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de la Universitat de Barcelona

2nd IBUB Early Career Day

16th May 2025

Faculty of Pharmacy and Food Sciences, UB

Book of abstracts

ISSN 3081-4510

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Dear IBUB researchers,

We are very happy to welcome you to the second IBUB early career day, which aims at encouraging students from our institute to share their experiences, great science and get to know each other. This is the second time organizing an event like this in our institute after the great success of last's year edition. We are excited to have such number of excellent early career researchers from our institute presenting their work.

During this symposium we will have a plenary lecture conducted by **Matías Rey** about scientific writing, 12 short talks of different PhD students, 15 flash presentations to introduce the posters and 29 posters that will be exposed during the coffee break and lunch, separated into two sessions. Finally, we have invited **Ricardo Moure** to discuss about science communication, with a hint of humor. This last session will be conducted in Spanish but we encourage everyone to stay for the fun time.

Thank you very much for joining and promoting the IBUB, and we want to wish you a very enriching career day.

On behalf of the organizing committee,

Anna Sampietro Pifarre, Coordinator, Post-Doc. Research
Verònica Casadó, Lecturer Professor
Roger Castaño, Pre-Doc. Research
Tuo Chen, Pre-Doc. Research
Ouldouz Ghashghaei, Adjunct Professor
Andreea Larisa Turcu, Lecturer Professor
Natàlia Llopart Jiménez, Pre-Doc. Research
Ana Mallo Abreu, Juan de la Cierva Researcher
Gemma Moreno Besora, Research Manager
Francisco Javier Pérez Areales, Lecturer Professor
Pol Rodríguez Martínez, Pre-Doc. Research
Maria Tarradas Alemany, Pre-Doc. Research

SCIENTIFIC PROGRAMME

08:00-08:30	REGISTRATION	
08:30-08:45	OPENING AND PRESENTATION	
08:45-09:45	OPENING PLENARY - MATÍAS REY	
09:45-10:00	ORAL SESSION 1	Selena Aranda
10:00-10:15		Andrea Bertran
10:15-10:30	FLASH POSTERS EVEN NUMBERS	
10:30-11:30	COFFEE BREAK & POSTER SESSION EVEN NUMBERS	
11:30-11:45	ORAL SESSION 2	Damián Antonio Antelo
11:45-12:00		Núria Corbella
12:00-12:15		Antonio Viayna
12:15-12:30		David López
12:30-12:45	FLASH POSTERS ODD NUMBERS	
12:45-13:00	ORAL SESSION 3	Nerea Ugartondo
13:00-13:15		Rama Haddad
13:15-13:30		Lídia Fortuny
13:30-14:45	LUNCH & POSTER SESSION ODD NUMBERS	
14:45-15:00	ORAL SESSION 4	Iván Sopena
15:00-15:15		Núria Nadal
15:15-15:30		Artur Navarro
15:30-16:30	CLOSING PLENARY - RICARDO MOURE	
16:30-17:00	CLOSING REMARKS AND AWARDS	

We kindly ask you to hang the poster during the registration time on the pannel with your number.
Be around your poster during your session (odd or even numbers) so people can ask you questions.
At the end of the day remove your poster or it will be thrown away.

FLASH POSTER

FP1	Aràntzazu	Alonso	**FP9**	Keerthi	Kurapati
FP2	Ariadna	Bada	**FP10**	Siyu	Li
FP3	Daniel Jireth	Castellanos	**FP11**	Brian	Medel
FP4	Shahrzad	Chitgaran	**FP12**	Marcel	Nos
FP5	Marc	Ciruela	**FP13**	Rabia	Idrees
FP6	Anna	Delgado	**FP14**	Fernando	Romero
FP7	Carla	Franco	**FP15**	Raul	Ventura
FP8	Piedad	Herrera			

POSTER

P1	Míriam	Acosta	**P16**	Alba	López
P2	Mar	Alujas	**P17**	Sergio	Martinez
P3	Irene	Álvarez	**P18**	Leonardo	Ortega
P4	Samia	Arshad	**P19**	Laura	Portell
P5	Dila	Bekiroglu	**P20**	Yanhao	Qiu
P6	Lucía	Caler	**P21**	Valeria	Rizzo
P7	Simge	Ceyhan	**P22**	Judith	Robles
P8	Tuo	Chen	**P23**	Cèlia	Rodríguez
P9	Magalí	Colomer	**P24**	Ayda	Roudi
P10	Ana	Corral	**P25**	Romaissa	Tafat
P11	Esnan Ece	Ertugrul	**P26**	Alejandro	Tapia
P12	Iratxe	Eskubi	**P27**	Edurne	Urquizu
P13	Tamara Madzi	Gonzalez	**P28**	Paula	Vidal
P14	Marc	Granje	**P29**	Juerui	Wang
P15	Raúl	Jiénez	**P30**	Celia	Escriche

Opening Plenary

Scientific Writing: Tips and Tricks

Matías Rey Carrizo, PhD

Key concepts regarding the three types of medical writing (publications, medical communications, and regulatory) will be introduced. The role and activities of the Spanish Association of Medical Writers (AERTeM) will also be explained. The main topic of the presentation will be on manuscript writing and will be divided into three sections. The first will deal with the rule of 4Cs to improve readability, the second will describe advice on writing order and structure of manuscript, and the last will introduce a tool (i.e., sentence outline) to bridge the gap between compiled data and manuscript format.

BCN Medical Writing

Closing lecture

Cómo divulgar y no morir en el intento

Ricardo Moure es biólogo, doctor por la Universidad de Barcelona y divulgador. Lleva más de diez años dedicándose a juntar la ciencia y el humor para hacer accesible y atractivo el conocimiento a todo tipo de públicos. Eso le ha llevado a formar parte del grupo de monologuistas científicos Big Van Ciencia, ser colaborador de programas de televisión como "En el aire con Buenafuente", "Dame veneno", "La Roca", "El club de la comedia" y "Órbita Laika". Además, ha sido un habitual de las radios y podcast, donde tiene su propio consultorio en "Serendipias" (la SER) o a ser uno de los creadores de "Materia absurda". Recientemente acaba de publicar su primer libre, "Sexo salvaje", donde hace un viaje lleno de humor por la reproducción en la naturaleza para darnos a conocer las reglas que rigen la biología.

En esta charla nos contará su experiencia como divulgador, sus secretos y los retos que se ha ido encontrando a lo largo de su extraña carrera.

Short talks

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Unraveling the genetic overlap between Autism Spectrum Disorder and Schizophrenia

Selena Aranda[1], Dora Koller[1], Sergi Papiol[2,3], Marta Cosin-Tomas[1], Mar Fatjó-Vilas[2,4,5], Javier González-Peñas[2,6], Javier Costas[7], Celso Arango[2,6], Elisabet Vilella[2,7], M. Dolores Moltó[2,8], Julio Bobes[2,9], Benedicto Crespo-Facorro[2,10], Ana González-Pinto[2,11], Lourdes Fañanás[2,5], Bárbara Arias[2,5], Renato Polimanti[12], Ditte Demontis[13], Anders Børglum[13], Thomas G Schulze[3], Bru Cormand[1,14], Marina Mitjans[1,2]

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Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by impairments in social interaction and presence of stereotyped behaviors. However, autistic traits can also be observed in other psychiatric disorders, including schizophrenia (SCZ) (Kästner et al 2015). Recent studies have reported a significant genetic correlation between ASD and SCZ (rG=0.24-0.26), suggesting a shared genetic architecture underlying their co-occurrence (Lee et al 2019). Therefore, our study aimed to investigate the common genetic basis of ASD and SCZ by integrating multiple genomic approaches.

Leveraging the most recent GWAS meta-analyses for SCZ (Trubetskoy et al 2022) and ASD (unpublished data), we studied the shared genetic risk factors between the two disorders through: 1) Global (LDScore; Bulik-Sullivan et al 2015) and local (LAVA; Werme et al 2022) SNP-based genetic correlation analyses; 2) Quantification and characterization of polygenic overlap (PolarMorphism; von Berg et al 2022); 3) Genetically informed causal inference analyses (Mendelian Randomization; Hemani et al 2018); 4) Polygenic Score analyses (PRS-Cs, Ge et al 2019) to assess whether ASD polygenic load is associated with SCZ diagnosis in the CIBERSAM sample (1,826 cases and 1,372 controls).

Our findings confirmed that ASD and SCZ are genetically correlated (rG=0.26) and identified 10 specific genomic regions with a higher local genetic correlation (|rG|>0.50). Genomic regions with positive correlations were enriched in developmental genes, whereas those with negative correlations were enriched in immune-related genes. We identified 13 loci with pleiotropic effects between the disorders, encompassing 24 genes. However, no causal relationship was found between ASD and SCZ. The ASD polygenic load was associated with SCZ diagnosis, explaining 0.60% of the variance ($p<10e-5$).

Altogether, our study confirms a shared genetic basis between ASD and SCZ, identifying specific genomic regions and pleiotropic loci. These findings may have clinical implications for early diagnosis and personalized treatment strategies targeting both disorders.

Enhancing the therapeutic efficacy of CAR cells using targeted protein degradation

A. Bertran-Mostazo[1,2], T. Chen[1,2], M. Burón[3], L. Cabellos[4], E. M. Arce[1,2], I. Puig[4], D. Muñoz-Torrero[1,2], H.G. Palmer[4], C. Eguizábal[3], C. Galdeano[1,2]

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Cancer immunotherapy based on genetically redirecting T cells has been used successfully to treat B cell malignancies. In this strategy, the T cell genome is modified by integration of viral vectors or transposons encoding chimeric antigen receptors (CARs) that direct tumour cell killing. Specifically, the efficacy of CAR-T cell therapy is very often limited by the extent of expansion and persistence of CAR-T cells. As an example, in large B-cell lymphoma around 50% of patients treated with CAR-T therapies relapse.

In parallel, there is a rapid growing interest in developing CAR-NK cells for cancer therapy, since they could offer some significant advantages, including better safety, more mechanisms of cytotoxicity and feasibility of “off-the shelf” therapies. However, NK cells represent a minor fraction of peripheral blood leukocytes, and thus the generation and expansion of sufficient and persistent numbers of NK cells remain a major challenge.

There is no doubt that new technologies to enhance the production and efficacy of the CAR cells and that can also expand its application to solid tumors are needed. Previous evidence has shown that the downregulation of TET2 with siRNA triggers an increase in T-cell proliferation and a change in the T-cell phenotype leading to central memory T cells, suggesting that TET2 downregulation could improve the efficacy of CAR therapies.

In this context, we designed a small molecule-based PROTAC to induce the degradation of TET2 to chemically-induce the silencing of TET2. Interestingly, TET2 has been considered undruggable for a very long time. So, we present the first-examples of small-molecule based PROTACs targeting TET2. We show that the addition of this TET2-based PROTAC during the ex-vivo expansion of the CAR cells (as a pre-treatment reagent) can lead to an improved expansion and an increased efficacy of current CAR therapies.

Hepatic Metabolic Disorder Associated with Ovarian-Uterine Dysfunction

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Recent findings highlight the significant role of the mitochondrial fusion protein Mfn2 in liver metabolism, particularly its involvement in metabolic dysfunction-associated steatohepatitis (MASH).

Loss of Mfn2 function disrupts this transfer, leading to impaired liver metabolism, oxidative stress, and mitochondrial dysfunction, all of which contribute to the progression of liver diseases. Our group has demonstrated that male Mfn2 liver knockout (L-KO) mice exhibit defective phospholipid transport between the ER and mitochondria, which is linked to a reduction in key enzymes involved in PS synthesis in mitochondria-associated membranes (MAMs). This deficiency results in altered lipid metabolism, ultimately leading to steatohepatitis.

There is a sexual dimorphism according to Mfn2 action in liver. We observed that ablation of Mfn2 in the liver of female mice is related to dysregulated estrus cycle, which can affect the proper development of various tissues, including the ovaries and uterus, where significant morphological disruptions are observed.

These findings suggest that Mfn2 deficiency in females interacts in a complex manner with hormonal regulation. Impaired Mfn2 function may impact lipid availability, thereby affecting hormonal synthesis and potentially influencing estrogen-related metabolic pathways.

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Effects of rRNA processing proteins kkz and clw in *drosophila* tracheal morphogenesis

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Defects in genes involved in ribosome biogenesis and function lead to a group of human disorders collectively known as ribosomopathies. A notable feature of these disorders is the occurrence of specific phenotypes in particular cell types, despite the universal requirements of ribosomes for protein synthesis. The prevailing hypothesis suggests that tissues with higher proliferative demands are more severely impacted by ribosomal dysfunction due to their greater need for ribosomes. In this study, we describe a *Drosophila melanogaster* mutant with a mutation in *kkz* gene, which encodes a non-ribosomal protein involved in rRNA processing. Surprisingly, *kkz* is highly expressed in the tracheal system, a non-highly proliferative tissue. Kkz mutant larvae are smaller and die shortly after reaching the L1 stage. Tracheal-specific knockdown of *kkz* leads to defects at the L3 stage, resulting in larval death. Interestingly, downregulation of polyploidy in the tracheal system mirrors the phenotypes observed in *kkz* RNAi experiments. Together, these results represent the first characterization of *kkz* in *Drosophila* and provide novel evidence that ribosomopathies may impact not only proliferating tissues but also polyploid tissues.

Expanding Hydrophobicity Scales: A Computational Approach to Non-Canonical Amino Acids

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The lipophilicity of amino acids is key property for understanding their physicochemical behavior, influencing solubility, binding affinity, and bioavailability—crucial factors for peptide-based therapeutics. Herein, we employed the integral equation formalism polarizable continuum model/Miertus-Scrocco-Tomasi (IEFPCM/MST) implicit solvation model[1,2] to compute the *n*-octanol/water partition coefficient as a lipophilic descriptor for non-standard amino acids, extending our prior hydrophobicity scale for canonical amino acids.[3]

Using our computational model, we analyzed two structural models differing only in their C-terminal capping groups: Model 1 (*N*-methyl) and Model 2 (*O*-methyl). In both cases**,** we observed strong correlations with experimental data,[4] confirming the reliability of our computed lipophilicity values. While most results were consistent across models, some differences emerged due to the capping group effect on side-chain hydrophobicity. Model 1, containing a hydrogen bond donor, exhibited lower statistical errors than Model 2, which features a hydrogen bond acceptor.

Our predictions successfully captured experimental hydrophobicity variations in peptide pairs with different numbers of acetylated lysines, as determined by HPLC. This suggests that our approach can be applied in proteomics, particularly for studying post-translational modifications beyond acetylation.

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Multi-Omics and Genome-Scale Metabolic Models Reveal Metabolic Vulnerabilities in FOLFOX-Treated Colorectal Cancer

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Colorectal cancer (CRC) remains the second leading cause of cancer-related deaths in Western nations and a major global health burden. While chemotherapy and targeted therapies have improved survival, most patients ultimately develop metastatic CRC (mCRC) due to disease progression following an initial response. Tumor metabolic reprogramming presents a promising therapeutic target, yet the substantial heterogeneity in CRC metabolic phenotypes necessitates precise characterization to enable effective therapeutic strategies.

To address this, we employed the CORDA (Cost Optimization Reaction Dependency Assessment) algorithm to construct high-confidence Genome-Scale Metabolic Models (GSMMs) from multi-omics data (fluxomics, metabolomics, and transcriptomics) in a panel of CRC cell lines spanning all four Consensus Molecular Subtypes (CMS). CORDA optimizes reaction network inclusion based on omics evidence thresholds, generating more physiologically relevant models than traditional approaches. This integrative framework enabled a robust mapping of metabolic pathways and regulatory networks critical for CRC progression and treatment response.

Our analysis revealed two fundamental metabolic phenotypes in CRC: a flexible subgroup capable of dynamically adapting its energy production pathways and a rigid subgroup with limited metabolic plasticity. By applying CORDA-optimized GSMMs to eight distinct CRC cell lines, we systematically classified them along this flexibility-rigidity spectrum by evaluating their flux adaptability under different conditions.

This metabolic classification paradigm provides a powerful framework for predicting CRC therapeutic responses to metabolism-targeting agents. Identifying whether a tumor exhibits metabolic flexibility or rigidity may enable precision oncology strategies tailored to individual adaptation capacities, offering new avenues to overcome therapeutic resistance in CRC management.

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scRNAseq analysis of hIPSC-derived neurons from Williams Beuren and 7q11.23 microduplication mirroring syndromes

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Williams-Beuren syndrome (WBS) and 7q11.23 microduplication syndrome (7DUP) are rare multisystemic disorders caused by, respectively, a deletion or a duplication at 7q11.23. Both syndromes affect neurodevelopment. Patients display some opposite and some overlapping phenotypical characteristics. The development of human induced pluripotent stem cells (hiPSC) and derived neuronal (iNeu) cultures has revolutionized the field of *in vitro* modeling for brain disorders.

We obtained hiPSCs from four WBS patients, four 7DUP patients and four controls and differentiated them into mature iNeus using a *NGN2* based lentiviral transfection protocol. At day 15 post-transfection, after FACS sorting, cells were processed to scRNAseq at CNAG-CRG (10x Genomics single cell 3' mRNA library preparation, NovaSeq 6000 library sequencing). We applied specific filtering, integration and quality control. Then, data was processed to group cells into clusters and identify conserved markers. The resulting UMAP of ~74000 sequenced cells clustered into 26 groups. We manually annotated the clusters into eight major cell identities ranging from iPSCs to mature neurons and intermediate groups representing different stages of neurogenesis.

Focusing on the mature neurons, we observed that four of the 30 genes in the WBS region (*EIF4H*, *BAZ1B*, *GTF2I* and *BUD23*) were differentially expressed genes (DEG) consistent with other publications. The analyses revealed 277 DEG Del*vs*Ctrl, and 122 DEG Dup*vs*Ctrl. Enrichment analyses indicated that WBS are enriched in ‘Synapse’ and ‘Cholesterol biosynthesis’ related pathways and GO terms, while 7DUP were enriched on ‘Signaling’ and ‘Protein Processing’. We also observed a large set of ASD-related genes.

When comparing 7DUP*vs*WBS samples, we found 53 overexpressed and 101 under-expressed genes. 27% were predicted targets of mir590-3p, disrupted in WBS/7DUP. These genes were also enriched in pathways related to axonal generation and synapsis.

Our comprehensive transcriptomic profile of WBS and 7DUP iNeus at single-cell level will allow the identification of novel therapeutic targets.

Grants RTI2018-101960-A-I00 and PID2021-128215OB-I00 funded by MICIU/AEI/10.13039/ 501100011033 and “ESF Investing in your future”. Grant RYC-2017-21636 by MICIU/AEI/ 10.13039/501100011033 and by “ESF Investing in your future”. Grants PID2021-128215OB-I00 and CEX2021-001139-M-20-4 by MICIU/AEI /10.13039/501100011033 and by FSE+. Institutional support to CNAG was provided by the Spanish Ministry of Science and Innovation through the Instituto de Salud Carlos III, and by the Generalitat de Catalunya through the Departament de Salut and the Departament de Recerca i Universitats.

Estimation of drug-human serum albumin binding using biomimetic chromatography

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The binding between drugs and plasma proteins, particularly human serum albumin (HSA), plays a crucial role in determining drug activity, potential toxicity, and adverse effects. Therefore, predicting this binding is essential in the early stages of the drug discovery process as it can prevent unsuitable drugs from reaching the in vivo experiments.

Biomimetic chromatography is an HPLC technique that employs a column with an immobilized protein as the stationary phase and a buffer at pH 7.4 as mobile phase to simulate the binding process in the human body. When an HSA column is used, it can mimic the in vivo interactions of drug molecules with HSA. The drugs are eluted according to their affinity for HSA and so, their retention times (t_R) have a relationship with their binding affinities.

In this study, a previously published method was adapted for use with a commercially available column of different dimensions by optimizing the mobile phase gradient conditions. The developed method was validated using a large set of compounds, and a strong correlation was found between our results and the published ones. The method succeeded in detecting drugs with binding percentages ranging from 20% to 95%. Next, a new test set of previously untested compounds was analyzed to estimate their HSA-binding parameters and expand the drug-binding data library.

The developed method enables the calculation of the apparent affinity constant of drugs from their retention times, which can be converted to the affinity constant that serves in comparing the binding results with other techniques, such as fluorescence.

Compared to other techniques, the binding affinity processes detected by chromatography correspond to weak interactions. Consequently, it is recommended to integrate this method with other complementary techniques for obtaining a comprehensive understanding of the drug-HSA affinity.

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

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

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

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Gut homeostasis and longevity: the ROS-Akt-p38 axis

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Scientific advances have extended human longevity, but a significant gap remains between lifespan and healthspan. Global research efforts are focused on improving healthspan by understanding the aging process. The intestine, a key organ in close contact with external agents, plays a crucial role in nutrient absorption and overall energetic state. During aging, the adult gut experiences a partial and progressive loss of tissue homeostasis. This phenomenon leads to oxidative stress and deregulated growth, as evidenced by increased proliferation of intestinal stem cells (ISCs). Reactive oxygen species (ROS) trigger Akt kinase and MAPK p38 signaling activation, key signals to drive regeneration and homeostasis.

Using *Drosophila* as a model system, we investigated the role of ROS, Akt and p38 signaling in age-related gut dysfunction. We found that both Akt and p38 are upregulated in aged guts. Genetic downregulation of Akt and p38 activity rescued the overproliferation of ISCs in aged guts and extended lifespan. Our results indicate that the ROS-Akt-p38 signaling axis is involved in increased ISC proliferation in aged guts. We conclude that modulating the ROS-Akt-p38 signaling pathway can “rejuvenate” the adult gut and extend longevity. This work contributes to our understanding of how maintaining a healthy gut is crucial for extending the healthspan of the entire organism.

The psychoplastogen 25C-NBF shows rapid antidepressant effects in mice

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Treatment-resistant depression is a major public health concern, with many patients failing to respond adequately to currently available antidepressant medications. Additionally, the delayed onset of therapeutic effects with current medications remains an issue. In this sense, psychedelics - a class of psychoactive compounds known for their ability to alter perception, mood and consciousness - are emerging as promising therapeutic alternatives. These compounds have been shown to induce neural adaptations, including the promotion of synaptic plasticity, which may underlie their therapeutic benefits. Therefore, psychedelics are often referred to as psychoplastogens – a term that describes compounds capable of rapidly promoting structural and functional changes in neural circuits. In this study we investigated the potential of 25C-NBF, a phenethylamine that has received limited attention to date, to promote neural plasticity and exert antidepressant effects in mice.

Male Swiss CD-1 mice received a single administration of 10 mg/kg of 25C-NBF, and spinogenesis in the prefrontal cortex was assessed using Golgi-Cox staining. Our results demonstrated increased spine density in the anterior cingulate cortex and in the prelimbic cortex 24h post-administration. The antidepressant-like effects were studied in two models of depression-induction in mice; acute restraint stress (ARS) and corticosterone (CORT)-induced depression models. In the ARS model, 25C-NBF significantly reduced immobility time in the tail suspension test (TST) at both 24 hours and one week post-administration. In the CORT model, a significant reduction in immobility was observed 24 hours post-treatment, but not at the one-week mark. Additionally, the sucrose preference test confirmed anhedonia in CORT-treated mice, which was reversed 24 hours after 25C-NBF administration.

To sum up, our findings suggest that 25C-NBF exhibits promising antidepressant properties with a rapid onset of action, supported by its ability to modulate neuroplasticity. This study offers value into the search of alternatives for treating individuals with treatment-resistant depression.

Crosstalk between cardiomyocytes and macrophages during the development of cardiac hypertrophy *in vitro*

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Cardiac hypertrophy is characterized by an increase of the heart ventricle walls and is one of the most common Cardiovascular Diseases (CVDs) worldwide. Although it is first conceived as a compensatory mechanism, persistent stress can cause the heart to switch to a maladaptive state and finally lead to heart failure. On a cellular level, the transition between a compensatory and a maladaptive state is not understood and other cellular types, other than cardiomyocytes, might also play a role in this transition. Here, we focus on the interaction between macrophages and cardiomyocytes during the development of cardiac hypertrophy. Macrophages are the most prominent immune cells present in this organ and they are crucial for the repairing of damaged heart tissue, however, their function during the development of cardiac hypertrophy has not been previously studied. In this study, we investigated the interaction between these cellular cell types during the development of cardiac hypertrophy *in vitro*. We found that pro-inflammatory M1 type macrophages alone were able to induce cardiac hypertrophy by increasing cellular area, hypertrophic markers and modulating metabolism markers. Moreover, posterior M2 anti-inflammatory conditioned medium treatment ameliorated hypertrophy on these cells, indicating the beginning of a potential reparative phase. Finally, M2 anti-inflammatory polarization was induced after cardiac hypertrophy development in our *in vitro* models, highlighting a potential reparative mechanism that these cells exert after the development of cardiac hypertrophy.

Flash poster talks

Beyond pain: how dopamine D1 receptor blockade could improve opioid effectiveness

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Chronic pain is a major health issue in Europe, further exacerbated by the opioid crisis due to the widespread use of opioids for severe pain management. While opioids alleviate pain by binding to the mu-opioid receptor (MOR), they also lead to adverse effects such as tolerance and addiction[1]. MOR can form higher-order complexes, known as heteromers, which modulate its function. Of particular interest is the MOR-dopamine D_1 receptor (D_1R) heteromer[2], which may contribute to adverse effects, including abuse liability[3]. This study aimed to elucidate the localization, structure, and function of the MOR-D_1R heteromer. First, ex vivo evidence of the complex in the mouse striatum was obtained using proximity ligation assays. Next, its interaction interface was investigated through bimolecular luminescent complementation (BiLC) assays with disruptive peptides. D_1R's TM4 and TM5 domains, along with MOR's TM4, disrupted the heterodimer. Given the symmetrical nature of these systems, a TM4/5 heteromeric interface was proposed and modeled. To assess signaling effects, cAMP accumulation assays were performed in transfected cells. As expected, D_1R activation increased cAMP levels, while MOR activation decreased them due to their respective Gα subunits. Interestingly, endomorphin-1 reduced the cAMP levels induced by SKF38393, indicating negative crosstalk at the adenylyl cyclase (AC) level. Additionally, bidirectional cross-antagonism between MOR and D_1R was observed, suggesting close proximity and potential oligomerization influencing AC activity. This cross-antagonism was also reversed by the same disruptive peptides identified in BiLC assays, further validating the proposed interaction interface. Notably, literature suggests that chronic D_1R blockade following spinal nerve ligation preserves MOR responsiveness to opioids, preventing tolerance. The findings of this study, particularly regarding cross-antagonism, provide valuable insights for designing improved chronic pain treatments with reduced opioid tolerance[4]. Moreover, the structural model paves the way for designing selective ligands, which are expected to be more effective and produce fewer side effects.

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Aknowledgments:
This work was supported by the grant PID2020-113938RB-I00, PID2023-146914OB-I00 (MCIN/AEI/10.13039/501100011033 and FEDER), and the grant 2021-SGR-00230 from "Generalitat de Catalunya", Spain (VC, NL, AAC, EM, VCA). AGAUR-FI fellowship from "Generalitat de Catalunya" (2023 FI-3 00065) (AAC) and UBPredocs fellowship from the University of Barcelona (NL).

A genomic approach to the Evolutionary Nature of Autism Spectrum Disorder

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Given the impairing nature and reproductive drawbacks of Autism Spectrum Disorder (ASD), its high prevalence in the population poses an evolutionary paradox. Natural selection removes alleles that reduce the fitness of their carriers –i.e. risk alleles with large effects on susceptibility to mental illnesses. However, a substantial fraction of ASD risk stems from polygenic predisposition driven by common variants of small to moderate effect. We aimed to investigate the evolutionary nature of ASD by applying state-of-the-art genomic tools to the latest ASD-GWAS meta-analysis, comprising 38,717 cases and 232,735 controls (unpublished).

We used MAGMA (Leeuw et al. 2015) to test whether ASD-associated variants are enriched in LoF-intolerant genes or genes nearby Human Accelerated Regions (HARs) (Girskis et al. 2021). ASD-associated variants were significantly enriched in LoF-intolerant genes (all p=6.64e-08, brain-expressed p=2.99e-07, highly-brain-expressed p=5.44e-07) as well as in HAR-associated genes (p=0.0005).

We also assessed whether ASD-risk alleles (stratified by MAF bins) tend to be enriched for the derived or ancestral allele (present in chimpanzee, *Pan Troglodytes*). Our results indicate that ancestral alleles generally reduce susceptibility to ASD compared to their derived counterparts.

Moreover, we examined the burden of ASD-risk-alleles in ancient genomes (Allentoft et al. 2024). Trend analysis of ancient DNA samples revealed a significant increase in the proportion of ASD-risk alleles toward the present (τ=-0.1421, p=0.0354).

Finally, we compared the proportion of ASD-related risk and protective alleles originating in *Homo* lineages versus *Anatomically Modern Humans* (AMH). *Homo* lineages displayed a relatively lower proportion of ASD-risk alleles compared to AMH (OR=0.38, p=0.023).

Our results show a gradual increase in ASD genetic risk across evolutionary time, with AMH genotypes exhibiting greater risk compared to ancestral genotypes. They also suggest a complex interplay of evolutionary forces contributing to the genetic architecture of ASD. Further analyses are needed to clarify the selective pressures shaping ASD.

Novel multitarget inhibitors with amyloid antiaggregating activity to treat Alzheimer's disease

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Alzheimer's disease (AD) remains the most prevalent cause of dementia globally. Its pathogenesis is complex, involving multiple interacting biological pathways. To address this complexity, the strategy of multitarget drug design has gained interest, aiming to simultaneously modulate several pathological processes. Among the various mechanisms implicated in AD, three interconnected factors stand out: neuroinflammation, cholinergic dysfunction, and protein aggregation. Accordingly, we proposed a therapeutic approach targeting these axes by: 1) mitigating neuroinflammation through inhibition of soluble epoxide hydrolase (sEH), thereby elevating levels of anti-inflammatory epoxyeicosatrienoic acids (EETs); 2) reducing cholinergic deficits by inhibiting acetylcholinesterase (AChE); and 3) interfering the spontaneous aggregation of amyloidogenic proteins, thus pursuing a so far unexplored target combination profile.

This study presents a first class of dual sEH and AChE inhibitors with antiaggregating capacity, developed by linking specific pharmacophores via a tether chain. These hybrids demonstrate highly potent inhibitory activity against both enzymes, achieving IC_{50} values in the subnanomolar range. Furthermore, they significantly reduce aggregation of β-amyloid and tau proteins, with inhibition rates exceeding 50% at 10 μM. Notably, certain candidates also inhibit aggregation of TDP-43, a protein associated with multiple neurodegenerative conditions, including AD and amyotrophic lateral sclerosis. These compounds exhibit low neurotoxicity in SH-SY5Y neuronal cells at 100 μM, possess the ability to permeate across the blood-brain barrier, and display acceptable aqueous solubility and microsomal stability. Ongoing pharmacokinetic and efficacy assessments in an AD mouse model aim to validate the therapeutic promise of a lead candidate as a disease-modifying agent for AD.

Identification of regeneration-specific elements required for posterior specification in planarians

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Regeneration is the process of generating new tissue that restores both the proportions and functionality of an organism. Planarians are whole-body regenerating animals and serve as an excellent model to study the molecular and cellular processes that enable the regeneration of missing body parts.

In our lab, we are interested in deciphering the mechanisms that specify proper anterior-posterior identity after amputation at any level of the planarian body. To address this, in previous studies we performed bulk and single-cell RNA-seq transcriptomic and genomic analyses of posterior versus anterior wounds at various time points during regeneration. From these studies, we identified new elements required for posterior identity specification, as their silencing leads to tailless or two-headed animals. One example is a newly identified secreted peptide, which we named Smed-tuck.

In this study, we aim to better understand the mechanism of action of Smed-tuck through functional analysis of its putative interactors. We also aim to identify regeneration-specific elements involved in posterior specification through an RNAi screen of candidates identified in the RNA-seq data.

These results will contribute to a deeper understanding of the mechanisms that trigger proper identity specification during complex regeneration processes, such as those observed in planarians in nature.

Improving Pain Management with Cannabis: Design of Small, Nonpeptidic Ligands that Disrupt 5HT2AR-CB1R heteromer to avoid side effects

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Cannabinoid agonists have proven their analgesic potential that could make them useful for the treatment of migraine, rheumatoid arthritis or cancer-related pain, among others, but their use has been hampered by their side effects such as memory impairment. The effects of cannabinoids are primarily mediated through two G protein-coupled receptors (GPCRs), cannabinoid CB_1 receptor (CB_1R) and CB_2 receptor (CB_1R). Recent findings linked the cognitive side-effects of cannabinoid agonists such as delta-9-tetrahydrocannabinol (THC) to complexes of CB_1R and serotonin 5-HT_{2A} receptor (5-$HT_{2A}R$), another GPCR, and the expression of CB_1R-5-$HT_{2A}R$ heteromers were demonstrated in native tissues and in behavioural studies in mice lacking 5-$HT_{2A}R$.

Notably, synthetic peptides capable to disrupt CB_1R-5$HT_{2A}R$ heteromerization *in vivo*, leads to a selective abrogation of memory impairment caused by exposure to THC. Using the synthetic peptides designed from CB_1R transmembrane helix (TM) 6, a virtual screening of commercially available "lead-like" identified compounds that mimic the pharmacophoric features of the synthetic peptides. We present three compounds that bind to the interface of 5-$HT_{2A}R$ according to molecular dynamics simulations and that are capable of altering CB_1R-5-$HT_{2A}R$ heteromerization in bimolecular fluorescence complementation assays and the heteromerization-dependent allosteric modulations in cell signalling experiments.

These results constitute a proof-of-principle for the design of optimized ligand-based disruptors of the CB_1R-5-$HT_{2A}R$ heteromer, opening new perspectives for *in vivo* studies.

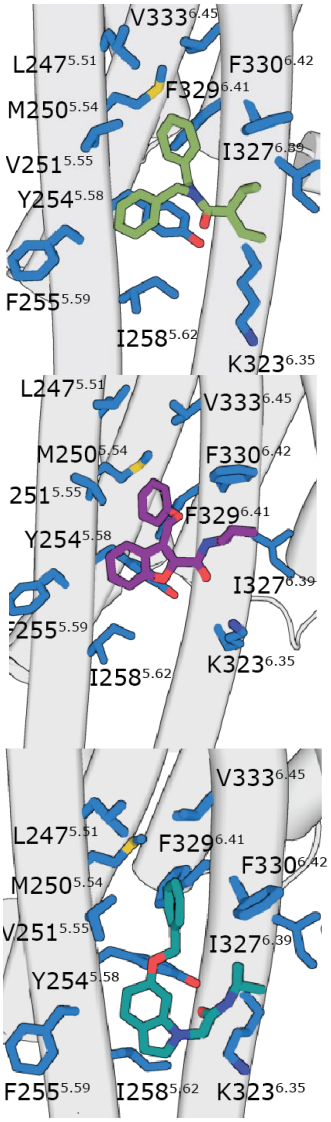

Fig1: Compounds **4**, **12** and **31** (green, purple and blue sticks) bound to 5$HT_{2A}R$ as predicted *in silico*.

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Preparation and characterization of a biomimetic chromatography column with bonded MALT1

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Evaluating the binding of drug candidate compounds to target proteins is crucial for identifying potential drugs for specific pathologies. A variety of techniques such as calorimetry, fluorescence or dialysis can be used to study drug-protein interactions, but there is still a need of development of high throughput methods for such kind of studies. High-performance liquid chromatography (HPLC) is a technique that offers several advantages including speed, precision, automation capability, low detection limits, and compatibility with a wide range of assay formats and detectors, which make it a reliable option for the study of drug-protein interactions.

Biomimetic chromatography methods are based on stationary and mobile phases that mimic the biological environment and physiological conditions. Proteins and phospholipids are some examples of biomimetic chromatography stationary phases. Using a mobile phase that emulate physiological conditions (for example, pH 7.4), the in-vivo partition and distribution of compounds can estimated through chromatographic measurements, facilitating the selection of optimal candidates for drug development.

In this study, a novel HPLC column with immobilized MALT1 (mucosa-associated lymphoid tissue lymphoma translocation protein 1) has been developed. In a first step, the resulting column has been characterized to evaluate its performance, including stability, reproducibility, and retention properties. In a second step, the retention of a set of compounds with different degrees of inhibitory activity to MALT1 has been measured in the column. Retention times have been used to establish a correlation between the retention factor (k) and the mean maximum inhibitory concentration (IC_{50}) with the aim to estimate the inhibitory concentration to MALT1 of new drug candidates.

Increased chaperone-mediated autophagy in adipose tissue of individuals with obesity: a novel contributor to adipose dysfunction in obesity

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Chaperone-mediated autophagy (CMA) is a selective lysosomal degradation pathway, distinct from macroautophagy that maintains protein homeostasis. While CMA plays a role in adipogenesis, its role in obesity is still unknown. Lamp2a, a key lysosomal receptor, regulates this pathway.

We performed RNA sequencing (RNAseq) analysis of subcutaneous (SAT) and visceral (VAT) adipose tissue from men and women with obesity, obesity combined with diabetes, and lean individuals (13 per group). We measured 39 anthropometric, body composition, and circulating parameters, as well as the CMA score, a validated indicator of CMA activity based on the combinatorial values of 18 transcripts (Bourdenx et al., 2021). Bulk RNAseq and single-nucleus RNA sequencing (snRNAseq) data from SAT and VAT of high-fat diet-induced obese mice were also analyzed. Alternative splicing-derived expression of *Lamp2a* was assessed using qRT-PCR, and Lamp2A protein levels were measured by immunoblotting.

Results showed a significant increase in CMA score in VAT—but not SAT—of individuals with obesity, particularly in women. *LAMP2A* mRNA levels were upregulated in VAT and correlated positively with body mass index (BMI), blood glucose, triglycerides, and HbA1c. Notably, bariatric surgery reversed the elevated CMA score in VAT. In mouse models, CMA activity was also elevated in VAT, involving adipocytes, and associated with increased Lamp2A protein levels.

Additionally, *LAMP2A* expression positively correlated with markers of endoplasmic reticulum (ER) stress, such as HSPA5, suggesting that ER stress may trigger CMA activation in adipose tissue.

These findings indicate that CMA is over-activated in adipose tissue in individuals with obesity, particularly in women, and this activation is associated with systemic metabolic dysregulation. ER stress appears to play a relevant role in the induction of adipose CMA in obesity. The role of adipose CMA over-induction in the etiopathogenesis of obesity deserves further investigation.

Ruthenium-based Photosensitizers for Cancer Therapy: Targeting Pancreatic and Colon Tumors

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Cancer remains one of the leading causes of death worldwide, affecting millions each year. In 2022 alone, nearly 20 million new cases were reported, with 9.7 million cancer-related deaths. This growing global burden highlights the urgent need for continued research and the development of more effective treatments. Bioinorganic chemistry plays a crucial role in developing novel treatment strategies, complementing traditional metallodrugs like cisplatin. Among these alternatives, photochemotherapies[1]—such as photodynamic therapy (PDT), offering targeted treatment by activating a photosensitizer (PS) through localized light irradiation, minimizing side effects (**Figure 1**)

Research on metal-based compounds has shown that the unique properties of metal ions can be leveraged for anticancer drug design. In this context, polypyridyl ruthenium (II) complexes have emerged as a highly promising class of photoactivatable agents.[2]

Herein, a novel family of ruthenium (II) was synthesized and characterized by photosical and chemical properties. Also, their *in vitro* photocytotoxic properties exhibited significant phototoxicity upon irradiation while non-toxic in the dark. To take a step further, the most promising complex was tested on pancreatic cancer spheroids and *in vivo* studies were initiated using an orthotopic xenograft mouse model. Notably, these photosensitizers are water-soluble, highly photostable, and can be synthesized through straightforward methods, making them attractive candidates for future cancer therapies.

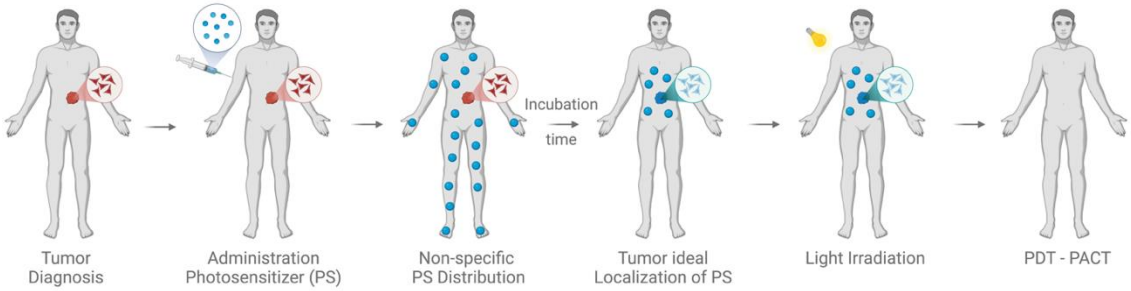

Figure 1. Schematic representation of PDT or PACT treatment. Dark red = tumor, blue = PS.

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Deciphering SCAN-1: The role of gkt in Drosophila organogenesis

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Spinocerebellar ataxia with axonal neuropathy type 1 (SCAN-1) is a debilitating peripheral neuropathy characterized by slowly progressive cerebellar ataxia and atrophy. All SCAN-1 patients identified to date carry the same mutation in tyrosyl-DNA phosphodiesterase 1 (TDP1). Like in humans, the homologue of TDP1 in Drosophila, a gene named *Tdp1* encodes a tyrosyl-DNA phosphodiesterase 1.

Previously, *Tdp1* was found to be essential for the formation of epithelial polarity and nervous system development. The fact that the *Drosophila* ortholog appears to have different functions, may help clarifying the complex aetiology of SCAN1.

Preliminary results from the laboratory indicate that *Tdp1* is expressed in tracheal cells and is important for tracheal formation in Drosophila melanogaster embryos.

Currently, our aim is to elucidate the function of Tdp1 in the tracheal system of *Drosophila melanogaster* and to further explore the connection between Tdp1 and other components in the pathway. So, we are using the *Drosophila* tracheal system as a model to study Tdp1 function in organogenesis. Furthermore, we will reintroduce a functional TDP-1 allele into a $Tdp1^{G85}$ genetic background to restore the normal wild-type tracheal phenotype. We are also testing genetic interactions between *Tdp1* and other *Drosophila* genes.

Long non-coding RNAs (lncRNAs) in white adipose tissue of children with obesity

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According to the WHO's 2024 report, 160 million children and adolescents (ages 5–19) were living with obesity in 2022 worldwide. Spain is the leading country in Europe in childhood obesity. Importantly, 80% of the children with obesity will remain with obesity until the adulthood facing elevated risks of chronic diseases and psychosocial difficulties. Effective prevention and treatment strategies are urgently needed to reduce the societal burden and improve public health. Owing to their lower side effects, gene-targeted therapies hold promise as future interventions. This study aims to identify and characterize molecular targets associated with childhood obesity, with a focus on long non-coding RNAs (lncRNAs), which are key regulators of obesity and metabolism. We performed transcriptomic analysis on subcutaneous (sWAT) and visceral (vWAT) white adipose tissue samples isolated from 35 children (boys and girls between 2-12 years old), including 26 with normal weight and 9 with obesity. In sWAT, 16 lncRNAs were upregulated and 33 were downregulated in the obesity group. In vWAT, 10 were upregulated and 1 downregulated. Enrichment analysis of vWAT lncRNAs revealed only the apelin signaling pathway, while sWAT lncRNAs were enriched in 67 pathways, primarily related to energy metabolism—including MAPK, cell cycle, insulin resistance, and adipokine signaling. These results reveal distinct transcriptomic profiles and metabolic pathway involvements in sWAT and vWAT in obesity, offering insights into tissue-specific mechanisms underlying childhood obesity.

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Synthon-Based Strategies Exploiting Molecular Similarity and Protein-Ligand Interactions for Efficient Screening of Ultra-Large Chemical Libraries

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The rapid expansion of ultra-large chemical libraries has revolutionized drug discovery, providing access to billions of compounds. However, this growth poses relevant challenges for traditional virtual screening methods. To address these limitations, synthon-based approaches have emerged as scalable alternatives, exploiting combinatorial chemistry principles to prioritize building blocks over enumerated molecules. In this work, we present exaScreen and exaDock, two novel synthon-based methodologies designed for ligand-based and structure-based virtual screening, respectively. In the former case, synthon selection is guided by the 3D hydrophobic/philic distribution pattern in conjunction with a specific synthon alignment protocol based on a quadrupolar expansion over the atoms that participate in the linking bonds between fragments. On the other hand, accommodation to the binding site under a geometrically restrained docking of synthon-based hybrid compounds is used in the selection of the optimal synthon combinations. These strategies exhibit comparable performance to the search performed using fully enumerated libraries in identifying active compounds with significantly lower computational cost, offering computationally efficient strategies for virtual screening in ultra-large chemical spaces.

Intron Characterization and Genomic Graph Annotation in Schmidtea mediterranea

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Increasing advances in DNA and RNA sequencing capabilities have allowed transcriptomics to reach previously unthinkable depth but generating huge amounts of data has made its processing more challenging. Assembling transcriptomes, updating them, and their presentation in a useful manner has proved a difficult resource-intensive task.
Sequence-based annotation necessitates recalculating assemblies on each transcriptome data set or projection over the reference genome, to integrate new RNA-seq experiments. As the amount of data available increases, constant reassembly is an costly impractical solution. We propose using graph-based genome structural annotation to allow easier integration of new data into existing assemblies. This requires updating only structurally differing regions for new data incorporation. Graph-based notation consists of the representation of genome elements by interconnected nodes which can be implemented in a database and made publicly accessible.
We have used genomic and transcriptomic data from Schmidtea mediterranea, a planarian studied due to its regeneration capability. Understanding function and expression of genes controlling the its regenerative potential will prove beneficial to biomedical research, especially stem cell therapy and regenerative medicine.
With a number of bioinformatic techniques and tools, some newly implemented, we have developed a protocol to identify all intronic regions within a genome from RNA-seq data. Gene structures for this genome were annotated and integrated into graphs, allowing for easy incorporation of data from new sequencing experiments and updating of databases to match recent increases in RNA-seq data available. Additionally, this pipeline could be implemented to analyze and project data from other non-model organisms.

Fast LFER: A new approach for rapid characterization of electrokinetic chromatographic systems intended for drug bioactivity screening

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The drug discovery process depends upon efficient prediction of drug candidates' behavior in biological systems. Traditionally, *in vitro* and *in vivo* testing is performed but it presents some drawbacks such as being time consuming and resource intensive. On its behalf, formulation of alternative models like biomimetic chromatography to emulate biological conditions in the laboratory settings is of great importance. This technique involves the use of physicochemical systems to surrogate biological partition processes such as skin partition or blood-brain permeation, and it has proven to be a promising alternative to animal testing and streamlines the drug development process.

Linear free energy relationship model by using Abraham solvation parameter model [1] allows characterization of several solute-solvent interactions and elucidation of related properties. This method relies on multilinear regression analysis of a number of a large set of solutes for a partitioning system characterization. However, in the present work, we have simplified the analysis by carefully choosing a limited set of pairs of reference compounds [2] to determine parameters like dipolarity, polarizability, hydrogen bond acidity, hydrogen bond basicity and cavity formation. Specifically, micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) systems [3] have been selected as viable tools to study solute-solvent interactions related to drug solubility, permeability or distribution. Owing to their tunability by adjusting buffer composition, nature of surfactant, pH and other properties, MEKC and MEEKC systems can mimic biological partitioning processes and assist in the drug discovery process. The fast LFER approach [2] not only enables rapid profiling of drug candidates by accelerating early stages of drug discovery but also saves time and resources for system characterization. Apart from this, the ethical aspect of the method to reduce reliance on animal testing is aimed at promoting a sustainable human approach in scientific research.

Acknowledgements

This work was supported by Grant PID2023-150245NB-I00 funded by MICIU/AEI/10.13039/501100011033 and by ERDF, EU.

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Doble Diastereoselective [3 + 2] Cycloaddition Strategy Toward Novel I2 Receptor Ligands

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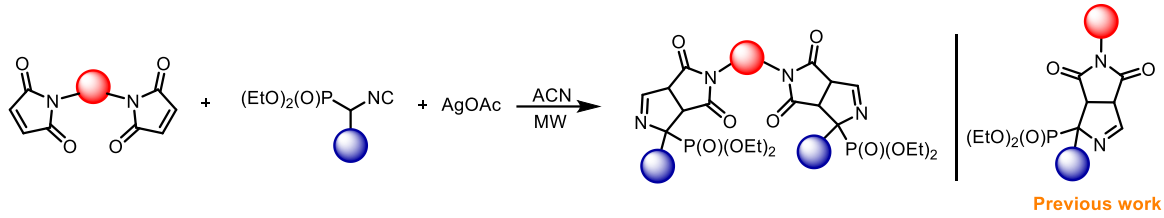
Imidazoline I_2 receptors (I_2-IR) are nonadrenergic binding sites, that emerged as relevant biological targets. The dysregulation of the levels of I_2-IRs is a hallmark in illnesses such as glial tumours, Alzheimer's disease (AD), Huntington's disease, Parkinson's disease, and depression, among others. To overcome the challenge of modulating I_2-IR activity, we developed a novel family of bicyclic δ-iminophosphonates, which exhibit high affinity and selectivity for these receptors[2].

Based on the structures of bicyclic δ-iminophosphonates, our ongoing efforts aim to design new molecules by applying the principles of molecular duplication. These novel structures have been synthesized through a diastereoselective [3 + 2] cycloaddition reaction[3] of PhosMic derivatives with dimaleimides in acetonitrile under AgOAc catalysis and microwave irradiation. The reaction was examined with dimaleimides bearing alkyl or aryl substituents and α-substituted PhosMic derivatives commercially available or synthesized according to published procedures. Analogous to our previous works, the main goal of this project is to study the doble [3 + 2] cycloaddition, clarify the relative configuration of the stereocenters, and evaluate the new family of compounds as I_2-IR ligands.



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The role of non-mitochondrial MFN2 and its implications in peroxisomal functionality

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Traditionally, Mitofusin 2 (Mfn2) was considered as a mitochondrial protein exclusively located in the mitochondria's outer membrane. However, it was also found in mitochondrial-associated membrane (MAMs). Here, we described the presence of Mfn2 in peroxisomes and in non-MAMs endoplasmic reticulum (ER) fraction. To assess the peroxisomal and ER Mfn2 localization, western blotting after cell subfractionations, co-localization studies with a super resolution confocal microscope, and electron microscopy images analysis were performed using mouse embryonic fibroblasts (MEF) and mouse liver. Due to the role of ER and mitochondria in the peroxisomal biogenesis, markers of this process and morphological studies were also assessed. Finally, functional studies of the peroxisomal activity such as catalase activity, or hydrogen peroxide accumulation were analyzed in control and Mfn2 ablated conditions. Mfn2 ablation also causes changes in peroxisomes size and number (there are more and smaller peroxisomes). Moreover, in our Mfn2 knockout models, several genes related to peroxisomal biogenesis are downregulated, impairing also the functionality of this organelle. All together indicates that Mfn2 located in the ER and peroxisomes is playing and important role in the peroxisomal biogenesis and dynamics.

Posters

Poster 1

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DNA methylation patterns and epigenetic aging in bipolar patients with childhood maltreatment

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Childhood maltreatment (CM) is a risk factor for bipolar disorder (BD), affecting around 50% of patients (Garno et al., 2005), that have been pointed to have a significant role in modifying DNA methylation patterns (DNAm) (Rubens et al., 2023). Moreover, CM has been linked to accelerated epigenetic aging (EA) (Harvanek et al., 2024). Hence, this study aims to investigate DNAm patterns and EA in BD patients with CM to identify whether specific methylation differences or accelerated EA are associated with CM scores. A sample of 80 bipolar type I (BD-I) and type II (BD-II) patients answered the Childhood Trauma Questionnaire (Bernstein et al., 2003), a retrospective screening self-assessment questionnaire. Our association analyses used the total CTQ score as an outcome measure. Raw DNAm data (InfiniumMethylationEPICBeadChip v1.0) from peripheral blood samples were processed with ChAMP (Tian et al., 2017). The association between individual CpGs and regions and total CTQ score was tested with a linear regression model adjusted for relevant covariates. EA was estimated using Horvath's calculator (https://dnamage.clockfoundation.org/) and DunedinPACE pipeline (Belsky et al., 2022). Benjamini-Hochberg correction for FDR was used for all analyses, and a $p<0.05$ was considered. We found no CTQ score-associated CpG sites or regions (FDR>0.05). However, we identified a suggestive association between GrimAge acceleration (GrimAgeAccel) and CTQ score (FDR p=0.058). Our results regarding EA align with a recent study that also identified an association between GrimAgeAccel and CM (Harvanek et al., 2024). Still, results should be further studied for a better understanding of the impact of CM on methylation and aging patterns.

Funding PI17/01122, PI22/01048, PI22/00431, LaMarató TV3 (202203-30-31-32), 2021SGR01093; 2021SGR00706; 2021SGR01358, and RYC2021-033573-I, PID2022-139740OA-I00

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Analysing the involvement of *Tyrosyl-DNA phosphodiesterase* and *sequoia* in neurodevelopment of *Drosophila melanogaster*.

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Analysing *Drosophila melanogaster* neurodevelopment is important for the understanding of neuronal disease. We focus on Spinocerebellar ataxia with axonal neuropathy type 1 (SCAN-1), a debilitating peripheral neuropathy characterized by cerebellar ataxia and progressive atrophy SCAN-1 patients present alterations in the coordination of movement due to the degeneration of the cells of the cerebellum. All these patients, so far, have a mutation in tyrosyl-DNA phosphodiesterase 1 (TDP1).

We also study *sequoia (seq)* that appears to be a universal regulator of morphology in Drosophila neurons. It is a transcription factor that influences dendrite and axon outgrowth. It is known that *seq* is necessary for the development of neuronal dendritic branches. However, its function has never been analysed in the development of the nervous system.

We are characterising two *Drosophila melanogaster* mutant phenotypes, *sequoia (seq)* and *Tyrosyl-DNA phosphodiesterase (Tdp1)*, in the central (CNS) and peripheral nervous Systems (PNS) during embryonic stages. The analysis is being done by immunostaining with the primary antibodies 1D4 and 22c10 which stain against Fasciclin II (Fas II) and Futsch, respectively. FasII is cell-adhesion molecule that in embryonic neurons and Fustch is MAP1B-like Protein Required for Dendritic and Axonal Development.

To carry out the study we are analysing $Tdp1^{G85}$ and $Tdp^{CO3958II}$ mutants and seq^{GA168} , seq^{C3101} , seq^{Z1241} , seq^{C022} and seq^{H156} mutants. This study will provide information of the molecular mechanisms involved in SCAN-1 disease and the functional relevance of *sequoia* in neural development.

Key Role of PrP_{Sc} in Aβ Peptide Oligomerization

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Recent evidence suggests a potential interaction between prion protein (PrP) and amyloid-beta (Aβ), the main hallmark of Alzheimer's disease. In this work, we studied the effect of preformed PrP^{Sc} fibrils on the aggregation of $Aβ_{40}$ peptide under physiological conditions. Kinetic assays revealed that the presence of PrP^{Sc} increases the lag phase and nucleation constant, while decreasing the elongation rate and final fibril concentration. These changes suggest a direct interaction between PrP^{Sc} seeds and Aβ monomers, which could alter the aggregation pathway. Interestingly, the presence of PrP^{Sc} promoted the accumulation of soluble oligomeric species, which are considered potentially more toxic and capable of spreading. In contrast PrP^{c} showed minimal impact on $Aβ_{40}$ aggregation. Fibrils formed in the presence of PrP^{Sc} showed increased resistance to denaturation, indicating a more stable structure.

Together, these results suggest that PrP^{Sc} not only reduces $Aβ_{40}$ fibril formation but also promotes the formation of more stable and potentially pathogenic oligomeric species. This supports a role for PrP^{Sc} in modulating Aβ aggregation and points to cross-seeding mechanisms as relevant contributors in the progression of neurodegenerative diseases.

Innovative biomimetic framework: utilizing chromatographic techniques for toxicity and permeability prediction

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Contemporary challenges in pharmaceutical and environmental risk assessment require predictive models that can emulate compound behaviour across multiple biological membranes. This study introduces an advanced mechanistic platform using tunable biomimetic chromatography systems based on liposome electrokinetic chromatography (LECK) and liquid chromatography (LC) [1].

In the present work, we aim to construct customized biological environments that mimic specific membrane characteristics of different body organs and tissues. The data from LEKC and LC is interrelated with permeability parameters such as skin permeation, membrane affinities, and toxicity, relying directly on Abraham's Solvation Model. This model is central to our study, specifically to analyse interactions, i.e., polarizability/dipolarity, cavity formation, and hydrogen bonding that underlie biological and chromatographic partitioning systems [2].

The integration of multiple chromatographic setups, such as immobilized artificial membranes, immobilized proteins or enzymes, liposomes, and micellar phases, provides a broad spectrum of chromatographic systems for the emulation of ADMET properties and toxicity profiles of the chemicals. The overarching goal of this work is, therefore, to design robust biomimetic chromatography systems that can predict ADMET parameters, reducing the need for in vivo and in vitro testing.

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Role of MFN2 in planarian regeneration

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Mitochondria are central regulators of cellular metabolism, and their dynamic behaviour through fusion and fission processes plays a critical role in adapting to physiological demands of the cell, specifically during the cell cycle progression. Among other few mitochondrial dynamics-related genes, Mitofusin 2 (mfn2) has also emerged as a key player in shaping mitochondrial networks by mediating mitochondrial fusion. Mfn2 has been shown to influence both cellular proliferation and differentiation, however, its function in large-scale regeneration remains poorly understood. Planarians, with remarkable regenerative capacity which is sustained by a population of pluripotent stem cells (neoblasts), offer a unique opportunity to study such connections in vivo. Building upon prior findings which suggest mfn2 influences cell cycle progression, cell differentiation, and cellular proliferation differently depending on metabolic state, our current study seeks to investigate the role of mfn2 during regeneration under two distinct metabolic states—starvation and feeding. By exploring how metabolic inputs influence mfn2 function, we aim to uncover whether mitochondrial fusion dynamics contribute to the coordination of regeneration and metabolic adaptation. This approach may shed light on how mitochondrial behaviour integrates environmental cues to regulate organismal plasticity in the regeneration context.

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KCNE4 is a potassium channel regulatory protein, which promotes the intracellular retention of Kv1.3. KCNE4 presents a polymorphism at the 145 residue, which has been correlated with several immune system pathologies. The aim of this project is to insert a lysine mutation, instead of an arginine, in the KCNE4 145 position. Next, the protein will be amplified, purified and expressed in a heterologous expression system. We will analyze whether the protein exhibits an aberrant behavior.

The KCNE4 145K vector was inserted in XL-10 gold ultracompetent bacteria by permeabilizing the plasma membrane and applying thermic shock, to then inoculate the bacteria into agar plates and incubate them overnight.

The optimal bacterial colonies are next selected and cultivated in growth medium throughout 24 hours. DNA is then extracted following a mini-prep kit procedure, in which the nucleic acid is purified and isolated from the rest of the cellular contents. Subsequently, the DNA is amplified by Sanger sequencing, in which a PCR mix is prepared using our sample of interest. An electrophoresis gel and the consequent fluorescent laser reading will then show our nucleic sequence in a chromatogram. By multiple alignment against a reference KCNE4 sequence, we should conclude that the sequence has properly incorporated our mutation of interest, and we will proceed to select the most base-per-base coincident sample to progress further with the experimentation.

Supported by the Ministerio de Ciencia e Innovación (PID2020-112647RB-I00 and PID2023-148085OB-I00) and AEI (10.13039/501100011033), Spain and European Regional Development Fund (FEDER).

Exploring organelle dynamics in subcellular branching

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Cell shape is intrinsically connected with cell function in tissues and organs, varying enormously throughout nature. Branched cellular networks are a common feature of multicellular animals and underlie the formation and function of numerous tissues and organs, including the nervous system, respiratory system, vasculature, and many internal glands. The production of branched structures by single cells, known as subcellular branching, involves complex organelle and cytoskeletal remodelling events. We aim to better understand single-cell branching beyond the dynamics of the cytoskeleton. Previous research from the Araújo laboratory showed that centrosomal and acentrosomal cytoskeletal changes affect subcellular branching in tracheal cells. We are now exploring how organelle dynamics can influence single-cell branching and identify the molecular partners involved. Ribosomes, involved in protein synthesis, have a homeostatic effect on cells during development. Since rapid protein transition is important for local stimuli response, ribosome localization and composition are expected to be important for single-cell branching. Additionally, protein synthesis, cell movement, and cytoskeletal dynamics are necessary for cell branching and require high ATP production. Mitochondria dynamics play a role in transporting mitochondria into axons and dendrites for neuronal activity. Yet, we do not know the function and effect of mitochondria and ribosomal localization on the subcellular branching of tracheal terminal cells.

We are using three different branched cell types as models: sensory and motor neurons and tracheal terminal cells, and focusing on the influence of organelles, such as the mitochondria and the ribosomes in the single-cell branching process. By analysing these cellular models in parallel we will be able to find common/shared mechanisms of subcellular branching as well as the specific mechanisms involved in each of these cell types.

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Development of TET2 PROTACs to enhance the efficacy of CAR-based cell therapies

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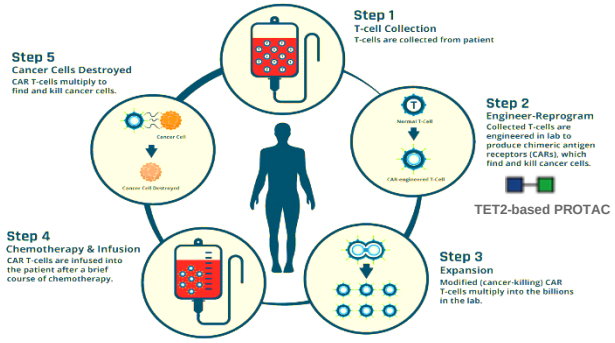
Genetically engineered CD8⁺ T-cells (CAR-T cells) have been progressively used as cancer immunotherapy to treat blood cancers, such as B cell malignancies and multiple myeloma. However, apart from the common side effects associated with CAR-T cell treatments, the efficacy of CAR-T cells is still insufficient. A high percentage of patients relapse some years after CAR-T cell infusion; mainly owing to deficient expansion and persistence of CAR-T cells. [1,2]

In this context, there are some recent studies demonstrating that TET2 might function as a governor of CD8⁺ T-cells differentiation by altering the epigenetic regulation of gene expression. The absence of TET2 results in hypermethylated regions of DNA that diverge the regulation of gene expression. Given this circumstance, the downregulation of TET2 terminates in a remodelled CD8⁺ T-cells memory phenotype, which is distinguished to persist longer in the body and respond faster upon re-exposure to antigens. [3,4] These results suggested us that a PROTAC-based molecule redirecting TET2 to proteasomal degradation could mimic the demonstrated effect provoked by TET2 downregulation. [5]

In this scenario, we aim to design, synthesize and evaluate TET2-based PROTACs that could be beneficial for CAR-based therapies using some of allosteric TET2 ligands that was previously identified by our group.



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Structural characterization of the Kv1.3-KCNE4 ion channel complex

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Voltage-gated potassium channels (Kv) are pore-forming transmembrane proteins that allow the flux of potassium ions through the plasma membrane. KCNE(1-5) proteins are a family of regulatory subunits that associate promiscuously with Kv channels and regulate their function in different aspects. Until now, only one structure of a complex between a Kv and a KCNE has been solved (Kv7.1-KCNE3). Our research focuses on Kv1.3, a channel expressed in a variety of tissues and cell types that plays a crucial role during the immune response. The channel is expressed in leukocytes jointly with KCNE4, an ancillary subunit that negatively regulates Kv1.3 activity. KCNE4 produces a decrease in the magnitude of Kv1.3 currents as a result of the intracellular retention of the channel. Moreover, KCNE4 also accelerates the inactivation of the channel. Our aim is to elucidate the structure of the Kv1.3-KCNE4 channel complex using cryogenic electron microscopy (cryo-EM). With tsA cells that express both Kv1.3 and KCNE4, we demonstrate how both proteins associate. After successful purification of the ion channel complex, we use protein samples to prepare grids and collect data used for 2D classification and reconstruction of a 3D structure. The low-resolution maps show a Kv1.3-KCNE4 complex with an extra density in the voltage-sensor domain (VSD) corresponding to a single KCNE4 subunit present in each tetramer. The experimental data is consistent with AlphaFold 2 predictions that locate the KCNE4 transmembrane close to the VSD or in the cleft between them. Our results pave the way into understanding the structural rearrangements of Kv channels when they associate with KCNE regulatory subunits.

Supported by MICINN/AEI, Spain (PID2020-112647RB-I00, PID2023-148085OB-I00 and 10.13039/501100011033) and FEDER to AF, and National Institute of Neurological Disorders and Stroke NS002945-28 grant to KJS.

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

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

We used the BB rats as a T1D model. BAT was dissected from WT and BB rats at 6, 7, 9, and 11 weeks of age, corresponding to preclinical, onset, and established stages of T1D in the BB model, respectively. The expression of markers of thermogenesis, lipid and glucose metabolism, inflammation, endoplasmic reticulum (ER) stress and ER-associated protein degradation (ERAD) was analyzed by qRT-PCR. BAT's morphology and macrophage infiltration were studied histologically. Our findings reveal that BAT in T1D BB rats is compromised even at the early stages of the disease. While BAT mass is reduced and exhibits increased macrophage infiltration, no significant changes were observed in ER stress, ERAD, or glucose metabolism during this period. However, as the disease progresses, BAT functionality becomes increasingly impaired. These results suggest that BAT may play a protective role in buffering the deleterious effects of T1D during the early stages, when normoglycemia is still maintained. Future studies will focus on a detailed characterization of BAT at the proteomic level to further elucidate these mechanisms.

Investigating Chronic Obstructive Pulmonary Disease through the *Drosophila melanogaster* Tracheal System

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Chronic Obstructive Pulmonary Disease (COPD) is an ongoing lung condition which commonly produces chronic bronchitis and a decrease in lung function. It is caused by damage to the lungs, most commonly through smoking. COPD symptoms such as airflow limitation and respiratory problems affect daily activities. Despite its high prevalence among the population, no effective treatments are available to cure the disease, leaving only treatments to ameliorate its symptoms. This can be considered to be due to a lack of proper understanding of its mechanisms at the molecular level. In previous studies, many genes have been observed to show altered expression levels upon the onset of COPD, and some promising candidates have been identified.

To further understand and characterize the molecular mechanisms behind this disease, we have employed several RNAi lines, targeting different candidate genes thought to be involved in this pathology, to assess their effect on the *Drosophila melanogaster* tracheal system and its development. Specifically, we have knocked down RIO Kinase I (RIOKI), Phosphodiesterase 12 (PDE12) and SAXO downstream of blistered (*sdb*). These genes play a role in many different cell signalling pathways involved in cell proliferation and apoptosis, such as TORC2, AKT, p53, and also in the regulation of smooth muscle function by alteration of the cGMP cycle, providing two plausible mechanisms through which the disease could occur.

In order to assess the effect of the induced genetic alterations, we have observed the development of the tracheal system in *Drosophila melanogaster* larvae at different stages. To this date, our RNAi lines have been able to produce different tracheal system development defects, providing promising perspectives for this research line. However, it will be necessary to establish whether these same pathways are a factor in the onset and maintenance of COPD.

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An in-situ perfusion protocol of rat mesenteric adipose tissue

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The mesenteric adipose tissue (mAT) drains directly from the intestine into the liver via the portal vein so the molecules it secretes could potentially contribute to lipid metabolism and accumulation in the liver. Therefore, it could play a crucial role in obesity and MASLD.

Due to the limited research on mAT and its direct drainage to the liver, the initial aim of this study was to develop a perfusion protocol in rats for mAT. Furthermore, we offer surgical solutions for a broad spectrum of challenges. Additionally, to validate the developed protocol, the functionality of mAT was assessed through monitoring its lipolytic response. Subsequently, mAT was characterized by analysing its lipid composition to ascertain any distinctions from the tissue to which it primarily drains its contents, the liver.

Although the mAT's lipidic content was similar to that of the other AT studied (epididymal and lumbar), it differed significantly from that of the liver. Noteworthy, in the mAT the precursors of active lipids such as linoleic acid (LA) and alfa-linolenic acid (ALA) were found, while higher relative concentrations of arachidonic (AA) and docosahexaenoic acid (DHA) were found in the liver. These results suggest that mAT might serve as a reservoir for those lipids that could modulate the inflammatory process in liver during MASLD evolution.

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Exploring Circadian Control of Extracellular Vesicles

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Extracellular vesicles (EVs) are key mediators of inter-organ communication, carrying bioactive molecules such as proteins, lipids, and RNAs between distant tissues. While their role in systemic physiological regulation is increasingly recognized, how EV release is regulated remains poorly understood. In particular, the connection between circadian clocks endogenous molecular mechanisms that drive 24-hour biological rhythms and EV secretion is still an emerging field of study. Based on preliminary data suggesting circadian clocks regulate EV-related genes in muscle, we hypothesize that EVs contribute to crosstalk between circadian clocks in metabolic tissues, especially between skeletal muscle and liver. Potentially, disruptions in clock-mediated EV crosstalk could play a role in diseases like obesity, type 2 diabetes, and cardiovascular disorders.

This project aims to investigate whether EV concentration varies in a circadian manner in skeletal muscle. To determine this, we will use FACS and western-blot based quantification techniques in 2 settings: in vitro, quantifying EVs released from circadian-synchronized C2C12 -derived myotubes (a cellular model of skeletal muscle) and in vivo, quantifying EVs released in extracellular fluid from mouse skeletal muscle harvested during the day or night. To firstly validate circadian synchronization in our C2C12 vitro models, we'll test synchronization protocols (serum shock, dexamethasone, forskolin) and validate them by qPCR of *ARNTL* and *DBP*, using *L14* and GAPDH as housekeeping genes. We aim with this project to profile the number of EVs released from muscle over 24 hours and follow up work will explore whether the EV cargo and function also follow circadian patterns and if these patterns are interfered in cases of cancer and other diseases.

Ultimately, this research aims to contribute to our understanding of how circadian clocks regulate inter-organ communication via EVs, and how this regulation may be utilized to better understand, diagnose, or even treat metabolic diseases.

Hitting a new target combination to cope with alzheimer's disease

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Alzheimer's disease (AD) is one of our most urgent unmet medical needs, in part due to its multifactorial nature, which makes very challenging the development of efficacious drugs. New therapeutic approaches, such as simultaneous modulation of multiple biological targets with a key pathogenic role are necessary. In this context, our group recently reported the discovery of a novel class of dual inhibitors of the enzymes soluble epoxide hydrolase (sEH) and acetylcholinesterase (AChE) [1], with a multitarget profile *in vitro* and beneficial *in vivo* effects against neuroinflammation and memory impairment. The lead compound showed well-balanced nanomolar potencies at both targets, good blood-brain barrier permeability and no cytotoxicity. However, its suboptimal solubility and metabolic stability might hamper its applicability for the treatment of AD. Here we report a lead optimization campaign, aiming to achieve more favourable DMPK properties, while retaining the high dual potencies and brain permeation of the initial lead. To this end, we have explored the effects of the introduction of different polar substituents in diverse positions of the molecule of the first-generation lead. Here we show the design and synthesis of the new analogues, as well as their *in vitro* activity, DMPK and early safety profiling, and *in vivo* efficacy studies in two mouse models of AD.

Acknowledgements:
This work was supported by grants PID2020-118127RB-I00 and PID2023-147004OB-I00 funded by MICIU/AEI/10.13039/501100011033 and FEDER, EU, and 2021SGR00357 funded by AGAUR.

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Unraveling the post-mitotic role of Rho GTPases during organogenesis

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Rho GTPases are small GTP-binding proteins that function as molecular switches, regulating cytoskeleton dynamics, cell adhesion, migration, and developmental programs. They alternate between an active (GTP-bound) and inactive (GDP-bound) state. When bound to the GTP-bound they interact with different effectors that orchestrate processes as cell migration, differentiation and neuronal development. Their dysregulation has been associated with neurodevelopmental disorders and cancer metastasis. Rho GTPases are highly conserved throughout evolution, sharing more than 80% between *Drosophila* and humans.

This study aims to characterize the role of Rho GTPases, specifically Rac1, Rho1 and Cdc42, which are the most studied members, during the embryonic development of *Drosophila melanogaster*. In particular, we are analysing the organogenesis of the tracheal, the central and the peripheral nervous systems under the effect of dominant-negative (DN) and catalytically active (CA) mutations using the GAL4-UAS system.

We are employing three GAL4 drivers, *btlGAL4 (trachea)*, *elavGAL4 (post-mitotic neurons)* and *p0163GAL4* (neurons of the peripheral nervous system) to drive UAS-transgene expression of DN and CA versions of Rac1, Cdc42 and Rho1. Our preliminary results showed that $Rac1^{V12}$ (CA), $Rac1^{N17}$ (DN) and $Cdc42^{N17}$ (DN) impact the formation of the tracheal system and the nervous system. The role of Rho GTPases in organ formation during embryogenesis may be a general feature conserved across eukaryotes.

Neuroprotective and analgesic activity of 3,4-dihydro-2H-pyrroles and pyrrolidine based-compounds

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Imidazoline I_2 receptors (I_2 -IRs) are altered in Alzheimer's disease (AD) patients and are associated with analgesia. Our research group have reported (2-imidazolin-4-yl)phosphonates derivatives were endowed with outstanding binding affinity and selectivity for I_2-IRs.[1] Herein, we describe the synthesis and the pharmacological evaluation of novel 3,4-dihydro-2*H*-pyrroles and pyrrolidines as I_2 -IRs ligands.

The 3,4-dihydro-2*H*-pyrroles family were synthesized by basic hydrolysis followed by decarboxylation of the starting bicyclic iminophosphonates prepared previously by procedures described by us.[2,3] The optimal ADME and pharmacokinetic profile of a selected compound secured its *in vivo* exploration in a SAMP8 mice revealing improvement in the cognitive impairment and unveiling the mechanism of action by analyzing specific AD biomarkers. In addition, the treatment of a capsaicin-induced mechanical hypersensitivity murine model with this compound reveals analgesic properties devoid of motor coordination issues.[4] To explore the reactivity of the above compounds, the imine function was reduced by catalytic hydrogenation, providing compounds containing a pyrrolidine core. However, this chemical modification leads to a drastic reduction of the affinity for I_2-IRs, demonstrating the importance of maintaining this imine-type double bond.

Overall, the selected 3,4-dihydro-2*H*-pyrrole is a promising preclinical candidate and highlights the I_2-IRs modulation as an innovative therapeutic approach.

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Gut-liver axis: Regulation of hepatic lipid metabolism and inflammation by microbiota extracellular vesicles

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Introduction: The gut-liver axis is a complex bidirectional communication network that regulates crucial metabolic processes, including lipid metabolism. The alteration of the gut microbiota and derived extracellular vesicles (EVs) contributes to the development and progression of various chronic liver diseases. Previous in vitro studies have shown the ability of certain probiotic strains to improve the hepatic lipid profile. **Aims:** Evaluate the ability of interventions based on EVs from the probiotic EcN and the commensal EcoR12 to modulate liver lipid metabolism, oxidative stress and inflammation in an in vivo model of suckling rats, and in the human hepatocyte cell line HepG2.

Methodology: In the in vivo model, equivalent doses of EVs from EcN or EcoR12 were daily administered to the pups by oral gavage, and blood and liver samples were collected on days 8 and 16 of life. Control rats received equal vehicle volume. Regarding the in vitro model, the HepG2 cell line was stimulated for 8 and 24 hours with EVs from both strains at different doses. Cytokine quantification was performed using ELISA, and gene expression analysis was conducted by RT-qPCR. The presence of EVs in the liver was assessed by quantification of LPS and OmpA by ELISA and dot blot, respectively.

Results: The pups receiving EVs from both strains experienced the most significant changes during the early stage (day 8). Administered EVs reached the liver and reduced the expression of inflammatory genes (TNF-A, IL-12), lipid synthesis genes (SREBP1c, FAS, ACC1), and ethanol metabolism genes (CYP2E1). On the other hand, the interventions activated the expression of lipid metabolism genes involved in fatty acid oxidation (CPT1A, PPARA) and antioxidant response (SOD1, CAT, GPX). The results from the in vitro model confirmed the findings observed in the suckling rats.

Conclusion: This study proves the ability of probiotic and microbiota strains to regulate inflammation and lipid metabolism, thus pointing to their potential application as postbiotics for the prevention or treatment of chronic liver diseases.

Mitochondrial Alterations and Impaired Mitophagy in the Development of Insulin Resistance: Insights from Muscle Models

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Metabolic disorders such as type 2 diabetes, obesity, and insulin resistance are characterized by mitochondrial dysfunction in affected tissues. These mitochondria often exhibit altered protein expression and accumulate within cells, contributing to oxidative stress and impaired metabolic function. However, the mechanisms underlying these mitochondrial alterations remain incompletely understood. Skeletal muscle is a key tissue in glucose homeostasis responsible for most insulin-stimulated glucose uptake and therefore, insuline resistance in skeletal muscle significantly contributes to systemic glucose imbalance.

A central hypothesis is that dysregulation of mitochondrial quality control leads to the accumulation of damaged mitochondria. Mitophagy—the selective removal of dysfunctional mitochondria—is a crucial quality control mechanism in this context. Its impairment may exacerbate mitochondrial damage and insulin resistance. To investigate this further, both in vitro and in vivo methods will be employed. C2C12 myotubes, a mouse skeletal muscle cell line, serve as an in vitro model. Treatment with palmitate, a saturated fatty acid elevated in obesity, induces insulin resistance and mitochondrial stress, mimicking key aspects of the diabetic muscle environment. For in vivo studies, mito-QC mice on the C57BL/6J background will be used. This strain allows fluorescent detection of mitophagy in tissues. Mice will be divided into four groups by sex and diet (control diet vs. high-fat). Over 8 weeks, they will undergo metabolic testing: glucose, insulin, and pyruvate tolerance tests, as well as indirect calorimetry to assess energy expenditure. At the end of the protocol, tissues will be collected to assess mitochondrial quality and mitophagy levels. This integrated approach will allow a comprehensive evaluation of the role of mitophagy in mitochondrial dysfunction and insulin resistance, contributing to a better understanding of metabolic disease progression and potential therapeutic strategies.

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Characterization of Greener Solvents for Biomimetic Chromatography

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Biomimetic chromatography is an analytical method that incorporates biological components, such as proteins and phospholipids, into the stationary phase. This approach enables the study of processes of biological and pharmaceutical interest, such as drug transport and cell permeation, and provides helpful information on drug distribution and absorption. However, in many instances biomimetic chromatography uses conventional toxic solvents as mobile phases, mostly acetonitrile and methanol. Because of that, it is necessary to search for greener alternatives to improve the sustainability of chromatographic methods.

In this study we have addressed the use of dimethyl carbonate (DMC) in combination with ethanol and water (2:1 v/v). The solvent properties of these mixtures have been characterized by the Kamlet-Taft solvatochromic parameters, in order to describe the hydrogen bond donor and acceptor ability (α and β) and the polarity (π) of solvents. Seven solvatochromic indicators have been used: 1-ethyl-4-nitrobenze, 4-nitroanisole, 2-nitroanisole, 4-nitrophenol, 4-nitroaniline and Reichartd's dyes ET(30) and ET(33). A spectrophotometer was used to determine the wavenumbers of these indicators in several solvent mixtures.

The results obtained showed that DMC is miscible with ethanol-water (2:1) in all proportions. As the DMC content increases, the hydrogen bond donor capacity (α) decreases rapidly, whereas the hydrogen bond accepting ability (β) and the polarizability (π) showed minor variations. The viscosity of the mixtures was also measured in this work, due to its relevance on the chromatographic back-pressure. Once the mixtures were characterized, we selected the one that could replace the acetonitrile:water as a mobile phase.

These findings demonstrate the potential of DMC as a greener solvent in biomimetic chromatography and also contribute to develop more sustainable analytical methods.

Pemafibrate treatment regulates Vdr levels in a tissue-dependent manner

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Pemafibrate treatment reduces hepatic lipid accumulation and improves insulin sensitivity in rats fed a high-fat diet and fructose-supplemented water. Activation of the vitamin D receptor (VDR) exerts a protective effect against the development of fatty liver and also improves insulin sensitivity. Therefore, the aim of this study was to determine whether the hypolipidemic drug pemafibrate modulates vitamin D receptor (VDR) expression in tissues and to explore its metabolic effects in a rat model of diet-induced metabolic dysfunction-associated steatotic liver disease. To this end, 24 female Sprague-Dawley rats were divided into three groups: Control (standard diet), HFHFr (high-fat diet supplemented with fructose 10% in drinking water (w/v)), and Pema (HFHFr diet + pemafibrate 0.5 mg/kg/day during the last month). Liver, subcutaneous (scWAT), and perigonadal adipose tissues (pWAT) were analyzed post-treatment. Pemafibrate administration significantly increased hepatic *Vdr* levels (1.75X vs. HFHFr, p=0.003), alongside with upregulated expression of fatty acid oxidation genes (*Cpt1a*, *Aco*, *Pdk4*), triglyceride hydrolase (Atgl) and glucokinase (GcK), all positively correlating with *Vdr* expression. Conversely, pWAT exhibited reduced *Vdr* (0.36X, p=0.0003) and *Atgl* (0.57X, p=0.0036) expression levels, after pemafibrate treatment, and both also correlated positively. Moreover, drug administration decreased proinflammatory cytokines (*Il1b*, *Il6*), without changing anti-inflammatory *Il10* in pWAT. However, scWAT *Vdr* expression remained unaffected. These findings demonstrate tissue-specific modulation of VDR by pemafibrate and suggest that VDR may mediate pemafibrate's metabolic effects, though further studies are required to confirm its mechanistic role. This highlights the complex interplay between pemafibrate, VDR signaling, and tissue-specific metabolic responses.

Design and evaluation of novel acetamides as potent inhibitors of soluble epoxide hydrolase

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Soluble epoxide hydrolase (sEH) is an enzyme involved in the metabolism of epoxyeicosatrienoic acids (EETs), with anti-inflammatory and analgesic properties. Thus, the inhibition of sEH is a promising approach for pain treatment, where effective and safe options remain limited.

Although numerous sEH inhibitors (sEHIs) have been developed, clinical progress has been limited. Most of the inhibitors are urea-based compounds — such as the standard inhibitor TPPU — whose poor aqueous solubility poses major challenges for formulation and bioavailability. Attempts to address this issue through the substitution of the urea moiety with an amide have generally led to a loss of potency[1,2].

To address this, our group previously modified the TPPU scaffold by: a) replacing the urea with an amide group; b) substituting the propionyl group with a benzyl moiety to introduce a protonable centre; and c) replacing the trifluoromethoxy (CF_3) group with a pentafluorosulfanyl (SF_5) substituent on the left-hand aromatic ring. While this restored activity against human sEH and preserved favourable physicochemical properties of the amides, it did not improve potency in mouse and rat enzymes, an essential step for preclinical and clinical development.

In the present study, we further optimized this scaffold to enhance cross-species potency. Following a Topliss approach, we introduced substituents on the right-hand benzyl group, generating derivatives with low nanomolar potency and balanced activity across human, mouse, and rat sEH.

The incorporation of the SF_5 group was key to these results and highlights the value of this substituent for developing next-generation sEH inhibitors for pain treatment.

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Subcloning KCNE4 in pcDNA

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In lymphocytes, Kv1.3, a voltage-gated potassium channel which belongs to the Shaker family (Kv1), plays a crucial role in a variety of physiological processes, including immune cell activation, proliferation and apoptosis. In addition, another Kv1 isoform, named Kv1.5 associates to Kv1.3 to form functional channels. Furthermore, KCNE4, a transmembrane protein which regulates the function of voltage-potassium channels is also present in leukocytes. KCNE4 modulates the biophysical properties, trafficking, and surface expression of Kv1.3. In order to decipher the molecular interactions between Kv1.3, Kv1.5, KCNE4 the laboratory has a series of cDNA constructs with fluorescent tags. However, some un-tagged proteins are needed for number of purposes. The primary objective of this project was to design a subcloning strategy to obtain a construct of KCNE4 in the pcDNA3.1 expression vector without fluorescent tags. The starting material included a KCNE4 which a C-terminal CFP tag. KCNE4 will be subcloned into a pcDNA3.1. Removal of the CFP tag was essential for future experiments that may be affected by the presence of a fluorescent protein, such as protein–protein interaction studies. The resulting untagged KCNE4-pcDNA3.1 construct will allow a more accurate investigation of KCNE4 interactions, especially in the context of heterotetrameric channel complexes formed by Kv1.3 and Kv1.5. Further work includes co-immunoprecipitation assays, which will help to understand the molecular mechanisms by which KCNE4 modulates these channel assemblies.

Supported by the Ministerio de Ciencia e Innovación (PID2020-112647RB-I00 and PID2023-148085OB-I00) and AEI (10.13039/501100011033), Spain and European Regional Development Fund (FEDER).

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Hunting for low-addictive opioid-drugs: ligands with weak potency on opioid-galanin receptor heteromers

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μ-opioid receptor (MOR) agonists are the most effective drugs to treat chronic pain[1]. MORs belong to the superfamily of G protein-coupled receptors (GPCRs) and mediate both the analgesic and addictive effects of opioids[1]. MOR and galanin 1 receptor (Gal_1R) have shown to form heteromers in both the ventral tegmental area (VTA) and the nucleus accumbens (NAc). Moreover, when opioids bind to MOR-Gal_1R heteromers, they modulate dopaminergic cell function in these areas, thereby activating the opioid reward pathway[2-4]. Therefore, the main aim of this project was to search for non-addictive opioid drugs useful to treat chronic pain. To achieve this objective, we used different pharmacological, biochemical, and functional techniques in transfected cells with MOR or MOR and Gal_1R, as well as in vivo assays in rodents. Using radioligand binding assays we have found that (S)-methadone was the MOR agonist with worst affinity in both MOR and MOR-Gal_1R cells. By G-protein BRET activation and cAMP production assays we have found that all MOR agonists tended to exhibit better potency in MOR cells compared to MOR-Gal_1R cells. Buprenorphine, PZM21 and (S)-methadone showed the worst potency on MOR-Gal_1R cells. Additionally, buprenorphine and PZM21 exhibited partial agonism in MOR-Gal_1R cells. Using several behavioral assays, such as drug self-administration and tests to assess the analgesic properties of (R)-methadone, (S)-methadone and (R,S)-methadone, we demonstrated that (S)-methadone induced analgesia with similar efficacy as (R)-methadone but was not self-administered, indicating low addictive profile. Our results suggest that, since MOR-Gal_1R heteromers mediate the dopaminergic effects of opioids, oligomerization-selective[5,6] opioids with low potency and/or efficacy for this heteromer may have low addictive profile while maintaining the same analgesic properties, and therefore could be used clinically as analgesics. In a future study, we will assess the analgesic and addictive properties of other opioids, such as PZM21 and buprenorphine.

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Acknowledgements:

This work was supported by the grant PID2020-113938RB-I00 and PID2023-146914OB-I00 (MCIN/AEI/ 10.13039/501100011033 and FEDER) and the grant 2021-SGR-00230 from “Generalitat de Catalunya”, Spain (VC, EM, VCA, NL, CRC). UBPredocs fellowship from the University of Barcelona (NL). National Institute on Drug Abuse, USA, intramural research funds (SF, MHB, MM).

Exploring the Role of Mfn1 Ablation in Modulating Lipid Droplet Metabolism

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The mitochondria are dynamic cellular organelles responsible for energy production. Mitochondrial morphology, dynamic and function are regulated by the fusion and fission processes. Mitofusins are one of the proteins that are involved in the outer membrane fusion of the mitochondria. At this regard, the pleiotropic role of mitofusin I (MFN1) is still unknown.MFN1 has been shown in recent research to have a wider range of roles outside mitochondrial dynamics, such as important role in lipid metabolism and inter-organelle communication. This study's objective is to investigate the possible effects of MFN1 on cell metabolism and mitochondrial function.In the present study, we employed mouse embryonic fibroblast (MEF) cells, both wild-type (WT) and knockout for MFN1(KO1) as in vitro model. For in vivo studies male C57BL/6 Mfn1 floxed mice (WT) and liver specific MFN1 knockout(L-KO1) were used that all were eleven weeks old. Microscopic analysis of cells and liver tissue revealed an increase in number and volume of lipid droplets in the absent of MFN1. Furthermore, liver samples from L-KO1 mice had higher level of lipid droplet -associated proteins like PLIN2 and PLIN5 than control group, based on western blot analysis. A decrease in beta-oxidation was observed in basal and starvation condition in KO1 cells, which explains the increase in the amount of lipids. Due to the increased requirement for fatty acid oxidation, confocal imaging of cells harvested 24 hours under starvation showed a reduction in the overlap between lipid droplets and mitochondria compared to the control group. Therefore, we hypothesize that MFN1 mediate this link during starvation.

Keywords: Mfn1, Lipophagy, Lipid

Identification of novel target genes differentially expressed in white adipose tissue of healthy and children with obesity

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Childhood obesity is a rising global health crisis, with Spain among the countries with the highest prevalence. White adipose tissue (WAT) plays a critical role in obesity-associated metabolic dysfunctions. Current research primarily focuses on adults and this created a significant gap in understanding the molecular mechanisms driving obesity in children (1,2).

To identify differentially expressed genes in WAT, we analyzed samples from a pediatric cohort of children aged 2 to 12 years. The cohort included 26 children with normal weight and 9 children with obesity.

RNA sequencing (RNA-seq) revealed 66 differentially expressed genes in WAT from children with obesity compared to their healthy peers—46 genes were upregulated and 20 were downregulated. Comparative analysis with adult WAT datasets uncovered distinct age-specific molecular signatures. Notably, we identified genes involved in adipogenesis, lipid metabolism, and inflammation. Some of these genes exhibited expression patterns similar to those seen in adults, while others showed opposing expression profiles, highlighting key age-dependent differences in the molecular mechanisms underlying childhood obesity.

Our findings suggest potential targets for early intervention in childhood obesity. Functional analysis using gene editing techniques will further investigate how these selected genes influence adipocyte biology and obesity-related dysfunctions.

[1] https://doi.org/10.3390/nu15183968

[2] https://doi.org/10.53435/funj.00925

Unveiling Metabolic Interactions Between Patient Derived Organoids and Tumour-Infiltrating Lymphocytes in Colorectal Cancer

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Metastatic colorectal cancer (mCRC) patients often develop resistance to standard therapies; chemotherapy (FOLFOX) for MSS tumours, and immunotherapy (Pembroluzimab, anti-PD-1) for MSI tumours; highlighting the need for new strategies. Previous results from our group suggests two main metabolic subtypes: IMC1 (glycolytic phenotype; Warburg effect) and IMC2 (flexible metabolic phenotype-mitochondrial and glycolytic). Moreover, recent studies suggest that the Tumour Microenvironment (TME) plays an important role in pharmacological response and development of treatment resistance. Tumour-infiltrated-lymphocytes (TILs) have a relevant role in tumour progression and, possibly, contribute to the development of therapy resistant in CRC. The aim of this project is to perform a metabolic characterization of patient-derived-organoids (PDOs), TILs and their interaction at the metabolic level to understand better the mechanisms behind the development of pharmaceutical resistance to immune-checkpoint inhibitors (ICI) therapies, providing us critical insights to progress on these therapeutic strategies. Moreover, all this experimental characterization, combined with computational tools that include GSMM and AI, will open a new spectrum of therapeutic opportunities such as a new prediction tool to design new therapeutic focuses, potentially increasing the efficacy of immunotherapy. Here we will present the procedures to obtain PDOs and TILs from patient biopsies, and the experiments carried out to optimize their co-culture conditions.

Acknowledgments

This work is part of the I+D+i project PID2023--150539OB-I00, financed by MICIU/AEI/10.13039/501100011033/-European Commission FEDER funds—"Una manera de hacer Europa", the Networked Researched Center on Liver and Digestive Diseases (Centro de Investigación Biomédica en Red en Enfermedades Hepáticas y Digestivas, CIBER-EHD; EHD20PI03 and CB17/04/00023), the Catalan Agency for Universities and Research (AGAUR) 2021-SGR-00350) and ICREA Foundation (ICREA Academia award granted to M. Cascante).

Neuroprotective effects of N-acetylcysteine-amide in a survival mouse model of acute paraoxon poisoning

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Organophosphorus compounds, such as paraoxon-ethyl (POX) are widely used in agriculture as pesticides and have also been employed as chemical warfare agents due to their potent neurotoxicity, mainly caused by acetylcholinesterase inhibition. Acute exposure can lead to organophosphorus poisoning (OPP), triggering a cholinergic crisis thus leading to neurodegeneration and long-term brain damage. Despite the severity of these effects, standard therapies have remained unchanged for decades and fail to address secondary toxic effects such as oxidative stress and inflammation.

This study investigates the therapeutic potential of N-acetylcysteine-amide (AD4), a brain-permeable antioxidant peptide, to mitigate the secondary neurotoxic effects of OPP in a murine model of acute POX intoxication. Male Swiss CD-1 mice were administered POX (4 mg/kg) subcutaneously, followed by atropine sulfate (4 mg/kg) and pralidoxime chloride (2-PAM) (25 mg/kg) intraperitoneally (i.p.) one minute later. Diazepam (5 mg/kg, i.p.) and a second dose of 2-PAM were given one hour later to manage seizures. A separate group received AD4 (150 mg/kg, i.p.) at 2- and 6-hours post-exposure.

Cognitive performance was assessed using the Novel Object Recognition Test (NORT) 9–11 days after treatment. Hippocampal tissue was collected 72 hours post-exposure to evaluate oxidative stress markers (4-HNE, GPx1) via western blot and ELISA assay. Furthermore, neuroinflammation was studied through GFAP immunostaining.

Results demonstrated that AD4 treatment significantly improved recognition memory ($p<0.001$), prevented the POX-induced rise in 4-HNE levels, and restored GPx1 expression ($p<0.001$). Additionally, AD4 attenuated GFAP upregulation in the dentate gyrus and CA3 areas of the hippocampus ($p<0.05$), though not in CA1.

In conclusion, these findings highlight the potential of AD4 as a neuroprotective agent capable of counteracting the secondary damage associated with acute organophosphate exposure, offering a promising complementary strategy to current treatments.

Characterization of liposomes for biomimetic chromatography to emulate the distribution of drugs in biological membranes

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The pharmacological potential of bioactive molecules is strongly influenced by their affinity for biological membranes, as this property governs key pharmacokinetic processes such as absorption, distribution, metabolism, excretion, and toxicity (ADMET). Consequently, the drug discovery process requires reliable and efficient analytical methods to assess membrane affinity at early stages. Traditional experimental approaches using biological systems often present technical limitations, high costs, time constraints, and ethical concerns associated with in vivo studies.

In this work, we propose an alternative approach by synthesizing liposomes with well-defined compositions to emulate different types of biological membranes. The studied liposomes are made of the phospholipids phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine, and contain also a given percentage of palmitic acid.

The interaction of selected drugs with these liposomal systems will be investigated using liposome electrokinetic chromatography (LEKC). In this technique, liposomes act as a chromatographic pseudostationary phase, and the distribution of drugs between an aqueous buffer at physiological pH and the liposomes is studied. To understand these interactions, the LECK system is characterized using the Abraham solvation model, widely applied for describing the distribution of compounds between two liquid phases:

$$\log k = c + eE + sS + aA + bB + vV$$

First, the work has focused on the selection of the electroosmotic flow and the liposome markers, needed in the determination of the retention factor (*k*) of solutes. Next, k has been determined for a set of compounds, and correlated with the Abraham descriptors (*E*, *S*, *A*, *B*, and *V*). This correlation provides the coefficients (*e*, *s*, *a*, *b* and *v*), that characterize the liposomal system and explain how the interaction between the solutes and the pseudo-stationary phase take place. Finally, the liposomal systems will be compared other biomimetic systems characterized with the same model.

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PPARβ/δ upregulates the insulin receptor β subunit in skeletal muscle by reducing lysosomal activity and EphB4 levels

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Background: The increased degradation of the insulin receptor β subunit (InsRβ) in lysosomes contributes to the development of insulin resistance and type 2 diabetes mellitus. Endoplasmic reticulum (ER) stress contributes to insulin resistance through several mechanisms, including the reduction of InsRβ levels. Here, we examined how peroxisome proliferator-activated receptor (PPAR)β/δ regulates InsRβ levels in mouse skeletal muscle and C2C12 myotubes exposed to the ER stressor tunicamycin.

Methods: Wild-type (WT) and Ppard$^{-/-}$mice, WT mice treated with vehicle or the PPARβ/δ agonist GW501516, and C2C12 myotubes treated with the ER stressor tunicamycin or different activators or inhibitors were used.

Results: Ppard$^{-/-}$mice displayed reduced InsRβ protein levels in their skeletal muscle compared to wild-type (WT) mice, while the PPARβ/δ agonist GW501516 increased its levels in WT mice. Co-incubation of tunicamycin-exposed C2C12 myotubes with GW501516 partially reversed the decrease in InsRβ protein levels, attenuating both ER stress and the increase in lysosomal activity. In addition, the protein levels of the tyrosine kinase ephrin receptor B4 (EphB4), which binds to the InsRβ and facilitates its endocytosis and degradation in lysosomes, were increased in the skeletal muscle of Ppard$^{-/-}$mice, with GW501516 reducing its levels in the skeletal muscle of WT mice.

Conclusions: Overall, these findings reveal that PPARβ/δ activation increases InsRβ levels by alleviating ER stress and lysosomal degradation

Keywords: ER stress; EphB4; GW5101516; InsRβ; PPARβ/δ

N-[(thiophen-3-yl)methyl]benzamides as influenza virus fusion inhibitors acting on H1 and H5 hemagglutinins

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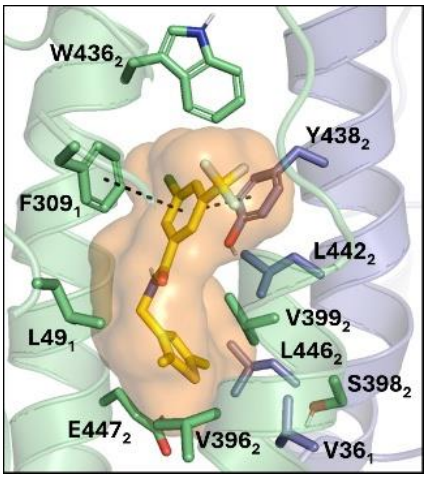
Influenza A and B viruses are highly contagious respiratory pathogens and the cause of annual epidemics with a high medical and economic burden. Although a few anti-influenza drugs are in clinical use, there is an increasing number of drug-resistant variants. Therefore, the development of newer anti-influenza drugs, preferably endowed with innovative mechanisms of action, is of the greatest relevance.

Hemagglutinin (HA), a homotrimer located on the viral envelope that is crucial for viral infectivity, has gained interest as a potential target for anti-influenza treatment[1]. In this work[2], we disclose a series of benzamides endowed with potent anti-influenza activity able to inhibit both H1 and H5 HAs. Antiviral assays have revealed that they act as fusion inhibitors. In conjunction with the analysis of resistance-associated mutations, computational studies have provided a structural model to rationalize the structure-activity relationships and selectivity.



Acknowledgements

We acknowledge the funding from the Spanish *Ministerio de Ciencia, Innovación y Universidades*, MICIU/AEI/10.13039/501100011033: Grants PID2020-118127RB-I00 (to SV) and PID2020-117646R-I00 (to FJL), and from *Fundació La Marató de TV3* (Grant 201832 to LN and SV).

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ISSN 3081-4510