP cells overexpressing vtRNA2-1/nc886 augments the dendritic cell differentiation after activation with extracellular polyI:C.

Our findings suggest the vtRNA2-1/nc886 functions as a positive modulator of native immunity in normal tissue and its loss during carcinogenesis weakens tumor immunity, in agreement with a tumor suppressor action. Although this vtRNA has been associated with immunity in other tissue contexts, the regulation we show in prostate cancer has not been previously recognized.

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Exploring Biomedical Applications of 2D Materials Using Omic Approaches

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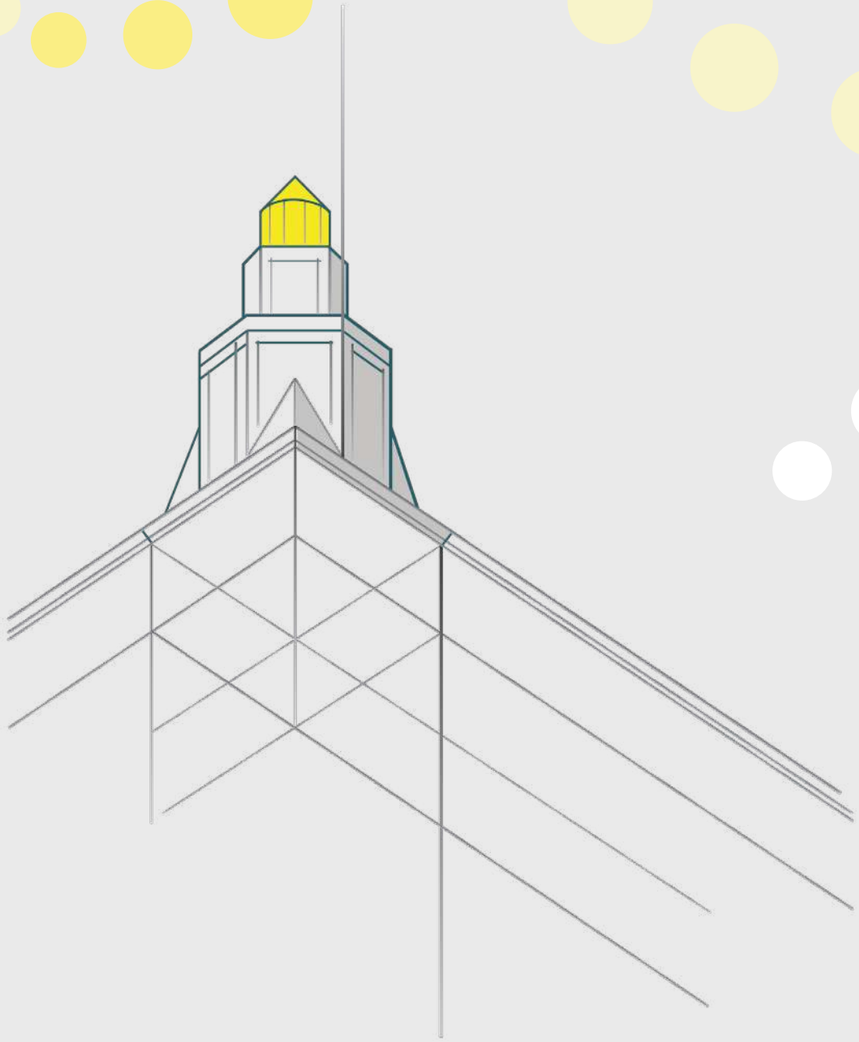
Nanomedicine involves applying nanoscale materials for the diagnosis, control, prevention, and treatment of diseases. These materials interact with biological systems at the molecular level, offering precise targeting and enhanced therapeutic effects. In this context, 2D materials are emerging as promising candidates for biomedical applications. Specifically, 2D pnictogens (P, As, Sb, and Bi) offer a compelling combination of structural flexibility and tuneable properties, including large drug loading capacity, NIR light absorption and excellent photothermal behaviour. Furthermore, their chemical reactivity, ability to stabilize organic molecules, and light-conversion properties can be exploited in nanomedicine for drug delivery systems, biosensors, and theranostic agents.

Despite their potential, the biological behaviour of these materials remains underexplored, with limited research on their pharmacological effects. To employ these materials in viable biomedical applications, it is crucial to comprehensively understand their overall impact in the organism. Omics studies provide an excellent approach to investigate the effects of these materials in cellular environments. Herein, we present the preliminary results of our study on the effects of 2D pnictogens—namely phosphorene, antimonene, and bismuthene—in three different cell lines, pimpointing the importance of omics studies in developing novel nanomedicine platforms.



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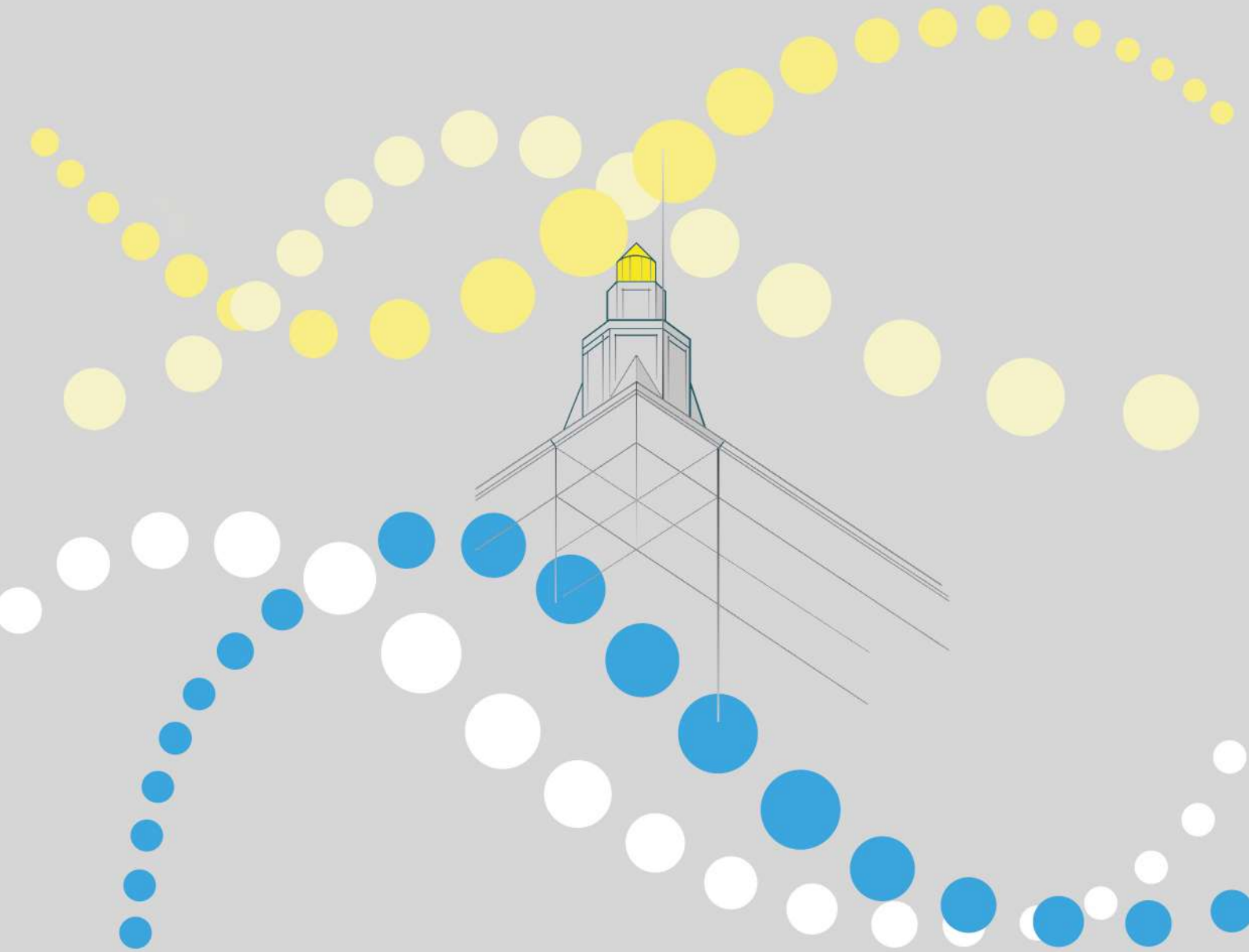

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