WORKSHOP IN HEALTH SCIENCE AND BIOMEDICINE

28 May 2024
School of Medicine
C/ Arzobispo morcillo 4, 28029 Madrid

WORKSHOP PROGRAM

9:30-9.55 REGISTRATION

10:00-10:15 WORKSHOP INAUGURATION (Aula Magna)

Prof. Pilar López Garcia, Dean of the School of Medicine, UAM Prof. Marta Ruiz Ortega, Vice-director of International Relations, EDUAM

10:15-12:00 ORAL COMMUNICATIONS I

AULA MAGNA **Moderated by Juan Arredondo and Juan Jose Berlanga**	**SEMINARIO 1** **Moderated by Fernando Rodríguez Artalejo and Susana Guerra**
TOTAL INTERNAL REFLECTION MICROSCOPY AS A POWERFUL TOOL TO STUDYCHEMOKINE RECEPTOR DYNAMICS.	PROTEIN INTAKE AND TRANSITIONS FROM MULTIMORBIDITY TO FRAILTY AND MORTALITY IN OLDER ADULTS
A CRISPR/CAS9 SCREENING FOR SYNTHETIC LETHALITY INTERACTIONS WITH LOSS OF PRIMPOL	PHTHALATE EXPOSURE AND THE METABOLIC SYNDROME: A SYSTEMATIC REVIEW AND META-ANALYSIS
IMPACT OF NRF2 SIGNALING ON THE ANGPT1/2-TIE2 PATHWAY: IMPLICATIONS FOR BLOOD-BRAIN BARRIER INTEGRITY	ANTITHROMBOTIC USE PATTERNS IN COVID-19 PATIENTS FROM SPAIN: A REAL- WORLD DATA STUDY
UNRAVELING RAF1 DEGRADATION AND ITS TUMORIGENIC ROLE USING THE DTAG SYSTEM	ASSOCIATION OF A HEALTHY BEVERAGE SCORE WITH TOTAL MORTALITY IN THE ADULT POPULATION OF SPAIN: A NATIONWIDE COHORT STUDY
DEVELOPMENT OF PREDICTIVE AND THERAPEUTIC TOOLS TO OVERCOME CHEMOTHERAPY RESISTANCE IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS	SURVIVAL OF THE PEDIATRIC AND ADOLESCENT POPULATION WITH CANCER IN THE COMMUNITY OF MADRID, 2015-2018
THE ROLE OF THE RNA/RBP BINDING ACTIVITY OF BRCA2 IN REPLICATION FORK DYNAMICS	COMPARATIVE ANALYSIS OF NOVEL 2022 OUTBREAK MPXV AND PREVIOUS CLADE II MPXV

12:00-12:30 COFFEE BREAK

12:30-14:00 ORAL COMMUNICATIONS II

AULA MAGNA **Moderated by Víctor Calvo and Oscar Martínez**	**SEMINARIO 1** **Moderated by Carmen Cavada and David Fernández de Sevilla**
CONSEQUENCE OF EXTRACELLULAR VESICLES FROM HYPOTHALAMIC ASTROCYTE ON LEPTIN SIGNALING PATHWAYS IN PROOPIOMELANOCORTIN (POMC) NEURONS.	IMPAIRED MODULATION OF TRIGEMINAL CAUDAL NUCLEUS SOMATOSENSORY RESPONSES BY THE LOCUS COERULEUS IN A MOUSE MODEL OF DIABETES: PARTICIPATION OF GABAERGIC AND GLYCINERGIC NEURONS
BIDIRECTIONAL RELATIONSHIP BETWEEN LONELINESS AND PSYCHOSIS SPECTRUM DISORDERS	EXPLORING GENDER-SPECIFIC THALAMIC TRANSCRIPTOMIC CHANGES IN A DUAL-HIT RAT MODEL OF SCHIZOPHRENIA
PREDIABETES PROFILES IN HIGH-RISK SUBJECTS STRATIFIED BY CLINICAL PHENOTYPES BASED ON 1-HOUR OGTT AND IMPAIRED GLUCOSE TOLERANCE	VOLUME ELECTRON MICROSCOPY ANALYSIS OF SYNAPSES IN ASSOCIATIVE AND PRIMARY REGIONS OF THE HUMAN CEREBRAL CORTEX
HTLV-1 SURVEILLANCE IN SPAIN, A NON-ENDEMIC COUNTRY	STUDY THE CELLULAR EFFECT OF NEW TAU ISOFORMS OVEREXPRESSION GENERATED BY INTRON RETENTION
A 37-COLOR FULL-SPECTRUM FLOW CYTOMETRY PANEL FOR THE IMMUNOLOGICAL CHARACTERIZATION OF CYSTIC FIBROSIS PEDIATRIC PATIENTS	QUANTITATIVE ULTRASTRUCTURE COMPARISON OF LAYER-SPECIFIC MICROCIRCUITS FROM FIRST AND HIGHER ORDER THALAMOCORTICAL PROJECTIONS INTO SOMATOSENSORY CORTICES

14:00-15:00 LUNCH TIME

15:00-16:00 POSTER SESSION

16:30-16:45 AWARDS AND WORKSHOP CLOSING (Aula Magna)

Organizing Committee: Víctor Calvo, Oscar Martínez, Ignacio Monedero y David F. de Sevilla.

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

WORKSHOP ABSTRACTS

1. ORAL COMMUNICATION I. AULA MAGNA

Total Internal Reflection Microscopy as a powerful tool to study chemokine receptor dynamics.

Noelia Santander Acerete[1], José Miguel Rodríguez Frade[1], Mario Mellado[1]

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Chemokine receptors are cell surface receptors that play fundamental roles in different physiological processes: embryogenesis, inflammatory response, development, leukocyte homing, etc. These receptors are embedded in the cell membrane and can form homodimers, heterodimers and oligomers1, all functional conformations. Chemokine receptor organization and dynamics at the cell membrane influences their behavior and how cells respond to chemoattractant gradients2,3. Alterations in actin cytoskeleton remodeling, lipid composition of the cell membrane or oligomerization impair normal cell responses. Some evidence indicates that heterodimers are functional and therefore it is necessary to analyze their dynamics on the cell surface, and how the ligands modify it.4,5 CXCR4, a conventional chemokine receptor, and ACKR3, an atypical chemokine receptor, form heterodimers. ACKR3 recognizes two ligands, CXCL11 and CXCL12, while CXCR4 only recognizes CXCL12. Thus, it is a very nice system to analyze the dynamic of these two receptors at the cell surface, and how the ligands modify it.4,5As CXCR4 and ACKR3 share one ligand, and signal through different pathways, this model may explain if chemokine receptor heterodimers have similar dynamics to dimers formed by a single receptor or on the contrary follow different profiles, how it affects the complexes when activated with their ligands and what are the functional consequences that derive. Total internal reflection microscopy (TIRF-M) is a new advanced fluorescent technique with great potential to study membrane processes.2,3 When the incident light of a microscope is totally reflected, an evanescent wave is created at the interface between coverslip and cell medium. This physical phenomenon allows excitation of cell fluorochromes in contact with the coverslip and therefore is ideal to investigate cell membrane-associated phenomena. Furthermore, TIRF-M allows single particle tracking (SPT). In our case cells transiently transfected with the chemokine receptor coupled to the monomeric green fluorescent protein (Ac-GFP), are sorted, to obtain a low receptor expression population of cells that simulates physiological conditions. Using human T lymphocytes as model, we have studied the dynamics of CXCR4 and ACKR3, when human T cells express both receptors (CXCR4 and ACKR3) and when only ACKR3 is expressed. ACKR3 oligomerization is much lower in response to CXCL12, the share ligand, when human T cells do not express CXCR4. These differences may influence the signaling properties and functional responses.

A CRISPR/Cas9 screening for synthetic lethality interactions with loss of PRIMPOL

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PRIMPOL (Primase-Polymerase) is a key player in DNA damage tolerance. Although PRIMPOL is not an essential gene, cellular exposure to toxic agents induces the recruitment of PrimPol protein to sites of DNA damage to promote re-initiation of DNA synthesis downstream of the lesions. Therefore, PrimPol potentially counteracts the efficacy of common chemotherapy drugs. We are carrying out a systematic study of potential genetic interactions between the loss of PrimPol and other genes or pathways. The rationale of the project is that the simultaneous loss of two independent pathways may cause synthetic lethality specifically in cancer cells, as in the known examples of BRCA mutations and PARP inhibition, or loss of WRN helicase in cells displaying microsatellite instability. This study is being performed in osteosarcoma-derived U2OS cells expressing normal levels of PrimPol and an isogenic cell line in which PRIMPOL gene expression has been ablated by CRISPR-Cas9. Initially, WT and PrimPol KO U2OS cells expressing Cas9 were transfected with a modular library (JM-MSKCC) of >2300 guide RNAs (gRNAs) targeting genes involved in DNA replication, replicative tolerance, recombination and DNA repair. While we were able to identify genes implicated in replication and repair that are essential in U2OS cells, no synthetic lethality partners could be identified within the genes in this library. For this reason, we are now conducting a genome-wide screening in the same cell lines transfected with the third version of the Toronto Knockout (TKO) CRISPR library. Hopefully, the ongoing screening will lead to genes or pathways whose loss or mutation is synthetic lethal with the loss of PRIMPOL, thereby identifying possible weaknesses in tumor cells that could be exploited in the clinic.

Impact of NRF2 signaling on the ANGPT1/2-TIE2 pathway: implications for blood-brain barrier integrity

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The selective permeability of molecules from the bloodstream to the brain is governed by the blood-brain barrier (BBB), which depends on several signaling pathways, including ANGPT1/2-TIE2 to maintain its properties. The integrity of the BBB is compromised under neuropathological conditions, in which endothelial cells are subjected to oxidative and inflammatory stress. To protect against such stress, NRF2 activates a genetic program, which may play a role in preserving BBB. In recent years, the transcription factor NRF2 is becoming a new target for neurodegenerative disorders, as it controls the expression of more than 250 genes involved in multiple homeostatic functions such as protection against oxidative, inflammatory or metabolic stress, as well as proteinopathy. However, its relevance in the protection of BBB integrity in this pathway has hardly been evaluated.

Preliminary studies, by obtaining targeted transcriptomic data of NRF2- regulated genes, suggest that they may have a role in BBB. Several experiments in the murine neuroendothelial cell line bEND.3 have revealed that NRF2 changes the balance of protein and mRNA levels of one of the major signaling pathways involved in BBB function, Angpt1/2-TIE2. This NRF2-mediated change has been addressed through natural inducers, such as sulforaphane isothiocyanate (SFN) and by overexpression techniques using lentiviral infections. In experimental models of NRF2 gene silencing, a reverse effect on TIE2/Tek levels is observed, increasing with respect to controls. This effect may be due to a transcriptional regulation mechanism, since the analyzed half-lives of TIE2 protein and mRNA did not evidence mechanisms of indirect regulation through miRNAs. Furthermore, we compared this same effect of NRF2, on an inflammatory condition described as destabilizing BBB integrity, by treating with lipopolysaccharide (LPS), and observed a similar effect of reduced TIE2 expression.

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.

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Development of predictive and therapeutic tools to overcome chemotherapy resistance in head and neck squamous cell carcinomas.

Acero-Riaguas Lucía[1,2], López-García Iván[1], Posse-Alonso Irene[1], Yáñez- Bartolomé Mariana[1], Griso-Acevedo Ana[1], Fernández-Díaz Sara[1], Ruiz Bravo- Burguillos Elena[3], Cebrián-Carretero Jose Luis[1,5], Castelo Beatriz[4], Sastre- Garzón Leandro[2], Sastre-Perona Ana[1,2].

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Head and Neck Squamous Cell Carcinomas (HNSCC) are the 8th most frequent cancer worldwide, with a poor prognosis and a 5-year mortality rate of 55% that has not improved in the last decade. First line treatment is surgery, combined with radiotherapy and/or chemotherapy. Advanced stage tumours which are unresectable or unsuitable for radiotherapy, are treated with standard chemotherapy. However, there are no molecular biomarkers that predict response to the various chemotherapies and treatment is still chosen based on patient characteristics.

In this project we have performed genome wide CRISPR/Cas9 screen to identify genes that confer cisplatin resistance, which is the most frequently administered chemotherapy in HNSCC. We have identified AMBRA1 (a gene involved in apoptosis and autophagy pathways) as a potencial biomarker to platinum-based therapies, as validation analyses showed that loss of this gene sensitizes cells not only to cisplatin but also to carboplatin in three HNSCC cell lines. Further mechanistic analyses are underway to elucidate which pathway is involved in conferring this sensitivity.

We are also considering extending these genome-wide CRISPR screens to other chemotherapies used in these tumours in order to better understand how oral tumors escape chemotherapeutic treatment and uncover new biomarkers and therapeutic targets to predict and overcome resistance.

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Unraveling RAF1 degradation and its tumorigenic role using the dTAG system

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Genetic interrogation of the KRAS signaling pathway in genetically engineered mouse models has shown that RAF1 ablation promotes tumor regression without significant toxicities, highlighting it as a promising therapeutic target. In this work, considering the unavailability of RAF1 specific degraders, we propose the use of the dTAG system as a proof of concept to pharmacologically reproduce those effects observed upon RAF1 genetic ablation. This strategy allows the study of the immediate consequences of protein loss and provides useful information about RAF1 degradation that could be used to develop a selective degron or PROTAC. To achieve this, we have generated a homozygous knock-in mouse with a FKBP12F36V tag inserted into the Raf1 locus, where lung adenocarcinomas are driven by KRASG12V and loss of Trp35. Phenotypically, some differences were observed in comparison with Raf1wt mice. Not only they seemed weaker and smaller than wild type mice, but tumor formation after adenoviral infection was 5-fold lower than expected. Since the key genetic modification lies in the expression of FKBP12-RAF1 instead of the WT protein, some differences between them are suspected. Moving on to the validation of target degradation under dTAGv1 treatment, we demonstrate a rapid and efficient systemic elimination of FKBP12-RAF1 without associated toxicities. However, protein degradation did not correspond to the expected anti-tumor effect. These findings may be linked to the aforementioned suspected differences, possibly associated with the kinase-independent function of RAF1. Interestingly, based on our results, our model offers a new approach to gaining insights into the major role of RAF1 protein in tumor development and progression by exploring the differences between this kinase and FKBP12-RAF1 through an interactome analysis. Moreover, the generation of RAF1 independent tumors allows the study of underlying resistance mechanisms. Altogether, this work opened up new possible strategies to clarify the tumorigenic role of RAF1.

The role of the RNA/RBP binding activity of BRCA2 in replication fork dynamics

Lucía Alvaro-Aranda 1, Aida Contreras-Pérez 1, Contreras-Pérez 1, Carmen Escalona-Noguero1, Stéphan Vagner2,3 and Aura Carreira 1

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Accurate DNA replication is crucial for the maintenance of genome stability and integrity. However, faithful replication is constantly challenged by numerous obstacles that can lead to the slowing or stalling of the replication forks. This situation, known as “replication stress”, can potentially result in genetic instability and it is considered as an important cause of human diseases, such as cancer [3]. For this reason, different molecular pathways are aimed at preserving the stability of the stalled replication forks and promoting their correct restart. Recently, RNA and RNA processing components have appeared as novel and unexpected regulators of these DNA repair pathways. However, they have emerged as having opposite roles in genome maintenance and their exact function in the replication stress response is not clear. Thus, there is an increasing need to elucidate whether DNA damage response factors interact with RNAs or RNA binding proteins (RBPs) and if such binding is important for their DNA repair functions. Several lines of evidence suggest that tumor suppressor BRCA2, a key player protein of the DNA repair pathway homologous recombination, could interact directly or indirectly with RNA. In particular, our group found that BRCA2 was able to directly interact with the RNA helicase DDX5 facilitating the resolution of RNADNA hybrids at DNA double- strand break sites of highly transcribed regions. Therefore, the aim of this project was to determine whether and how RNA/RBP- binding activities of BRCA2 impact replication fork dynamics and shed light on the possible regulatory role of RNAs at replication forks. Using purified protein and radiolabeled substrates to perform electrophoretic mobility shift assays (EMSA) we found that BRCA2 directly interacts with RNA through its C-terminal DNA binding domain (BRCA2CTD). Strikingly, the affinity of this interaction is higher for ssRNA than for ssDNA. BRCA2CTD shows preference for purines over pyrimidines. Further experiments are ongoing to confirm these findings with the full-length BRCA2 protein. Importantly, using a battery of recombinant BRCA2CTD fragments expressing different variants identified in breast cancer patients, we have uncovered a variant that reduces the RNA binding activity while keeping a similar affinity for DNA. We plan to use this variant in the context of the full-length protein in cells to determine the specific role of BRCA2- RNA interaction in the replication fork dynamics and/or in its DNA repair function using proximity ligation assay, DNA combing and other relevant functional assays.

2. ORAL COMMUNICATION I SEMINARIO 1

Protein intake and transitions from multimorbidity to frailty and mortality in older adults

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Background: Total protein intake has been associated with a reduced risk of frailty and mortality; however, evidence is conflicting as to which type of protein has a greater protective effect. The aim of this study was to examine the effect of different protein sources in individuals with multimorbidity and their transitions to frailty and mortality.
Methods: This study included 1868 individuals >65 years from the Seniors-Enrica cohort with prevalent multimorbidity. Habitual diet (2008-2010) was assessed with a validated dietary history. Participants underwent physical examinations (2012, 2015, and 2017) for frailty assessment (≥3 Fried index criteria: low physical activity, slow walking speed, muscle weakness, weight loss, and fatigue). All-cause mortality was recorded until January 2022. Analyses were performed using Cox proportional hazards models and multistate models adjusted for sociodemographic, lifestyle, and other dietary factors.
Results: Mean protein intake was 90.2 [standard deviation: 26.8] g/day, which represented 18.7% of the total energy intake and 1.23 (0.39) g per kg of body weight per day. Plant protein accounted for 6.16% of the energy intake, while animal protein accounted for 12.5%. During a median follow-up of 12.9 (range: 11.7 - 13.9) years, we documented 196 incident cases of frailty and 490 deaths; of these mortality cases, 83 individuals died after a frailty diagnosis. Higher total protein intake was associated with a reduced risk of frailty [hazard ratio (HR) for tertile 3 vs. tertile 1: 0.66 (95% CI: 0.45, 0.96); p trend: 0.03]. In multistate models, higher fish protein intake decreased the risk in the transition from multimorbidity to frailty [HR per 1-SD increment: 0.81 (95% CI: 0.68, 0.97)]; and higher plant protein intake decreased the risk of transitioning from multimorbidity to mortality [0.86 (0.75, 0.98)]. For the transition from frailty to mortality, estimates for total, plant, and animal protein showed an increased risk [HR for 1 SD increment in total protein: 1.38 (1.05, 1.81); HR for plant protein: 1.29 (1.01, 1.67); HR for animal protein: 1.41 (1.04, 1.92)]. No significant associations were found between meat protein and dairy protein in any transition.
Conclusions: In individuals with multimorbidity, higher protein intake, especially fish, was associated with a lower risk of frailty, whereas vegetable protein intake was associated with a lower risk of mortality.
Keywords: multimorbidity, frailty, mortality, protein intake, older adults

Phthalate exposure and the metabolic syndrome: a systematic review and meta-analysis

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Phthalates are chemicals widely used in plastic-based consumer products, and human exposure is universal. They are classified as endocrine disruptors, and specific phthalate metabolites have been associated with an increased risk of cardiometabolic diseases. The aim of this study was to assess the association between phthalate exposure and the metabolic syndrome in the general population. A comprehensive literature search was performed in four databases (Web of Science, Medline, PubMed, and Scopus). We included all the observational studies that evaluate the association between phthalate metabolites and the metabolic syndrome available until January 31st, 2023. Pooled Odds Ratios (OR) and their 95% confidence intervals were calculated by using the inverse-variance weighted method. Nine cross-sectional studies and 25,365 participants aged from 12 to 80 were included. Comparing extreme categories of phthalate exposure, the pooled ORs for the metabolic syndrome were: 1.08 (95% CI, 1.02- 1.16, I2=28%) for low molecular weight phthalates, and 1.11 (95% CI, 1.07-1.16, I2=7%) for high molecular weight phthalates. For individual phthalate metabolites, the pooled ORs that achieved statistical significance were: 1.13 (95% CI, 1.00-1.27, I2=24%) for MiBP; 1.89 (95% CI, 1.17-3.07, I2=15%) for MMP in men; 1.12 (95% CI, 1.00- 1.25, I2=22%) for MCOP; 1.09 (95% CI, 0.99-1.20, I2=0%) for MCPP; 1.16 (95% CI, 1.05-1.28, I2=6%) for MBzP; and 1.16 (95% CI, 1.09-1.24, I2=14%) for DEHP (including ΣDEHP and its metabolites). In conclusion, both low molecular weight and high molecular weight phthalates were associated with an 8 and 11% higher prevalence of the MetS, respectively. The exposure to six specific phthalate metabolites was associated with a higher prevalence of the MetS.

Antithrombotic use patterns in COVID-19 patients from spain: a real-world data study

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Background: Antithrombotics have been widely used to treat and prevent COVID-19-related thrombosis; however, studies on their use at population levels are limited. Objectives: We aimed to describe antithrombotic use patterns during the pandemic in Spanish primary care and hospital-admitted patients with COVID-19. Methods: A real- world data study was performed. Data were obtained from BIFAP's electronic health records. We investigated the antithrombotic prescriptions made within ±14 days after diagnosis between 03/2020- 02/2022, divided their use into prior and new/naive, and reported their post-discharge use. Results: We included 882,540 individuals (53.4% women), of whom 78,499 were hospitalized. The median age was 44.7 (IQR 39-59). Antithrombotics were prescribed in 37,183 (4.6%) primary care subjects and 42,041 (53.6%) hospital-admitted patients, of whom 7,505 (20.2) and 20,300 (48.3%), respectively, were naive users. Prior users were older and had more comorbidities than new users. Enoxaparin was the most prescribed in hospitals, with higher prescription rates in new than prior users (2348.2, IQR 2390- 3123 vs. 1378 IQR 1162-1751 prescriptions per 10,000 cases, p=0.002). In primary care, acetylsalicylic acid was the most used, having higher use rates in prior than in naïve users (139.9 ± 45 vs. 331±47). Post-discharge use occurred in 6,686 (15.9%) subjects (median use 10 days, IQR 9-30). Conclusions: Our study identified consensus on prescribing antithrombotics in COVID-19 patients; however, hospital use rates were lower than other national and international reports.

Association of a healthy beverage score with total mortality in the adult population of Spain: A nationwide cohort study

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Despite the substantial evidence of the relationship between diet and mortality, the role of beverage consumption patterns is not well known.The aim of this study was to assess the association of the adherence to a Healthy Beverage Score (HBS) and all-cause mortality in a representative sample of the Spanish adult population. We conducted an observational cohort study using data from the Study on Nutrition and Cardiovascular Risk in Spain (ENRICA), which included 12,161 community-dwelling individuals aged ≥18 years recruited in 2008 to 2010 and followed until January 2022. At baseline, food consumption was collected using a validated diet history. The HBS consists of 7 items, each of which is scored from 1 to 4 (highest adherence) (Table 1). The HBS ranges from 7 to 28 points with a higher score representing a healthier pattern. Adherence was assigned as a higher consumption of low-fat milk, and coffee and tea, a lower consumption of whole-fat milk, no consumption of fruit juice, artificially sweetened beverages, or sugar-sweetened beverages, and no or moderate consumption of alcohol. Total mortality was ascertained by linkage to the Spanish National Death Index. Statistical analyses were performed with Cox models and adjusted for the main confounders, including sociodemographic, lifestyle, dietary variables, and morbidity (Stata, version 18.0).

Table 1. Scoring criteria for the HBS in the ENRICA Study (2008–2010).

Components	Minimum score			Maximum score
Adequacy				
Low fat milk	1 (No consumption)	2 (Tertile 1 among consumers)	3 (Tertile 2 among consumers)	4 (Tertile 3 among consumers)
Coffee and tea	1 (Quartile 1)	2 (Quartile 2)	3 (Quartile 3)	4 (Quartile 4)
Moderation				
Whole-fat milk	1 (Tertile 3 among consumers)	2 (Tertile 2 among consumers)	3 (Tertile 1 among consumers)	4 (No consumption)
Fruit juice	1 (Any consumption)	--	--	4 (No consumption)
Artificially sweetened beverages	1 (Any consumption)	--	--	4 (No consumption)
Sugar-sweetened beverages	1 (Tertile 3 among consumers)	2 (Tertile 2 among consumers)	3 (Tertile 1 among consumers)	4 (No consumption)
Alcohol[b]	1 (Heavy drinkers)	--	--	4 (No consumption or moderate drinkers)
Range	**7**			**28**

After a mean follow-up of 12.5 years (SD: 1.7; range: 0.5 to 12.9), a total of 967 deaths occurred. For all-cause mortality, the fully adjusted hazard ratio (HR) for the highest versus lowest sex-specific quartiles of HBS was
0.72 (95% confidence interval [0.57, 0.91], p linear-trend = 0.015), corresponding to an 8.3% reduction in the absolute risk of death. A linear relationship between the risk of death and the adherence to the HBS was observed using restricted cubic splines. The results were robust to sensitivity analyses. In this study, we observed that higher adherence to the HBS was associated with lower total mortality. Adherence to a healthy beverage pattern could play a role in the prevention of premature mortality.

Survival of the pediatric and adolescent population with cancer in the community of madrid, 2015-2018

Authors: Raquel López-González1,2, David Parra-Blázquez2, Daniel Moñino3, Candela Pino-Rosón1,2, Marina Pollán4,5, Nuria Aragonés2,5

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Background and objectives: Cancer is the leading cause of death among children over 1 year old and the second leading cause among adolescents aged 15 to 19 in the Community of Madrid (CM)1. Despite treatment improvements, close epidemiological surveillance of survival remains necessary. This study analysed the 1, 3, and 5-year survival rates of individuals aged 0-19 diagnosed with cancer between 2015- 2018 in the CM.

Methods: Data from the Population-Based Cancer Registry in Childhood and Adolescence of the CM were used. Vital status was updated until February 2023. Survival functions were calculated with corresponding 95% confidence intervals using the Kaplan-Meier method, the log-rank test was used to identify significant differences between groups, and Cox regression was used to quantify the hazard ratios. Stratified analyses were conducted by sex, age, tumour group/subgroup, and stage at diagnosis.

Results: A total of 993 cases were included: 862 malignant tumours and 131 non-malignant central nervous system (CNS) intracranial and intraspinal tumours. The overall 5-year survival rate was 85.92% (83.41 – 88.08) for malignant tumours. There were no differences in survival by sex or age group (childhood/adolescence). The 10 to 14- year-old and under 1-year-old groups showed lower survival rates: 77.89% (71.3 – 83.15) and 84.13% (72.51 – 91.13) respectively.

Malignant CNS tumours had the worst survival rate: 66.93% at 5 years (57.24 – 74.91), compared to 98% (93.84 – 99.60) for non-malignant tumours in the same location. Patients diagnosed at metastatic stages had a 3.3 times higher risk of death than those diagnosed at initial stages. This risk was significantly higher for acute lymphoblastic leukaemia, Hodgkin lymphoma, neuroblastomas, and soft tissue sarcomas.

Conclusions: Survival following a cancer diagnosis in children and adolescents in the Community of Madrid is high, with an estimated six out of seven patients with malignant tumours surviving at least 5 years. However, survival is lower for malignant CNS tumours and advanced stages of the disease.

Comparative Analysis of Novel 2022 Outbreak MPXV and Previous Clade II MPXV

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The 2022-2023 outbreak of monkeypox (MPOX) is an important worldwide public health issue that caused significant concern in the scientific community (1). MPOX, caused by the monkeypox virus (MPXV) belonging to the Poxviridae family, presents a multifaceted challenge due to the diverse viral forms it produces. Notably, the Intracellular Mature Virus (MVs) primarily contribute to localized lesions and direct host-to-host transmission, while the Extracellular Enveloped Virus (EVs) are associated with systemic infection (2). Clinically, MPOX manifests as a vesiculopustular rash that initially emerges on the face and trunk, subsequently spreading throughout the body, with heightened severity observed in immunocompromised individuals (3).

In the present work we have done a comparative analysis of two previous clade II strains, specifically WRAIR 7-61 and USA 2003, with the recent strain from the 2022-2023 outbreak. Our results indicate that that the novel 2022-2023 outbreak MPXV has a significantly slower viral cycle compared to previous Clade II strains, with WRAIR 7-61 being more intermediate and USA 2003 producing the highest viral titers. Additionally, a proteomic analysis demonstrates differences in protein expression between the three Clade II strains. These finding highlight differences in the growth and in the protein pattern between the current Lineage B.1 MPXV and previous strains. Further studies will be undertaken to demonstrate if these differences are important for the novel transmission mechanisms observed in the 2022-2023 outbreak.

3. ORAL COMMUNICATION II AULA MAGNA

Consequence of extracellular vesicles from hypothalamic astrocyte on leptin signaling pathways in proopiomelanocortin (POMC) neurons.

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The hypothalamus is the central regulator of homeostasis with proopiomelanocortin (POMC) neurons in the arcuate nucleus playing a fundamental role (1). These neurons release neuropeptides that promote energy expenditure and satiety (1) and are a target for leptin (2), an anorexigenic hormone of adipose tissue origin. POMC neurons communicate with hypothalamic astrocytes via extracellular vesicles (EVs) that contain proteins, lipids, and nucleic acids, and relay information regarding metabolic status (3). Our hypothesis is that astrocytes affect neuronal function and leptin signaling pathway 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 and 24 h in response to leptin and EV-OA, but treatment with EV-PA stimulated it only at 4 h. However, co-treatment with leptin and EVs at 4 h did not increase POMC expression, whereas at 24 h it induced POMC expression much more than the isolated treatments. This may be due to the effect of EVs on leptin signaling pathways. STAT3 is a transcription factor of POMC activated by leptin (4). EV-PA and EV-OA activate STAT3 at 24h, whereas co-treatment with leptin and EVs did not increase p-STAT3. However, EV-V activates STAT3 both with and without leptin. Also, leptin can activate PI3K-Akt pathway too (5). Our results suggest that at 4h EVs increased the Akt activation (p-Akt) and decreased p-mTOR. Thus, EVs from hypothalamic astrocytes could contain biological molecules, such as miRNAs, that modulate the leptin signaling pathway in these neurons.

Bidirectional relationship between loneliness and psychosis spectrum disorders

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Loneliness, defined as a subjective feeling of perceived isolation, has garnered significant attention in the context of mental health research. This paper aims to systematically review the bidirectional relationship between loneliness and psychosis spectrum disorders, addressing two fundamental questions: (1) Is loneliness a risk factor for the development of psychosis? (2) Does psychosis increase the risk of experiencing loneliness?

A comprehensive search of five electronic databases yielded a total of 3607 records, from which six articles were included for review. Three studies addressed the first research question, indicating a potential association between loneliness and the incidence of psychosis. Conversely, four studies examined the second research question, suggesting that individuals with psychosis spectrum disorders may be at higher risk of experiencing loneliness. Methodological quality assessment revealed moderate to high-quality studies overall.

The findings underscore the need for further research, particularly longitudinal and prospective studies, to validate the observed associations. Additionally, exploring potential mediators or moderators, such as stigma or neurobiological mechanisms, could provide deeper insights into the complex relationship between loneliness and psychosis. Addressing these gaps in knowledge could inform the development of targeted interventions to prevent and alleviate the adverse effects of both loneliness and psychosis spectrum disorders.

Prediabetes profiles in high-risk subjects stratified by clinical phenotypes based on 1-hour OGTT and impaired glucose tolerance

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BACKGROUND: Early intervention in subjects with prediabetes depends upon adequate individual risk stratification. The characterization of the intermediate hyperglycemia status may help to define the risk of these subjects. 1-hour post-load glucose (1h-PG) level during an oral glucose tolerance test (OGTT) may predict future type 2 diabetes (T2DM) and cardiovascular disease equal or more accurately than Impaired Glucose Tolerance (IGT). However, different prediabetes phenotypes may be associated with specific metabolic abnormalities.

AIM: We evaluated the incidence of new cases of T2DM after seven years (7yrs) in high- risk sample, underwent an OGTT, among subjects with 1h-PG hyperglycemia and those with IGT.

METHODOLOGY: 421 Peruvian subjects with impaired fasting glucose (IFG), based on the definition of the American Diabetes Association (ADA), were selected to perform an OGTT. Among them, a cohort of 321 individuals were follow-up during seven years for detection of new-onset T2DM. The outcome assess was diagnosis of T2DM, and the exposure were the condition 1h-PG≥155mg/dL and IGT. In the pooled groups we describe the frequencies and the association between T2DM diagnosis with the exposures using chi2 test. Relative risk (RR) was estimated using Poisson regression with 95%CIs. Also, the area under the curve (AUC) was estimated for the three exposures to predict T2DM.

RESULTS: The mean age of the sample was 56.1±11.6 years-old, 196 (61.1%) were female and 151 (47%) were overweigh/obese. At the baseline 242 (75.4%) individuals had NGT(2h-PG < 140 mg/dL), 142 (58.7)% showed 1h-PG ≥155mg/dL and 100 (41.3%) exhibited 1h-PG < 155mg/dl. Also, 79 (24.6%) had IGT.

After 7yrs follow-up 24.3% of the sample developed T2DM (4.6 years average). In NGT subjects 10.6 % of them with 1h-PG ≥155mg/dL and 37.0% with 1h-PG ≥155mg/dL(p<0.001). In patients with IGT 32.9% of them developed T2DM (p<0.001). Positive associations were found between T2DM with 1h-PG ≥155mg/dL (RR 3.50, CI95%1.92 – 6.38) and with 2-h PG (RR 3.11, CI95% 1.65 – 5.88) (p< 0.05). After adjusted the regression model by age, sex, and body mass index the association was maintained RR 3.25 (1.77 – 5.98) and 2.94 (1.54 – 5.60), respectively.

CONCLUSION: High incidences of new cases of T2DM at seven years were found in subjects with intermediate hyperglycemia and other cardiometabolic risk factors. 1h-PG ≥155mg/dL is shown as independent risk marker contributing to develop T2DM in individuals with NGT. The OGTT curve, considering the 1hr measure, may help to identify different categories of subjects at risk to develop T2DM or clinical phenotypes.

Key words: Type 2 diabetes, Oral Glucose tolerance test, 1hr Post load Glucose, Risk Factors, Cardiometabolic, Phenotypes.

HTLV-1 surveillance in Spain, a non-endemic country

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Background: HTLV-1 was the first discovered human retrovirus. Over 10 million people are infected worldwide but this figure may well underestimate the real number. Spain established a national registry for HTLV-1 cases in 1989 after identifying the first infected individual. Herein, we report the main features of HTLV-1+ individuals until the end of year 2023.

Methods: The national HTLV registry involves 50 centers across the country's geography. Distinct surveys are conducted annually in sentinel groups, including pregnant women, blood donors, organ donors, and individuals with sexually transmitted infections, among others. Participating centers report newly diagnosed cases and provide specimens to a central lab repository, and complete standardized case report forms.

Results: A total of 482 cases of HTLV-1 had been reported until Dec 31st 2023. Most cases are migrants from Latin America (66.2%) and Africa (10.8%). However, native Spaniards account for 17.1%, suggesting that horizontal transmission is ongoing locally.

HTLV-1 in Spain is more prevalent in women (64.4%) and median age at diagnosis is 41 years-old. In cases for which the route of infection is known, 32% acquired the virus following sexual exposure and 10% after vertical transmission.

Symptomatic cases at diagnosis represented 22.1% of total, being TSP/HAM in 62, ATL in 35, and Strongyloides stercoralis infestation in 9. New diagnoses of HTLV-1 infection in Spain increased sharply since 2008 (376 compared to 106 earlier), following the introduction of HTLV screening in blood banks. A total of 156 blood donors positive for HTLV-1 have been notified. As comparison, 11 HTLV-1+ pregnant women have been identified to date.

During year 2023, a total of 30 HTLV-1+ individuals have been identified, the second highest year historically. Three patients had HAM/TSP and 3 were coinfected with HIV-1.

Conclusions: HTLV-1 underdiagnosis must be high in Spain, given the disproportionate high rate (>22%) of symptomatic cases in the national registry. Given the large flux of migrants and visitors from HTLV-1 endemic regions to Spain, HTLV-1 screening should be expanded. In the meantime, in accordance with WHO proposals, HTLV testing of blood donors and pregnant women from endemic areas will contribute to unveil asymptomatic HTLV-1 carriers unaware of their infection.

A 37-color full-spectrum flow cytometry panel for the immunological characterization of cystic fibrosis pediatric patients

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Cystic Fibrosis (CF) is a rare chronic disease that affects 1 to 5000 newborns in Spain. It is an autosomal recessive disease caused by the presence of pathogenic variants in the gene encoding the protein known as Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). The CFTR gene has more than 2000 pathogenic variants associated with different clinical manifestations, leading to a wide range in the severity of symptoms among patients (Myer et al., 2023). The dysregulated function of this protein causes a wrong functionality of the chloride and sodium channel at the surface epithelium (Guggino & Banks-Schlegel, 2004). Due to that, CF is characterized by mucus accumulation leading to frequent pulmonary bacterial infections that compromise respiratory functionality, inducing severe lung damage that leads patients to lung transplantation or early death (Myer et al., 2023). The goals of treatment primarily include preventing and controlling this lung infections and the airway inflammation, among other afflictions. Nevertheless, not all pediatric patients with CF have access to the therapies due to age or the absence of the genetic variant in which the medication is indicated.
In this project we aim to evaluate to an unprecedented depth the immunological profile of circulating immune cells in pediatric patients suffering from CF. Furthermore, considering that adult CF patients present endotoxin tolerance (Fresno et al., 2008), we will evaluate whether this is also the case in pediatric patients and whether bacterial immunotherapies that induce trained immunity can revert this tolerance as shown in alternative contexts (Novakovic et al., 2016). We aim to achieve a new innovate preventive therapy for these children with CF, aiming to prevent infections and lung damage, enhancing the quality of life of these patients.

Here, starting from 1mL of peripheral blood of CF pediatric patients, a wide and deep characterization of the immunological state will be analyzed using a 37-color full-spectrum flow cytometry panel that we have set up in the laboratory. Including lineage, activation and immune-checkpoints markers, the panel provides a detail assessment of human blood immune cells, in which are included conventional T lymphocytes, B cells, NK cells, NKT cells, monocytes, dendritic cells, neutrophils and myeloid-derived suppresor cells (MDSC). Applying this panel to whole blood samples from both healthy donors and CF pediatric patients, the aim will be to look for immune descriptors that differ between CF pediatric patients and healthy donors.

4. ORAL COMMUNICATION II SEMINARIO 1

Impaired modulation of trigeminal caudal nucleus somatosensory responses by the Locus Coeruleus in a mouse model of diabetes: Participation of GABAergic and glycinergic neurons

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The trigeminal system plays a crucial 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), a key brainstem nucleus that plays a critical role in many cognitive and physiological functions. Previous electrophysiological investigations have revealed a diminished ability of the LC to inhibit somatosensory responses in the caudal nucleus of the trigeminal nerve (Sp5C) of diabetic mice. The administration of noradrenaline agonists and antagonists successfully modulated the inhibitory function of the LC. Our hypothesis is that GABAergic and glycinergic neurons in the Sp5C may be also participate in the modulatory action of the LC. Using electrophysiological recordings, we examined the activity of GABAergic and glycinergic neurons within the LC while monitoring trigeminal somatosensory responses to whisker stimulation in both control and streptozotocin-induced diabetic mice, anesthetized with isoflurane. Our results reveal that GABAergic and glycinergic are under the control of noradrenergic projections from the LC. In diabetic mice, the functionality of these inhibitory circuits appears to be compromised, contributing to the observed deficiency in LC-mediated modulation and probably in the generation of neuropathic pain. We also conducted immunohistochemical experiments supporting the presence of noradrenergic receptors in GABAergic and glycinergic neurons that were altered in diabetic mice. In conclusion, the modulation exerted by the LC on the somatosensory responses in the Sp5C is not only through noradrenergic receptors on projection neurons, but also exerts its control through projections to inhibitory interneurons.

Exploring Gender-Specific Thalamic Transcriptomic Changes in a Dual-Hit Rat Model of Schizophrenia

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This study aims to elucidate the transcriptomic changes underlying thalamic alterations in an animal model of schizophrenia. We have used a 'dual hit' rat model of schizophrenia, which combines an injection of the non-competitive NMDA receptor antagonist MK-801 at P7 and 8 weeks of postweaning social isolation. The model was developed in both male and female rats. At 3 months, behavioral tests and RNA sequencing (RNAseq) of the thalamus were performed. Differential expressed genes (DEGs) and enriched categories were validated in silico via MAGMA using GWAS summary statistics of the Psychiatric Genomics Consortium of schizophrenia (67,390 cases and 94,015 controls).

Regarding behavioral measurements, male model rats showed a decreased preference for sucrose and increased locomotion and time spend in the center in the open field test when compared to controls. The same trend was found in female rats, but with no statistical significance. In the RNAseq analysis, combined male and female enriched categories after validation pointed to synaptic membranes and glutamatergic synapse, attention deficit hyperactivity disorder (ADHD), seizures, and long-term potentiation. These results indicate that our 'dual hit' rat model of schizophrenia does recapitulate some of the symptoms seen in schizophrenia patients. RNAseq results highlight the importance of the synaptic function in schizophrenia, suggesting possible shared genetic pathways between schizophrenia, ADHD and epilepsy. This study provides a valuable model of sex-specific molecular alterations for further investigations in the pathophysiology of schizophrenia.

Volume Electron Microscopy Analysis of Synapses in Associative and Primary Regions of the Human Cerebral Cortex

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Functional and structural studies investigating macroscopic connectivity in the human cerebral cortex suggest that high-order associative regions exhibit greater connectivity compared to primary ones [1]. However, the synaptic organization of these brain regions remains unexplored due to the difficulties involved in studying the human brain at the nanoscopic level [2]. In the present work, we conducted volume electron microscopy using Focused Ion Beam/ Scanning Electron Microscopy [3] to investigate the synaptic organization of the human brain obtained at autopsy. Specifically, we examined layer III of Brodmann areas 17, 3b, and 4, as representative areas of primary visual, somatosensorial, and motor cortex. Additionally, we conducted comparative analyses with our previous synaptic datasets of layer III from temporopolar and anterior cingulate associative cortical regions (Brodmann areas 24, 38, and 21) [4]. 9,690 synaptic junctions were 3D reconstructed, showing that certain synaptic characteristics appeared to be specific to particular cortical regions. The number of synapses per volume, the proportion of the postsynaptic targets, and the synaptic size may distinguish one region from another, regardless of whether they are associative or primary cortex. By contrast, other synaptic characteristics were common to all analyzed regions, such as the proportion of excitatory and inhibitory synapses, their shapes, their spatial distribution, and a higher proportion of synapses located on dendritic spines. These observations may be included within the general rules of synaptic organization of the human cerebral cortex. The present results on nanoscopic characteristics of synapses provide further insights into the structural design of the human cerebral cortex.

Study the cellular effect of new Tau isoforms overexpression generated by intron retention

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Aims Truncated by intron 12 retention CW-Tau isoform could have protective function for Alzheimer's disease on a cellular level. To prove this, will be studying its subcellular localization compared to other canonical Tau isoforms, analyzing its role in protein aggregation, mitochondrial morphology and microtubule function, and characterizing its effect on vesicle trafficking, with special emphasis on the autophagic flux and vesicles exportation. Methods SH-SY5Y cells were infected with lentiviral vectors containing T30 canonic tau, TIR-30 CW-Tau and GFP as control and mitochondria, microtubules and vesicles involved in the autophagic flux were studied by immunofluorescence. In addition, cells were subcellular fragmented to analyze the nuclear presence of CW-Tau. Concurrently, HEK293T cells were transfected with psG5 eucaryotic expression plasmids encoding different Tau isoforms and were treated with CCCP and Bafilomycin A1 to analyze in detail the autophagic flux and the vesicle exportation upon on overexpressing different Tau isoforms. In addition, HEK293T FRET-TAU cells were used to analyze the effect of different Tau isoforms on the aggregation of Tau induced by human brain seeds. Results We first validated the specifity of a new antibody against CW-Tau for immunofluorescence assays. Once the specifity of detection was confirmed, we saw that CW-Tau shows homogeneous localization throughout the cell, with high nuclear representation, comparing with canonic Tau isoforms, with a lack of presence on the nucleus. We further demonstrated that CW-Tau overexpression does not affect the microtubule net area neither affect the mitochondrial morphology. CW-Tau overexpression induced an increase in EEA1 clusters suggesting enhanced endocytosis, a maintained autophagic flux with LC3 and p62 synthesis and degradation ratios. In contrast, the overexpression of canonic tau isoforms induced unbalanced synthesis and degradation ratios. CW-Tau also showed similar expression levels of exocytosis marker CD63, recycling vesicles marker Rab11 and mature lysosomal marker LAMP1 comparing to control. In addition, lysosomal acidity measured with Lysotracker Red was also similar to control, in contrast to canonic tau isoforms which showed and accumulation of acidic compartments. Finally, CW-Tau demonstrated the ability to prevent tau aggregation, but also the capacity to reduce the aggregation induced by canonical isoforms of the protein. Conclusions The obtained results point out that CW-Tau maintain proper cell function regarding to vesicle trafficking and autophagy and more over ameliorate tau pathology associated to tau aggregation in Alzheimer's disease. In conclusion, CW-Tau is proposed as a therapeutic target and gene therapy strategy against this dementia and other tauopathies.

Quantitative ultrastructure comparison of layer-specific microcircuits from first and higher order thalamocortical projections into somatosensory cortices

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Thalamocortical synapses are key cellular links in sensory, motor and cognitive information processing. In rodents, ventral posteromedial thalamic nucleus (VPM) axons innervate both layer 4 of primary and secondary somatosensory cortex (S1, S2) [1]. Posterior thalamic nucleus (Po) projects to the same somatosensory areas as a "higher order" nucleus following a different layer pattern [2]. Despite "primary" and "secondary" functional characterization, it is unknown if differences exist in VPM and Po axon synapse structure and their postsynaptic targets in secondary cortices [3]. Here, we set out to 3D measure and compare the ultrastructure of VPM and Po synapses on these two areas and compare them as well as with previous datasets from our lab [4,5].
We microinjected adult male C57B/L6 mice iontophoretically with biotinylated dextran amine (BDA) in to selectively label thalamocortical axon arborizations. Following a 5 day survival, mice were perfused, and their brains sectioned (50µm) into two parallel series. BDA-labeled cortical arborizations were located on a light-microscopy section series. Adjacent sections were stained for BDA and included for electron microscopy. Area and layer-specific serial images samples were obtained with serial-sectioning TEM or FIB/SEM electron microscopy and subsequently 3D reconstructed and measured [6,7].

Our results are consistent with previous evidence that VPM axon synapses are mostly located (83-85%) on spiny cell dendrites, while the remaining are dendrite shafts which probably correspond to interneurons [4,5]. VPMc-S2L4 synaptic boutons are significantly smaller in volume than VPMr-S1L4. Surprisingly, Po-S2L4 boutons show the highest synaptic vesicle count among all datasets. Progress in the description of synaptic circuitry in such areas has a direct impact in sensory integration models [8].

5. POSTER SESSION

Adverse childhood experiences and cognition: A cross-sectional study in Xhosa people living with schizophrenia and matched medical controls

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Background: Adverse childhood experiences (ACEs) are associated with impaired cognitive function in adult life in the general population as well as in people living with schizophrenia (PLS). Research on cognitive function in PLS in low- and middle-income countries (LMIC) is, however, limited. The objectives of this study were to investigate the association between ACE types and various cognitive domains in a sample of PLS and matched medical controls, and to determine the moderating effect of group membership (PLS vs. medical controls) on these associations, in the South African setting. Methods: Participants (n PLS = 520;n medical controls = 832) completed the Childhood Trauma Questionnaire Short Form, the Structured Clinical Interview for DSM-IV (SCID-I), and the University of Pennsylvania Computerized Neurocognitive Battery (PennCNB). An efficiency or speed score was used to assess performance across 9 cognitive domains. The association between exposure to different ACE types and 9 cognitive domains was examined using partial correlations and multiple linear regression models, adjusting for sex, age and education years. Finally, potential moderating effects of group membership (PLS vs. medical controls) on the association between ACEs and cognitive domains were tested. Results: In the entire sample, emotional and physical abuse predicted worse performance on sensorimotor and emotion identification domains. Also, emotional abuse was negatively associated with motor function, physical abuse was negatively associated with spatial processing, and physical neglect was negatively associated with fase memory and emotion identification. In contrast, emotional neglect was related to better performance on abstraction and mental flexibility. No moderating effect of group membership was found on any of these associations. Conclusion: Exposure to ACEs was associated with social and non-social cognition in adulthood, although the magnitude of these relationships was small and similar between PLS and matched medical controls. The nature of these associations differed across ACE subtype, suggesting the need for a nuanced approach to studying a range of mechanisms that may underlie different associations. However, a number of ACE subtypes were associated with worse performance on emotional identification, indicating that some underlying mechanisms may have more transversal impact. These findings contribute to the sparse body of literature on ACEs and cognition in PLS in LMIC.

Tau protein: the key to fighting brain metastases

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Intracranial tumors are difficult to treat and have a poor prognosis, affecting the brain and surrounding areas. Despite their low prevalence, they represent a major health challenge due to their poor response to treatment. Intracranial tumors can be divided into two main groups, primary and secondary. Among primary tumors, gliomas are the most common and are characterized by an immunological environment devoid of lymphocyte infiltrates, also known as a desert, which facilitates their escape from immunotherapies. On the other hand, secondary tumors, commonly known as brain metastases (BM), are the most common intracranial tumors, accounting for about 20% of adult cancers that metastasize to the brain. In contrast to gliomas, they have a primary origin outside the central nervous system, subsequently, colonize it, and can present with an immune-excluded phenotype or an immune-inflammatory phenotype. The immune-excluded phenotype is characterized by the localization of immune cells in the stromal region surrounding the tumor. The immune-inflammatory phenotype is defined by the ability of immune cells to infiltrate the tumor tissue. The present work aims to establish a link between gliomas and BrM to find effective treatments for these pathologies. Results: Using RNAseq transcriptional studies of a cohort of intracranial tumors (n= 12 gliomas; n= 14 BrM), we differentiated the transcriptional profile that defines each tumor type. Surprisingly, we identified a BrM phenotype intermediate between gliomas and BrMs, which we termed BrM-like gliomas. BrM-like gliomas were characterized by an immune response profile common to both tumors, a response linked to resident immune cells (microglia) similar to gliomas, and an interferon (IFN)-linked immune response common to BrMs. However, transcriptionally, the only thing that differed between BrM and BrM-like gliomas was the differential expression of neurodevelopmental genes by BrM-like gliomas as in gliomas. Following an analysis of neurodevelopmental genes, we singled out the MAPT gene as a marker to group our BrM cohort of human patients because of its direct involvement with patient survival. Using immunohistochemistry, we were able to differentiate tumors with high amounts of tau protein (encoded by the MAPT gene) from tumors with low amounts of tumor tau. Thanks to this clustering, we were able to observe a direct correlation between the amount of tumor tau and patient survival in BrM. We then characterized the immune microenvironment produced in the brain when invaded by these two tumor phenotypes. What we observed was a high myeloid influence, especially microglial involvement in tumors with high tau by cd68, iba1, and p2ry12 markers. In addition, tumors showed more normalized vasculature with fewer dilated vessels compared to low tau tumors. Having established the influence of neurodevelopmental genes, we analyzed the relationship on the IFN-mediated immune response. In this sense, STING is the mediator of the IFN response involved in neuroinflammation, immune recruitment, and vascular normalization. Gene and protein expression levels were measured, showing a direct relationship between tau and sting in the tumor only at the protein level. Knowing these results and the possible lymphocyte distribution phenotypes, we examined the infiltration of both CD8 lymphocytes and IBA1 macrophages and observed a direct relationship between STING levels and immune infiltration, as expected from previous studies (Yang et al., 2019). The next steps were to determine the cell type and nature of both myeloid and lymphoid immune activation. For the myeloid study, morphometric analysis of infiltrating cells within the tumor was performed to quantify their branching complexity, as well as molecular analysis of pro- (mhc-II) and anti-inflammatory markers (cd163) and microglial cell type (p2ry12) or bone marrow-derived macrophages (BMDMs) (cd49d). In these analyses, a greater proinflammatory response mediated by resident myeloid cells and BMDMs was determined in tumors with high tumor tau. In terms of lymphocyte status, tumors with high tau revealed differential regulation of the immune response between the two groups. Finally, we used C57BL/6 mouse models intracranially injected with metastatic breast cells (E0771-BrM) overexpressing tau protein, showing the same phenotype as observed in patients with high tumor tau. Conclusions: The effect of tumor tau on survival could be produced by the change in the immune microenvironment influencing sting stabilization at both tumor and vessel levels, precipitating increased maintenance of the key IFN-mediated immune response in the anti-tumor response.

Exploring quiescence sub states of neural stem cells during the development of the hippocampal neurogenic niche

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During embryonic and postnatal development of the dentate gyrus (DG), neural stem cells (NSCs) proliferate, migrate and generate mature granule neurons. Unlike in other brain regions, NSCs remain in the adult DG, mostly in a state of deep quiescence, with the ability to re-enter cell cycle and generate new neurons. The transitions back and forward from a proliferative state to a temporal shallow quiescence or primed state ensure the maintenance of stem cell population throughout life. NSCs enter quiescence during early postnatal development, before the adult hippocampal neurogenic niche is fully established, thus marking the transition from embryonic to adult NSCs (Morales and Mira, 2019). However, the mechanisms controlling NSC first quiescence entry and the correct level of quiescence are largely unknown.

Recently, we have determined that transcription factor Sox5 is required for the transition from quiescence to activation in adult NSCs and for the generation of new neurons (Li et al., 2022). Now, using conditional developmental mutant mice for Sox5 (Sox5Nestin), we describe an impairment in the acquisition of quiescence during DG development. As a result, adult Sox5Nestin mice present an over activation of NSCs and an excess in neurogenesis that ultimately leads to a premature reduction of the NSC pool and a decrease in the generation of new neurons. Further analysis of quiescence sub states revealed an excess in primed quiescent NSCs in P14 mice that could underlie the alterations observed in the neurogenic niche of adult mice, defining consequently a critical window when NSCs may build up a shallow or primed quiescent state. In addition, we show that Sox5 prevents an excess of BMP/Smad1/5/9/Id4 activation, which we find associated to primed quiescent NSCs. Given that BMP signalling pathway is a well- known inductor quiescence in adult NSCs, we are currently exploring in further detail BMP and TGF-β involvement in the acquisition of quiescence sub-states during DG development, both in vivo and in vitro.

Directed evolution of a fungal peroxygenase from Daldinia sp. EC12 for functional expression in yeast and synthesis of indigoids

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Indigoids are a class of bis-indoles with an important commercial presence since the textile and denim industries rely heavily on indigo as a vat dye, and they have projection as pharmaceuticals and semiconductors [1]. Indigoids can be obtained from natural sources (plants, mollusks), but they are mainly produced by chemical synthesis generating harsh pollutants in the process [2]. A great amount of research has been carried out into developing more sustainable processes with genetically modified organisms and enzymes [3]. However, the turnover numbers of these processes do not reach still industrial standards [1,3].

The easiest way to obtain indigoids by enzymatic methods is hydroxylating indole to form 2-oxindole (indoxyl), which spontaneously oxidizes and dimerizes into indigo [3]. In this regard, fungal unspecific peroxygenases (UPOs) are oxygen-transfer biocatalysts with outstanding potential for industrial application: They are stable, extracellular enzymes that perform selective and efficient oxyfunctionalization reactions by using hydrogen peroxide as the only oxygen donor and electron acceptor, overcoming the use of complex cofactors or living cells as classical cytochrome P450 monooxygenases do need [4].

In this work, we have engineered a novel UPO (Daldinia sp. EC12 UPO, DspUPO) to increase its expression titers in yeast and improve the enzymatic synthesis of indigo and 6,6'- dibromoindigo (Tyrian purple) from different indoles (Fig 1). After two rounds of random mutagenesis and screening to increase its recombinant expression levels in Saccharomyces cerevisiae, the amino acids lining the heme-access channel were studied. Two independent libraries of saturation mutagenesis over five different residues were screened against the production of indigo and Tyrian purple. The best mutants were purified and biochemically characterized in order to study their potential for indigoid synthesis.

A de novo FMR1 frameshift variant in a female patient affected by Fragile X syndrome

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Fragile X syndrome (FXS, MIM #300624) is an X-linked disorder caused by the absence or deficiency of fragile X mental retardation 1 protein (FMRP), encoded by FMR1 gene. Most FXS (98%) cases result from expanded trinucleotide repeat (CGG) in the 5'UTR of FMR1. If the number of repeats is greater than 200, the disease occurs [1]. The remaining cases are due to pathogenic pointed mutations in FMR1 [2]. Many male patients have been reported in scientific literature with this type of mutations [3], however, to our knowledge, only one female patient with a pointed mutation has been published [4]. Herein, we describe a clinical and molecular report of the second FXS female patient due to a pointed genetic variant in FMR1.

Whole exome sequencing analysis revealed a de novo frameshift variant in FMR1. This was followed by an X-chromosome inactivation study, which resulted in moderate XCI skewing (84:16). Therefore, we are carrying out studies to elucidate whether the variant is located in the maternal allele or in the paternal allele and which allele is the inactivated one. In order to achieve these goals, we are performing additional techniques such as, long reads sequencing or expressions studies.

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Mechanosensitive and metabolic pathways regulate plasma membrane ATP synthase trafficking in mouse embryonic fibroblasts

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*This work was directed by AE and MAP

Multiple studies have provided evidence suggesting that mechanotransduction and metabolic pathways significantly crosstalk. Caveolin-1 (Cav1), the main caveolae component, is tightly associated with those pathways, but the molecular mechanisms by which Cav1 connect mechanical and metabolic signals are poorly understood. Here, we provide evidence showing that the functional relationship between Cav1 and ATP synthase trafficking is part of a stress response connecting mechanical and metabolic pathways. Using proximity labeling (BioID), we have identified the interaction between all caveolar components and the mitochondrial ATP synthase. Cav1 is involved in lipid and carbohydrate metabolism, including defects in ATP generation from fatty acids in mitochondria in Cav1KO mice, however, the significance and the molecular mechanisms of the caveolar components-ATP synthase association are not understood. ATP synthase has also been described at the plasma membrane (PM), a pool called ecto-ATP synthase, and the pathways involved in its translocation from mitochondria to the PM remain elusive. Here we show that depleting Cav1 levels and nutrient deprivation increase ecto-ATP synthase levels. Cell tension conditions, that lead to cell stress, also increase ecto-ATP synthase levels, while impairment of mitochondrial dynamics promotes a higher increase of ecto-ATP synthase levels upon amino acid starvation. In addition, we show that ecto-ATP synthase levels are dependent on Atg5 but do not increase with rapamycin induced autophagy. To further identify involved pathways, we have generated the proximity interactome of the α and β subunits of the ATP synthase in MEFs using BioID in steady state and upon amino acid starvation, identifying proteins involved in contacts with other organelles, lipid trafficking and autophagy. Altogether, our results suggest that the trafficking of ecto-ATP synthase could be part of a stress response initiated by mechanical and metabolic challenges.

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Defining CAF-induced tumor cell plasticity as a positive switch for tumor progression and therapy resistance.

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Oral squamous cell carcinomas (OSCCs) remain one of the most common head and neck cancers, classified into distinct malignancies depending on HPV infection status. HPV-unrelated OSCCs have a low survival rate and a high risk of recurrence and metastasis. Higher infiltration of stromal microenvironment cell populations such as cancer-associated fibroblasts (CAFs) have been linked to higher metastatic potential by inducing cell plasticity programs, such as epithelial-to-mesenchymal transition (EMT). However, their role in promoting, maintaining or inducing partial EMT (pEMT) -highly plastic and metastatic intermediate states between epithelial and mesenchymal phenotypes- in the context of OSCC remains understudied. One of the more documented mechanisms of CAF-induced classic EMT is mainly dependent on TGFβ signalling, which is known to be highly expressed by both populations, creating a bidirectional feed-forward loop maintaing pathway activation. To study if CAF-induced metastatic potential in OSCC is due to pEMT rather than classic EMT, and w relies on paracrine secretion or cell-cell interaction at the invasive front, biologically relevant 2D and 3D OSCC-CAF coculture models are employed. These will allow accurate in vitro representation of CAF-OSCC crosstalk and further marker expression analysis to evaluate CAF contribution to the pEMT state. We expect to describe CAF-induced gene expression dependency to TGFβ signaling as well as discerning differential signalling pathways exclusive to CAF exposure, as well as further characterization of CAF phenotypic and functional heterogeneity.

Longitudinal study of the adult hippocampal neurogenic niche in a mouse model of Alzheimer's disease

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In the hippocampal dentate gyrus (DG), new neurons are generated throughout life in a process known as adult hippocampal neurogenesis (AHN) which takes place in an environment called the neurogenic niche, composed of an extensive vascular network and glial cells, primarily astrocytes and microglia. The objective of this project is to unravel the cellular mechanisms responsible for the failure of the AHN process in Alzheimer's disease (AD) (1) by in-depth studying the components of the neurogenic niche in the DG (2).

We have used a mouse model of AD (mice that overexpress glycogen synthase kinase 3 beta) (3), and conducted a longitudinal study at 4, 12, 18 and 30 months of age. Our results indicate alterations in the numbers of $Iba1^+$ microglias and $GFAP^+$ astrocytes in the AD model which include an increase in the density of both cell types in the granule cell layer (GCL) at 4 months of age, as well as a decrease in the density of both cell types in the subgranular zone (SGZ) at 12 months of age. We also performed 3D reconstructions of astrocytes and microglia located at the SGZ and obtained from mice of 12 months of age. Sholl´s analysis revealed morphological alterations in both cell types in the AD model.

To measure the vascularization of the DG sub-regions, we analysed the area occupied by capillaries (stained with UEA-1) and found an increase in vascularization in the AD model, both in the SGZ at 12 and 18 months of age, and in the GCL at 12 months of age. These vessels were also thicker in the GCL at 12 and 30 months of age in the AD model compared to control mice. These results indicate the existence of disrupted DG neurogenic niche homeostasis in this mouse model of AD that could underlie the observed AHN alterations.

New cell models to assess connections between HSV-1 infection and Alzheimer's disease

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Cell models are research tools to approach specific scientific questions that contribute to the current understanding of human diseases. The recent development of two- and three- dimensional platforms that better resemble the environment of the central nervous system have shed some light on the complexity of neurodegenerative diseases, such as Alzheimer's disease (AD). On the other hand, increasing evidence of the implication of herpes simplex virus type I (HSV-1) in AD progression supports the use of infection models to study the neuropathological mechanisms involved in the disease. In this line, we propose the development of new models of infection and neurodegeneration using human neuronal stem cell lines, such as ReNcell VM and LUHMES, which are able to differentiate into neuron and glial-like cells in vitro. So far, we have characterized HSV-1-induced infection and neurodegeneration in several 2D models, in which we have been able to observe the main alterations associated with AD: intracellular accumulation of amyloid beta and hyperphosphorylated tau protein, the inhibition of beta-amyloid secretion and alterations in autophagy-lysosome system. Furthermore, we have initiated the development of 3D models in which we also aim to study infection and neurodegeneration induced by HSV-1. Overall, our goal is to establish more complex models of HSV-1 infection which overcome the limitations of current study platforms and could lead to a better understanding of the disease and the role of one of its risk factors. In the future, this kind of systems could contribute to the assessment of mechanisms that are thought to mediate connections between viral infection and neurodegeneration (e.g., cholesterol metabolism), or drive the discovery of new therapeutic targets and biomarkers for such a high-impact disease.

Defining the role of the NRF2 transcription factor in synaptic maintenance in Alzheimer's disease

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Alzheimer's disease is characterized by the progressive decrease in the number of synapses within the neuronal circuits. In this study, we will focus on the fact that oxidative alteration of lipids (LPO), crucial components of the synaptic vesicle cycle, neurotransmitter release, and signaling pathways, might deeply impact in synapsis homeostasis. Transcription factor NRF2 regulates the expression of over 250 genes, including those related to protection against oxidative stress.

Employing microarray technology, we have analyzed 25,000 transcripts in brain samples from a proprietary AD mouse model based in transgene expression of hAPP(V717I) and TAU(P301L) in a wildtype background or null for NRF2 expression. Comparison of the results from both genotypes identified 16 clusters (formed by 122 genes) whose expression was altered by the absence of NRF2 and are related indirectly or directly to synapsis homeostasis. Since we have previously described that absence of NRF2 exacerbates LPO production in connection with a reduction to synaptic transmission in the neurons of the dentate gyrus in these mice (Rojo et al. 2017), we next evaluated the levels of 544 distinct lipid species from 26 lipid subclasses in NRF2-null or wildtype samples by untargeted lipidomics. We found that NRF2 deficiency leads to the dysregulation of several lipid species from phospholipid, neutral lipids and sphingolipids subclasses. Interestingly, the levels of 3 ether-linked phospholipids (plasmalogen, associated with AD human pathology) were increased in the hippocampus of NRF2 null mice compared with the wildtype littermates. Moreover, when administered to primary cultures, the number of synaptic contacts, as measured by the colocalization of vGLUT1 and PSD95, were altered. However, when NRF2 was upregulated by the novel 6-MSITC, synapses were protected. Therefore, we examined whether the absence of NRF2 induced the same changes in the composition of excitatory synapses. Our findings revealed that there were changes in the molecular composition of the synapse, caused by alterations in the quantity and size of synaptic contacts. Moreover, the lack of NRF2 induced impairments in calcium homeostasis, crucial to synaptic function. In conclusion, NRF2 emerged as a crucial modulator of synaptic homeostasis, providing a new avenue for exploring its potential as a therapeutictarget for neurodegenerative diseases characterized by progressive synaptic loss, such as AD.

Optimizing Bio-Based Plastic Degradation: Engineering Thermostable Chimeras by SCHEMA-RASPP homologous Recombination

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Cutinases (EC 3.1.1.74) belong to the α/β hydrolase superfamily and function as serine esterases, catalyzing the hydrolysis of ester bonds. These enzymes are highly sought after for their potential applications in various industrial processes, including cotton scouring and plastic degradation [1,2]. Recent advancements in cutinase research have led to the discovery of numerous enzymes from bacterial and fungal sources, each with unique features such as high thermostability and the ability to degrade different types of plastic derivatives [3]. Given this diversity, combining the properties of these enzymes could lead to more efficient plastic degradation. Enzyme chimeragenesis offers a powerful approach to engineer robust enzymes with improved thermostability and broader substrate specificity. The SCHEMA-RASPP algorithm, developed by the Frances Arnold group [4], has emerged as a successful strategy for generating chimeric proteins across various enzyme systems. [5]

In this study, we explored the potential of chimeras using three cutinase enzymes as parental types, sharing as low as 55% sequence identity and therefore exhibiting diverse biochemical characteristics. Our SCHEMA-RASPP library comprised 7 SCHEMA blocks, yielding 2187 possible combinations. After screening this library, we selected 14 chimeric designs demonstrating significant enhancements in thermostability and substrate promiscuity. These findings represent a promising avenue for future protein engineering endeavours in the field of plastic degradation and beyond.

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Systematic WES reanalysis and rapid functional studies in a skeletal dysplasia cohort yield new diagnoses.

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Introduction. Exome analysis (WES) is a pivotal tool for genetic diagnosis of skeletal dysplasias (SD). Despite its utility, the continual discovery of novel causal genes highlights the necessity for periodic re-evaluation of unresolved cases.

Objective. To combine WES reanalysis and rapid functional studies to enhance the diagnostic yield in a cohort of 53 SDs without diagnoses.

Methods. Exome analysis (singletons/duos/trios) was performed using VarSeq (Golden Helix). Further genomic studies were also performed when necessary (SNP-arrays, breakpoint PCRs). Functional and/or transcriptomic studies were conducted using patient serum, lymphocyte RNA or in vitro minigene assays.

Results. A probable molecular etiology was elucidated in 16/53 cases (31%). Of these, 4 had variants in genes identified posterior to the initial analysis (MBTPS1, COLEC10, RPL13, EMILIN1) and 12 had newly identified or characterized variants in SD-associated genes. These included: 1) The identification of an intronic MYH3 variant, NM_002470.4:c.348+39A>G in a girl with multiple pterigium and vertebral synostosis in whom only one MYH3 variant had been previously identified. Although in silico predictions were negative, the variant resulted in aberrant splicing, thus confirming Spondyocarpotarsal synostosis syndrome with contractures and pterygia, MYH3-related. 2) We detected a splice site variant, NM_003791.4:c.2353+3A>G, confirmed to alter splicing, in MBTPS1, and a whole gene deletion in a boy with a spondyloepimetaphyseal dysplasia (SEMD). 3) In a child with severe pre- and postnatal bone fractures, we identified a homozygous pathogenic variant in EMILIN1 due to chromosome 2 uniparental isodisomy. 4) We detected and delimited a hemizygous intragenic PLS3 duplication in a patient with bone fragility. 5) Loeys-Dietz syndrome was diagnosed in a patient with microcephaly and scoliosis and his similarly affected mother, who both had a missense variant in the last base of TGFB3 exon 5 which altered splicing.

Conclusions. We advocate for routine WES reanalysis every 1-2 years to maximize diagnostic yield in unresolved cases. Secondly, rapid functional assays need to be incorporated into routine genetic diagnosis, so that variants of unknown significance can be reclassified as pathogenic and the patient's diagnosis can be confirmed.

Unraveling the role of enzyme promiscuity in driving new metabolic traits in Pseudomonas putida

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An important application of microbial biotechnology is to develop microorganisms with new metabolic capabilities that help us to confront the current challenges of our era. An alternative to increasing the metabolic space of a given organism by heterologous systems is to exploit the so-called underground metabolism [1]. The underground metabolism is the collection of enzyme promiscuous activities in a cell, which serves as starting point for the development of new metabolic traits [2]. In order to generate a computational tool for predicting the environmental conditions to expand the metabolic space of the microbial chassis Pseudomonas putida KT2440, we have included the underground metabolism in the genome-scale metabolic model iJN1462 of P. putida KT2440 based on the available biochemical information. Following the model predictions, we have been able to expand the metabolic versatility of P. putida KT2440 towards the use of N-acetyl-L-alanine as a carbon source through Adaptive Laboratory Evolution (ALE). The mutations found in the evolved strains coincided with the model predictions. Characterization of such mutations revealed that the evolved phenotypes have arisen from the increase in both enzymatic activities and genetic dosage of those enzymes. Overall, we show here how to increase the metabolic versatility in P. putida KT2440 by exploiting the underground metabolism making use of genome-scale metabolic models

Study of PBMC-mediated apoptotis in ASC product: ASC susceptibility & therapeutical implication

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ASC (Adipose-derived mesenchymal Stem Cells) interaction with host cells is one of the key elements of understanding ASC mechanism of action. Its role in immunomodulation and interaction with immune system cells are still a relevant research focus in this field. These characteristics make ASC an attractive treatment for pathologies with chronic or exacerbate inflammation as Crohn's Disease and acute sepsis. Yet, ASC immunoprivilege has been questioned, as it is demonstrated that host immune system recognizes and even attacks MSC. In fact, it is becoming evident that some ASC-derived immunomodulatory and regenerative mechanisms act through host phagocytosis of ASC.

Our cell product is Alofisel: an ASC-based cell therapy that targets Crohn's Disease developed by Takeda. We studied Alofisel susceptibility to lymphocyte-mediated killing by mimicking an inflammatory environment. To evaluate PBMC degranulation and prostaglandin-E2 (PGE2) role in ASC:PBMC interaction, we used inhibitors for both degranulation and PGE2. We demonstrated that ASC apoptosis by PHA-activated PBMCs is contact-dependent, strongly inhibited after blocking degranulation. Additionally, apoptosis is modestly increased when blocking PGE2 secretion pathway.

We have also interrogated the role of apoptosis in the clinical setting. We have found a correlation between ASC apoptosis levels and a positive clinical response in Crohn's disease patients treated with Alofisel.

All in all, apoptosis evaluation of ASC may represent a useful tool for treatment response prediction.

Activation of innate immune system by endogenous production of double-stranded RNAs in Dido3 mutants

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Introduction

DIDO3 protein interacts with H3K4me3 through an amino-terminal PHD domain [1] and RNA polymerase II through central TF2S and SPOC domains [2]. This suggests its involvement in transcription. The carboxy-terminal COIL domain (coded by exon 16) interacts with SFPQ, participating in alternative splicing. Transcriptomic data show that DIDO3 mutants without exon 16 present an increase in readthrough and expression of innate immunity genes [3,4,5]. Our current objective is to describe the link between these two observations. We propose that readthrough results in the transcription of both DNA strands of converging genes. Transcription of the sense strand results in an unprocessed precursor, while antisense in a processed messenger. Finally, hybridization of the two strands may produce a double-stranded RNA that acts as substrate for the innate immune system.

Materials and methods

Cells. MEFs WT and exon 16 mutant were cultured in DMEM supplemented with 10% fetal bovine serum, 1% Penicillin/Streptomycin and 1% L-Glutamine. Kept in cell culture incubators at
37.C and 5% of CO2.

RT-PCR. Total RNA was prepared with a SPLIT RNA extraction kit (Lexogen) according to the manufacturer's protocol. Amplifications were performed with a Verso One-step RT-PCR Kit (ThermoFisher), using specific primers for the amplified genes.

Results

For the TMEM94 and GPS1 genes, no cDNA bands from unprocessed RNA are observed in WT cells and in the case of RAC3 this band is weaker in WT than in mutant cells.

Conclusions

RT-PCR confirms transcriptomic data, which indicate that DIDO3 mutant cells produce higher amounts of unprocessed RNA from the antisense DNA strand due to increscent events of readthrough. Thus, the presence of transcripts from both strands may result in double-stranded RNAs that mimic viral RNAs, triggering the innate immune system response.

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

Broad therapeutic benefit of myosin inhibition in hypertrophic cardiomyopathy

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ABSTRACT

Myosin inhibitor mavacamten is the only targeted treatment available for hypertrophic cardiomyopathy (HCM), a disease caused by hundreds of genetic variants that affect mainly sarcomeric myosin and its negative regulator cardiac myosin-binding protein C (cMyBP-C, encoded by MYBPC3). Here, we have examined whether the reported limited efficacy of mavacamten in a fraction of HCM patients can result from dissimilar HCM pathomechanisms triggered by different genetic variants, a scenario particularly relevant for MYBPC3-associated HCM. To this aim, we have generated knock-in mice including missense pathogenic variant cMyBP-C p.R502W, which, different from patients who carry truncations in the protein, develop progressive pathogenic myocardial remodeling in the absence of alterations of cMyBP-C levels and localization. Mechanistically, we find that mutation R502W reduces the binding affinity of cMyBP-C for myosin without inducing a shift towards more active myosin conformations as observed when cMyBP-C levels are reduced. Despite these diverging molecular alterations, we show that mavacamten blunts myocardial remodeling both in R502W and cMyBP-C-deficient, knock-out hearts. These beneficial effects are accompanied by improved tolerance to exercise only in R502W animals. Hence, our results indicate that myosin inhibition is effective to treat HCM caused by both truncating and missense variants in MYBPC3 regardless of the primary pathomechanisms they elicit.

Expanding the genetic evidence of genes with low proof of association reported to cause pulmonary arterial hypertension

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Pulmonary Arterial Hypertension (PAH) is a serious disease characterized by elevated blood pressure in the pulmonary arteries causing progressive heart failure and leading to premature death if untreated. Genetic testing is currently recommended for adults and children diagnosed with PAH. Variants in at least 27 genes have putative evidence for PAH causality. An international panel of experts in PAH applied a scoring system to classify the relative strength of evidence supporting PAH gene-disease relationships based on genetic and experimental evidence.

According to this classification, twelve genes were classified as having definitive evidence, three with moderate, six were classified as having limited evidence, five genes were disputed because of a paucity of genetic evidence over time. Therefore, there are several genes that require further study to definitively associate them with PAH.

This study aimed to detect genetic defects in a cohort of PAH patients, using a panel of 14 genes with low evidence of association with PAH. A total of 554 patients were studied, including 499 patients clinically diagnosed with PAH and 55 family members.

We observed 26 variants in 32 patients (27 probands and five relatives): 11 pathogenic/likely pathogenic variants in three different genes and 16 variants of unknown significance (VUS) in 11 genes.

We provide further genetic evidence of association of these genes and the development of PAH, highlighting ABCC8 gene where 10 different variants have been detected. However, further studies are required to confirm the functional impact of the variants and their involvement in PAH.

Co-infection with two strains of the parvovirus Minute Virus of Mice selected chimeric viruses with enhanced tropism toward human glioblastoma cells.

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Tumor cells provide conditions that facilitate the replication of some oncolytic viruses. Virotherapy in cancer relies on the natural capacity of some viruses to interfere with tumor progression by cell lysis (oncolytic viruses) or by eliciting a secondary immune response. This is a field of intensive research with more than 40 active clinical trials and two licensed recombinant viruses. Rodent Parvoviruses are being investigated as oncolytic agents in several preclinical and clinical trials. Our group is exploring the oncolytic potential of the parvovirus Minute Virus of Mice (MVM) against high-grade glioblastomas, the deadliest form of brain cancer. Here we show preliminary attempts to enhance MVM oncotropism toward glioblastoma cell lines U87 and U373. Our targeted-evolution strategy con- sisted in producing genetically diverse populations by forcing genomic recombination between the MVMp and MVMi strains. Emerging viruses were then subjected to serial blind-passages in glioblastoma cell lines. A high multiplicity of coinfection with these strains produced viruses that did not propagate in U87 cells, but rapidly adapted to U373 cells developing extensive cytopathic effects in a few passages. We monitored recombination and evolution by Sanger sequencing of molecular clones and Next Generation Sequencing of three PCR-amplified regions. Chimeric viruses rapidly emerged in culture passages with dominant genomes contained NS genes from MVMi and structural genes from MVMp. We have demonstrated that one of these chimeric viruses infects, propagates and kills better glioblastoma cells lines. Interestingly, mutants with deletions in a region with predicted strong DNA-secondary structure progressively accumulated. Transfection of U373 or highly permissive NB324K cells with an engineered infectious plasmid harbouring one of these deletions failed to express viral proteins, suggesting a defective nature. Our results reveal that emerging defective interfering genomes may hamper targeted-evolution strategies based on repeated blind- passages on culture. Importantly, recombination between natural MVM strains may give rise to viruses with enhanced oncotropism.

Pharmacogenetic analysis of 35 Wolf-Hirschhon Syndrome patients treated with antiepyleptic drugs.

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Wolf-Hirschhorn Syndrome (WHS) is a congenital malformation syndrome caused by a terminal deletion of the short arm of chromosome 4, characterized by pre- and postnatal growth, developmental disability of variable degree, characteristic craniofacial features and seizures. These seizures are highly heterogenic and variable. Nowadays, it is not known why the eficacy of the treatment of the seizures differs in patients with WHS.
The aim of this work is to evaluate whether a pharmacogenetic recommendation in patients treated with antiepleptic drugs could have avoided the administration of ineffective drugs and/or the occurrence of adverse effects.

These patients were sequenced using Whole Genome Sequencing. The bioinformatic analyses were done using BWA, Sambamba, Picard, GATK, DELLY, Control-FREEC and ANNOVAR. The pharmacogenetic analyses were done using xHLA, StellarPGx and PharmCat.

The pharmacogenetic test established that all patients had at least one recommendation in the prescription of the analysed drugs. Drugs as tacrolimus, warfarin, escitalopram, tramadol, clopidogrel had prescribing recommendations in some patients. Specifically, three patients (8%) were phenytoin poor metabolizers (CYP2C9 with decreased function), drug used to treat the seizures.

Pharmacogenetic analyses are essential for the correct pharmacologic management of many diseases. In the literature, there are no pharmacogenetic studies on WHS patients. Because of that, these analyses increase the knowledge of terapeutic guidelines of epilepsy treatment and allow physicians to avoid drug adverse events and inefficacy.

Analysis of the dendritic spines of principal neurons of the CA1, CA3 and entorhinal cortex regions in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is characterized by cognitive deficits and synaptic dysfunction. The hippocampal trisynaptic circuit, which receives afferent connections from the entorhinal cortex (EC) and includes the dentate gyrus, and the hippocampal CA3 and CA1 regions, plays a critical role in learning and memory. Within this circuit, dendritic spines—small protrusions on dendrites— are crucial for synaptic plasticity and function. These spines vary in shape and size, being mushroom spines mature and stable, thin spines immature and dynamic, and stubby spines short without distinct necks. The present study investigated alterations in dendritic spine morphology and density in the apical and basal dendrites of hippocampal principal neurons of the CA1, CA3, and EC regions. For that purpose, intracellular injections of Lucifer yellow were performed in wild-type (WT) mice and a murine model that overexpresses GSK-3β, a kinase that plays a crucial role in AD pathology. We first in-depth studied the distribution of dendritic spines in WT mice. The results show marked differences between apical and basal dendrites. In the CA1 region, basal dendrites showed a higher density of mushroom and stubby spines. In the CA3 region, the density of thin and mushroom spines, as well as the percentage of the former spines, were also reduced. In the EC, the percentage of thin spines was decreased. Conversely, both the density and percentage of mushroom spines were increased. Additionally, region-specific alterations in dendritic spines were found in the AD model. In both the apical and basal CA1 regions, these animals exhibited reduced spine density alongside a shift towards a higher proportion of thin spines and fewer mushroom spines.

Additionally, spines in apical dendrites showed smaller heads, while those in basal dendrites exhibited a tendency towards being shorter and having bigger heads. The apical dendrites of the CA3 region contained an increased percentage of stubby and mushroom spines, as well as less thin spines. Basal dendrites of the CA3 region displayed bigger spine heads. Additionally, they showed a lower percentage of thin spines alongside an increase of mushroom and stubby spine density. In the EC, the spines of both apical and basal dendrites showed smaller heads. Moreover, less stubby spines were observed in the apical dendrites, whereas more thin and less mushroom spines were seen in basal dendrites. Therefore, the observed shift from mature mushroom spines to immature thin spines in some hippocampal regions suggests disruptions in synaptic plasticity and stability, potentially leading to synaptic impairments, halted long-term potentiation, and cognitive decline. Overall, this study highlights the importance of understanding regional differences in dendritic spine alterations as it suggests a selective impact of AD pathology on distinct hippocampal areas.

Adult hippocampal neurogenesis, the post-mortem delay, and prolonged aldehyde fixation: the enemies within

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ABSTRACT

Adult hippocampal neurogenesis (AHN) gives rise to new neurons throughout life. This phenomenon takes place in more than 120 mammalian species, including humans, yet its occurrence in the latter was questioned after a putative absence of neurogenesis markers in the adult human hippocampus was suggested. In this regard, we showed that prolonged fixation impedes the visualization of Doublecortin$^+$ immature neurons in this structure, whereas a dilated post-mortem delay (PMD) has been suggested to underlie these discrepancies. Nevertheless, the individual and/or additive contribution of fixation and the PMD to the detection (or lack thereof) of other AHN markers has not been investigated to date. To address this pivotal question, we used a tightly controlled experimental design that included three groups of mice, two of which were subjected to artificially generated PMD of 6 h and 24 h, whereas a control group lacked any PMD. In those mice, the right hemisphere was fixed for 24 h whereas the left hemisphere was fixed for 2 months. This experimental design allowed dissecting the relative contribution of the aforementioned factors to the visualization of markers of individual AHN stages. Fixation emerged as the most prominent factor globally impeding the study of this process in mice. Moreover, the visualization of other especially sensitive epitopes was further prevented by prolonged PMD. These results are crucial to disambiguate current controversies related to the occurrence of AHN not only in human beings but also in other mammalian species.

Keywords: Adult hippocampal neurogenesis, post-mortem delay, fixation, doublecortin

Radiobiology in Proton Therapy: relative biological effectiveness, range verification with nanoparticles and combination with immunotherapy

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Proton therapy is a radiotherapy technique based on the use of protons for tumor management. The specific deposition of energy at depth, known as Bragg Peak, by protons confers properties that affect the tumor tissue, spreading healthy tissues from the surroundings [1-5]. Treatment is based on the use of a factor called Relative Biological Effectiveness (RBE), which is the ratio between the absorbed dose needed with a studied radiation, in this case protons, to produce the same biological effect than with a reference photon irradiation (250 kV X-rays, ^{60}Co source, or 6 MV photons) [6-8]. Currently, the value used in the clinic is 1.1 [9], but several studies have indicated the dependence of this value on several factors, such as cell type and linear energy transfer (LET) [10]. LET is one of the principal characteristics that affects cell death, changing through the Bragg Peak curve and being higher just after the peak, which produces a higher RBE in this area. Consequently, healthy tissues can be affected by this high LET and investigations are being performed to improve proton therapy in this sense [11,12]. This study addresses three different perspectives. First, the use of 250 kV and 6 MV photons as reference radiation in RBE was evaluated in five different human and mouse cell lines (A549, U251, V79, 4T1 and Renca) using clonogenic assays. The results showed how different variables (technical procedures, energies and dose rate) affected each cell line differently. Moreover, the effect of different LETs of protons along the Bragg Peak was also tested in the radioresistant Renca cell line, showing a high dependence on survival by clonogenic assay in LET for these cells, reaching RBE values of 3 after the Bragg Peak. Second, verifying the proton range in patients, overcoming the uncertainties on the Bragg Peak, is essential for ensuring the accuracy and effectiveness of proton therapy [13,14]. Different techniques have been proposed, such as online or offline verification methods that detect secondary radioactive particles using imaging techniques [13,15]. In this study, we developed a Zinc-Doped Iron Oxide Nanoparticle (IONP@Zn-cit) with a hydrodynamic size of 10 nm and stability in serum, which could be suitable for prompt-gamma detection. We tested their cytotoxicity, defined as half of the surveillance, which was 100 µg Zn/mL in U251 cells. The effect on clonogenic cell death was tested after X-ray irradiation, which suggested a radioprotective effect of these nanoparticles at low concentrations (1–10 µg Zn/mL). To evaluate the production of positron emitters and prompt-gamma signals, IONP@Zn-cit were irradiated with protons, obtaining prompt-gamma signals at the lowest measured concentration (10 mg Zn/mL). Finally, the biodistribution of the radiolabeled nanoparticles with ^{67}Ga (^{67}Ga-IONP@Zn-cit)was evaluated, showing accumulation in the liver and spleen, and an accumulation in the tumor tissue of 0.95% ID/g in a glioblastoma mouse model of U251 cells. These results suggest the possibility of using Zn nanoparticles as proton-activatable agents to verify the range by prompt gamma detection and to face the challenges of prompt gamma detection in a specific biological situation, opening different avenues to go forward in this field. In the end, proton therapy can be improved by irradiating with lower doses, reducing toxicity. This can be achieved by combining radiotherapy with immunotherapy [16-18]. The use of antibodies with immunostimulatory effects can be combined with the effects of radiotherapy to achieve a better tumor control. Therefore, we studied how the use of an immune checkpoint inhibitor (ICP), anti-PD-L1, could impact both tumor growth and the immune system response in a renal adenocarcinoma (Renca) mouse model. Mice were treated with either anti-PD-L1 or control IgG isotype and with or without radiotherapy (X-rays or protons). Tumor growth was evaluated by computed tomography (CT), and blood and tumor tissues were collected after 10 days to study the presence of different immune cell populations by hematological analysis and flow cytometry. Tumor infiltration of immune cells increased when αPD-L1 was used in combination with both proton and X-ray irradiation. Radiotherapy alone produced an immunosuppressive phenotype, whereas anti- PD-L1 blocked this signal. The lowest NLR (neutrophils-to-lymphocyte ratio) value was observed in the combination of 7 Gy X-ray irradiation with anti-PD-L1, which coincided with the best tumor control. In conclusion, more studies are needed to better understand how proton therapy affects the tumor microenvironment and how these techniques, including the use of a variable RBE and nanoparticles for range verification, could be exploited to achieve the best outcomes for patients.

Comparative evaluation of optical genome mapping and next-generation sequencing technologies to identify structural variants and copy number variants in pediatric acute lymphoblastic leukemia

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Background:Structural variants (SVs) and copy number variants (CNVs) are prevalent genomic alterations intricately linked to the oncogenic processes observed in pediatric patients diagnosed with acute lymphoblastic leukemia (ALL). The elucidation of these variants offers valuable insights into the underlying pathways of cancer progression and development. Conventional next-generation sequencing (NGS) techniques, such as whole exome sequencing (WES), have been widely adopted for genomic profiling, albeit facing limitations in accurately detecting variants across the entire spectrum of sizes. Novel techniques such as optical genome mapping (OGM) present a promising avenue for addressing these limitations, offering superior resolution compared to conventional methods like fluorescence in situ hybridization (FISH), karyotyping, and chromosomal microarray (CMA). This shift towards OGM heralds a new era in genomic analysis, poised to provide unprecedented clarity in delineating complex structural and CNVs associated with cancer.

Aims:The aim of this study is to compare the performance, accuracy, and reliability of various standard methods in WES for classifying SVs and CNVs, categorized by variant type, in contrast to the results yielded by the OGM protocol.

Methods: Thirteen somatic samples from diagnostic bone marrow aspirates (>80% blasts) from pediatric patients with ALL were studied. OGM data was analyzed utilizing the Rare Variant pipeline provided by Bionano. WES data was analyzed for CNVs and SVs using four distinct software applications in each case. The comparisons between OGM data and WES-generated data was conducted using the caret package in R, both at the patient and cohort levels.

Results: The analysis revealed variations in performance among the callers and types of variations investigated. When OGM did not detect variants, the performance of WES callers showed satisfactory results, potentially reaching 100% accuracy and specificity depending on the software utilized and the type of variation. In these scenarios, WES software effectively managed variant mapping with sufficient precision. However, when OGM detected variants, the accuracy of the callers varied based on the type of variation identified, the specific caller employed, and the sample. The cohort's percentage of accuracy ranged between 0-84.67% for SVs and 0-30.77% for CNVs. Notably, the results from these callers lacked the precision necessary to confidently confirm the presence of the variation. The correlation plots of the cohort revealed a stronger tendency for clustering between WES callers and Bionano independently in the case of SVs, although this correlation was contingent upon the variant type. Conversely, this clustering trend was not observed in the case of CNVs, where certain WES callers clustered with Bionano. In contrast, in the case of CNVs, the correlation between WES callers and Bionano was mixed, with no clear trend observed in separate groups.

Summary/Conclusion: The analysis reveals variations in performance among different callers and types of variations, with superior results observed when OGM does not detect variants. However, when OGM detects variants, the accuracy of callers varies, and the precision required for confident confirmation of the variation is often lacking. The utility of WES techniques for CNVs or SVs analysis shows poor results in comparison to OGM, marking a potential shift in genomic analysis towards OGM for improved resolution and clarity in delineating complex structural and copy number variations associated with cancer.

LGI3 and LGI4 electrophysiological effects on k_V4.3 and k_V4.2 potassium channels

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K_V4.2 and K_V4.3 voltage-dependent potassium channels generate the transient potassium outward current (I_{to}), which determines phase 1 repolarization in cardiac action potentials. The characteristics of this current are only observed when these channels interact with auxiliary subunits that develop a modulating function on their activity, forming signaling complexes or channelosomes. Abnormal changes in the expression levels of the channelosome components and/or in their biophysical properties are related to the development of cardiac pathologies such as atrial fibrillation (AF), the most common type of arrhythmia. Lgi1 and Lgi3, members of the leucine-rich glioma inactivated protein family, have proven to modulate the activity of K_V1 and K_V4 channels in neurons, modifying their trafficking, stability, and/or biophysical properties. We have demonstrated that Lgi3 and Lgi4 are the only members of this protein family present in the human myocardium. Consequently, we are interested in unraveling the electrophysiological effects that Lgi3 and Lgi4 induce on the activity of K_V4.2 and K_V4.3 channels. For this purpose, potassium currents were recorded in CHO cells transiently transfected with cDNA encoding the channel and the auxiliary protein of interest, using the whole-cell configuration of the patch-clamp technique. Our results show that Lgi3 and Lgi4 induce important changes in the electrophysiological properties of Kv4.3, while their effects on K_V4.2 channels are not biologically significant. Specifically, the magnitude of the K_V4.3 current in the presence of Lgi3 and Lgi4 is, respectively, 3- and 2-fold greater than that observed in the absence of these proteins. Also, Lgi3 slows down the activation and the inactivation kinetics of K_V4.3; whereas Lgi4 accelerates the recovery kinetics of this channel. Thus, the increase in the magnitude of the K_V4.3 current produced by Lgi3-4 could be explained by: i) the delay in the channel inactivation for Lgi3 and ii) the acceleration of the recovery kinetics for Lgi4. From this study, we establish Lgi3 and Lgi4 as relevant modulators of K_V4.3 channels to be considered in the study of cardiac electrophysiology.

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Study of Epigenetic Tumor Plasticity through the carcinogenesis and progression of Non Small Cell Lung Cancer (NSCLC) in liquid biopsy. PI21/00145 FORT23/00006

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Introduction: Non-small cell lung cancer (NSCLC) is one leading cause of death worldwide, with a 5-year survival rate of only 24%. Late diagnosis and the innate or acquired anti- cancer drug resistance are the main causes of this high mortality among men and women. Despite advances in lung cancer therapy, only 15% of patients can benefit from the use of targeted therapies (EGFR/ALK mutations <15% and PDL-1 overexpression~20%), with a low clinical response rate and a high associated cost, hindering its clinical adoption. This means that the molecular alterations responsible for 85% of NSCLC remain unidentified. However, the challenge of discerning tumor-derived molecules amidst a milieu of non-tumor elements in the bloodstream, compounded by the absence of robust normalization methods, has hindered marker identification and yielded disparate results. Our study leverages epigenetics and liquid biopsy as promising avenues for identifying potential biomarkers, enabling longitudinal examination of molecular changes throughout carcinogenesis, tumor progression, and treatment responses within individual patients.

Methods: We performed an innovative approach encompassing comprehensive analysis of the transcritome (mRNA and miRNAs) and DNA methylation profiles (via EPIC methylarrays) across adjacent-normal and tumor tissue explants together with their extracellular vesicle contents secreted to the culture medium, totalling 60 samples, to isolate tumor-specific characteristics. Subsequently, we tracked the identified profiles in blood samples collected longitudinally from the same patients throughout their disease trajectory, spanning various treatments until relapse or decease. Additionally, validation will extend to a separate cohort of over 400 NSCLC patients and healthy controls.

Results: We are validating the miRNome identified in liquid biopsy, consisting of 28 miRNAs across 480 patient plasma samples using Taqman OpenArray technology. Simultaneously, bioinformatics analysis on EPIC arrays will decipher DNA methylation profiles in both tissue and liquid biopsy samples. Additionally, we have integrated omics data obtained from the smallRNAseq and RNAseq analyses conducted on paired tumor-adjacent lung tissue samples. This comprehensive approach has enabled us to uncover 158 miRNA-mRNA pairs involving 77 miRNAs and 99 distinct genes, followed by subsequent functional enrichment analyses.

Conclusion: In conclusion, this endeavour represents a pioneering cross-sectional studies aimed at establishing a longitudinal epigenetic signature for NSCLC carcinogenesis and progression, offering crucial insights for personalized therapeutic interventions.

Ca10 proteoglycan antigen as a new diagnostic and prognostic marker in colon cancer

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The word “cancer” condenses a huge variety of pathologies, nonetheless, sharing all of them have some featured characteristics. Among those, the capacity of the tumor cells to avoid cell death, even when they have accumulated a variety of mutations, is one of the most amazing characteristics. Interestingly, the accumulation of different mutations in the cell genome usually implies that the cell will synthetize and/or fold in an inappropriate manner many different cellular components such as proteins or glycans. Those atypical and aberrant proteins or glycans are generally named as tumor (neo)antigens. The role of tumor antigens has been widely studied trying to find an application in diagnosis or prognosis in many different oncological conditions.

Here we describe a novel proteoglycan (Ca10), which is identified as a tumor antigen produced by Ehrlich tumor cells, showing its potential use as a biomarker in colon cancer.

We have recruited two different cohorts (colon cancer patients n=133 and healthy donors n=37). In those cohorts we have measured the plasmatic levels of Ca10 by ELISA as well as different immunological parameters, all of that, combined with clinical data in the case of patients. Our study showed higher levels of plasmatic Ca10 in colon cancer patients compared to healthy donors. Moreover, considering the clinical data obtained from these patients, there is a negative correlation between Ca10 levels and the degree of the pathology. In line with that, our data showed that low levels of Ca10 in plasma associate with shorter survival periods, allowing to propose this marker as a potential prognostic marker for colon cancer patients.

We aimed to find some extra evidence to explain the role of Ca10 in colon cancer pathophysiology. Considering a recent publication in a mouse model linking the production of Ca10 by Ehrlich tumor with the generation of T regulatory cells (Tregs), we performed a spectral flow cytometry profiling of peripheral blood mononuclear cells from patients. We showed that the frequency of Tregs in blood positively correlate with circulating levels of Ca10, as well as other Tregs markers such as soluble CD25.

Considering our data, we can propose that this novel proteoglycan, Ca10, can constitute an interesting tool as a prognostic biomarker in colon cancer.

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Targeted evolution of parvovirus Minute Virus of Mice towards human glioblastoma cells selects mutants at the receptor binding site with improved oncotropism.

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Glioblastoma is the deadliest form of brain cancer (less than 5% of survival rate at five years). Due to its high resistance to current clinical treatments, several potential alternative therapies are being assessed. Virotherapy based on oncolytic viruses are one of the most promising alternative strategies, a field of intensive research with many active clinical trials and two licensed recombinant viruses. Rodent Parvoviruses show natural preference for proliferative cells, elicit a strong secondary immune response and are safe for humans in clinical trials. The protoparvovirus Minute Virus of Mice (MVM) infects target cells via recognition of sialic acid (SIA) residues exposed on the cell surface. This interaction takes place in a small depression at the 2-fold axis of symmetry of the capsid called “dimple”. We have previously demonstrated that amino acids surrounding this region are key determinants of tropism and pathogenicity. Tumour cells show important glycan surface alterations including a glycocalix richer in SIA residues including some tumour-markers. Here we have followed a directed evolution strategy to enhance MVM oncotropism by retargeting its capsid to SIA glycans specifically expressed on glioblastoma cells. Three libraries of 13,600; 10,000 and 8,700 MVM mutants at the SIA binding domain of the capsid (1-2 coding changes per genome) were obtained by saturation mutagenesis (MVMp) and error-prone PCR (MVMp and MVMi), respectively. Targeted evolution was addressed by serial blind-passages in the U87 and U373 human glioblastoma cell lines. Neither of these libraries of mutants propagated in U87 cells. In contrast, they adapted to U373 cells, developing extensive cytopathic effects in a few passages. Evolution was monitored by Sanger and Next-generation sequencing showing a rapid restriction of genetic diversity metrics that parallels selection of single or double coding mutants at VP2 residues. These amino acid changes were exposed at the capsid surface and localized nearby the SIA-receptor binding site. We have used a plethora of tumour and non-tumour cell lines to demonstrate that recombinant viruses engineered with some of these mutations showed improved oncotropism toward human glioblastoma cells.

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Functional interplay between PrimPol and WRN helicase upon replication stress

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The process of DNA replication can be impaired by different reasons, creating situations of replicative stress (RS) that compromise genomic integrity and cell survival. Specific mechanisms that counteract RS include the stabilization of stalled forks by temporary fork reversal, the activation of dormant origins and the repriming of DNA synthesis by PrimPol primase[1]. Our laboratory has described the role of PrimPol-mediated repriming at several types of DNA lesions, e.g., UV-C-induced adducts or intra- and inter-strand crosslinks as well as during the cellular recovery from hydroxyurea-induced nucleotide depletion[2–3].

To gain mechanistic insights into the regulation of the repriming mechanism, we have focused our interest on WRN, a member of the RecQ family of DNA helicases with a 3'-5' exonuclease activity. WRN is implicated in several aspects of DNA metabolism, including unwinding of DNA secondary structures, remodelling of reversed forks, protection of collapsed forks, base excision repair and double-stranded breaks repair[4–5].

We have checked the potential functional interaction between PrimPol and WRN during DNA replication using DNA fibre assays. In a normal S phase or in the presence of hydroxyurea or G4 stabilisers, the individual downregulation of PrimPol and WRN reduced fork progression rate to a similar extent. However, the co-depletion of both proteins did not enhance the phenotype, suggesting an epistatic relation between them. The same behaviour was observed in the fork restart capacity upon complete fork stalling induced by higher concentrations of hydroxyurea. Our working hypothesis is that PrimPol and WRN operate in the same pathway to restart DNA synthesis, with the remodelling of reversed forks by WRN taking place before PrimPol-mediated repriming, and experiments supporting this will be shown.

Furthermore, we are currently investigating the importance of this interaction in the context of cells with high microsatellite instability (MSI), to study the implication of PrimPol in the known synthetic lethality between MSI and the absence of WRN, which might be a potential cancer therapy.

The role of physical activity in healthy aging

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The regular practice of physical activity constitutes a protecting factor against the onset of multiple diseases, including diabetes or cardiovascular problems. Also, exercise modulates cognitive abilities such as learning or stress resistance. However, its mechanisms of action remain poorly understood, even though microRNAs seem to play a role in most species. Drosophila melanogaster, the fruit fly, presents some advantages to study aging: it shows some aging hallmarks at 25 days old and the different behavioral protocols and developed techniques allow to address cognitive functions easily. This project is aimed to analyze in depth the effect of exercise on brain health during aging and involved mechanisms using Drosophila as a model. After four weeks of training, aged male flies showed a total nocturnal sleep more similar to the one of young flies than their untrained, age-matched controls. We also tested their social learning abilities in aged flies by keeping them grouped or isolated for seven days after training. Under this protocol, grouped flies move less than their isolated counterparts. Our results showed that aged trained males exhibited greater difference in movement between isolated and grouped animals when compared to their untrained, age-matched controls, suggesting better learning abilities. Our results indicate that exercise may prevent the age-associated reduced sleep and help to maintain learning abilities during aging. Future experiments will include the use of animals with reduced microRNA levels to elucidate the role of microRNAs as well as to determine whether or not the exercise benefits on cognition can be inherited in Drosophila.

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Epigenetic regulation of MAFG and its implication in the clinical management of patients with early stages of non-small cell lung cancer

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Non-small cell lung cancer (NSCLC) has one of the highest incidence and mortality rates among all cancers (1). This is in part due to it is usually diagnosed in advanced stages and these patients frequently develop resistance to chemotherapy treatment (2). We have recently found that MAFG derepressed after miR-7 hypermethylation to promote platinum resistance in NSCLC and ovarian cancer cell lines
(3). While the role of MAFG as a regulator of oxidative stress is well known (5), an alternative role for MAFG as a methylator-phenotype regulator has been found in colorectal cancer (6) and melanoma (7). However, how MAFG reshapes the lung cancer epigenome hasn't been determined yet.

Here we studied the role of MAFG as a regulator of DNA methylation by combining deletion of MAFG by CRISPR/Cas9 and CpG-Methyl-Array followed by expression (qPCR) and methylation (qMSP) analysis in tumor cell lines. Our translational approach combined aptahistochemistry at early stages with specific aptamers of the MAFG protein in 127 patients, methylation analysis in 35 fresh frozen tumors and 40 FFPE samples as well as the use of methylation and expression TCGA databases of NSCLC patients.
Our results indicate that loss of MAFG reduces the promoter methylation of LIF and MAFG itself. Moreover, we found that reduced methylation of MAFG in TCGA and increased protein levels of MAFG are associated to worse prognosis. Finally, transcriptional levels of MAFG can predict KRAS mutated NSCLC adenocarcinomas surveillance.

In summary, our work shows that detection of MAFG mRNA, DNA methylation or protein levels are a potent prognostic biomarker for NSCLC, specifically adenocarcinomas.

Different Exercise Modalities in Cardiac Rehabilitation Post Myocardial Infarction: a Systematic Review with Network Meta-Analysis

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Background. According to the World Health Organization, cardiovascular diseases are the leading cause of global mortality, representing 31% of total deaths. Among these, ischemic heart disease accounts for 16% of the total, with myocardial infarction presenting the highest mortality rate. Secondary prevention through cardiac rehabilitation has been established as the treatment of choice for these patients by the primary European and American cardiology guidelines. Exercise is considered a fundamental component; however, despite extensive research into the optimal prescription of aerobic exercise, there remains a lack of consensus regarding the prescription of strength training and other modalities, which have also demonstrated benefits in patients with myocardial infarction.

Question. What are the most effective exercise interventions in post-myocardial infarction cardiac rehabilitation to improve mortality rates, recurrence of major adverse cardiac events, quality of life, and physical condition?

Design. Systematic review with network meta- analysis of randomized controlled trials.

Search strategy. We screened MEDLINE via PubMed, EBSCO via CINAHL and SPORTDiscus, Web of Science and Google Scholar without language or year of publication restrictions. The review began with 7888 results. After removing duplicate articles, two reviewers independently evaluated the articles based on their title and abstract. In case of conflicts regarding any of the articles, they were discussed among the five members involved in the research. Subsequently, the same process was repeated with full-text reading.

Participants. Individuals over 18 years of age who have experienced a myocardial infarction in the year preceding the study, who do not have significant comorbidities (such as cancer, stroke, major depressive disorder...), and are suitable to participate in a cardiac rehabilitation program that includes at least one modality of exercise. Studies focusing on the intra-hospital phase 1 related to early mobilization or the acute effects of exercise were excluded.

Interventions. Any kind of exercise modality isolated or combined with other interventions. To consider the inclusion of the article, it must include at least the exercise modality used, the total intervention time, and the weekly frequency. In the case of aerobic, strength, or concurrent exercise, the intensity must also be specified. Comparators were pharmacological interventions alone, general physical activity recommendations, lifestyle education, other exercise interventions, no treatment or usual care.

Outcome measures. Cardiovascular and general mortality rates, recurrence of major cardiac adverse events, quality of life and physical condition.

Results. The review is expected to be completed by the end of 2024.

Registration. PROSPERO: CRD42024482757.

Keywords: cardiac rehabilitation, exercise, myocardial infarction, mortality, major adverse cardiac events, quality of life, physical condition.