Universidad Autónoma de Madrid | Escuela de Doctorado | Facultad de Medicina

Workshop in Health Science and Biomedicine

27 May 2025 School of Medicine UAM

WORKSHOP PROGRAMME

9:00-9.40 REGISTRATION

9:45-10:00 WORKSHOP INAUGURATION (Aula Magna)

Prof. Pilar López Garcia, Dean of the School of Medicine, UAM

Member of EDUAM

10:00-13:00 ORAL COMMUNICATIONS I

Aula Magna	Moderated by Teresa Iglesias and Antonio Castrillo
Seminario 1	Moderated by Mercedes Sotos and Luisa Borell
Seminario 2	Moderated by Carmen Cavada and Maria Josefa Calzada

13:00-14:00 LUNCH TIME

14:00-15:00 POSTER SESSION AND COFFEE IN DECANATO HALL

15:00-17:30 ORAL COMMUNICATIONS II

Aula Magna	Moderated by Teresa Iglesias and Alejandro Khalil
Seminario 1	Moderated by Víctor Calvo and Ignacio Monedero

17:30-18:00 AWARDS AND WORKSHOP CLOSING (Aula Magna)

Scientific Committee: Teresa Iglesias Vacas, Antonio *Castrillo Viguera*, Víctor Calvo, Oscar Martínez, Ignacio Monedero, María Bailén Andrino y David Fernández de Sevilla.

Oral Communications I Aula Magna (10:00 -13:00)
Moderated by Teresa Iglesias and Antonio Castrillo

Title: NEUROGENIC NICHE DYSFUNCTION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE: A LONGITUDINAL ANALYSIS OF GLIAL AND VASCULAR ALTERATIONS
Author: Ana Victoria Prádanos Senén
Title: The Role of MCT8 in Brain Development and its Implications for Allan-Herndon-Dudley Syndrome
Author: Beatriz Muñoz Falder
Title: Analysis of the effect of a mutant variant of E2F4 in oligodendrocytes in a mouse model of Alzheimer´s disease
Author: Cristina González Bragado
Title: Defining the role of transcription factor NRF2 in the maintenance of synaptic homeostasis
Author: Daniel Carnicero Senabre
Title: E2F4-based gene therapy demonstrates a safe profile for Alzheimer's disease treatment
Author: Irene Camacho Olmos
Title: Defining the Role of NRF2 in ALS-Driven Stress Granule Pathology
Author: José Jiménez Villegas
Title: Human adult hippocampal neurogenesis is shaped by neuropsychiatric disorders, demographics, and lifestyle.
Author: Marta Gallardo Caballero
Title: Evaluating neuroinflammation in-vivo in a mouse model using multiparametric MRI, with ex-vivo insights from immunofluorescence and HRMAS spectroscopy
Author: Raquel González Alday
Title: Refining transcription factor enrichment analysis with the upgraded TFEA.ChIP
Author: Yosra Berrouayel Dahour

Title: Impact of a respiratory rehabilitation program on the quality of life **o**f children and adolescents diagnosed with persistent bronchial asthma. **Author:** Silvia Cordoba fuente

Oral Communications I Seminario 1 (10:00 -13:00)
Moderated by Mercedes Sotos and Luisa Borell

Title: Macronutrient content and quality, and risk of multimorbidity in the UK Biobank
Author: Aitana Vázquez-Fernández
Title: Breast cancer survival by subtype, stage at diagnosis and socioeconomic status among young women in Madrid, Spain
Author: Candela Pino Rosón
Title: PROSPECTIVE ASSOCIATION BETWEEN PLASMA AMINO ACIDS AND HEALTHY AGING IN OLDER ADULTS
Author: Damián González Beltrán
Title: Cardiovascular health, as per Life's Essential 8, and impaired lower-extremity function in older adults
Author: David Gómez Ángel
Title: The Association between Plant-Based Diets and Chronic Kidney Disease Incidence: A Prospective Study from the UK Biobank Cohort
Author: Javier Maroto Rodríguez
Title: Sin titulo enviado
Author: Julián Puente-Ferreiro
Title: HEALTH AND ENVIRONMENTAL DIETARY IMPACT: PLANETARY HEALTH DIET VS. MEDITERRANEAN DIET. A NATIONWIDE COHORT IN SPAIN
Author: Maria del Carmen Aznar de la Riera
Title: Association between a Planetary Health Diet and Changes in Intrinsic Capacity in Older Adults: The Seniors-ENRICA cohorts
Author: Mercedes Gómez Cao
Title: Secondhand Smoke exposure in outdoor settings assessed by airborne and biomarkers of tobacco: a systematic review
Author: Manuel Badino
Title: Home and school urban food environment and childhood obesity in the city of Madrid
Author: Luis Carmona-Rosado

Oral Communications I Seminario 2 (10:00 -13:00)
Moderated by Carmen Cavada and Maria Josefa Calzada

Title: Extracellular vesicles from hypothalamic astrocytes modify transcription factors of the leptin signaling pathway in proopiomelanocortin (POMC) neurons
Author: Alfonso Gómez Romero
Title: Action of different fatty acids on communication between astrocytes and neurons mediated by TGFβ
Author: Amanda Alóndiga Mérida
Title: TITLE: The Impact of Childhood Adversities on Disability in Adulthood: Exploring the Role of Psychosis and Loneliness
Author: Ana Ortiz-Tallo
Title: Understanding Loneliness in Spain: trajectories and predictors from the Edad con Salud longitudinal study
Author: Blanca Dolz del Castellar
Title: NUTRITIONAL PARAMETERS AS PREDICTORS OF COMPLICATIONS IN ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION
Author: Javier Cornago Navascués
Title: FROM PREDIABETES TO NORMOGLYCEMIA: WICH PREDICTORS ARE INVOLVED?
Author: Juan Carlos Lizarzaburu Robles
Title: NEUROPATHOLOGICAL VALIDATION OF THE BOSTON CRITERIA V2.0 FOR CEREBRAL AMYLOID ANGIOPATHY IN ALZHEIMER'S DISEASE DEMENTIA
Author: Mario Emiliano Ricciardi Serra
Title: Effect of Virtual Reality Distraction on Anxiety, Pain, Readiness, and Satisfaction in Patients Undergoing Cardiac Catheterization in Palestine: A Randomized Controlled Clinical Trial.
Author: Murad Jkhlab
Title: Validation of the NECPAL 4.0 prognostic model in a primary care dependence program in Chile: a study protocol using multiple methods
Author: Pamela Turrillas Villagra
Title: Outpatient Care Reduces Complications
in Stem Cell Transplantation
Author: Javier Cornago Navascués

Oral Communications II Aula Magna (15:00 -17:30)
Moderated by Teresa Iglesias and Alejandro Khalil

Title: Adipose tissue mitochondrial dysfunction promotes obesity cardiomyopathy
Author: Ana Belén Alonso Aguado
Title: Targeting tumor resistance to Raf1 deletion in NSCLC
Author: Ana María Fernández Rodríguez
Title: Gut microbiota ecology differs across metabolic and obesity phenotypes
Author: Blanca Lacruz Pleguezuelos
Title: Molecular mechanisms of high-fat diet as a risk factor for pancreatic ductal adenocarcinoma (PDAC) initiation and progression in adult mouse models.
Author: Ana Galván
Title: Targeting cGAS/STING for the treatment of genomic instability driven pathologies.
Author: Mario López Prieto
Title: Validation of STAT4 polymorphism as a biomarker in patients with early arthritis
Author: Marisa Pardines Ortiz
Title: Exploring the contribution of STAG1 and STAG2 terminal regions to their
specific functions as part of Cohesin
Author: Ana Rita Marques
Title: The motor adaptor protein Miro1 is critical in the intercellular mitochondrial transfer to glioblastoma cells
Author: Néstor Ruisánchez Gómez
Title: SIN TITULO

Author: Rosa María Andreu Martínez
Title: Urea cycle upregulation is a metabolic adaptation driving liver-specific metastatic organotropism
Author: Víctor Manuel Cruz Vilchez

Oral Communications II Seminario1 (15:00 -17:30)
Moderated by Víctor Calvo and Ignacio Monedero

Title: Impaired Locus Coeruleus Inhibitory Pathway in the Trigeminal System in Diabetes: Mechanisms and IGF-1-Dependent Rescue
Author: Alberto Mesa Lombardo
Title: Studying infection and neurodegeneration induced by HSV-1 in 3D human neuronal cultures
Author: Blanca Salgado Fuentes
Title: New intron retention tau isoforms fights proteinopathy everting classic tau impairments and disminishing tau seeding activity.
Author: Francisco Vallejo Bedia
Title: The NS2 protein of the parvovirus Minute Virus of Mice (MVM) disrupts the Rae1-mediated mRNA nuclear export machinery
Author: Jorge Martínez Ortega
Title: Heat-killed bacterial immunotherapies induce trained immunity in pediatric cystic fibrosis patients
Author: Laura Bravo Robles
Title: Diferencias moleculares entre machos y hembras en modelos preclínicos de fracaso renal agudo
Author: Lucía Miño Izquierdo
Title: Fenotipo renal de ratones Fosl2 knock-out
Author: Marta Ribagorda Bermejo
Title: Evaluación del papel de la sestrina1 en células tubulares y en modelos murinos de fracaso renal agudo
Author: Natalia Villar Gómez
Title: Directed evolution of mouse parvovirus towards human glioblastoma provides receptor binding-site mutants with enhanced oncotropism
Author: Pedro Arroyo Gil
Title: Accelerated and refined genomic analysis, coupled with epidemiological interventions, to optimize tuberculosis transmission control
Author: Sheri Bakheit Gadelkarim

Oral and Poster Communication Abstracts

Universidad Autónoma de Madrid | Escuela de Doctorado | Facultad de Medicina

Oral Communications I Aula Magna (10:00 -13:00)

Defining the role of transcription factor NRF2 in the maintenance of synaptic homeostasis

Daniel Carnicero-Senabre [1], Mariana A. Barata [2], Cláudia Guimas Almeida [2], Ana I. Rojo [1].

1 Department of Biochemistry and Biomedical Research Institute "Sols-Morreale", Faculty of Medicine, Autonomous University of Madrid; Centro de Investigacion Biomédica en Red Sobre Enfermedades Neurodegenerativas (CIBERNED); Instituto de Investigación Sanitaria La Paz (IdiPaz), Madrid, Spain;
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Background: Failure to translate successful neuroprotective preclinical data to a clinical setting in Alzheimer's disease (AD) indicates that amyloidopathy and tauopathy provide an incomplete view of disease. Here, we evaluated the relevance on synapses of additional homeostatic deviations that result from loss of activity of NRF2, a transcription factor that regulates the expression of over 250 genes, including those related to protection against oxidative stress, whose activity declines with ageing.

Methods: We have employed immunofluorescence techniques to quantify synapses both in primary cultures and in brain slices. To analyse synaptic composition by western blotting, we have isolated synaptosomal fractions. To determine lipidic composition, we have carried out untargeted lipidomics of whole hippocampus or the synaptosomal fraction.

Results: We decided to uncover the role of NRF2 on lipid levels in the brain and their impact on synapses. In order to do that, we evaluated the levels of over 700 distinct lipid species in NRF2-null or wild type hippocampi by untargeted lipidomics and found that NRF2 deficiency led to the dysregulation of several lipid species including 5 types of ether-linked phospholipids (related to LPOgeneration). To examine the impact of NRF2 deficiency in the synaptic contacts, we analyzed excitatory synapses, both in brain slices and primary neuronal cultures. Our findings revealed that the absence of NRF2 modified the molecular composition of the synapse, both at a protein and lipidic level. Relevantly, when primary neuronal cultures were treated with an ether-linked lipid precursor, synaptic contacts were impaired. However, an earlier induction of NRF2 with the novel 6-MSITC (6-methylsulfonyl isothiocyanate), prevented the synaptic loss.

Conclusion: In conclusion, NRF2 emerged as a crucial modulator of synaptic homeostasis, providing a new avenue for exploring its potential as a therapeutic target for neurodegenerative diseases characterized by progressive synaptic loss, such as AD.

NEUROGENIC NICHE DYSFUNCTION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE: A LONGITUDINAL ANALYSIS OF GLIAL AND VASCULAR ALTERATIONS

Ana Victoria Prádanos-Senén[1,2], Carla B. Rodriguez-Moreno[2,3], María Llorens-Martín[1,2,3]

1. *Department of Molecular Biology, Faculty of Sciences, Universidad Autónoma de Madrid, Madrid, Spain.*
2. *Department of Molecular Neuropathology, Centro de Biología Molecular Severo Ochoa (CBM), Spanish Research Council (CSIC)—Universidad Autónoma de Madrid (UAM), Madrid, Spain.*
3. *Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Madrid, Spain.*

ABSTRACT (300 words max)

In the hippocampal dentate gyrus (DG), adult hippocampal neurogenesis (AHN) occurs within a specialized environment, the neurogenic niche, comprising an extensive vascular network and glial cells. This study aims to identify the cellular mechanisms underlying AHN failure in Alzheimer's disease (AD) by longitudinally studying the components of the DG neurogenic niche.

We used a mouse model of AD (mice that overexpress glycogen synthase kinase 3 beta (GSK-3β)), and conducted a longitudinal study at 4, 12, 18, and 30 months of age (mo). Our results reveal significant alterations in glial populations and vascularization. In AD mice, astrocyte and microglia densities increase in the granule cell layer (GCL) at 4 mo but decrease in the subgranular zone (SGZ) by 12 mo, accompanied by morphological changes observed through 3D reconstructions. Oligodendrocyte precursor cells (OPCs) decrease in the GCL at 4 mo, while mature oligodendrocytes remain stable in the GCL but decline in the SGZ from 12 mo onwards.

These glial changes correlate with increased DG vascularization, particularly in the SGZ at 12 and 18 mo and the GCL at 12 mo. GCL blood vessels exhibit increased thickness at 12 and 30 mo, with elevated CD31 immunoreactivity at 30 mo.

The putative interaction of newborn dentate granule cells with glial cells was also examined. Astrocytes show increased overlapping with 1-week-old neurons in the AD model, which decreases in 8-week-old neurons in both AD and wild-type conditions. Conversely, overlap with UEA1+ blood vessels increases in 8-week-old neurons compared to 1-week-old ones in AD mice.

Finally, a detailed analysis of the hippocampal expression of 96 cytokines revealed a variable imbalance between pro- and anti-inflammatory signals in the AD model.

These findings bring to light the disruption of the DG neurogenic niche homeostasis in this mouse model of AD, potentially contributing to AHN impairments.

The Role of MCT8 in Brain Development and its Implications for Allan-Herndon-Dudley Syndrome

B Muñoz-Falder[1], A Pérez-Pestourie[1], A Montero-Pedrazuela[1], A Guadaño-Ferraz[1], S Bárez-López[1]

Biomedical Research Institute Sols-Morreale Consejo Superior de Investigaciones Científicas (CSIC) – Universidad Autónoma de Madrid (UAM), Madrid, Spain

ABSTRACT

Thyroid hormones (THs) are essential for CNS development, regulating processes such as neurogenesis, cell migration, synaptogenesis, and myelination. THs need specific transmembrane transport proteins like MCT8 to cross the cell membrane. Mutations in the *SLC16A2* gene, which encodes MCT8, lead to the Allan-Herndon-Dudley syndrome (AHDS), a rare X-linked disorder that mainly affects males. MCT8 dysfunction disrupts THs transport to the brain, causing cerebral hypothyroidism and peripheral hyperthyroidism; resulting in profound intellectual disability (IQ <30), neurodevelopmental delays, and central hypotonia with spastic paraplegia, among others. Despite advances in understanding this syndrome, there is still no effective treatment for its neurological symptoms. These alterations arise during neurodevelopment, as shown in published studies from our laboratory. While the mouse is commonly used in preclinical studies, the role of MCT8 in mouse neurodevelopment remains unexplored.

This study aimed to clarify MCT8's function during murine neurodevelopment, to improve understanding of AHDS pathophysiology, and determine the optimal timing for treatment. Using immunohistochemistry, we examined MCT8 expression in wild-type mouse brains during perinatal stages. Our findings indicate that MCT8 is expressed from prenatal stages in key brain regions, including the cortex, hippocampus, and brain barriers, mirroring human patterns.

We also studied an avatar mouse model of AHDS with a point mutation in *SLC16A2* (the P253L avatar mouse), using RNA sequencing, RNAscope and immunofluorescence. Our results confirm AHDS brain disorders begin prenatally, emphasizing the need for an early and effective treatment to address neurological problems early on. Additionally, as transient perinatal cerebral hyperthyroidism may affect various brain cells differently, therapies could target affected cells to reduce collateral damage. Thus, administration of TH analogues targeting specific brain cell types in the fetus is suggested as a potential therapeutic approach to alleviate the neurological problems arising during prenatal stages in AHDS.

Defining the Role of NRF2 in ALS-Driven Stress Granule Pathology

José Jiménez-Villegas[1,2,3], Daniel Carnicero-Senabre[1,2,3], Adrià Sicart[4,5], Antonio Cuadrado[1,2,3], Ludo Van Den Bosch[4,5], Ana I. Rojo[1,2,3]

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[2] Instituto de Investigación Sanitaria La Paz (IdiPaz), Madrid, Spain
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[4] Department of Neurosciences, Experimental Neurology and Leuven Brain Institute (LBI), KU Leuven-University of Leuven, Leuven, Belgium
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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the loss of upper and lower motor neurons and remains without effective therapeutic interventions. A major pathological hallmark of ALS is the cytoplasmic aggregation of TDP-43, associated with the aberrant persistence of stress granules: ribonucleoprotein condensates that form in response to cellular stress. Altered redox homeostasis and oxidative stress have been implicated in stress granule formation in ALS, suggesting a potential role for NRF2, a key transcription factor involved in redox control, whose response we have previously found to be compromised in ALS models. This study investigates the impact of NRF2 deficiency on stress granule dynamics. We observed that NRF2-deficient neurons exhibited increased sensitivity to stress granule formation. Mechanistic studies in NRF2-deficient mouse embryonic fibroblasts revealed that this increased sensitivity was not due to enhanced phosphorylation of eIF2α, a principal upstream regulator of stress granule formation. Furthermore, NRF2 deficiency resulted in delayed stress granule clearance, due to diminished p62 recruitment to stress granules. Additionally, the accumulation in stress granules of disease-associated proteins such as FUS and a constitutive cytosolic TDP-43 mutant was promoted by NRF2 deficiency. Importantly, NRF2 activation with the natural compound sulforaphane normalized the elevated stress granule formation in iPSC-derived motor neurons from a C9orf72-ALS patient. In conclusion, our findings highlight the role of NRF2 in modulating the formation and clearance of stress granules, suggesting that NRF2-targeted therapeutic strategies may mitigate persistent stress granules and TDP-43 aggregation in ALS.

Evaluating neuroinflammation *in-vivo* in a mouse model using multiparametric MRI, with *ex-vivo* insights from immunofluorescence and HRMAS spectroscopy

Raquel González-Alday[1], Carla Dávila-Yagüe[1], Nuria Arias-Ramos[1], Blanca Lizarbe[1,2], Pilar López-Larrubia[1]

[1] *Instituto de Investigaciones Biomédicas Sols-Morreale (CSIC-UAM), Madrid, Spain;*
[2] *Departamento de Bioquímica (UAM), Madrid, Spain*

ABSTRACT (300 words max)

INTRODUCTION: Systemic administration of lipopolysaccharide (LPS) is a widely used murine model for studying neuroinflammation, with established microglia activation and cytokine production [1]. The objective of this work is to characterize this model using a multiparametric MRI approach to identify non-invasive biomarkers of neuroinflammation.

METHODS: 34 C57BL/6J adult mice were subjected to an intraperitoneal injection of either LPS from *Escherichia coli* (10mg/kg) or saline. MRI studies were conducted in a 7T system, baseline and at 3h and 24h post-administration, including T_2W images, T_2 and T_2* maps, magnetization transfer imaging and diffusion tensor imaging. Parametric maps were processed using an in-house Python pipeline. Statistical analysis was performed using linear mixed-effects models with R. Analyzed brain areas include cortex, hippocampus, thalamus and hypothalamus. Mice were sacrificed by high-power focused microwave for ^{1}H-HRMAS spectroscopy or by intracardiac perfusion for immunofluorescence assays (Iba1 to detect microglia and GFAP for astrocytes). Cell morphology was analyzed with an in-house ImageJ macro.

RESULTS & DISCUSSION: A significant decrease of mean diffusivity (MD) was observed in LPS-treated mice after injection in all brain areas, while MD increased in control mice (**Figure-1**). Immunofluorescence assays (**Figure-2**) showed higher number of astrocytes and microglia in LPS-treated mice, being microglia cells bigger and more circular, meaning that diffusivity is hindered by these reactive cells, therefore the MRI results of LPS-mice. The increased diffusivity in controls suggests a vasogenic effect caused by the saline injection, which might be masking a higher decrease in MD in LPS-mice. These results confirm that diffusion metrics are the most sensitive to neuroinflammation effects. Regarding HRMAS, lower concentration of glucose was observed in all brain areas of LPS-mice than controls, and higher concentration of glutamate in the cortex (**Figure-3**). These differences are probably caused by the toxic effect of LPS and the reactive state of astrocytes and microglia.

FIGURE 1

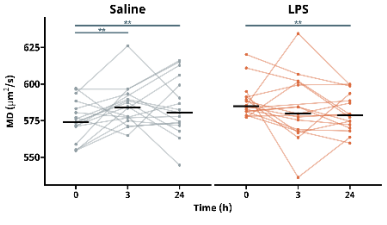

FIGURE 2

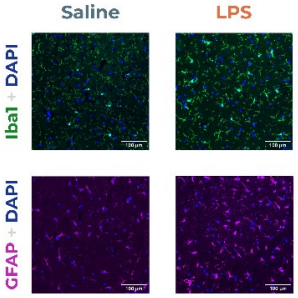

FIGURE 3

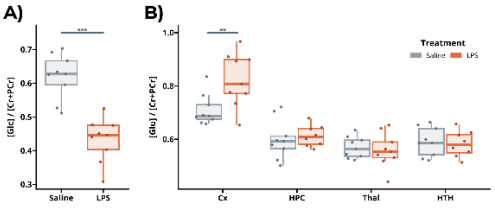

Figure 1 – Mean diffusivity before and 3h and 24h after injection (average of all brain areas for each subject is represented).
Figure 2 – Immunofluorescence images of microglia (Iba1) and astrocytes (GFAP) in a section of hippocampus.
Figure 3 – Relative metabolite concentrations obtained by HRMAS spectroscopy of ex-vivo samples. A) Average of glucose concentration along all brain areas. B) Glutamate concentration by brain area.

References: 1. A. Skrzypczak-Wiercioch, K. Sałat, *Molecules*. 27, 5481 (2022)

Human adult hippocampal neurogenesis is shaped by neuropsychiatric disorders, demographics, and lifestyle.

Berenice Márquez-Valadez [1,2,3¥]; Marta Gallardo-Caballero [1,2,3¥]; María Llorens-Martín [1,2,3].

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3 Department of Molecular Biology, Faculty of Sciences, Universidad Autónoma de Madrid; Madrid (Spain).

¥ These authors contributed equally.

The mammalian hippocampus is one of the few regions of the brain to host the addition of new neurons during adulthood, a phenomenon known as adult hippocampal neurogenesis (AHN). As a result, newly formed dentate granule cells incorporate into hippocampal circuits, modulating hippocampal-dependent functions. Patients with neuropsychiatric disorders show structural and functional abnormalities in the hippocampus. However, whether AHN impairments underlie these alterations remains unknown. To address this question, we aimed to determine whether major depression (MD), schizophrenia (SCH), and bipolar disorder (BD) compromise the integrity of human AHN and the homeostasis of the dentate gyrus neurogenic niche – a specialized micro-environment in which new neurons grow. With this objective, our study examined a cohort of 59 human subjects (24 females and 35 males). Coded fresh frozen glass-slide-mounted 14µm hippocampal sections were obtained, including tissue from 14 neurologically healthy control subjects and 45 patients (15 per diagnosis group: MD, SCH, and BD). Using immunohistochemistry and confocal microscopy imaging, we conducted an in-depth analysis of hippocampal sections to assess alterations across individual AHN stages in neuropsychiatric disorders. Additionally, we evaluated alterations of the neurogenic niche components including astrocytes, microglia, and blood vessels. Morphometric characterizations and unbiased stereological cell quantifications were used to characterize distinct cell populations. Demographic and lifestyle data were also included in our analysis. Our findings demonstrate that human AHN is shaped by distinct neuropsychiatric disorders and that it is further influenced by demographic and lifestyle factors. These data might be relevant for the design of future therapeutic strategies to prevent or treat mental health disorders.

Analysis of the effect of a mutant variant of E2F4 in oligodendrocytes in a mouse model of Alzheimer´s disease

C. González-Bragado[1], A. Lozano-Ureña[1], J. M. Frade[1]

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ABSTRACT

Alzheimer's disease (AD) is the most common age-related neurodegenerative disease and form of dementia. Recent studies have highlighted the role of non-neuronal cells and their potential contribution to the disease progression. Oligodendrocytes (OL) are generated throughout life from oligodendrocyte progenitor cells (OPCs), making OPCs a potential source to repair myelin defects in AD. Previous studies in our lab have shown that the expression of a dominant negative form of the transcription factor E2F4 (E2F4DN), unable to become phosphorylated in a conserved threonine-conserved motif, reduces microgliosis and astrogliosis in the homozygotic mouse model 5xFAD, but there is not yet studies related to OL population. This study aims to characterize the oligodendrocytic lineage in this AD model and assess the impact of E2F4DN on these cells. The analysis of these populations reveals an increase in the number of OPCs in the cortex and corpus callosum of 5xFAD animals. In contrast, the CA1 region of the hippocampus, another well-known area involved in AD, shows a decrease in the number of proliferative OPCs in the 5xFAD animals. Notably, the expression of E2F4DN in these animals tends to attenuate the alterations of these regions to control levels. The gene expression of different OL lineage markers show changes in the pan-marker *Olig2* and the OL mature marker *Mbp* in the 5xFAD cortex. The expression of *Olig2* is downregulated at early and late stages of the pathology. Instead, the expression of *Mbp* decreases at 3 months but is upregulated at 6 months, suggesting a dynamic shift in the oligodendrocytic lineage population during the pathological progression. These results emphasize the importance to understand how OL are affected and to investigate new tools, such as E2F4DN, as therapeutic strategies in AD.

E2F4-based gene therapy demonstrates a safe profile for Alzheimer's disease treatment

I. Camacho-Olmos[1,2], C. Sánchez-Puelles[1,2], J.M. Frade [1,2]

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ABSTRACT

Alzheimer's disease (AD) is an irreversible neurodegenerative disorder and the leading cause of dementia, accounting for 60% to 80% of all cases. It affects over 50 million individuals worldwide, and its prevalence is expected to continue rising. Accordingly, the World Health Organization classifies AD as a global public health priority. Despite decades of research, effective therapeutic options are limited. We have focused on the transcription factor E2F4, which regulates cellular quiescence and modulates gene networks involved in AD. Using gene therapy, we developed a dominant-negative, non-phosphorylatable form of human E2F4 (hE2F4DN). This therapeutic strategy has been patented and is licensed by Tetraneuron, a biotech company spin-off from the Cajal Institute and currently is about to enter clinical phase. When delivered via an adeno-associated viral vector (AAV.hE2F4DN) in homozygous 5xFAD mice (h5xFAD) has shown memory improvement, reduced reactive astrocytosis and microglial activation, among other benefits. Previous studies demonstrated no adverse effects when hE2F4DN was expressed *in vitro* in primary hippocampal neurons or *in vivo* in control animals, with no impact on neuronal survival, synaptic function, or memory performance, while showing therapeutic benefits in AD models. The objective of this study was to further evaluate the safety profile of hE2F4DN-based gene therapy for AD. We investigated whether hE2F4DN expression affects neuronal process complexity by analysing neuronal morphology in both wild-type mouse pyramidal neurons and human induced pluripotent stem cell (iPSC)-derived hippocampal neurons. Our data confirmed that AAV.hE2F4DN expression does not alter neuronal complexity in either model. Importantly, this evaluation in human iPSC-derived neurons represents a translational step bridging murine studies and clinical application, reinforcing the safety profile of hE2F4DN across species. These findings provide further support for the safety and translational potential of hE2F4DN. We propose E2F4DN-based gene therapy as a promising, multifactorial, and safe approach for the treatment of AD.

Refining transcription factor enrichment analysis with the upgraded TFEA.ChIP

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ABSTRACT (300 words max)

Identifying transcription factors (TFs) that regulate co-expressed gene sets is a critical step in interpreting differential gene expression and uncovering underlying biological mechanisms. TFEA.ChIP is a key tool for TF enrichment analysis, using publicly available ChIP-seq data. The original version used GeneHancer to link TF binding sites to target genes, a necessary yet challenging aspect of enrichment analysis. It then used ChIP-seq data from ReMap2022 to identify TFs bound to these regions, highlighting potential master regulators. However, this approach is limited by its dependence on predicted interactions, with minimal grounding in raw experimental data and lacking the resolution needed for tissue- or cell type-specific contexts.

To address these limitations, we present a major update to TFEA.ChIP. By incorporating cell type–specific associations from the rE2G ENCODE resource, the tool now captures regulatory patterns across diverse cellular contexts, enhancing biological relevance. The rE2G database is built on high-resolution experimental data, including CRISPR and Hi-C, providing a robust foundation for mapping regulatory interactions. Additionally, the updated version filters out TFs not expressed in specific cell types, reducing noise and providing more accurate results. Benchmarking against gene expression profile datasets demonstrates that the updated TFEA.ChIP outperforms the original in accuracy and predictive power.

In summary, this upgraded version of TFEA.ChIP offers a more precise, context-aware framework for TF enrichment analysis, facilitating deeper exploration of the transcriptional networks underlying complex cellular responses.

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Oral Communications I seminario 1 (10:00 -13:00)

PROSPECTIVE ASSOCIATION BETWEEN PLASMA AMINO ACIDS AND HEALTHY AGING IN OLDER ADULTS

Damián González-Beltrán[1], Esther Lopez-Garcia[1,2,3], and Francisco Félix Caballero[1,2].

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ABSTRACT

Background: Most studies have compared plasma amino acids profiling across different age groups using a cross-sectional design, but no previous research has assessed the relationship between specific amino acid species and healthy aging.

Objectives. This study aims to explore the relationship between plasma concentrations of nine amino acids and healthy aging in an older Spanish population.

Methods: This longitudinal study uses data from the Seniors-ENRICA 2 Spanish cohort, which comprises community-dwelling individuals aged 65 and older. Plasma amino acid concentrations were measured at baseline and after a five-year follow-up period (n = 859). Healthy aging has been defined as the delay on the onset of chronic conditions, optimal physical functioning, and no cognitive impairment. Mixed-effect logistic models were used to examine the prospective association proposed, after adjusting for age, sex, socioeconomic status, and lifestyle behaviors.

Results: The baseline mean age of the participants was 70.9 years (SD = 4.0), and 51.6% were men. In the fully adjusted models, lower plasma concentrations of alanine [odds ratio (ORs) per 1-SD increase (95% CI) = 0.78 (0.72, 0.86)], isoleucine [0.70 (0.63, 0.78)], leucine [0.78 (0.71, 0.86)], and valine [0.79 (0.71, 0.86)], were prospectively associated with healthy aging (p-value < 0.001 in all cases). No significant associations were observed for glutamine, glycine, histidine, and aromatic amino acids.

Conclusion: Lower concentrations of alanine and branched-chain amino acids were prospectively associated with healthy aging in the older population.

The Association between Plant-Based Diets and Chronic Kidney Disease Incidence: A Prospective Study from the UK Biobank Cohort

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ABSTRACT (300 words max)

Background: Chronic kidney disease (CKD) affects to 9% of population worldwide. Only a few studies have investigated the role of diet on the risk of developing CKD in European populations and have mainly focused on the Mediterranean diet. This is the first study to evaluate the association between plant-based diets and CKD incidence in British adults.

Methods: 106,870 participants from the UK Biobank, a prospective cohort, followed from 2009 to 2012 through 2021. Food consumption was obtained from at least two 24-h dietary assessments. Diet was assessed with the healthful Plant-based Diet Index (hPDI) and the unhealthful Plant-based Diet Index (uPDI). Incident CKD was obtained from clinical records, death registries, and self-reports. To analyze the study associations, multivariable Cox regression models were used, and 3-knots cubic splines to evaluate linearity.

Results:2934 cases of CKD were ascertained after a median follow-up of 9.27 years. Hazard ratios (95% confidence interval) of CKD for the highest compared with lowest tertile of adherence was 0.79 (0.72, 0.87) for the hPDI and 1.27 (1.16, 1.40) for uPDI. The association was linear and in a dose-response manner. Results were robust: 1) among older adults, 2) including participants with CVD and cancer, 3) excluding participants with major risk factors for CKD, 4) excluding diagnoses within the first two years of follow-up; 5) including only participants with three or more diet assessments, 6) further adjusting for nephrotoxic medications.

Conclusions: In British adults, higher adherence to the hPDI was associated with lower risk of CKD, whereas greater adherence to the uPDI was associated with greater risk.

Secondhand Smoke exposure in outdoor settings assessed by airborne and biomarkers of tobacco: a systematic review

Authors:

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ABSTRACT

Background

Exposure to secondhand smoke (SHS) is associated with cardiovascular and respiratory diseases and certain types of cancer. The World Health Organization recommends extending smoke-free policies to outdoor public places. This study aimed to conduct a systematic review to summarize SHS exposure in outdoor settings assessed by airborne and biomarkers of tobacco.

Methods

Studies measuring airborne and biological markers of tobacco to assess exposure to SHS in outdoor spaces were screened. A search for open-access articles was carried out in the PubMed, Scopus, and Ovid-Medline databases between the years 2000 and 2025. The search strategy included the following MeSH terms and keywords: (Secondhand smoke OR environmental tobacco smoke OR passive smoking OR secondhand smoke OR Tobacco Smoke Pollution) AND (outdoor* OR outdoor setting OR outdoor place OR terraces).

Results

35 articles met inclusion criteria. A total of 27 studies used PM2.5 concentration, 6 used airborne nicotine and 2 salivary cotinine as SHS markers. 19 studies out of 35 assessed

SHS in outdoor hospitality venues; 4 measured SHS in building entrances and 3 of them measured SHS in hospital campuses. Other outdoor settings included were outdoor smoking facilities (n=2); beaches (n=1); music festivals (n=1); children's playgrounds (n=1); university campuses (n=1); entrances of primary schools (n=1); airport terminals (n=1); and bus stops (n=1). Mean PM2.5 concentration ranged from 4.1 to 101.5 μg/m^3 when smokers were not present and from 17.9 to 233.6 μg/m^3 when smokers were present. Most studies indicated a significant association between SHS measures and the presence of smoking bans, walls, humidity, season temperature, wind, and the number of smokers present.

Conclusions

We found high SHS levels in some outdoor areas. In some cases, these levels were comparable to those obtained indoors when smoking was allowed. Health authorities should consider extending smoke-free policies to outdoor settings.

Home and school urban food environment and childhood obesity in the city of Madrid

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ABSTRACT

Unhealthy food retailers are considered a key component of the obesogenic environment. However, no studies have quantitatively assessed their impact on children's health in Spain. We aim to evaluate the association between the density of unhealthy food outlets around children's homes and schools and their weight status in Madrid.

We carried out a multilevel study. Individual data came from a representative survey of 5961 children aged 3-12. Our outcome variables were overweight/obesity, measured using objective anthropometric data. We assessed the density of unhealthy food retailers around the home-, school-, and both food environments combined. Using GIS, we calculated 400m and 800m street network buffers and operationalised them into quartiles (Q4 the highest count). We estimated Prevalence Ratios (PR) and confidence intervals (CI95%) using Poisson regression models with robust errors - adjusted for age, family socioeconomic status, sex, and population density. We stratified models by sex and family affluence.

After adjusting for covariates, a higher count of unhealthy food outlets for an 800 m buffer in the school and combined environments was associated with a higher prevalence of overweight for those in Q4 of access to unhealthy foods (PR = 1.24 95% CI 1.02 – 1.50) and (PR = 1.20 95% CI 1.01 – 1.41), respectively. For obesity, in the combined food an association was observed for those in Q2-Q4 ([Q2, PR=1.50 95% CI 1.08-2.07]; [Q3, PR=1.41 95% CI 1.01-1.97]; [Q4, PR=1.58 95% CI 1.11-2.24] with a 400 m buffer, and in Q2 and Q4 [Q2, PR=1.47 95% CI 1.07 - 2.03; Q4, PR=1.65 95% CI 1.19 - 2.29] with an 800 m buffer. Stratified analyses showed a positive association among girls for obesity.

The density of unhealthy food outlets around home and school, both together and individually, may play an important role in childhood overweight/obesity in the city of Madrid.

Macronutrient content and quality, and risk of multimorbidity in the UK Biobank

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ABSTRACT (300 words max)

Background: Multimorbidity is one of the main determinants of health span among older adults. The impact of diet on multimorbidity onset has been studied, addressing only specific diet patterns or nutrients. We aimed to examine the prospective association between overall, healthy and unhealthy Low-Carbohydrate diets (LCD), and Low-Fat diets (LFD), and incidence of multimorbidity.

Methods: 112,710 individuals from the UK Biobank cohort. Food consumption was assessed using up to five 24-hour dietary recalls. LCD and LFD scores were computed, with unhealthy and healthy versions of both scores (high-quality vs. low-quality carbohydrate or fat, and plant vs. animal protein). Multimorbidity was defined as the coexistence of two or more of nine chronic diseases including cancer, chronic obstructive pulmonary disease, dementia, Parkinson's disease, stroke, depression, osteoarthritis, diabetes, and coronary heart disease.

Results: 8,387 multimorbidity cases occurred during a median follow-up of 10.7years. Overall LCD and LFD scores were not associated with higher risk of multimorbidity. For unhealthy LCD score, a higher risk of multimorbidity was found for individuals in the highest quintile vs. the lowest quintile [fully-adjusted hazard ratio (HR): 1.07, 95%CI: 1.01, 1.15, p-trend=0.16]; analyses among non-tobacco smokers showed an estimate of 1.11 (1.00, 1.23, p-trend=0.09). The unhealthy LFD score was associated with multimorbidity among the general population [fully-adjusted HR Q5 vs. 1: 1.07 (1.00, 1.14), p-trend=0.07] and among never smokers [1.12 (1.01, 1.24); p-trend=0.01]. Results for healthy scores were less consistent. The plant protein component in the scores showed an inverse association with the risk of incident multimorbidity, whereas the low-quality fat component and the animal protein component were associated with higher risk of multimorbidity.

Conclusions: Diets defined only by the total amount of carbohydrates or fat were not associated with incident multimorbidity. However, unhealthy versions including low-quality macronutrients and animal protein were associated with increased multimorbidity risk.

Cardiovascular health, as per Life's Essential 8, and impaired lower-extremity function in older adults

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ABSTRACT (300 words max)

Background and objectives: Cardiovascular health (CVH) is a broad construct that encompasses multiple behavioral and biological factors. A decline in CVH has been associated with various adverse health outcomes, but its role in impaired lower-extremity function (ILEF), a major contributor to disability, diminished quality of life and mortality in older adults, is unknown. Therefore, we examined the cross-sectional and prospective association between CVH and lower-extremity function.

Methods: Using data from 2,487 individuals aged ≥65y from the Seniors-ENRICA-2 cohort, we estimated CVH at baseline using the American Heart Association's Life's Essential 8 (LE8) score (range 0 to 100, with higher values indicating better CVH). We assessed ILEF at baseline and at 2.4-year and 5.2-year follow-ups using the Short Physical Performance Battery (SPPB). Statistical analyses were conducted with logistic regression with adjustment for the main confounders.

Results: ILEF was present in 26.8% of participants at baseline (666 events). The cumulative incidence over 2.4 and 5.2 years was 24.8% (278 events) and 22.5% (157 events), respectively. A 10-point higher LE8 score at baseline was associated with lower prevalence of ILEF (SPPB ≤9) at baseline (odds ratio [OR] 0.75, 95% confidence interval [CI]: 0.69-0.80), and lower risk of incident ILEF over 2.4 years (OR 0.77, 95% CI: 0.68-0.87) and 5.2 years (OR 0.76, 95% CI: 0.65-0.89). Physical activity, glucose levels,

body mass index and nicotine exposure stood out as major contributors to the lower risk of incident ILEF associated with a higher LE8 score.

Conclusions: A higher LE8 score was associated with both a lower prevalence and incidence of ILEF in older adults. Comprehensive evaluation of CVH offers insight into older adults' lower-extremity function and how it may progress over time, identifying opportunities for early intervention.

Association between a Planetary Health Diet and Changes in Intrinsic Capacity in Older Adults: The Seniors-ENRICA cohorts

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ABSTRACT (300 words max)

Background: The Planetary Health Diet (PHD) benefits health and the environment. However, its impact on healthy ageing, estimated by intrinsic capacity (IC), remains unexplored.

Objective: To examine the association between adherence to a PHD Index (PHDI) and changes in IC in older adults.

Methods: Data were collected from 2519 adults aged ≥60 years from the Seniors-ENRICA-1 (2012-2015), and 3273 aged ≥65 years from the Seniors ENRICA-2 (2017-2019) Spanish cohorts. Food consumption was collected with a dietary history, and the PHDI was based on 15 food groups. IC was measured across six domains: cognition, psychology, vitality, hearing, vision, and locomotion (ranged: 0-18, lower score equals better IC). Adjusted multinomial logistic regressions were used, and data from both cohorts were pooled.

Results: Over a 2.6-year median follow-up, IC worsened for 32.0% participants, improved for 27.7%, and remained stable for 40.3%. Participants in the highest vs lowest tertile of adherence to the PHDI were more likely to improve vs worsen IC (relative risk ratio [RRR] 1.36; 95% confidence interval [95% CI] 1.05-1.77; P-trend= 0.021). Higher PHDI scores were significantly

associated with improvement vs worsening in the hearing domain (RRR 1.37; 95% CI 1.04-1.82; P-trend= 0.025). Higher adherence to PHDI's recommendations regarding nuts (RRR 1.05; 95% CI 1.01-1.09) and starchy vegetables (RRR 1.09; 95% CI 1.01- 1.17) were independently associated with improvement vs worsening IC.

Conclusion: In these older-adult cohorts, higher adherence to the PHDI was associated with improvement in overall IC, and in its hearing domain. Adherence to nuts and starchy vegetables recommendations were particularly beneficial.

HEALTH AND ENVIRONMENTAL DIETARY IMPACT: PLANETARY HEALTH DIET VS. MEDITERRANEAN DIET. A NATIONWIDE COHORT IN SPAIN

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ABSTRACT (300 words max)

Background: Plant-based diets, such as the Planetary Health Diet (PHDI) and the Mediterranean Diet (Med), offer notable advantages for human and planetary health. However, knowledge on the PHDI's benefits is limited, particularly in Southern European countries where the Med is culturally rooted and is an environmentally sustainable dietary pattern.

Objective: to evaluate the association of both PHDI and Med with mortality and assess their environmental burden in the adult population of Spain.

Methods: Data were taken from the study on Nutrition and Cardiovascular Risk in Spain (ENRICA) comprising 13,105 participants representative of the Spanish adult population. The PHDI score (0–140 points) was based on 15 food groups, while adherence to Med was assessed with the 14-item MEDAS score (0–14 points). Environmental impact was assessed using the SHARP-ID database (including greenhouse gas emissions and land use). Analyses were performed with Cox regression and adjusted for main confounders.

Results: During a mean 14.4-year follow-up, 1157 all-cause deaths occurred. The mortality hazard ratio (95% CI) for the highest vs lowest tertile of the PHDI score was 0.78 (0.66, 0.91) but reached a plateau level at 90 points of PHDI. For the MEDAS, the corresponding results for the highest vs lowest tertile was 0.79 (0.68, 0.93) with a continuous inverse dose-response association. Adherence to some components of the PHDI (fruits, dairy, and unsaturated oils) and of MEDAS (nuts, and low consumption of soda and pastries) was independently and significantly associated with lower mortality. Results remained robust in sensitivity analyses. In terms of environmental impact, both plant-based diets had similar low footprints, with dairy and meat products being the largest contributors.

Conclusion: In this large cohort of Spanish adults, higher adherence to the PHDI and MEDAS was similarly associated with lower all-cause mortality and showed comparable low environmental impact.

Association of a healthy lifestyle and social frailty predictors with the risk of multimorbidity

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ABSTRACT (300 words max)

Background: Multimorbidity is defined as the coexistence of multiple chronic conditions, and it is becoming increasingly common among older adults.

Objective: To assess the prospective association between lifestyle factors and social determinants and the risk of developing multimorbidity among community-dwelling older adults.

Methods: This study included 1288 adults aged ≥65y from the Seniors-ENRICA II cohort. Lifestyle and social characteristics were measured at baseline (2015-2017) and summarized into the Healthy Lifestyle Index (HLI) and Social Frailty Index (SFI). Medical diagnosis information was obtained from electronic health records in Primary Care. These conditions were grouped across 45 categories and within 4 body systems to assess multimorbidity (≥6 chronic conditions) and complex multimorbidity (≥4 chronic conditions affecting ≥1 body systems). Cox proportional hazard models adjusted by potential confounders were used.

Results: 1288 participants [mean(SD) age, 70.8(4.17); 59.7% men] were followed up for a median of 6.5 years. We identified 611 cases of multimorbidity and 460 cases of complex multimorbidity. Participants in the highest tertile of healthy lifestyle adherence versus the lowest had a decreased risk of developing multimorbidity [fully-adjusted HR (95%CI): 0.79 (0.64-0.98), P-trend: .03]. A 2-point HLI score increment was associated with a 7% (95%CI: 2%-12%) reduction on the risk of multimorbidity and 12% (95%CI: 7%-17%) less risk of complex multimorbidity. No significant associations were found for the SFI and multimorbidity but, among participants with above-average adherence to healthy lifestyles, greater social robustness was associated with a lower risk of complex multimorbidity [HR in comparison with below-average adherence to healthy lifestyles and low social robustness: 0.72 (95%CI: 0.52-0.98) for medium social robustness, 0.67 (95%CI: 0.48-0.92) for high social robustness].

Conclusions: Greater adherence to a healthy lifestyle was associated with a reduced risk of developing multimorbidity, while social frailty was relevant on multimorbidity development only for those with above-average adherence to healthy lifestyles.

Breast cancer survival by subtype, stage at diagnosis and socioeconomic status among young women in Madrid, Spain

Author Candela Pino-Rosón[1]; David Parra-Blázquez[2]; Sonia Ávila-Arroyo[2]; Daniel Moñino[3]; María José Soto Zabalgogeazcoa[4]; Clotilde Sevilla-Hernández[2]; Raquel López-González[1]; Cristina González-Blázquez[5]; Miguel Martín[6]; Nuria Aragonés [2,7]

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Objective: The aim of this study was to determine 5-years survival rates of breast cancer diagnosed in young adult women (20-49 years) in 2018 by grade, molecular subtype, stage at diagnosis and socioeconomic deprivation.
Methods: Invasive breast cancer cases diagnosed in young adult women (20-49 years) in 2018 in Madrid were extracted from the Population-Based Cancer Registry of the Community of Madrid. Descriptive analyses were performed for sociodemographic and tumour characteristics. For survival analysis, observed survival rates (OS) were calculated using the Kaplan–Meier method, and net survival and age-standardized net survival rates using the Pohar Perme estimator. These were calculated at 1, 3, and 5 years, both globally and stratified by grade, molecular subtype, stage at diagnosis and deprivation quartile. Flexible parametric models were adjusted to determine differences in the instantaneous death risk between molecular subtypes.
Results: In 2018, 1,049 new cases of invasive breast cancer were registered among 1,432,392 women. Half of the patients were aged 45-49 years. Grade II, Luminal B subtype and early stages diagnosis were the most common characteristics. The global 5-year OS was 95%. Stratified survival analysis showed that grade III tumours [90.5% (95% CI: 86.0-93.7)], triple negative subtype [85.4% (95% CI: 77.0-91.0)] and stage IV at diagnosis [53.8% (95% CI: 37.2-67.9)] showed the lowest survival rates. Incidence rates were highest among least deprived women, with no differences in survival observed across deprivation quartiles. Women with triple negative tumours had a 6.45 times higher death risk than those with Luminal A tumours ($p<0.01$).
Conclusions: Although young women with breast cancer have excellent prognoses, there are differences in survival rates by molecular subtype and stage. This study offers comprehensive epidemiological data on breast cancer characteristics and survival in young women in the Community of Madrid.

Universidad Autónoma de Madrid | Escuela de Doctorado | Facultad de Medicina

Oral Communications I seminario 2 (10:00 -13:00)

Extracellular vesicles from hypothalamic astrocytes modify transcription factors of the leptin signaling pathway in proopiomelanocortin (POMC) neurons.

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ABSTRACT (300 words max)

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. Hypothalamic astrocytes communicate with POMC neurons via extracellular vesicles (EVs) that contain proteins, lipids, and nucleic acids, and relay information regarding metabolic status. Our hypothesis is that astrocytes affect neuronal function and leptin signaling pathway via EVs 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. At 24 h POMC expression raised in response to leptin and EV-OA, and the increase in POMC expression was significantly higher with the combined treatments. This could be due to an effect of EVs on leptin signaling pathways. FoxO1 is a repressor of POMC transcription and when phosphorylated (p-FoxO1) it does not repress POMC transcription. Levels of p-FoxO1 were unchanged in response to EV-PA, whereas EV-OA increased them at both 4 and 24h. FoxO1 forms complexes with PGC-1α to regulate some genes and PGC-1α was decrease in response to EV-PA at 4 and 24 h; however, with EV-OA these levels were increased at 4 and 24 h. Therefore, EVs from hypothalamic astrocytes could contain biological molecules, such as miRNAs, that modulate the leptin signaling pathway affecting POMC transcription in these neurons.

Universidad Autónoma de Madrid | Escuela de Doctorado | Facultad de Medicina

Semana del Doctorado 2025 en la Facultad de Medicina

Outpatient Care Reduces Complications in Stem Cell Transplantation

Javier Cornago Navascués[1], Juan Carlos Caballero Hernáez[1], Amalia Domingo-González[1], Jose L. López Lorenzo[1], Laura Pardo Gambarte[1], Jesús Ignacio Merlo Luis[1], Alberto Sánchez Donaire[1], Ana Rodríguez Calvo[1], Carmen López Álvarez[1], Pilar Llamas Sillero[1] and Laura Solán Blanco[1]

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ABSTRACT

Home-based hematopoietic stem cell transplantation (HSCT) is an emerging approach that allows patients to undergo treatment outside the hospital, with close medical supervision. This model aims to improve patient comfort, reduce hospital-related complications and optimize healthcare resources. We conducted a retrospective analysis of 135 adults undergoing auto-HSCT between march 2021 and november 2024.

Median time to neutrophil engraftment was significantly shorter in inpatient (IP) group: 11 days (range: 10–13) compared to 14 days (range: 12–16) in the outpatient (OP) group. This difference may be explained by the absence of G-CSF administration and less frequent blood testing in the OP group. No significant differences were observed in time to platelet engraftment.

Significantly fewer febrile episodes were observed in OP (41.18% *vs.* 87.13%, $p < 0.001$) and a lower incidence of infections (2.9% *vs.* 26.73%, $p = 0.003$). Mucositis was significantly less frequent in OP compared to IP (20.59% *vs.* 82.18%, $p < 0.001$). The need for parenteral nutrition was markedly lower in the OP group (8.82% *vs.* 75%, $p < 0.001$). Diarrhea occurred less frequently in OP (64.7% *vs.* 82.17%, $p = 0.034$). None of the OP required morphine infusion, compared to 11% of inpatients ($p = 0.065$).

Despite a slightly delayed engraftment, outpatient care is a safe and effective approach. Patients in the outpatient regimen have lower rates of febrile episodes, infections, and mucositis, as well as lower needs for parenteral nutrition.

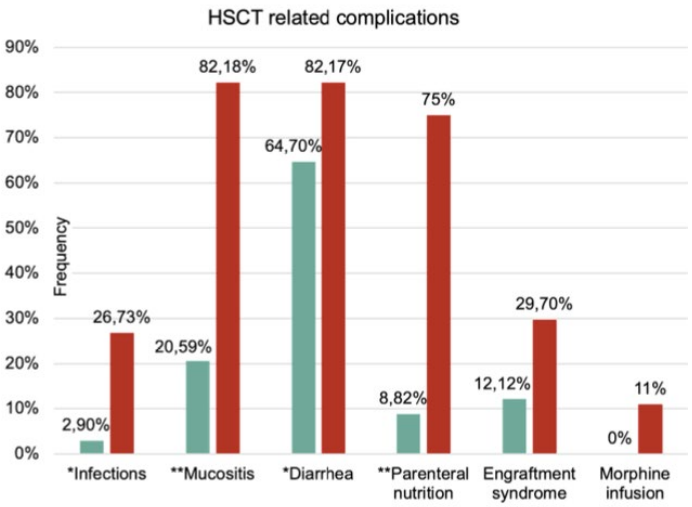

Figure 1. HSCT related complications. * p<0.05; ** p<0.001

Outpatient / Inpatient

TITLE: Validation of the NECPAL 4.0 prognostic model in a primary care dependence program in Chile: a study protocol using multiple methods.

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ABSTRACT (300 words max)

Introduction: Prognostic models help clinicians identify individuals with limited life expectancies who may need a palliative approach. The NECPAL 4.0 prognostic model uses six palliative need indicators plus age and has been shown to perform well in predicting 24-month mortality in a Catalonia cohort, identifying three prognostic stages to aid clinical decision-making. The Primary Care Division in Chile recommends this model to identify palliative care beneficiaries. This thesis aims to externally validate the NECPAL 4.0 prognostic model in home care program for individuals with severe dependence in Chile's South Metropolitan Health Service.
Methods and analysis: Multiple-methods studies. Two prospective cohort studies utilise secondary data from all patients (N≈2100) screened for palliative needs in 2022-2023 across primary centers delivering the program in SSMSO, with a follow-up period of 24 months. Data collected includes sociodemographic variables of patients and caregivers, Surprise Question (SQ), and NECPAL 4.0 variables. The first study aims to evaluate SQ's predictive ability for mortality at 12 and 24 months, comparing it to NECPAL 4.0 stages. It will analyse sensitivity, specificity, and predictive value, along with survival analysis using Cox regression and Kaplan-Meier curves with Log-Rank tests. The second study aims to externally validate the model in patients with SQ positive. It will assess 24-month mortality as primary outcome and evaluate model's calibration and discrimination, along with Kaplan-Meier analysis for risk groups. Besides, one qualitative study with multiple case design using semi-structured interviews and thematic analysis will explore model's acceptability and identify barriers and facilitators to its use in clinical practice by generalist and specialist providers of palliative care (N≈20).
Discussion: Data collection and cleaning are underway. Mortality follow-up finishes by December 2025. Qualitative study approval is pending.
Ethics and dissemination: quantitative studies were approved by SSMSO ethics committee. Results will be shared in four articles and at conferences.

Keywords: Prognosis, Palliative Care, Advanced diseases

TITLE: The Impact of Childhood Adversities on Disability in Adulthood: Exploring the Role of Psychosis and Loneliness

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ABSTRACT (300 words max)

Introduction: Adverse childhood experiences (ACEs), including abuse, neglect, and loneliness, have been linked to various long-term health outcomes, including psychiatric disorders and disabilities. Psychotic disorders, among the most debilitating psychiatric conditions, significantly impair cognitive and social functioning, leading to substantial disability. While the link between ACEs and disability is well-established, less is known about how specific forms of childhood adversity impact functional impairment, especially in individuals with first episodes of psychosis (FEP). This study examines the mediating role of psychosis and explores how different types of childhood adversity influence adult disability.

Methods: The study sample included 431 individuals with a first episode of psychosis (FEP) and 346 healthy controls, recruited from two observational studies in Madrid. Participants were assessed for sociodemographic factors, childhood trauma (using the CECA questionnaire), and disability (using the WHO Disability Assessment Schedule 2.0). Linear regression models examined the relationship between childhood adversity and disability, with interactions for psychosis status and control for age, sex, and education.

Results: The results revealed that higher educational levels were associated with lower disability, while older age was linked to higher disability. Psychosis status was significantly associated with increased disability. Among the various forms of childhood adversity, loneliness showed the most pronounced impact, particularly for individuals with psychosis. The loneliness-psychosis interaction was significant, suggesting that loneliness exacerbates disability in those with psychosis. Other forms of trauma, such as sexual abuse, also contributed to disability, but their effects were not moderated by psychosis status.

Discussion: This study highlights the significant impact of childhood loneliness on adult disability, particularly in individuals with psychosis. The findings emphasize the need for targeted interventions that address loneliness in at-risk populations, including those with psychotic disorders. Childhood trauma, especially loneliness and sexual abuse, should be considered in both clinical and preventive mental health strategies to mitigate long-term disability. Future research should explore the role of loneliness in other psychiatric disorders and the potential benefits of early intervention programs.

NEUROPATHOLOGICAL VALIDATION OF THE BOSTON CRITERIA V2.0 FOR CEREBRAL AMYLOID ANGIOPATHY IN ALZHEIMER'S DISEASE DEMENTIA

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ABSTRACT

BACKGROUND AND OBJECTIVES
Cerebral amyloid angiopathy (CAA) is closely linked to Alzheimer's disease (AD) and increases the risk of Amyloid-Related Imaging Abnormalities (ARIA) in patients receiving anti-amyloid therapies. While brain MRI is the standard for detecting CAA, its effectiveness in this population remains understudied. This study aimed to evaluate the diagnostic performance of the Boston criteria version 2.0 for CAA diagnosis in individuals with advanced AD and neuropathological confirmation.

METHODS
The study included 63 individuals from the VARS cohort of the Alzheimer's Centre Reina Sofía - CIEN Foundation, a clinicopathological cohort of dementia patients, with no history of intracranial haemorrhage. Each underwent antemortem MRI with T2 flair and GRE sequences, and a brain autopsy assessing CAA with the modified Vonsattel scale. The accuracy of the Boston v2.0 criteria was evaluated against neuropathologically confirmed CAA.

RESULTS
The mean age at MRI was 84.7 years, with a median time from MRI to death of 2.5 years. Most participants were women (86%), and 73% had neuropathologically confirmed moderate or severe CAA. Boston v2.0 criteria were not met by 60.3% of subjects, yet 45% of them had moderate or severe CAA. The criteria had a sensitivity of 40.8% and specificity of 64.2% for probable CAA (AUC 0.52), and a sensitivity of 83.7% and specificity of 35.7% for possible CAA (AUC 0.51).

CONCLUSIONS
The Boston v2.0 criteria have low accuracy in patients with AD dementia. New biomarkers are needed to improve the diagnosis of CAA in this population in order to optimize the safety of treatment with anti-amyloid drugs.

NUTRITIONAL PARAMETERS AS PREDICTORS OF COMPLICATIONS IN ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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ABSTRACT

Advanced anthropometric study and determination of nutritional parameters is not routinely performed as part of the assessment prior to haematopoietic stem cell transplantation (HSCT). We wanted to identify those morphofunctional parameters that, in advance, allow us to stratify patients into different risk groups for complications or transplant-related mortality (TRM). A retrospective analysis of 48 adult patients undergoing allogeneic HSCT between February 2021 and June 2023 in a tertiary hospital was performed.

A significant relationship was found between low albumin levels on admission and the development of acute graft versus host disease (GVHD) ($p<0.05$). At 6 months post-transplant, patients with admission albumin levels <4 g/dL had a cumulative incidence (CI) of 84% for acute GVHD, while those with a higher threshold had a CI of 22% (HR 13.9, $p<0.0001$). A trend towards statistical significance was identified in the relationship between lower calf circumference and the presence of acute GVHD.

Age showed a relationship with TRM ($p<0.05$). The 1 year CI of TRM was 31% in patients older than 58 years versus 1% in patients younger than this age (HR 4.6, $p=0.03$). A significant difference was also observed between TRM and obesity. The 1 year CI of TRM was 42% in obese patients, compared to 12% in patients with a body mass index below 30 (HR 4.6, $p=0.03$). On the other hand, Pearson's correlation test results revealed a significant association ($p<0.01$) between mid-upper arm circumference at admission and length of hospital stay, suggesting a relationship between lower muscle mass with longer hospitalisation stay.

Malnutrition and increased basal catabolism result in an inflammatory state that predisposes to the development of acute GVHD. Optimising body composition by reducing the fat component to avoid obesity and promoting muscle mass can reduce complications, hospitalisation time and even TRM.

FROM PREDIABETES TO NORMOGLYCEMIA: WHICH PREDICTORS ARE INVOLVED?

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Background:
Prediabetes carries a risk of cardiometabolic-complications including type-2 diabetes (T2DM). However, a considerable number of patients regresses to normoglycemia (RNG), but this response and the associated markers are not completely elucidated. We evaluated potential predictors for RNG in a sample of high-risk patients at the outpatient clinic.

Methodology:
621 patients with history of Impaired Fasting Glucose (IFG) from Madrid and Lima were evaluated in a longitudinal study for 5-years follow-up for the incidence of RNG. Prediabetes was considered at baseline in patients with fasting glucose ≥ 100 mg/dL and/or HbA1c ≥ 5.7%, according to the American Diabetes Association (ADA). The outcome RNG was defined when the new glycemia measurement exhibited both a fasting glucose < 100mg/dl and HbA1c < 5.7% (ADA).

Results
Among 621 selected patients, 311 exhibited prediabetes and completed the follow-up. The mean age of the population was 57±11.5 and 69.5% of them were females. After 5 years, 54 (17.4%) subjects showed RNG, 169 (54.3%) remained as prediabetics, and 88 (28.3%) progressed to T2DM. Interestingly, we found a significant negative association between basal glycemia, HbA1c, liver enzymes (AST/TGP) and plasma creatinine with the development of RNG [OR 0.94 CI 95% (0.91 –0.97), p< 0.001; OR 0.90 CI 95% (0.82–0.98), p= 0.014; OR 0.78 CI 95% (0.56–1.00), p= 0.047 and OR 0.77 CI 95% (0.60– 0.97), p= 0.022, respectively]. In addition, we observed a positive association of the glomerular filtration rate (CKD-EPI) [OR 1.47 CI 95% (1.04–2.20); p= 0.008)] and RNG.

Conclusion:
Lower levels of basal glycemia, HbA1c, liver enzymes and plasma creatinine, but an increased glomerular filtration rate may be potential determinants of RNG in prediabetes.

Key words: *Prediabetes; T2DM; Normoglycemia; Regression*

FROM PREDIABETES TO NORMOGLYCEMIA: WICH PREDICTORS ARE INVOLVED?

Juan Carlos Lizarzaburu-Robles (1), Flor Vento (2), Ignacio Mahillo-Fernandez (5), Amalia Paniagua (3), Clotilde Vázquez (3), Maite Ortega, Sebastián Mas-Fontao (6), Alonso Garro-Mendiola (4), Blanca Timón (3), Sacramento Martínez-Albaladejo (6), Oscar Lorenzo (6)

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Background:
Prediabetes condition carries a risk of cardiometabolic-complications and a high-risk state for T2DM progression. Despite regression from prediabetes to normoglycemia (RNG) is accompanied by an improvement in cardiometabolic risk factors, determinants for this process are not completely defined. We evaluated predictors of RNG in a sample of high-risk patients at the outpatient clinic of two general hospitals.

Methodology:
621 patients, with history of Impaired Fasting Glucose (IFG) from Madrid-Spain and Lima-Peru, were selected to evaluate in a longitudinal study for 5-years follow-up. Prediabetes was considered at baseline in patients with fasting glucose ≥ 100mg/dL and/or HbA1c ≥ 5.7%, according to the American Diabetes Association (ADA). The outcome RNG was defined when the new glycemia measure exhibit a fasting glucose < 100mg/dl and HbA1c < 5.7% (ADA).

Results
Of the 621 selected-patients, 311 subjects exhibit prediabetes and complete the follow-up. The mean age of the sample was 57±11.5 and 69.5% were female. After the follow-up, 54 (17.4%) subjects RNG, 169 (54.3%) remained in prediabetes and 88 (28.3%) progressed to T2DM. We found a significant association with low levels of basal glucose (BG), HbA1c, AST/TGP and creatinine measurement and the RNG [OR 0.94 CI 95% (0.91 –0.97), p< 0.001; OR 0.90 CI 95% (0.82–0.98), p= 0.014; OR 0.78 CI 95% (0.56–1.00), p= 0.047 and OR 0.77 CI 95% (0.60– 0.97), p= 0.022 respectively]. Interestingly, our findings show a positive association with high levels of Glomerular filtration rate (CKD-EPI) [OR 1.47 CI 95% (1.04–2.20); p= 0.008)] and the RNG.

Conclusion:
Several heterogeneous individual characteristics may contribute towards regression to normoglycemia in subjects with prediabetes. However, according to our results the low levels of basal glucose, HbA1c, Liver enzyme AST/TGO and a high level of Glomerular filtration rates may be potential determinants of regression to normoglycemia in high-risk individuals.

Key words: *Prediabetes; T2DM; Normoglycemia; Regression*

TITLE: Understanding Loneliness in Spain: trajectories and predictors from the Edad con Salud longitudinal study

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ABSTRACT (300 words max)

Background: In recent years, research has shown that loneliness negatively affects both mental and physical health and has been declared a public health issue. Moreover, loneliness is a dynamic phenomenon that, in some cases, can become chronic, leading to greater health consequences. Therefore, it is important to examine whether heterogeneous patterns in loneliness experiences exist and to identify profiles of individuals who follow different trajectories. This study aimed to identify distinct loneliness trajectories among Spanish adults and to explore a range of potential determinants—sociodemographic, social, psychological, and health-related—associated with these trajectories.

Methods: Using data from 4,537 adults participating in a nationally representative longitudinal study in Spain over a 12-year period, we applied growth mixture modeling to identify distinct loneliness trajectories. Logistic regression analyses were then conducted to examine predictors of trajectory membership.

Results: Two trajectory classes were identified: a *low-stable* group (87.86%) and a *high-unstable* group (12.14%). Being unmarried, divorced or widowed, living alone, being migrant, having social isolation, depression, suicidal ideation, and cognitive complaints significantly increased the likelihood of belonging to the high-unstable group. In contrast, social support, social trust, and life satisfaction were protective factors.

Conclusion: This study revealed the existence of heterogeneous loneliness trajectories, each with distinct characteristics and associated factors. The findings underscore the need for tailored strategies in the assessment, prevention, and management of loneliness.

TITLE

Effect of Virtual Reality Distraction on Anxiety, Pain, Readiness, and Satisfaction in Patients Undergoing Cardiac Catheterization in Palestine: A Randomized Controlled Clinical Trial.

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ABSTRACT

Background: Cardiovascular diseases (CVDs) are a leading cause of global mortality, with coronary artery disease (CAD) being a significant concern in Palestine. Patients undergoing cardiac catheterization frequently experience anxiety and pain, which can adversely affect both their clinical outcomes and overall procedural experience. Virtual reality (VR) has emerged as a promising non-pharmacological intervention to alleviate anxiety and pain in medical settings.

Aim: To evaluate the effect of a pre-procedural virtual reality distraction intervention on anxiety, pain, readiness, and satisfaction levels in patients undergoing cardiac catheterization in Palestine.

Method: A prospective, randomized controlled clinical trial will be conducted among patients scheduled for elective cardiac catheterization. Participants will be randomly assigned to either the intervention group, which will receive a single VR distraction session before the procedure, or the control group, which will receive standard pre-procedural care. Anxiety, pain, readiness, and satisfaction will be assessed using validated measurement scales before and after the intervention.

Expected Result: It is hypothesized that patients in the VR group will report significantly lower levels of anxiety and pain, along with higher levels of readiness and satisfaction, compared to those receiving routine care.

Conclusion: The findings could inform the implementation of innovative, patient-centered interventions to enhance the quality of care and procedural outcomes for individuals undergoing cardiac catheterization in Palestine.

Action of different fatty acids on communication between astrocytes and neurons mediated by TGFβ

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ABSTRACT (300 words max)

Obesity is a globally widespread multisystemic disease, and to combat it understanding the mechanisms that regulate homeostasis is necessary. Neuronal activity can be regulated by various factors released by astrocytes. Proopiomelanocortin (POMC) neurons in the arcuate nucleus detect and respond to nutritional changes. Their activity is regulated by, among others, transforming growth factor β1 (TGFβ1). Extracellular vesicles (EVs) have been shown to be involved in astrocyte-neuron communication. Our aim was to determine whether the nutritional environment affects the production of TGFβ1 and whether it modulates the effect of astrocyte EVs on POMC neurons.

Our results show that treatment with oleic (OA) or palmitic (PA) fatty acids stimulates TGFβ1 expression in primary hypothalamic astrocytes of male mice, with no effect in females; however, astrocytes of female mice release higher levels of TGFβ1 to the medium. We also analysed the NF-κB pathway, which is key in inflammation and cell survival, and may act as a signalling pathway for TGFβ1. We observed that p-IκB levels increased in astrocytes of both sexes treated with PA, whereas with OA, p-IκB levels decreased in females.

In addition, we studied the effect of TGFβ1 on POMC neurons. We found that at low concentrations, the expression of POMC is reduced, but when treated with high concentrations, POMC expression is increased. Finally, TGFβ1 was found to interact with EVs isolated from astrocytes to modulate POMC expression. EVs treated with OA or PA, increased POMC expression; however, the greatest effect occurred with EVs from astrocytes not exposed to fatty acids in conjunction with TGFβ1 resulting in a significant synergistic effect.

We conclude that the nutritional environment modulates the expression and secretion of TGFβ1 in astrocytes, with differences according to sex. This factor also affects POMC neurons, and interacts with astrocyte EVs regulation in a nutrient-dependent manner.

Universidad Autónoma de Madrid | Escuela de Doctorado | Facultad de Medicina

Oral Communications II Aula Magna (15:00 -17:30)

Adipose tissue mitochondrial dysfunction promotes obesity cardiomyopathy.

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Obesity, a condition resulting from an excess of adipose tissue, is a serious health problem worldwide and an important factor in the development and progression of cardiovascular diseases. It is well established that mitochondrial dysfunction in adipose tissue might contribute to obesity-related diseases. We found that adipose tissue of obese patients showed a reduced expression of PGC1α, an important mitochondrial regulator. Echocardiographic analysis of mice lacking PGC1α specifically in adipose tissue fed with normal diet showed that these mice develop a cardiac dysfunction like the one observed during obesity. However, this cardiomyopathy was not accompanied by diabetes, hypertension or increased adiposity. Recovery of adipose tissue functionality with AAV encoding PGC1α or BAT transplantation from C57BL/6 mice can reverse cardiac dysfunction. Proteomics analysis of plasma from mice lacking PGC1α in adipocytes and obese participants from a second human cohort revealed several promising adipokines, mainly from the brown adipose tissue, that could be driving obesity cardiomyopathy.

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Exploring the contribution of STAG1 and STAG2 terminal regions to their specific functions as part of Cohesin

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ABSTRACT

Cohesin is a ring-shaped complex that mediates chromatin-loop formation. These loops are major players in genome folding during interphase, facilitating contacts between distal genomic regions. Cohesin is composed of subunits SMC1A, SMC3, RAD21 and either STAG1 or STAG2. Complexes carrying STAG1 or STAG2 are present in all cells and either one is sufficient to maintain cell viability, but they have both common and specific roles. Our group has shown that the STAG1 is more important for Topologically Associated Domain (TAD) border maintenance, while STAG2 is central in maintaining local chromatin contacts, such as those between enhancers and promoters, thereby supporting cell-type specific transcription.

To this day, it is unclear what confers the STAG paralogs with such different behaviors. The central regions of the two proteins are 80% identical, but they differ in the amino- and carboxi-terminal regions, which are largely unstructured. Thus, we hypothesize that the terminal regions of the STAG proteins determine their specialized behavior as part of Cohesin.

To test this hypothesis, we plan to assay the behavior of chimeric versions of the STAG subunit, combining the terminal regions of STAG1 and STAG2. To avoid interference with endogenous proteins, these will be eliminated when inducing the expression of the chimeras. We have generated a cell line in which STAG1 and STAG2 genes have been edited to add FKBP and AID degron tags, respectively, which will allow for rapid and reversible degradation of either or both proteins. We will next introduce cDNAs of the chimeric proteins in a safe-harbor locus for their expression under the control of a Tet-on system. By analyzing the chimeric proteins' behavior in terms of association to chromatin and genome-wide distribution, we intend to dissect the structural determinants underlying the specific functions of Cohesin-STAG1 and Cohesin-STAG2.

Programa de Doctorado en *Biociencias Moleculares*

Validation of STAT4 polymorphism as a biomarker in patients with early arthritis

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Background: Previous results from the group described that patients with early arthritis (EA) who are homozygous for the minor allele (TT) of the rs7574865 polymorphism in STAT4 have higher disease activity and disability in their evolution[1]. However, validation of this biomarker is needed prior to implementation in clinical practice. Our group belongs to the Inflammatory Diseases Network (REI; RD24/0007)whose main objective is sample sharing processes for biomarker validation.
Objective: To validate the association between TT genotype of rs7574865 in STAT4 and severity in EA patients from REI's cohorts.
Methodology: A total of 385 patients (300 fulfilling RA criteria and 85 with undifferentiated arthritis), 317 new patients from PEARL study and 68 patients from IMIBIC EA cohort, with a total of 1444 visits were included in the study. DNA was obtained from peripheral blood and the samples were genotyped for the rs7574865 genetic variants using a predesigned SNP-Genotyping Assay Disease activity was assessed with HUPI[2] and disability by HAQ[3] We fitted two population-averaged model by generalized linear models nested by patient and visit in which HUPI and HAQ were the dependent variables and the independent variables those included in the original work[1].
Results: After adjusting for confounding variables such as gender, age and anti-citrullinated proteins antibodies, the TT genotype was associated with a trend to increased HUPI values (beta coefficient=0.66, p=0.143) as compared with the GG genotype. However, we did not observed association between homozygosity for the T allele of rs7574865 in STAT4 and greater disability as compared with the GG genotype (beta coefficient=0.054, p=0.613).
Conclusions: Our new data seems to confirm that patients with EA who are homozygous for the T allele of rs7574865 in STAT4 may develop a more severe form of the disease with increased disease activity. These results agree with those obtained previously, however a higher population should be required to validate rs7574865 in STAT4 as a severity biomarker in patients with EA.

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Targeting tumor resistance to *Raf1* deletion in NSCLC

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ABSTRACT (300 words max)

Lung tumors are the leading cause of death among cancer patients. At the molecular level, oncogenic mutations in KRAS are present in one-third of all cases of lung adenocarcinoma. Although these mutations have been studied for four decades, effective therapies targeting the oncogenic downstream signaling of KRAS have not yet been approved. In 2018, Sanclemente et al. found that targeting RAF1 induces significant regression of advanced *Kras*^{G12V}/*Trp53*^{KO} mutant lung tumors. This effect occurs through a mechanism that induces massive apoptosis without affecting canonical MAPK signaling.

RAF1 is an effective therapeutic target for advanced KRAS mutant tumors, leading to partial regression of most tumors, and a high percentage of complete regressions. However, we found that resistance to *Raf1* ablation emerges after two months. Over time, tumor cells can adapt to the absence of RAF1, activating alternative signaling pathways or compensatory mechanisms that restore tumor growth. This acquired resistance limits the long-term efficacy of *Raf1* ablation, highlighting the need for combination therapies or additional approaches to overcome or prevent resistance.

Through RNA sequencing data from *Kras*^{G12V}/*Trp53*^{KO}/*Raf1*^{KO} resistant tumors, we have identified various targets to overcome *Raf1* deletion resistant tumors. One significant finding is the upregulation of KRAS, which suggests that even after targeting *Raf1*, the tumors may activate compensatory mechanisms that enhance KRAS signaling. This upregulation of KRAS underlines the need to determine and validate suitable pharmacological compounds targeting these pathways. Furthermore, additional research is needed to fully understand the molecular mechanisms underlying this resistance.

The motor adaptor protein Miro1 is critical in the intercellular mitochondrial transfer to glioblastoma cells

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ABSTRACT (300 words max)

Glioblastoma (GBM) is the most common and lethal glioma, due to its high ability for tissue infiltration and chemoresistance. Recent discoveries reveal that GBM cells form tunneling nanotubes (TNTs) that communicate with surrounding neural cells, promoting GBM infiltration and tumor progression. TNTs enable GBM cells to establish coordinated networks and communications, ranging from intercellular signaling to the sharing of intact and functional mitochondria. In this context, the dynamics of mitochondrial transfer require active transport mediated by the motor adaptor Miro1, which is critical for the anterograde movement of organelles to the periphery of the donor cells. However, whether and how Miro1 orchestrates the transfer and integration of mitochondria into GBM cells remains unaddressed.

In this work, live microscopy, flow cytometry, and morphometric analysis show that, as expected, Miro1 is critical for the intercellular transfer of mitochondria to GBM cells. Genetic knockout models effectively block the anterograde transport of mitochondria, and we demonstrate for the first time that this process can be modulated by pharmacologically reducing Miro1 levels. As a result of Miro1 loss of function, mitochondria are absent in TNTs, and mitochondrial transfer to GBM cells is drastically reduced, even though the number of TNTs remains unaltered. Notably, Miro1 appears to be irrelevant in acceptor cells to incorporate exogenous free mitochondria. On the other hand, donor cells lacking Miro1 may still release mitochondrial content, which can be captured by adjacent GBM cells.

In summary, our results place Miro1 as a key regulator of mitochondrial transfer via TNTs and highlight its importance in understanding the mechanisms governing this process in GBM progression. Importantly, the genetic and pharmacological modulation of Miro1 strongly endorses this protein as a potential therapeutic target to halt brain tumor development.

Mechanisms underlying vascular impairment in COPD: new unsuspected role of nAChR

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ABSTRACT (300 words max)

Tobacco smoke is the main risk factor for the development of chronic obstructive pulmonary disease (COPD). Despite current therapies alleviate symptoms there are limitations in the efficacy of treatments to curb its cardiovascular morbidities, particularly pulmonary hypertension. Our previous studies demonstrate that cigarette smoke directly contributes to pulmonary arterial dysfunction. However, further characterization of the molecular basis involved is needed for more effective targeted treatment. We have performed *in vitro* analysis with human pulmonary artery smooth muscle cells challenged with cigarette smoke extract, and *in vivo* approaches of tobacco exposure in murine models and transgenic mice. Additionally, we extrapolated our results in pulmonary arteries from human samples. These approaches allowed us to explore the molecular pathways contributing to the harmful effects from oxidative stress calcium dysregulation and disruptions to the contractile machinery of pulmonary artery smooth muscle cells. Interestingly, these effects were triggered by the activation of α7 nicotinic acetylcholine receptors (nAChRs) in these cells. Additionally, we demonstrated that nAChR antagonists or α7 nAChR deletion in a murine model effectively protected pulmonary artery function from damage. Most importantly, α7 nAChR expression in pulmonary arteries of COPD patients rose with disease severity and showed an inverse correlation with respiratory function. These findings have important clinical implications, indicating that nAChR-targeted antagonists could be a promising therapeutic strategy for COPD-related pulmonary hypertension.

Urea cycle upregulation is a metabolic adaptation driving liver-specific metastatic organotropism

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ABSTRACT (300 words max)

Metastatic cells preferentially colonize specific organs, a phenomenon known as metastatic organotropism. Lung and liver are primary targets for disseminated cells, yet the metabolic adaptations enabling survival in these tissues remain unclear.

To investigate these adaptations, we engineered A375 and 4T1 cells to express an OMP25-bound HA tag on their mitochondrial membrane. These cells were injected into mice and mitochondria was isolated and immunoprecipitated from primary tumors, lung, and liver metastases for proteomic analysis using TMT isobaric labelling. Additionally, we conducted an in vivo CRISPR screening with a LOF sgRNA library targeting nuclear-encoded mitochondrial genes in MDA-MB-231 cells.

Our proteomic analysis revealed distinct proteomic profiles between primary tumors, lung, and liver metastases. Notably, liver metastases exhibited an upregulation of urea cycle enzymes, particularly CPS1, corroborated by CRISPR screening showing selective depletion of CPS1-targeting sgRNAs in liver metastases. CPS1 expression was significantly elevated in liver metastases across multiple tumor models and patient datasets. Functionally, CPS1 deficient cells exhibited reduced metastatic capacity to the liver, with no impact on their growth in 2D or 3D culture, primary tumor formation, or metastatic burden in the lungs. Since CPS1 is essential for ammonium detoxification, we found that liver ammonium levels were significantly higher than in tumors, plasma, lung, or brain. CPS1-depleted cells were more sensitive to physiological ammonium concentrations, exhibiting impaired proliferation and reduced mitochondrial respiration in its presence. These results suggest that CPS1 deficiency sensitises metastatic cells to the high ammonium concentrations present in the liver environment, thus affecting their ability to colonise this tissue.

Our findings reveal that metastatic cells undergo distinct metabolic adaptations depending on the secondary tissue, with CPS1 playing a key role in liver-specific colonization. These insights refine our understanding of metastatic organotropism and postulate CPS1 as a potential therapeutic target to disrupt liver metastasis.

Targeting cGAS/STING for the treatment of genomic instability driven pathologies.

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ABSTRACT (300 words max)

The accumulation of free DNA from DNA repair deficiencies has been shown to trigger activation of the cGAS/STING pathway. In particular, several works indicated that the activation of cGAS/STING by genomic instability was due to micronuclei, which would release DNA during their futile and asynchronous DNA replication. However, these works were primarily done in vitro, and the relevance of this phenomenon to disease remains to be addressed.

Previous work from our group revealed that deficiency in NSMCE2, part of the SMC5/6 complex, leads to a substantial accumulation of micronuclei due to chromosome segregation errors. In mice, this is linked to an increased incidence of cancer and accelerated ageing. Given the links between micronuclei and cGAS/STING, we hypothesized that some of the pathologies of NSMCE2-deficient mice could derive from the accumulation of cytoplasmic DNA. Specifically, these mice suffer from Karyomegalic Interstitial Nephritis (KIN). Our work indicates that, indeed, the onset of KIN is associated to cGAS/STING activation. scRNAseq analyses provided the first picture of the cellular changes that occur during KIN and further supported the driver role of cGAS/STING in this disease. Accordingly, the use of the STING inhibitor H-151 significantly alleviated kidney inflammation in NSMCE2-deficient mice.

Together, our work provides the first in vivo characterization of a disease that is linked to genomic instability-driven activation of the cGAS/STING pathway.

Gut microbiota ecology differs across metabolic and obesity phenotypes

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ABSTRACT (300 words max)

Obesity is a complex disease that can severely impact patient health and well-being in the long term. Metabolically healthy obesity (MHO) is a transient phenotype defined by the lack of metabolic alterations and risk of comorbidities that usually accompany an increased body mass index. Given that the gut microbiota (GM) participates in metabolic and inflammatory processes related to obesity, it may play a relevant role in this condition. Here, we aim to describe whether there is an "MHO microbiome" by using network science methodologies.

Our sample comprises 959 subjects with MHO, metabolically healthy non-obese (MHNO), metabolically unhealthy obese (MUO) and metabolically unhealthy non-obese (MUNO) phenotypes. To achieve this sample size, we have used whole genome sequencing data from the AI4Food cohort, formed by 98 Spanish patients with overweight and obesity, together with publicly available data from the R package curatedMetagenomicData.

After characterizing their GM through alpha- and beta-diversity measurements, differential abundance analysis, and machine learning, we moved on to a systems biology approach based on co-occurrence networks. These networks allow us to go past taxonomic relative abundances and obtain information about potential microbial interactions. We evaluated topological properties, identified keystone taxa, and assessed network robustness as a measurement of ecosystem stability.

Metabolically healthy microbiomes were characterized by more robust and functionally cohesive microbial networks, where the most influential microbes are SCFA producers, while metabolically compromised communities show a dysbiotic state with reduced connectivity and are dominated by low-abundance, ectopic, and potentially pro-inflammatory species. Our results highlight metabolic disorders as a driver of ecological changes in the human gut microbiota and showcase the potential of network-based methods for the study of microbial communities.

This study was funded by AI4FOOD-CM (Y2020/TCS-6654), CD3DTech-CM (TEC-2024/BIO-167), PID2023-150146OA-I00, IMPaCT-Data (IMP/00019), COST Action CA18131, FPU22/04053, and MSCA (101105645).

Molecular mechanisms of high-fat diet as a risk factor for pancreatic ductal adenocarcinoma (PDAC) initiation and progression in adult mouse models.

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ABSTRACT (300 words max)

Pancreatic ductal adenocarcinoma (PDAC) is the seventh leading cause of cancer-related deaths, due to late diagnosis and limited treatments. High fat diet (HFD)-induced obesity is a significant risk factor for PDAC. Yet, the mechanisms linking obesity triggered by high-fat diets to tumor initiation and progression remain poorly understood. Previous research has shown that *KRAS* mutant cells and precursor lesions are relatively common among healthy individuals. Most of genetically-engineered mouse models (GEMMs) for PDAC bear these mutations from embryonic development. However, we showed that expression of *Kras* mutation in adult acinar cells alone does not lead to ADM or PanIN/PDAC, but in the presence of chronic pancreatitis a subset of these cells gets transformed. Our goal is to delineate the molecular mechanisms underlying the effects of HFD on adult pancreas tumorigenesis.

Using *Kras*$^{+/LSLG12Vgeo}$; *Trp53*$^{lox/lox}$; *Elas-tTA/tetO-Cre* (KpeC) mice, we induced oncogenic expression postnatally (P21) and fed them HFD. Unlike mice with embryonic *Kras* activation which develop tumors by 12–16 weeks, postnatal expression reduced tumor incidence, supporting adult resistance to tumor formation (Guerra et al., 2007). HFD-treated mice had worse overall survival compared to controls. HFD-induced tumors exhibit a mesenchymal phenotype, in contrast with the epithelial phenotype in controls. Primary HFD-derived PDAC cells displayed mesenchymal morphology yet were enriched in EpCAM+Sca1+ cancer stem cells (CSCs). RNAseq analysis revealed an enrichment in gene signatures related to epithelial-mesenchymal transition (EMT), invasiveness, and cancer stemness. Indeed, functional assays confirmed enhanced migration capacity in HFD tumor cells. HFD cells exhibited *Gata6* downregulation, suggesting that HFD induces a hybrid subtype expressing both epithelial and mesenchymal markers.

These findings suggest that HFD promotes aggressive PDAC with hybrid epithelial and mesenchymal phenotypes and enhanced tumor cells plasticity. We aim to elucidate obesity-mediated molecular mechanisms and identify preventive or therapeutic strategies for high-risk obese patients.

Universidad Autónoma de Madrid | Escuela de Doctorado | Facultad de Medicina

Oral Communications II seminario 1 (15:00 -17:30)

NEW INTRON RETENTION TAU ISOFORMS FIGHTS PROTEINOPATHY REVERTING CLASSIC TAU IMPAIRMENTS AND DISMINISHING TAU SEEDING ACTIVITY

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ABSTRACT (300 words max)

Recently, a novel MAPT transcript variant resulting from intron 12 has been described (CW-Tau). This new Tau isoform exhibits microtubule-binding properties, a reduced propensity to aggregate, and is notably decreased in the brains of Alzheimer's disease (AD) patients. Cells overexpressing CW-Tau, unlike those expressing canonical Tau isoforms, displayed normal vesicle trafficking and optimal autophagy flux, akin to control cells. This contrasts with the disrupted vesicle trafficking and impaired autophagy flux observed in cells expressing canonical Tau isoforms. Moreover, the export of Tau to the extracellular space, a process linked to intercellular transmission impaired Tau clearance, varied among different Tau isoforms. We demonstrated that canonical Tau isoforms can transmit proteinopathy between cells, a process partly mediated by exosomes. However, CW-Tau was able to prevent the transfer of proteinopathy between cells between cells, conferring this novel Tau isoform with a highly efficient anti-seeding capacity.

Furthermore, co-expression of CW-Tau with canonical Tau isoforms prevents the cellular effects typically induced by the canonical forms, indicating that CW-Tau can effectively inhibit canonical Tau activity and its aggregation and seeding capabilities.

The existence of additional Tau isoforms originated by intron retention has been demonstrated, specifically those generated by the retention of intron 3 (referred to as NW-Tau) and the retention of both introns 3 and 12 (referred to as DW-Tau). These isoforms are predominantly expressed in the central nervous system with higher levels than CW-Tau, and also contribute to maintaining cellular homeostasis. Notably, they also exhibit anti-aggregative properties and are capable of inhibiting Tau seeding activity and propagation.

All these findings put all CW-Tau, NW-Tau and DW-Tau as promising tools for gene therapies of Alzheimer's disease.

Evaluación del papel de la sestrina1 en células tubulares y en modelos murinos de fracaso renal agudo.

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ABSTRACT (300 words max)

El fracaso renal agudo (FRA) es una afección en la cual hay una disminución de la función renal resultando en un aumento de la creatinina sérica. Tiene una alta mortalidad y los tratamientos son limitados, de ahí la importancia de encontrar nuevas dianas terapéuticas. Las sestrinas son proteínas de respuesta a estrés que están implicadas en varias enfermedades y estudios de transcriptómica previos del grupo sugieren un posible papel de la sestrina1 en el FRA.

- *In vitro*: se utilizó una línea celular tubular proximal (MCTs) de ratón. Se estudió la expresión de la sesn1 estimulando las células con la citoquina TWEAK, lipopolisacárido bacteriano (LPS) y ácido aristolóquico. Además, se hicieron estudios de viabilidad y citotoxicidad silenciando la sesn1 con un siRNA específico. Asimismo, se evaluó el efecto del silenciamiento sobre la dinámica mitocondrial.
- *In vivo*: se realizaron varios modelos de FRA: uno nefrotóxico inducido por sobredosis de ácido fólico, uno de tormenta de citoquinas inducido por inyección con LPS y, por último, uno por administración de ácido aristolóquico.

En células los estímulos causaron una disminución de la expresión génica de la sesn1. Por otro lado, el silenciamiento de la sesn1 produjo una disminución en la viabilidad y un aumento en la citotoxicidad de las MCTs. Asimismo, la ausencia de sesn1 resultó en un aumento en la expresión del marcador de biogénesis mitocondrial pgc1α y en una disminución de la producción de ATP y el potencial de membrana mitocondrial de las células; entre otras alteraciones. Por otro lado, la expresión de la sesn1 disminuía en todos los modelos de FRA respecto a los ratones control.

La sesn1 es importante para la viabilidad celular de las MCTs y podría tener un papel importante en la dinámica mitocondrial. Por otro lado, su disminución en los ratones con FRA sugiere un papel de esta proteína en esta afección.

Studying infection and neurodegeneration induced by HSV-1 in 3D human neuronal cultures

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ABSTRACT

Alzheimer's disease (AD) is a devastating neurodegenerative disease as well as the most common form of dementia. The increasing incidence and the huge complexity of the disease underscore the need to develop more sophisticated tools that better resemble the heterogeneous and dynamic environment of the human brain, as well as integrate other influential factors, such as, viruses, which have been proposed to have a role in neurodegeneration. The results obtained so far support the so-called infectious hypothesis of AD and the potential implication of herpes simplex virus type I (HSV-1). In this line, the development of new study platforms aims to fill the gap between the lab and the clinic, thus improving the transferability of experimental results. This is the reason why we are currently working with human neuronal stem cells—which are able to differentiate into neurons and glial cells—and three-dimensional cultures—which reflect features such as cell-extracellular matrix interactions or spatial orientation. We have already characterized HSV-1 infection in both progenitors and differentiated 2D cultures, and observed the main alterations associated with AD neuropathology. We have also initiated the development of 3D models composed by Matrigel with embedded cells, which maintain the differentiation capacity and their susceptibility to HSV-1 infection. These new tools will serve to study different molecular mechanisms that link HSV-1 infection and AD-like neurodegeneration, especially those related to changes in cholesterol homeostasis and neuroinflammation. In conclusion, these new strategies could lead to a better understanding of the disease and the role of one of its risk factors, shed some light on the controversy surrounding the infectious hypothesis of AD, and even contribute to the development of a model of AD that, in contrast to the vast majority of current study platforms, is induced by an environmental factor rather than genetic mutations.

The NS2 protein of the parvovirus Minute Virus of Mice (MVM) disrupts the Rae1-mediated mRNA nuclear export machinery

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ABSTRACT (300 words max)

Many cellular mRNAs exit the nucleus to the cytoplasm by the Rae1/Nup98 transport complex, thereby this system is targeted by protein factors of important human pathogens as Influenza, Herpesvirus, or SARS-CoV2, to maximize viral mRNA translation. It is unknown whether small ssDNA viruses are able to interfere with this host mRNA transport machinery. Previous data showed that the Rae1/Nup98 complex is a major hit of the NS2 protein of the canine parvovirus. Here, we have studied if the highly related NS2 protein of the parvovirus Minute Virus of Mice (MVM) also harbours Rae1 interactive properties, and the putative significance in virus-host interaction. In MVM infection, a strong nuclear accumulation of polyA+ mRNA was observed in mouse A9 and human NB324K fibroblasts, which are common MVM cell hosts but with distinct NS2 requirement. Structural AlphaFold-3 NS2-Rae1 model comparison among the Rae1 interacting viral factors mentioned above identified a motif in NS2 with potential Rae1 binding capacity. Mutations at this specific NS2 motif allowed the recovery of mutant viruses grown in NB324K cells showing altered NS2 subcellular distribution and impaired nuclear mRNA polyA+ retention. Importantly, these mutations further severely restrict NS2-mutants plaque forming capacity and propagation in A9 mouse fibroblasts. We suggest that the NS2-mediated inhibition of Rae1-mediated mRNA nuclear export may counteract host defense mechanisms and contribute to parvovirus fitness and pathogenicity.

Accelerated and refined genomic analysis, coupled with epidemiological interventions, to optimize tuberculosis transmission control

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Background

Whole genome sequencing (WGS) is essential in tracking tuberculosis (TB) transmission, assuring highest precision to assign genetically clustered cases. The conventional approach relies on quantifying SNPs between isolates to assign clusters. Alternatively, evolutionary approach determines more precisely the relationships between cases in genomic networks, paying attention to the distribution of differential SNPs between cases. This offers insights into ongoing transmission dynamics, reactivations, and diagnostic delays. Based on these interpretations, we enable tailored epidemiological interventions, which require acceleration of the availability of genomic data.

Methods

To accelerate genomic results, we leveraged immediate analysis of each incident single TB case, using the flexibility of the nanopore sequencing platform, on 33 consecutive primary cultures and 45 clinical samples (sputa). Additionally, to exploit the data obtained from the sputa with suboptimal sequences, we designed a rescue pipeline based on the assessment of the presence/absence of key strain marker SNPs, unique to circulating strains in our population. Finally, we designed a nanopore-based amplicon sequence scheme (targeting strain-marker SNPs) to fast-track new cases of relevant clusters, without the need to perform WGS.

Results

All primary cultures achieved optimal results (≥ 90% genome coverage ≥ 20X). It allowed us to rule in 27 new cases to clusters and perform precise genomic network analysis within the 21 days of ongoing contact tracing, enabling reorientation of the epidemiological investigation. Among the 45 sputa, 24% offered optimal results (≥ 90% genome coverage ≥ 10X) while another 13% were suboptimal (≥ 20% genome coverage ≥ 10X). By applying our rescue pipeline, we could rule in new cases to 4 clusters (56-88% of marker SNPs of the 4 clustered strains were successfully called), despite their suboptimal WGS coverages. Based on this strategy, we identified and tracked an interterritorial wide transmission chain, involving a super-spreader case. A fast nanopore-based amplicon sequence scheme, targeting strain marker SNPs, was prospectively applied to identify new cases of this cluster in the two involved populations.

Conclusions

Our study presented several rapid and flexible alternative paths to couple with genomic analysis to enhance genomic epidemiology programs.

Impaired Locus Coeruleus Inhibitory Pathway in the Trigeminal System in Diabetes: Mechanisms and IGF-1-Dependent Rescue

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ABSTRACT (300 words max)

The trigeminal system plays an important role in processing somatosensory information, and its modulation is crucial for maintaining sensory homeostasis. This study investigates the inhibitory modulation of trigeminal somatosensory responses by the locus coeruleus (LC), which plays a critical role in many cognitive and physiological functions. Previous electrophysiological investigations have shown a diminished ability of the LC to inhibit somatosensory responses in the caudalis division of the spinal trigeminal nucleus (Sp5C) of diabetic mice. We hypothesize that GABAergic and glycinergic neurons in the Sp5C participate in the modulatory action of the LC. Using unit recordings in isoflurane-anesthetized control and streptozotocin-induced diabetic mice, we examined the effect of LC electrical stimulation on these inhibitory neurons during vibrissal stimulation. Antagonists of GABAergic and glycinergic receptors revealed that LC noradrenergic projections modulate these neurons via α1 and α2 adrenergic receptors, respectively. In diabetes, this inhibitory control is reduced, likely contributing to abnormal sensory processing.

We further explored LC activity in response to peripheral noxious stimulation. While formalin injection in the vibrissal pad activated the LC in control mice, this response was absent in diabetic animals. We hypothesized that impaired LC activation results from deficient insulin-like growth factor 1 (IGF-1) signaling, and that addition of IGF-1 restored LC activity and its ability to modulate inhibitory circuits in the Sp5C. Immunohistochemical experiments studied changes in the expression of IGF-1 receptors and α1 and α2 adrenergic receptors in the LC. The results revealed significant alterations in the expression of these receptors in diabetic mice, which could contribute to the loss of LC function and the breakdown of descending inhibitory control. These findings suggest that IGF-1 plays a critical role in maintaining LC functionality, and its deficiency leads to sensory dysfunction and potentially neuropathic pain.

Diferencias moleculares entre machos y hembras en modelos preclínicos de fracaso renal agudo

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ABSTRACT

El fracaso renal agudo (FRA) es un síndrome clínico de alta prevalencia, asociado a una considerable morbilidad y mortalidad, caracterizado por un deterioro rápido de la función renal para el cual, hasta la fecha, no existe un tratamiento específico. En los últimos años, se ha identificado que tanto la incidencia como la progresión del FRA pueden estar influenciadas por el sexo de los pacientes. De manera similar, estudios en modelos animales han demostrado que la respuesta al daño renal puede variar según el sexo.

En el presente estudio, basándonos en investigaciones previas de nuestro grupo y otros grupos, evaluamos si la respuesta al FRA y su severidad pueden diferir en función del sexo, y si estas diferencias podrían estar asociadas a una activación diferencial de mecanismos patogénicos como la ferroptosis, necroptosis y la inflamación.

Para ello, utilizamos tres modelos experimentales de FRA en ratones: uno inducido mediante sobredosis de ácido fólico durante 48 horas, otro por exposición a lipopolisacárido bacteriano (LPS) durante 24 horas y el tercero por exposición a cisplatino durante 72h. En dichos modelos, se analizó el deterioro de la función renal, el daño histológico, la expresión génica de los marcadores implicados en muerte celular e inflamación, así como la expresión proteica de los cambios observados a nivel génico, comparando siempre las diferencias entre machos y hembras.

Los resultados mostraron diferencias moleculares entre sexos, especialmente en términos de daño renal e inflamación. En particular, observamos que las hembras presentaron un mayor deterioro de la función renal y daño histológico en comparación con los machos en el modelo de nefrotoxicidad causado por sobredosis de ácido fólico. Mientras que, en el modelo de toxicidad por cisplatino, los machos presentaron mayor deterioro de la función renal y daño histológico que las hembras. El análisis detallado de estas diferencias, y su comparación en diversos modelos experimentales, permitirá avanzar en la comprensión del papel del sexo en el desarrollo y progresión del FRA.

Fenotipo renal de ratones Fosl2 knock-out

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ABSTRACT

Fosl2 es un factor de transcripción perteneciente a la familia AP-1 que ha demostrado tener un papel crucial en múltiples procesos incluidos inflamación y fibrosis. Se ha demostrado que la sobreexpresión de *Fosl2* en modelos murinos induce un desarrollo de esclerosis sistémica espontánea. Por otro lado, la deficiencia de *Fosl2* ha reducido la severidad de la inflamación y fibrosis. Sin embargo, el papel de *Fosl2* en la enfermedad renal no se ha caracterizado.

Para estudiar el papel de *Fosl2* durante el daño renal generamos un ratón *knock-out* de *Fosl2* específico de células tubulares proximales ($Fosl2^{\Delta tub}$). Contrariamente a lo esperado, la deficiencia de *Fosl2* en los túbulos proximales dio lugar a un fenotipo patológico basal. Si bien los niveles de creatinina y urea, utilizados como medidores de la función renal, no se vieron alterados respecto a los ratones *wild type* control, los ratones $Fosl2^{\Delta tub}$ presentaban una expresión aumentada de *Ngal*, un marcador temprano de daño renal, así como citoquinas proinflamatorias asociadas a una mayor infiltración de células inmunes. De la misma manera, se observó el desarrollo de un fenotipo fibrótico incipiente mediante el aumento de expresión de *Fn1* y de una disminución de la expresión de los factores asociados a la nefroprotección *Klotho* y *Cpt1a*. Se corroboró el mismo patrón de expresión en cultivos primarios de células tubulares renales murinas. Por otra parte el análisis transcriptómico de la expresión renal permitió identificar una expresión aumentada de *Ccn2*, el cual codifica para CTGF un factor de crecimiento conocido por desempeñar un papel central en la fibrosis renal y con un gran potencial terapéutico.

Por tanto, la deficiencia de *Fosl2* en células renales tubulares proximales de ratón parecen generar un daño renal subclínico. Estos resultados resaltan la versatilidad de las funciones de *Fosl2* en diferentes tejidos y tipos celulares, y abren nuevas líneas de investigación para el tratamiento de la enfermedad renal.

Semana del Doctorado 2025 en la Facultad de Medicina

Heat-killed bacterial immunotherapies induce trained immunity in pediatric cystic fibrosis patients

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Cystic Fibrosis (CF) is a rare chronic disease that affects 1 to 5000 newborns in Spain. It is characterized by frequent pulmonary bacterial infections that compromise respiratory functionality, inducing severe lung damage that can lead to lung transplantation or early death. We demonstrated that circulating monocytes from adult patients suffering from CF exhibit a defect in their inflammatory capacity in response to bacterial agents (del Fresno, C. *et al*. 2008). Trained Immunity (TI) refers to a form of innate immune memory in which innate immune cells, in a heterologous manner and mediated through epigenetic and metabolic changes, can enhance their responses upon a second encounter with pathogens. This concept holds clinical relevance in CF as it represents a heterologous immunomodulatory approach for enhancing host defense mechanisms in conditions where innate immunity is compromised. Bacterial immunotherapies can induce TI, a therapeutic approach that has an extensive clinical track record, to the extent that standardized formulations are now available. These formulations comprise bacterial compositions that reflect the representative bacterial composition of most patients with recurrent respiratory tract infections. Consequently, we aim to evaluate the impact of these immunotherapies on the innate immune responses of pediatric CF patients. We are examining to an unprecedented depth the immunological profile and functionality of circulating immune cells in pediatric patients suffering from CF. Using 10ml of peripheral blood from CF patients attended at La Paz University Hospital, we have deeply characterized their immunological profile using a 37-color full-spectrum flow cytometry panel designed for this project. Furthermore, we have analyzed their inflammatory functionality and antibacterial functions in phagocytosis and bacteria-killing assays. These parameters have been studied in the presence or absence of bacterial immunotherapies, consisting of preparations based on heat-inactivated bacteria. These immunotherapies may enhance the immune response in these patients by inducing TI. These findings could serve as a starting point for an innovative preventive therapy for pediatric patients suffering CF, with the potential to prevent infections and potential pulmonary damage.

Comentado [CD1]: "With Cystic Fibrosis" suena muy españolizado. Te propongo esto o también podría ser "(…) pediatric patients suffering from cystic fibrosis"

Comentado [CD2]: Non-specific… uy lo que ha dicho!!!

Directed evolution of mouse parvovirus towards human glioblastoma provides receptor binding-site mutants with enhanced oncotropism

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ABSTRACT (300 words max)

Glioblastoma is the most common form of malignant brain tumour and also the one with the worst prognosis. The lack of effective treatments makes alternative therapies urgent. Cancer virotherapy is one of the most promising approaches, with many clinical trials underway and two recombinant viruses licensed. Rodent protoparvoviruses, as the Minute Virus of Mice (MVM), have natural preference for proliferative cells, including human tumour cells. These viruses exhibit significant oncolytic activity, and the safety of their administration in humans has been recently demonstrated in clinical trials. Importantly, these viruses elicit a strong immune response which is normally depressed in central nervous system tumours. MVM tropism is primarily determined by the capsid binding to sialoglycans through a shallow depression at the twofold axis of symmetry called the "dimple". Glioblastoma glycocalix displays an altered pattern of sialoglycans, including well-known tumor-markers. To improve MVM oncotropism toward glioblastoma, we address several strategies of directed evolution that combine random mutagenesis of the capsid dimple and serial blind-passages in two glioblastoma cell lines. For this, three infectious plasmids libraries were produced by error-prone PCR (MVMp and MVMi) and by saturation mutagenesis of key codons involved in tropism and sialoglycan-interaction (MVMp). These libraries, developed cytopathic effects upon transfection and few passages in U373MG cells, but not in U87MG cells. In total, six different mutants were selected with changes in exposed residues near the sialoglycan binding-site of the capsid dimple. Accordingly, their sensitivity to neuraminidases activities were different to wild type viruses. Their fitness improvement in the infection of U373MG cells was demonstrated by higher viral genome production and viral protein expression. The oncotropism of selected mutants was assessed in a panel of human and rodent cell lines. These results demonstrate that directed evolution targeting the sialoglycan-receptor binding-site of parvovirus is a successful strategy to enhance oncotropism.

Abstract: 299 words

Universidad Autónoma de Madrid | Escuela de Doctorado | Facultad de Medicina

Poster Communications Abstracts
(Decanato Hall 14:00 – 15:00h)

625 – Programa de Doctorado en Biociencias Moleculares
Elimination of Hyaluronic acid in Pancreatic Ductal Adenocarcinoma (PDAC) as a potential strategy for immunotherapy

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths, with a five-year survival rate around 5–7%. In PDAC, stromal cancer-associated fibroblasts (CAFs) play a vital role in promoting the desmoplastic and immunosuppressive tumor microenvironment (TME), as well as tumor growth and malignancy, and have emerged as cancer targets (1). Has proteins are responsible for the production of hyaluronic acid (HA). Has overexpression results in accumulation of HA which leads to high pressure on neighboring structures as well as elevated interstitial fluid-pressure (IFP) within PDAC, which can interfere with drug delivery (2) and has also been linked with tumor escape from immune surveillance. Previous results from our lab showed that Has1 and Has2 was differentially expressed between PDGFRa+ CAFs and PDGFRα+ normal pancreatic fibroblasts (NPFs) (3).
We have developed developed genetically modified mouse models (GEMMs) of Has triple knocked-out (Has1/2/3 TKO) based on Has2 conditional KO mice (4) to study the role of HA in PDAC development and progression. We aim to explore the influence of HA in tumor development and in the desmoplastic and immunosuppressive TME. In allograft studies, the TKO mice environment significantly inhibited proliferation of tumor cells competent for these genes. These studies may help to design future therapeutic strategies.

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COHESIN AS A DYNAMIC COMPLEX: UNRAVELING INDEPENDENT ROLES OF ITS SUBUNITS

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ABSTRACT

Beyond its role in mitosis of ensuring proper sister chromatid cohesion and segregation, Cohesin is essential for 3D genome organization by actively extruding chromatin loops. Cohesin is a complex formed by four subunits, i.e. RAD21, SMC1, SMC3 and STAG (either STAG1 or STAG2 variant).

Cohesin has always been conceived to work as a complex, without independent functions of its different subunits. Nevertheless, recent work seems to indicate that this might not be the case. In a RAD21 degron working model, STAG subunits have been reported to localize at DNA. In a different set up, in which they study Cohesin loading by creating inducible loading sites, SMC3 has been reported to load in absence of RAD21. These findings point towards a model in which some subunits of Cohesin might present certain degree of independence from the rest of complex. Nevertheless, it is yet poorly understood the biological relevance of these findings.

In this study, we use a RAD21 AID2 degron system to assess the behaviour of the remaining Cohesin subunits upon RAD21 degradation. So far, we have observed that during G1 entry in absence of RAD21, SMC1 and SMC3 subunits load on chromatin, but not STAG1 or STAG2. In asynchronous cells, after RAD21 degradation, SMC1 and SMC3 also remain in chromatin forming a SMC1-SMC3 complex. Further work of the study will focus on discerning the possible functions of SMC1 and SMC3 at chromatin, independent from the Cohesin complex; and their relevance in a physiological cellular context, as well as in Cohesin related pathologies, e.g. cancer, cohesinophathies.

Phosphorylation-dependent regulation of FMNL1β at S1086 during immune synapse-mediated exosome secretion

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ABSTRACT (300 words max)

T-cell receptor (TCR) stimulation by antigen-presenting cells (APCs) triggers a cascade of intracellular events essential for immune responses. Among these, immune synapse (IS) formation leads to protein kinase C (PKC) activation, filamentous actin (F-actin) depletion at the central IS (cIS), and polarization of multivesicular bodies (MVB) and the microtubule-organizing center (MTOC) toward the IS, culminating in polarized exosome secretion. These exosomes are key mediators of immune regulation, influencing cytotoxicity and activation-induced cell death (AICD). However, the molecular mechanisms coordinating actin cytoskeleton remodeling and secretory polarized traffic remain incompletely understood.

Based on experiments utilizing transient transfections followed by IS formation and image analysis, we have explored the role of formin-like 1 β (FMNL1β), an actin-regulatory protein, in orchestrating MTOC/MVB polarization and exosome secretion in a phosphorylation-dependent manner. Our findings reveal that IS formation transiently recruits FMNL1β to the IS independently of PKCδ activity. RNA interference of FMNL1 isoforms disrupts MTOC/MVB polarization and exosome secretion, effects that are rescued by FMNL1β wild-type (WT) expression. Strikingly, the non-phosphorylatable FMNL1βS1086A mutant fails to restore these processes, whereas the phosphomimetic FMNL1βS1086D mutant successfully rescues them. Nonetheless, FMNL1βS1086D mutant is unable to compensate for PKCδ depletion, indicating that S1086 phosphorylation alone is insufficient for MTOC/MVB polarization and exosome secretion.

Furthermore, FMNL1 knockdown impairs F-actin depletion at the cIS, a prerequisite for MTOC/MVB polarization. Restoration of F-actin depletion at the cIS is observed by expressing FMNL1βWT and FMNL1βS1086D, but not with FMNL1βS1086A, emphasizing the functional significance of S1086 phosphorylation in cytoskeletal remodeling. These findings highlight FMNL1β as a pivotal regulator of actin cytoskeleton dynamics at the IS, governing IS-evoked exosome secretion through a FMNL1β phosphorylation-dependent mechanism. Understanding these pathways offers novel insights into T-cell immune regulation and may provide new therapeutic avenues for immune modulation.

Introducción: La enfermedad coronaria es la primera causa de muerte, con el infarto agudo de miocardio (IAM) como forma más frecuente de muerte. El IAM de tipo I es causado por la ruptura de una placa de arteriosclerosis llena de colesterol de una de las arterias coronarias y la formación súbita de un coágulo (trombo) en su interior, cerrándola e impidiendo el flujo de la sangre hasta el corazón (miocardio), cuya parte dependiente de esa arteria deja de recibir oxígeno y muere en 6-12 horas (infarto). Cuanto mayor sea el infarto más dañado quedará el corazón y más probable que el paciente muera a corto plazo o a largo plazo.

Por este motivo, en el presente trabajo se ha analizado el papel que los neutrófilos, a través de la liberación de NETs en el proceso conocido como Netosis, juegan en pacientes con IAM de tipo I.

Métodos: Se realiza la cuantificación de tres componentes de NETs (elastasa, mieloperoxidasa (MPO) y ADN libre en plasma) mediante la técnica ELISA en plasma libre de plaquetas procedente de la arteria coronaria responsable del infarto (ARI), arteria contralateral y arteria periférica. Se realizará un análisis según presentación clínica y según fisiopatología de la trombosis.

Resultados: Se analizan 58 pacientes (IAMSEST n=17, SCACEST n=31). Las variables de riesgo cardiovascular de la población se incluyen en resumen en la tabla adjunta. De los pacientes incluidos, 5 (18,8%) presentan trombosis del stent, 3 (9,7%) presentan una fisiopatología embólica y 37 (77,08%) presentan una fisiopatología aterotrombótica. En el análisis de marcadores no existe asociación entre el incremento de los marcadores analizados en la ARI ($p=0.82$, $p=0.17$ y $p=0.46$) ni tampoco en la arteria periférica ($p=0.53$ y $p=0.06$) a excepción de una leve correlación entre el aumento de MPO y DNA ($p=0.04$) (Figura 1). A pesar de que existe un incremento significativo de MPO en la ARI en ambos tipos de presentación ($p<0.001$), no se observan diferencias en la concentración del resto de marcadores entre esta arteria y la periférica (SCACEST, $p<0.395$ y $p<0.094$; IAMSEST, $p<0.892$ y $p<0.341$) (Figura 2). El análisis de la arteria contralateral en comparación con la ARI no revela diferencias entre las concentraciones de los marcadores de estudio independientemente de la forma de presentación (SCACEST, $p<0.640$, $p<0.817$ y $p<0.640$; IAMSEST, $p<0.566$, $p<0.911$ y $p<0.236$) (Figura 3). El análisis de la concentración de los marcadores en función de la fisiopatología del infarto, tampoco evidencia diferencias significativas ($p<0.246$, $p<0.868$ y $p<0.436$) (Figura 4).

Conclusiones: En este análisis inicial, el papel de la netosis en la trombosis coronaria no es evidente a nivel local ni sistémico en el infarto, independientemente de la presentación clínica del infarto y la fisiopatología de la trombosis.

Programa de Doctorado en *Biociencias Moleculares*

Exploring the role of hnRNPK in post-transcriptional regulation of hypothalamic neurometabolism

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Post-transcriptional regulators such as microRNAs (miRs) and RNA-binding proteins (RBPs) play a critical role in fine-tuning gene expression, ensuring metabolic homeostasis.

In this context, miR-7 is a prototypical neuroendocrine microRNA known to contribute significantly to pancreatic function and insulin production. It is encoded by three different alleles located at separate loci. However, our research focuses on miR-7-1, which independently impacts glucose and lipid metabolism. This specific form of miR-7 is encoded within the gene for heterogeneous nuclear ribonucleoprotein K (hnRNPK), an RBP that appears to act coordinately with miR-7.

Our group has been investigating their role in metabolic, neurodegenerative disorders, and glioblastoma. We have partially unravelled the mechanisms by which this regulatory pair modulates insulin signalling. Together, miR-7 and hnRNPK may act as central metabolic regulators, bridging insulin resistance and amyloid-beta (Aβ) metabolism, two key pathological features shared by type 2 diabetes and Alzheimer's disease [1].

Beyond insulin signalling, miR-7 also regulates key genes in cholesterol biosynthesis and efflux, such as DHCR24, an enzyme also known as Seladin-1 (Selective Alzheimer's Disease Indicator-1) for its involvement in this condition [2].

In this project, we aim to further explore the therapeutic potential of hnRNPK in metabolic and brain disorders, focusing on its previously unexplored role in the hypothalamus and its influence on feeding behaviour and energy balance. This poses a significant challenge, as hnRNPK has been extensively studied in cancer, but its function in the nervous system remains poorly understood.

To address this, we have developed a hnRNPK floxed mouse model, which will be crossed with hypothalamic cell-specific *Cre* lines to generate knockout mice. We will induce obesity through diet and characterise their metabolic phenotype and feeding behaviour. We will then perform spatial transcriptomics across the hypothalamus to gain a broad view of the changes caused by hnRNPK deficiency, followed by single-cell transcriptomics of the most affected neuronal populations. Finally, we will identify and validate new direct and indirect targets of hnRNPK in other brain regions using bioinformatics tools and in vitro assays.

Definitely, this study will enhance the understanding of miR-7/hnRNPK pair and its potential as a therapeutic target linking metabolic and brain impairment, contributing to the development of novel treatments that improve life expectancy and quality of life.

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Unlocking the Potential of Regulatory T Cells: A Novel Approach to Allogeneic Cell Therapies

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ABSTRACT (300 words max)

Regulatory T cells (Tregs) possess the ability to maintain immune system balance and prevent harmful immune responses through their inherent immunosuppressive capacities. This unique property makes them valuable for therapeutic applications by mitigating exacerbated immune responses, such as those observed in transplant rejection, autoimmunity, and graft-versus-host disease.

Regulatory T cells isolated from thymic tissue (thyTregs) have emerged as a groundbreaking innovation. These cells have demonstrated impressive success in the world's first autologous clinical trial, conducted by our group, aimed at preventing rejection in heart-transplanted children. Building on this milestone, their potential use in allogeneic contexts is now being explored, where challenges like allo-reactivity and cell persistence in recipients remain critical areas of study.

The ***Thy-G-002*** molecule (a provisional name used to safeguard the identity of the actual product, which is currently undergoing patent application) is a cutting-edge protein with immunomodulatory properties that physiologically promotes immune privilege in specific human tissues. This study aims to investigate whether genetically modified thyTregs with hypoimmunogenic molecules, such as ***Thy-G-002***, can enhance their properties for allogeneic applications without compromising their immunosuppressive phenotype.

Using *in vitro* co-culture assays with various primary cells and flow cytometry analysis, we demonstrated that the genetic modification of thyTregs with ***Thy-G-002*** preserves their phenotype and suppressive functions, maintaining their efficacy in immune-associated disorders. Moreover, the results assessing the impact of ***Thy-G-002*** on thyTreg allo-recognition in allogeneic settings, such as mixed lymphocyte reactions or co-culture with allogeneic NK cells or iDCs, are promising, laying a promising foundation for future research.

These exciting results suggest a transformative strategy in allogeneic cell therapies. By combining the potent immunosuppresive properties of thyTregs with the unique capability of ***Thy-G-002,*** this approach holds the potential to redefine therapeutic possibilities, offering new hope for patients in need of innovative regulation solutions.

Effects of Aurora kinase A (AURKA) overexpression in DNA replication

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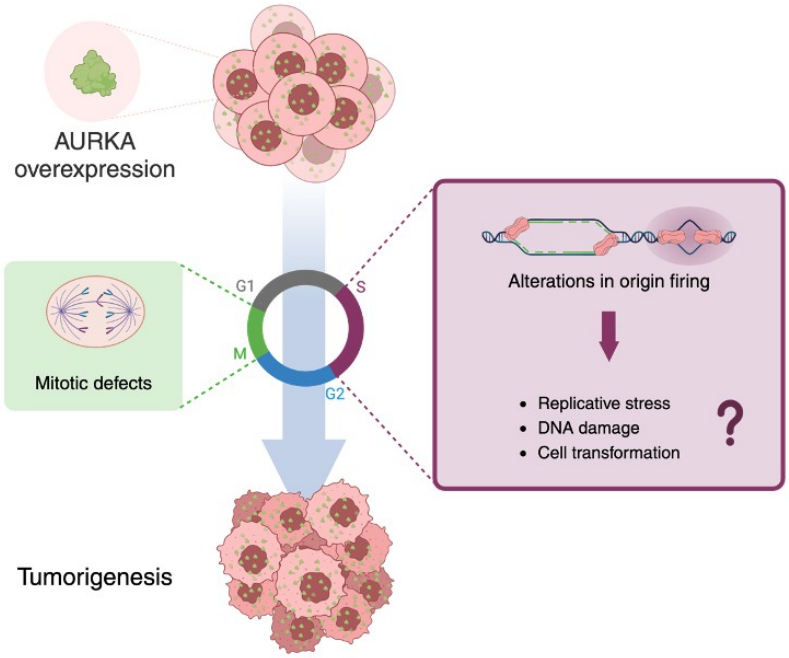
Aurora kinase A (AURKA) participates in chromosome segregation during mitosis. Abnormally high levels of AURKA correlate with malignancy in various tumor types. While the role of AURKA overexpression in carcinogenesis has been traditionally linked to mitotic defects, recent evidence shows that AURKA has functions beyond mitosis, including a new role in DNA replication that is independent of its kinase activity. Inhibition of AURKA blocks the G1/S transition and a kinase-dead version of AURKA can rescue this blockage, allowing DNA replication to continue. It has also been reported that PROTAC-mediated AURKA degradation results in impaired S phase progression. Together, these findings suggest that AURKA participates in the initiation of DNA replication. Origins of replication are licensed during the G1 phase, but only 20-30% of licensed origins are activated during the S phase. When fork progression is impeded, however, additional 'dormant' origins are activated to ensure complete genome duplication. We have recently observed that cells overexpressing AURKA exhibit defects in origin firing, particularly in the activation of dormant origins. Our model is that this source of replicative stress may contribute to the transformation phenotype promoted by AURKA overexpression.



Masters of the Microenvironment: The Role of Liver X Receptors in Tissue-Resident Macrophage Diversity and Function in Immune and Hematopoietic Tissues.

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The liver X receptors (LXRα and LXRβ) are members of the nuclear receptor superfamily of transcription factors. In macrophages, LXRs play essential roles in the coordination of both metabolic and immune responses, such as the transcriptional control of lipid metabolism or the modulation of innate and adaptive immune responses. Tissue resident macrophages are professional phagocytes that orchestrate innate immune responses but also participate in the maintenance of tissue homeostasis by regulating different metabolic processes. Consequently, they acquire considerable genetic and phenotypic diversity at different anatomical locations. Macrophage precursors localize in tissues during embryo development but which transcriptional cues organize their identity and unique functions in different tissues in their adulthood is a matter of intense research. During this workshop, we will present and discuss our recent results that point to an important role of LXRs in the transcriptional control of specific subpopulations of macrophages in tissues, especially in immune and hematopoietic tissues. We will show our recent results using high resolution confocal imaging microscopy to understand the participation of LXRs in the maintenance of tissue-resident macrophage subpopulations.

IMMUNOMETABOLIC ACTIONS OF NEW CHEMOTHERAPEUTIC DRUGS ON HUMAN MACROPHAGES: RELEVANCE FOR THEIR ANTITUMORAL ACTION

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The recent development of new chemotherapeutic drugs has led to trying to unveil the immunometabolic actions of these drugs, especially on human immune system cells. The attention has been focused on tumour-associated macrophages (TAMs) because these cells are among the most abundant in the tumour microenvironment. Here, we used a state-of-the-art approach in vitro macrophages (hMφ) that integrates transcriptomics and metabolomics to assess the immunometabolic effects of trabectedin (TRB) and lurbinectedin (LUR), two compounds whose source comes from Ecteinascidia turbinata, an ancestral marine urochordate which inhabits the Caribbean Sea, among others. Because both compounds' mechanism of action is to bind to the DNA, they have been previously tested against tumour activity. Our results show that these drugs effectively modulate the macrophages in vitro response, inducing a stratification in terms of hMφ viability, showing two different populations; either resistant macrophages which are slightly affected by these drugs or sensitive ones. Furthermore, hMφ mitochondria is also affected as OXPHOS pathway is repressed and mitochondria biogenesis is activated, as we observed an upregulation of nuclear-DNA mitochondrial transcripts. Moreover, we observed that these drugs induce a metabolic switch in human macrophages towards a pro-inflammatory phenotype explained by the increase in the transcription of pro-inflammatory pathways as well as the enhancement of the production of mitochondrial superoxide ROS. It is important to highlight that both drugs showed to have a similar effect on human macrophages because both of them upregulate MHC I class protein and lipid catabolic processes. Taken together these facts, we could suggest that both marine-based drugs could exert an antitumoral effect in human macrophages.

Impact of a respiratory rehabilitation program on the quality of life of children and adolescents diagnosed with persistent bronchial asthma.

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Introduction: Bronchial asthma is a chronic disease, characterized by a complex interaction between genetic, environmental and immunological factors[1]. According to the World Health Organization, 300,000,000 people[1] suffer from it, resulting in 450,000 deaths/year[1]. In pediatrics, it is the most prevalent disease with significant repercussions on quality of life and socio-health costs[2,3]. Respiratory rehabilitation programs (RRP) are the indicated therapeutic strategy, although there is controversy as to which techniques are more adherent and effective[4-6].

Objective: To evaluate the impact of a respiratory rehabilitation program on the quality of life in children and adolescents diagnosed with asthma.

Methods: Randomized clinical trial. Subjects: Asthmatics aged 6 to 17 years. Control group (CG): 9-week RRP that included educational sessions, respiratory physiotherapy, and aerobic training (video game platform). Experimental group (EG): RRP identical to that of the CG plus home-based respiratory muscle training (5 days/week, using a threshold resistance valve). Muscle strength and endurance were incrementally trained, using pre-intervention peak inspiratory and expiratory pressures (PIM-PEM) as reference values. Variables: Dyspnea (Modified Medical Research Council Scale), quality of life (Childhood Asthma Control and Paediatric Asthma Quality of Life Questionnaires), peak expiratory flow, respiratory muscle strength (PIM-PEM), exercise tolerance (Six-Minute Walk Test (6MWT)), and exacerbations. Pre-intervention and post-intervention measurements were performed, as well as a follow-up at 6 and 12 months.
Results: Thirty-four subjects were analyzed: CG (n=16) and GE (n=18). The mean age was 9.18 years; 35.3% were male. Group homogeneity was verified at baseline in all variables ($p>0.05$). A clinically significant improvement in quality of life was observed in both intergroup and intragroup comparisons.

Conclusion: In the PRR evaluated, quality of life appears to improve; further studies with larger sample sizes are needed to establish statistically significant difference

Programa de Doctorado en *Biología*

Regulation of metabolism by hnRNPK

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Persistent insulin resistance causes many pathologic states and it leads to the development metabolic disease, including diabetes, obesity, cardiovascular disease, or nonalcoholic fatty liver disease (NAFLD) [1]. In addition to classical transcriptional factors, posttranscriptional control of gene expression exerted by miRNAs and RNA-binding proteins constitutes a new level of regulation with important implications in metabolic homeostasis. Here we aimed to explore whether hnRNPK could also metabolic signaling pathways *in vitro* and *in vivo*. Our initial bioinformatic analysis showed a number of consensus binding sites for this RBP insulin signaling effectors. Further experiments demonstrated that modulation of hnRNPK expression both in neuronal cell lines as well as in mouse primary hepatocytes blunted intracellular insulin cascade. *In vivo* studies were performed in mice injected with AAV-sh-hnRNPK-GFP showed changes in glucose metabolism, body weight, and lipid accumulation in the liver. Overall, these results describe the novel contribution of hnRNPK in regulating metabolism at the posttranscriptional level [2].

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Semana del Doctorado 2025 en la Facultad de Medicina

Gold Nanoparticle-Based Therapeutic Strategies for Duchenne Muscular Dystrophy: Functionalization and Delivery Approaches

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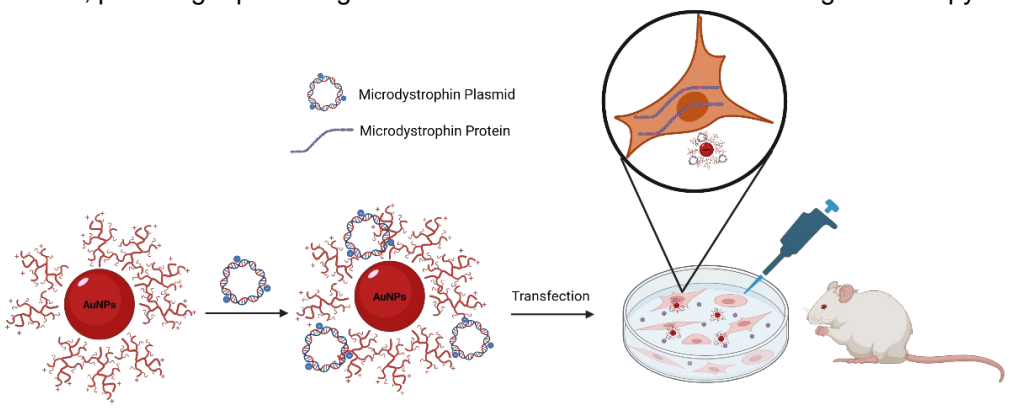
Duchenne muscular dystrophy (DMD) is a severe genetic disorder caused by mutations in the dystrophin gene, leading to progressive muscle degeneration. Current gene therapies, including exon skipping, CRISPR/Cas9-based genome editing, and the use of adeno-associated virus (AAV) vectors, face significant challenges, such as high costs, immunogenicity, off-target effects, and limited cargo capacity. Additionally, nucleic acid-based therapies have several limitations, including poor cellular uptake, rapid degradation by nucleases, and inefficient nuclear targeting. To address these challenges, we developed a gold nanoparticle (AuNP)-based delivery system for microdystrophin gene replacement therapy. AuNPs are promising nanocarriers due to their tunable size, biocompatibility, and ability to functionalize with biomolecules for efficient delivery. In this study, we synthesized citrate-stabilized AuNPs (~13 nm) and modified them with thiol (-SH) groups to generate cationic AuNPs, facilitating electrostatic conjugation with nucleic acids. The physicochemical properties of the resulting complexes were characterized using UV-Vis spectroscopy, dynamic light scattering (DLS), zeta potential analysis, and agarose gel electrophoresis. In vitro studies using C2C12 cells and an eGFP reporter gene demonstrated efficient gene delivery and expression. qPCR and Western blot analysis confirmed successful microdystrophin expression and dystrophin regeneration. These findings highlight the potential of AuNPs to enhance nucleic acid stability, improve cellular uptake, and reduce off-target effects compared to conventional delivery methods. Future studies will focus on enhancing muscle-specific uptake by introducing aptamers and conducting in vivo validation using DMD mouse models to assess biodistribution and therapeutic efficacy. This work advances nanomedicine-based strategies for DMD treatment, providing a promising alternative for safer and more effective gene therapy.



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Acknowledgments

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Mislocalized cytoskeletal protein biogenesis within TDP-43 aggregates derails sarcomere organization in human myocytes induced from fibroblasts

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A key challenge in the study of amyotrophic lateral sclerosis (ALS) is that the affected cells, mainly human motor neurons and muscle cells, are difficult to isolate and culture. To address this limitation, we evaluated cultured dermal fibroblasts as a potential cellular model for ALS investigations, given their accessibility via minimally invasive biopsies and their capacity to be reprogrammed into ALSrelevant cell types, including neurons and muscle cells. Morphological and biochemical analyses of patient-derived fibroblasts revealed prominent cytoplasmic mislocalization and aggregation of TAR DNA-binding protein 43 (TDP-43), along with substantial disruption of F-actin and 4A-alpha-tubulin fibers, indicating compromised cytoskeletal integrity, which are pathological features of the disease. Intriguingly, cytoskeletal fibers partially colocalized with cytoplasmic TDP-43, including within its aggregates, suggesting that TDP-43 may participate in their synthesis or assembly in control cells and contribute to their mislocalization and disorganization in the ALS context. To further investigate these observations, we reprogrammed fibroblasts into induced myocytes (iMCs) via retroviral expression of the muscle lineage determinant MyoD. Consistent with previous findings, control iMCs exhibited TDP43 not only in the nucleus but also in association with myosin within sarcomeric structures, implying a role in muscle skeletal protein biogenesis, particularly within the sarcomere. Moreover, in patientderived iMCs, myosin colocalized with TDP-43 aggregates while sarcomeres were disrupted, supporting the notion that these aggregates misdirect myosin synthesis and disrupt sarcomere organization. Finally, we investigated whether preventing TDP-43 aggregation could reduce myosin aggregation and restore sarcomere structure. To this aim, we used protein kinase inhibitors, as our previous work demonstrated their ability to restore TDP-43 nuclear localization and promote aggregate clearance. Treatment with these inhibitors rescued TDP-43 distribution and alleviated cytoskeletal defects. Overall, our findings reveal a novel mechanism that may be central to ALS, deepening our understanding of disease pathogenesis and identifying new avenues for therapeutic intervention.

Pax6 isoforms label distinct stages of neuronal differentiation in the mouse brain

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The transcription factor Pax6 is a key regulator of complex developmental processes in both the embryonic and adult brain. During early development, it is widely expressed across eye, telencephalon, diencephalon, and midbrain, marking both progenitor and differentiated cells. In the adult brain, Pax6 expression persists in neural stem cells within neurogenic niches, as well as in mature neurons of the olfactory bulb and piriform cortex, and specific astrocyte populations. Interestingly, overexpressing Pax6 in mature astrocytes can reprogram them into progenitor-like cells, reinforcing its role in neural stem cells. However, in mature neurons, Pax6 endogenous expression does not alter their differentiated state, suggesting the presence of **cell-type-specific regulatory mechanisms governing its function**. How can the same gene perform such different roles in progenitor cells compared to mature neurons? While cellular context could lead to different responses to the same gene, a simpler explanation is **alternative splicing**. This process generates variants (isoforms) of the same gene with distinct structures and functions. Pax6 produces three major isoforms, each differing in their DNA-binding properties; however, their precise contributions to Pax6's functional diversity remain largely unexplored. To define their specific role, we used CRISPR to engineer different mouse knock-in lines in which each isoform is tagged with a short epitope, enabling their individual detection. Our findings revealed a dynamic shift in isoform expression during neuronal differentiation: progenitor cells predominantly express the canonical Pax6 isoform, but as differentiation progresses, its levels decline, while Pax6(5a) becomes the dominant variant in mature neurons. These results suggest a functional transition, where the **canonical Pax6 isoform supports proliferation, while Pax6(5a) plays a role in differentiation and neuronal identity maintenance.** This shift in isoform dominance sheds light on how Pax6 regulates neuronal fate, opening new avenues for understanding its broader role in brain development, cell therapy and neurodevelopmental disorders.

OXPHOS activity dictates the tumorigenic and metastatic capacity of cancer stem cells through extracellular vesicles

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ABSTRACT (300 words max)

Cancer is a group of genetic diseases characterized by the loss of cell adhesion, sustained proliferation, and evasion of programmed cell death. Warburg observed that cancer cells, despite having sufficient oxygen, preferentially generate energy through aerobic glycolysis rather than oxidative phosphorylation (OXPHOS).

However, it has been noted that during tumor development, a subset of cells plays a particularly critical role: cancer stem cells (CSCs), which rely more heavily on the OXPHOS system compared to other tumor cell populations.

In the laboratory, it is possible to generate cybrids—cells with identical nuclear DNA but different mitochondrial DNA (mtDNA)—to study the role of mitochondria in tumor development. Previous studies have shown that mtDNA influences the tumorigenic capacity of cells. Cells harboring severe mtDNA mutations that completely abolish OXPHOS function are unable to form tumors in mice, whereas cells with moderate mutations, which only partially impair OXPHOS, retain the ability to do so.

Recent advances have deepened our understanding of extracellular vesicles (EVs) and their role in intercellular communication, pre-metastatic niche formation, and tumor progression. Based on this information, we hypothesize that the tumorigenic and metastatic capacity of CSCs is determined by the OXPHOS activity of tumor cells, and that the necessary signaling is mediated by extracellular vesicles.

Dissecting the role of liver mTORC1 on the beneficial effects of dietary restriction in systemic metabolism and liver cancer

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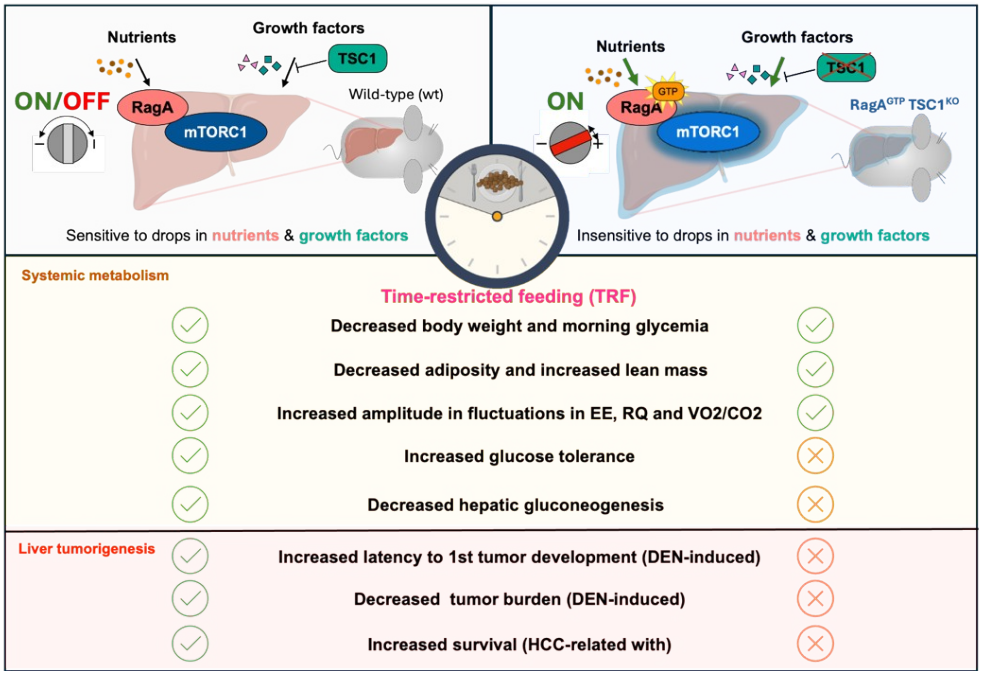
ABSTRACT

Obesity is considered a pandemic whose prevalence is steadily increasing in many countries worldwide. Effective weight management measures are urgently needed considering the evidence from epidemiologic studies that obesity is directly linked to an increased risk of chronic diseases, including metabolic disorders and different types of cancer, as hepatocellular carcinoma (HCC).

Dietary restrictions have emerged as strategies for cancer prevention and for addressing metabolic disorders. Time-restricted feeding (TRF) is a popular dietary approach that involves limiting the consumption of calories to a specific window of time in the day. Accumulating evidence has suggested a promising effect of TRF in partially alleviating effects of metabolic syndrome as reducing body weight, adiposity, glucose intolerance and a potential cancer-fighting effect. Nonetheless, the molecular mechanisms underlying this protection remain poorly understood.

The mechanistic target of rapamycin complex 1 (mTORC1) is a master regulator of cell metabolism by the integration of two major regulatory inputs: nutrients, which activate mTORC1 pathway through Rag GTPases, and growth factors, which inhibit the Tuberous Sclerosis Complex 1 (TSC1) to allow its activation. In our lab, we have observed that activation of mTORC1 by nutrient and hormone signaling specifically in the liver (Li-RagA^{GTP} TSC1^{KO} mice) abolishes some aspects of the metabolic and HCC protection reported by TRF. However, mice with only constitutive insulin signaling to mTORC1 (Li-TSC1^{KO} mice) and wild type (wt) mice presented a positive response to TRF in terms of improved systemic metabolism and tumor development, even when HCC is induced chemically by the carcinogen dyethilnitrosamine (DEN).

Together, our work identifies that the modulation of liver mTORC1 activity is essential for some of the reported TRF benefits as the potential preventive role in HCC development. Now, we are trying to elucidate this novel pathway that connects liver metabolism with TRF and HCC, that could be also exploited to develop preventive and therapeutic strategies for patients at risk of progression from liver dysfunction to HCC.



CEREBRAL MAGNETIC RESONANCE IMAGING AND IMMUNOFLUORESCENCE REVEAL THE ANTI-INFLAMMATORY EFFECTS OF AN OBESITY TREATMENT IN MICE

Adriana Ferreiro[1], Irene González-Villena[1], Maya Holgado[1], Sara González-Soto[3], Lidia Martinez[3], Ángeles Vicente[3], Pilar López-Larrubia[1] and Blanca Lizarbe[1,2]

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ABSTRACT

Obesity is a chronic disease associated with several pathologies such as type 2 diabetes, cardiovascular risk and neurodegeneration. Obesity triggers inflammation affecting multiple organs, including the brain, and specifically in regions that regulate energy homeostasis. These changes can be quantified using magnetic resonance imaging (MRI) and correlated with histological data[1]. The aim of this study is to parametrize the cerebral and physiological effects of celastrol, a compound known to induce weight loss in animals and improve leptin and insulin sensitivity as well as glucose tolerance, though its mechanisms remain unclear[2,3]. On these grounds, we designed an experimental setup in which mice were fed either a high-fat high-sugar or low-fat low-sugar diet for 20 weeks, followed by treatment with celastrol or a vehicle solution for three consecutive days. Brain alterations were assessed using MRI, regional neurochemical profiles using High Resolution Magic Angle Spinning *(*HRMAS), glial markers through immunofluorescence and appetite-related hormones levels via ELISA. Celastrol administration resulted in a significant reduction in both food intake and body weight. These physiological changes were accompanied by alterations in brain diffusivity, as assessed in vivo by MRI. These findings may be associated with a decrease in the size, number, and occupied area of astrocytic and microglial cells, on agreement with a successful reversal of the inflammatory state. Furthermore, ^{1}H HRMAS analysis demonstrated that treatment with celastrol resulted in reduced concentrations of taurine and myoinositol, consistent with reduced brain osmolytes and supporting celastrol's anti-inflammatory potential.

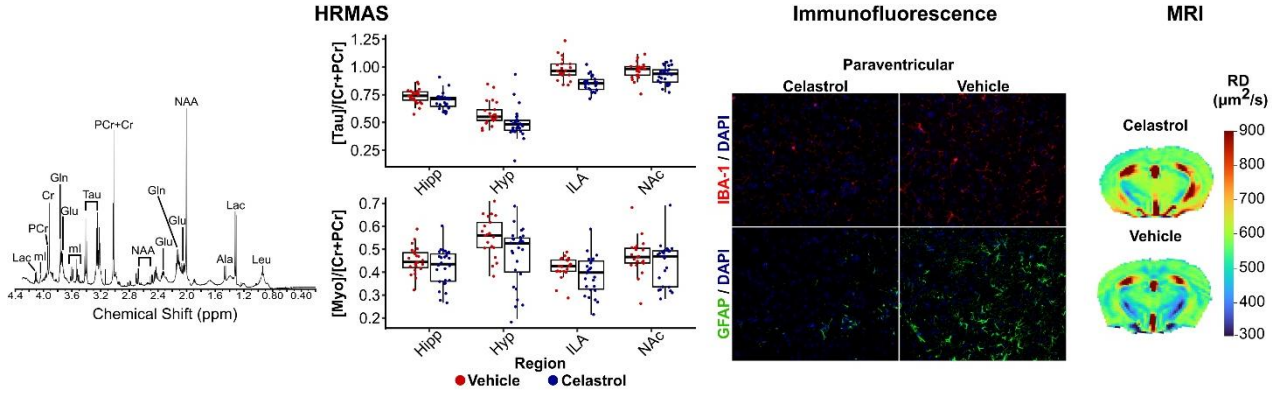

Figure 1. *Brain changes during obesity treatment detected by HRMAS, Immunofluorescence and MRI. Abbreviations: Hipp, hippocampus; Hyp, hypothalamus; ILA, infralimbic area; NAc, nucleus accumbens; RD, radial diffusivity.*

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Neuroinflammation and Metabolic changes induced by medium-term high-fat diet in an IL-1R1KO murine model: An MRI-based study

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ABSTRACT

Obesity is a pathological condition with rising prevalence in society and associated comorbidities such as diabetes [1]. Chronic inflammation is associated with obesity, moreover, high-fat diets (HFD) activate pro-inflammatory cascades in the brain. Interleukin-1-receptor 1 (IL-1R1), a pro-inflammatory mediator plays a central role in this context, as previous studies have shown that absence of IL-1R1 prevents insulin resistance in diet-induced obesity (DIO) models [2]. Our objective is to characterize neuroinflammation using *in vivo* multiparametric MRI in IL-1R1 knockout (KO) and *wild-type* mice (WT) under standard diet (SD) and HFD. Also, we study the differences in mouse phenotyping between groups by using an indirect calorimetry.

12 male C57BL/6J WT-mice and 19 male IL-1R1KO mice with the same genetic background, aged 7-8 weeks, were assigned to HFD or SD. In week 10, multiparametric MRI studies were acquired using a Bruker Biospec 7T scanner, including magnetization transfer, diffusion tensor imaging, T_2 and T_2* sequences. Subsequently, parametric maps were processed with a Python-based software and, using ImageJ, 4 brain regions of interest were quantified: cortex, hippocampus, thalamus and hypothalamus. Statistical analysis was done using linear mixed effects models to assess the impact of genotype and diet, on imaging parameters. Moreover, a metabolic and motor analysis system (Phenomaster) provided data on indirect calorimetry, motor activity and food intake.

WT mice gained weight faster than KO and both groups with HFD lost circadian oscillations of respiratory exchange ratio (RER). MRI revealed higher T_2 values in KO mice, suggesting vasogenic edema, while T_2* was increased in the cortex of obese WT mice, potentially indicating microvascular changes. Significant differences in T_2* values between genotypes, particularly in hippocampus and thalamus, were independent of diet. These findings suggest that *ilr1r1* deletion modifies neuroinflammatory responses in DIO. Further MRI studies with additional WT mice are ongoing to expand on these results.

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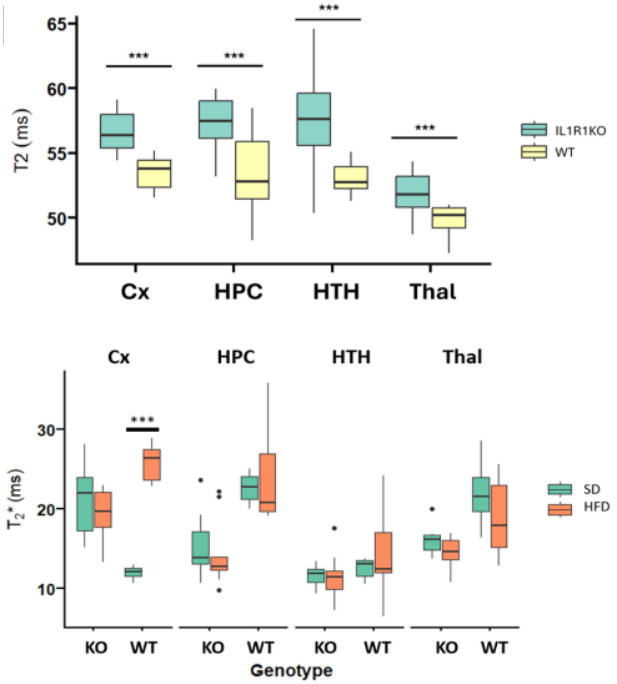

Figure 1. Boxplots representing the quantification of T_2 values in the 4 ROIs comparing both genotypes (Above) and the quantification T_2* values in the 4 ROIs according to genotype and diet (Below).

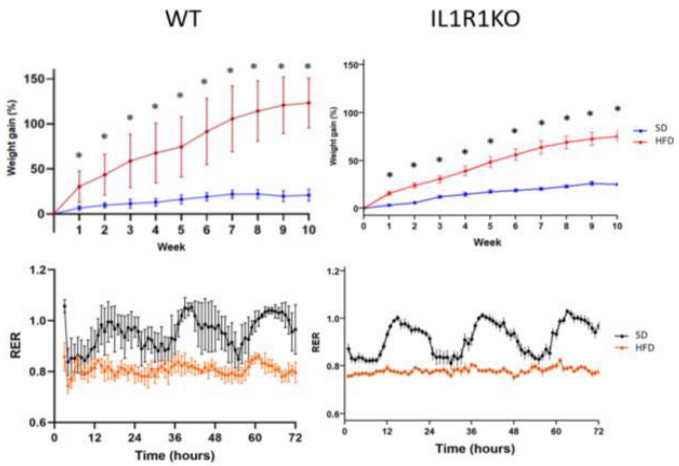

Figure 2. Representations of body weight increase in groups relative to the start of the diet (Above). Representations of Respiratory Exchange Ratio (RER) adjusted to 72 hours (Below).

Complete Deletion of Kras in Adult Young Mice: a genetic model

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KRAS is the initiating oncogenic event in 30% of lung adenocarcinomas and in 90% of human PDACs (1, 2). Both illnesses have low 5 years survival rate due to late diagnosis and lack of effective treatments (3, 4). Significant advances are being made with the development of KRAS inhibitors against specific KRAS mutations (G12C and G12D) and against all KRAS mutations (Pan-KRAS) (5).

Total ablation of all three RAS genes is lethal (proved in our previous studies) making Pan-RAS drugs inevitably toxic (6). Since Pan-KRAS inhibitors are under development, we aim to study the potential side effects of total KRAS inhibition. To do so, we have developed genetically modified mice that allow us to genetically ablate Kras, without affecting the other isoforms of Ras, Hras and Nras.

We have observed that systemic genetic deletion of Kras in adulthood (2 and 12 months of age) does not lead to major adverse effects, as mice appear to be healthy up to 12 months of Kras elimination. However, a detailed histopathological analysis identified the presence of myelomonocytic metaplasia in all the mice. Our studies will unravel adverse effects of the Pan-KRAS inhibitors.

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An Ehrlich-based perspective on the potential of tumour proteoglycan antigens as vaccines

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Introduction: Therapeutic cancer vaccines hold promise for boosting immune responses against tumors and offer potential as new treatments, though improvements are still needed.
When carbohydrate antigens, either proteoglycans or glycoproteins, arise from cellular neoplastic transformation are called Tumor-Associated Carbohydrate Antigens (TACAs). TACAs have been extensively studied in various tumors for their potential to induce immune responses against tumors and their diagnostic and prognostic potential. Their potential as antineoplastic vaccines of clinical use has been explored, although their induction of immune responses is considered to be less potent than with protein antigens. Here we study Ehrlich tumor (ET) cells as a source of a TACA called Ca10

Objective:

1. Evaluation of TACA Ca10 as leading antigen for an antitumor vaccine candidate.

Methods: C57BL6 female mice (6-8 weeks) were immunized with Ca10 through intramuscular route. One month after the final dose, ET cells were grafted subcutaneously into the groin. Tumor growth and survival were monitored. For immune infiltrate analysis, tumors were collected 20 days post-inoculation, enzymatically digested, and stained for FACS using the Aurora Cytek spectral flow cytometer.
Results: We showed that Ca10 antigen administration effectively protects against Ehrlich solid tumors in terms of growth and survival, across a range of antigen doses and grafted tumor cells. Therefore, mice were protected against ET when vaccinated both intramuscularly and intranasally. Spectral flow cytometry of tumor infiltrates revealed that intramuscular Ca10 increased total $CD45^+$ cells, particularly B cells, $CD4^+$ and $CD8^+$ T cells, monocyte-derived Dendritic Cells (moDCs), and neutrophils. Depletion of this populations generated a negative impact in mice survival indicating that play a critical role in the immune response against ET.

Iron Oxide Nanoparticles for Renal Clearance: Synthesis, Radiolabeling and *In vivo* Evaluation

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Keyword
Iron Oxide Nanoparticles, Renal Clearance, Nano-radiopharmaceuticals

ABSTRACT (300 words max)

Iron oxide nanoparticles (IONP) developed by our group can incorporate various radiometals into their crystalline structure[1], allowing the synthesis of nano-radiopharmaceuticals for PET, SPECT, and therapy. Biodistribution studies of ^{89}Zr-IONP showed high accumulation in liver and spleen. We hypothesised that reducing the hydrodynamic size (D_H) of IONP could promote renal clearance, potentially reducing hepatic toxicity.

IONP were synthesised by dissolving $FeCl_3 \cdot 6H_2O$ and sodium citrate in a high-pressure microwave flask. The reaction was carried out in a silicone oil bath, so that when the reaction temperature was reached (120 °C), 1 mL of hydrazine monohydrate was added, and the reaction was stirred for 45 min. To modulate the D_H of the IONP, the synthesis conditions were varied. D_H and z-potential were measured over time to assess colloidal stability in water and PBS. ^{68}Ga was incorporated into the IONP, and radiolabelling yield (RLY) and stability in human serum were evaluated to determine suitability for *in vivo* applications.

Standard conditions (120 °C, 45 min, 0.27 mmol citrate) produced IONP of ~10 nm D_H and ~80 % RLY. Increasing the citrate concentration reduced the D_H but also significantly lowered the RLY (~20 %). Higher temperatures (120–140 °C) with shorter reaction times (1 min) produced ~5 nm D_H with ~35 % RLY. Lower temperatures (65–80 °C) with reaction times of 5–10 min gave ~3 nm D_H and ~15 % RLY, while longer reaction times (30 min) gave better RLY (~30 %) at 65 °C. D_H and zeta potential showed that the IONP remained stable under physiological conditions. The best candidates were selected to assess stability in human serum over time. Taking all the results into account, the 65 °C/30 min candidate was selected for *in vivo* biodistribution by ^{68}Ga PET/MRI, showing renal clearance.

In conclusion, IONP size and radiolabelling efficiency can be finely tuned by synthesis parameters. The *in vivo* studies with an optimal renal clearance candidate (65° C/ 30 min; ~3 nm D_H) demonstrate the ability to shift clearance from the hepatic to the renal pathway.

RESOLVIN D2 PREVENTS ANGIOTENSIN II-INDUCED CARDIAC HYPERTROPHY, FIBROSIS, INFLAMMATION AND AUTOPHAGY IN OBESE MICE

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ABSTRACT

Angiotensin II (AngII) and obesity are closely linked to cardiac remodeling, hypertrophy, fibrosis, and hypertension. These effects involve the participation of inflammatory mediators, altered autophagy, oxidative stress, and endoplasmic reticulum stress, among others. On the other hand, resolvin D2 (RvD2) is a potent anti-inflammatory lipid mediator that plays a protective role in various cardiovascular diseases by promoting the resolution of inflammation. Therefore, the study aimed to evaluate the effect of RvD2 administration on the cardiac structure of obese mice infused with AngII.

The study used left ventricular samples from male C57BL/6J mice fed with standard or high-fat diet. Mice fed with high-fat diet were also infused with AngII (1.44 mg/kg/day, for 28 days) via subcutaneous pumps, in the absence or presence of RvD2 treatment (4 µg/kg, every other day, intraperitoneally, starting 1 day before pump implantation). Different cardiac parameters were studied using histology (Wheat Germ Agglutinin, WGA, and picrosirius red staining), immunohistochemistry, qPCR and western blotting.

Hearts from obese hypertensive mice showed upregulation of different markers of hypertrophy, fibrosis and inflammation. RvD2 reduced cardiomyocyte hypertrophy, downregulated the TGFβ1/pSMAD2/collagen pathway, thereby improving cardiac fibrosis, and downregulated the expression of contractile proteins αSMA and SM22α suggesting phenotypic switching. RvD2 also reduced the expression of proinflammatory markers such as IL6 (interleukin 6) and Ccl5 (chemokine ligand 5) and reversed the increase in macrophage infiltration caused by AngII and obesity. At molecular level, RvD2 does not reverse the upregulation of oxidative and endoplasmic reticulum stress markers but might improve cardiac autophagy induced by AngII.

In conclusion, treatment with resolvin D2 prevents cardiac alterations caused by obesity and angiotensin II by reducing hypertrophy, fibrosis, inflammation and autophagy.

Uncovering the Role of Ferroptosis in Myocardial Ischemia-Reperfusion Injury

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ABSTRACT (300 words max)

INTRODUCTION: Heart failure (HF) is a complex clinical syndrome and a major public health concern, with more than half of patients failing to respond to treatment, despite the introduction of various therapeutic interventions. Among the underlying mechanisms contributing to HF, myocardial ischemia-reperfusion injury (MIRI) is particularly significant. Ferroptosis a newly recognized form of regulated cell death driven by iron-dependent phospholipid peroxidation, plays a critical role in the development of cardiovascular diseases. In this study we characterized a murine model of MIRI, with a particular-focus on elucidating the role of ferroptosis in cardiac tissue damage.

METHODS: Myocardial ischemia was induced in 8–10-week-old male mice by ligating the left anterior descending (LAD) coronary artery for 45 minutes, followed by reperfusión. Mice were sacrificed at 6 hours, 24 hours, or 7 days after reperfusion. Genes associated with ferroptosis were analyzed in cardiac tissue by RT-qPCR. Oxidative stress, lipid peroxidation, and mitochondrial ultrastructure were evaluated in cardiac tissue using immunofluorescence for 8-hydroxy-2'-deoxyguanosine (8-OHdG), high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS), and transmission electron microscopy (TEM), respectively. Cardiac magnetic resonance imaging (CMRI) was used to analyze cardiac function and structure seven days post-reperfusion while Sirius red staining was performed to assess fibrosis.

RESULTS: Seven days post-reperfusion, Sirius Red staining revealed fibrosis, and CMRI analysis indicated ischemic dilated cardiomyopathy, characterized by increased left ventricular volumes and reduced ejection fraction. At 24 hours post-reperfusion, RT-qPCR analysis showed upregulation of SLC7A11, Alox15, and Hmox1, and downregulation of GPX4 and Trf1. Oxidative stress levels were significantly increased. HPLC-MS analysis revealed an increase in lipid peroxidation products, while TEM analysis demonstrated mitochondrial disorganization.

CONCLUSIONS: Our results indicate that 24 hours post-reperfusion, membrane lipid peroxidation increased, mitochondrial disorganization was evident, and several genes associated with ferroptosis were differentially expressed, in accordance with published reports. These findings strongly support the involvement of ferroptotic cell death in MIRI.

B lymphocytes need of DGKz for optimum CD40 mediated immune synapse and mTORC1 activation: a sensor of signal quality?

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ABSTRACT (300 words max)

The ζ isoform of the Diacylglycerol kinases (DGKζ) limits B cell receptor (BCR) signaling by consuming the second messenger diacylglycerol to generate phosphatidic acid (PA). PA has an important role beneath BCR signaling, promoting actin rearrangements and mechanical forces at the BCR-driven immune synapse of B lymphocytes. Those events facilitate antigen extraction and its presentation in the context of the major histocompatibility complex class-II (MHC-II) to cognate $CD4^+$ T follicular helper lymphocytes, which in return give co-stimulatory signals via CD40. Herein we investigated if DGKζ has a role downstream CD40-mediated immune synapse and signaling. We set up an experimental model using planar lipid bilayers to study the interaction of B lymphocytes through CD40. We found that CD40 stimulation promotes LFA-1/ICAM-1 interactions and actin cytoskeleton reorganization, inducing a migratory behavior in the B lymphocyte. We observed that DGKζ participates in the CD40 signaling cascade by reducing cell ruffling and peripheral LFA-1-mediated adhesion. In contrast, DGKζ enhances F-actin polymerization under the CD40 cluster in a PA and scaffold-dependent manner, limiting cell motility. Also, DGKζ participates in the reorganization of the tubulin cytoskeleton by promoting MTOC recruitment along with lysosomes and mitochondria to the CD40-mediated immune synapse in a PA dependent manner. The CD40 early signaling is also regulated by DGKζ. We found that DGKζ diminishes the activation of PKCβ and PI3K-Akt pathways, while the activation of the mTORC1 is promoted by DGKζ function in a kinase and scaffold activity dependent manner. The mTORC1 action depends on its association with lysosomes and our colocalization studies revealed that this association is impaired in the absence of DGKζ. Altogether, our data suggests that DGKζ is essential downstream CD40 for the assembly and function of the immune synapse and thus for the B lymphocyte activation. We propose that DGKζ acts as a control of quality for the CD40 response.

Dual regulation of NRF2 Signaling and Angpt1/2-TIE2 pathway: Implications for Blood-Brain Barrier Integrity

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ABSTRACT (300 words max)

The blood-brain barrier (BBB) is crucial for maintaining brain homeostasis and protecting against harmful substances from the bloodstream. However, under neuropathological conditions such as Alzheimer's disease (AD), endothelial cells endure oxidative and inflammatory stress, compromising BBB integrity. NRF2 has emerged as a potential protector of BBB integrity in neurodegenerative disorders. Yet, its specific involvement in the ANGPT1/2-TIE2 pathway, a key pathway that maintains the BBB integrity, remains poorly understood.

We confirmed that NRF2 regulates TIE2 levels in a mouse brain endothelial line (bEND.3), through the induction of NRF2 by chemical compounds or lentiviral induction, in which it was shown to downregulate the Angpt1/2-TIE2. Notably, this regulatory mechanism appears to operate independently of BACH1, a canonical inhibitor of the transcription of many NRF2 targets. Thus, while most ARE genes are activated by NRF2, our findings suggest that *TEK*/*Tek* belongs to a small number of genes that exhibit the opposite regulation.

Moreover, we have also observed a regulation of NRF2 by TIE2, wherein stimulation with its ligand, Angpt1, leads to NRF2 upregulation, potentially modulating TIE2 levels. Ongoing experiments involve assessing whether these molecular changes correlate with alterations in BBB integrity using an in vitro model and evaluating in AD mouse models the TIE2 levels to confirm the relevance of NRF2 in maintaining them, revealing a reduction at older ages. To translate the changes seen in the in vitro model we also performed an Evan's Blue assay and analyzed the fluorescence signal in brain tissue.

In summary, there is a dual regulation of NRF2 signaling and the ANGPT1/2-TIE2 pathway, suggesting a feedback loop, offering insights into NRF2 as a potential therapeutic target for BBB integrity in conditions when it's compromised, such as neurodegenerative diseases.

Functional characterization of the interaction between BRCA2-PARP1 in DNA repair

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ABSTRACT (300 words max)

BRCA2 is a tumour suppressor essential for DNA repair by homologous recombination (HR). Loss-of-function mutations in BRCA2 impair HR, leading to genome instability and cancer predisposition.

Using a proteomic screening under DNA damage conditions in the lab, PARP1 was identified as a potential binding partner of BRCA2 N-terminal region (first 1,000 aa). PARP1 senses various types of DNA lesions and catalyzes the synthesis of ADP-ribose polymers (PAR), promoting the recruitment of repair factors. In BRCA2-defective cells, PARP1 becomes essential by preventing SSBs to be converted into DSBs, which require HR for repair. This synthetic lethality underlies the clinical use of PARP inhibitors (PARPi) for treating HR-deficient tumors. However, many tumours become resistant to PARPi.

Preliminary results from the lab indicate that BRCA2 and PARP1 form a complex in cells which is stimulated under DNA damage conditions and is favored by PARylation (Proximity Ligation Assay and co-immunoprecipitation). This interaction is direct and involves the N-terminal region of both proteins as shown by pull-down with the purified fragments *in vitro*. Moreover, BRCA2 promotes PARP1 activity and stimulates its release from DNA (electrophoretic mobility shift assay) *in vitro*, suggesting a role in promoting HR by stimulating PARP1 turnover.

To map the interaction site, we generated truncated BRCA2 cDNA constructs lacking different N-terminal regions and used GFP-trap pulldown in human cells. BRCA2 bound specifically to PARylated PARP1, confirming our previous results. Deletion of a 100-aa region abolished the interaction, identifying this domain as essential. Interestingly, BRCA2 itself is not PARylated.

Finally, by using UV laser-induced DNA damage, we observed reduced recruitment of GFP-PARP1 in BRCA2-depleted cells, suggesting BRCA2 influences PARP1 dynamics at DNA damage sites. Once confirmed, we plan to perform similar experiments to explore the effect of disrupting BRA2-PARP1 interaction both in PARP1 recruitment to DNA damage but also in HR repair.

TITLE

Search for predictors to the occipital nerve stimulator in patients with refractory chronic cluster headache

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ABSTRACT (300 words max)

Objectives: To identify through a panel of biomarkers the response to treatment with occipital nerve stimulation (ONS) in patients with refractory chronic cluster headache (rCCH).

Methodology: Prospective observational study carried out at the Hospital Universitario La Paz in patients with rCCH who are candidates for treatment with ENO. Prior to device implantation, all patients will undergo a multidimensional study of different biomarkers: clinical, neuroimaging, biochemical, neurophysiological and algometric. Then, in the usual device implantation test phase, a "sham window" will be randomly performed in which the participants will have the device connected to a subthreshold stimulation for 2 weeks followed by 2 weeks of "washout" period and then 2 weeks of normal stimulation with the intention of assessing the possible placebo effect of the therapy and reducing the biases associated with this effect as much as possible. During these periods the patient will record his weekly attacks and will be evaluated by a clinician blinded to the stimulation received. Once the possible placebo effect has been determined and following the usual clinical practice, patients with a positive response will be implanted with the definitive generator and will undergo the usual follow-up. Subsequently, they will be divided into responders (estimating, according to the available literature, a reduction ≥ 30% of attacks per week with respect to baseline) and non-responders at 3, 6 and 12 months of follow-up. Finally, the differences existing between both groups in the initial evaluation of the different examinations performed will be analyzed, in order to develop a predictive model capable of establishing the success of this neuromodulation therapy.

TIGIT: New biomarker in obstructive sleep apnoea

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Obstructive sleep apnoea (OSA) is a syndrome characterized by the partial or complete obstruction of the upper airways during sleep, leading to intermittent hypoxia among others conditions. These physiological disturbances are associated with an increased risk of various diseases, such as cardiovascular disease and cancer. In fact, the prevalence of cancer in individuals with OSA is 1.53 times higher than in patients without OSA. This increase could be due to the disruption of immune surveillance caused by the effect of certain immune checkpoints (IC) on T cells. One of this IC is TIGIT, an inhibitory receptor expressed in lymphocytes that interacts with the ligands CD155 and CD112, which are present on antigen-presenting and tumor cells. The binding of this receptor to its ligands downregulated T cell functions.

Thus, we aim to explore TIGIT and its ligands expression on lymphocytes and monocytes respectively of patients with OSA, assessing the role of intermittent hypoxia in this context. This study includes a cohort of patients with and without OSA who underwent a sleep study. We explore TIGIT, CD155 and CD112 expression from OSA patients and control subjects and the role of intermittent hypoxia with in vitro models. We obtained these results through flow cytometry and qPCR.

Our findings show that TIGIT expression in lymphocyte T membrane is upregulated in patients with severe OSA and correlates with parameters indicating OSA severity, suggesting an association between OSA and TIGIT expression. Finally, in vitro model data suggested intermittent hypoxia were associated with upregulation of TIGIT in patients with severe OSA.

In conclusion, TIGIT expression in T cell membrane is mediated by intermittent hypoxia in OSA patients.