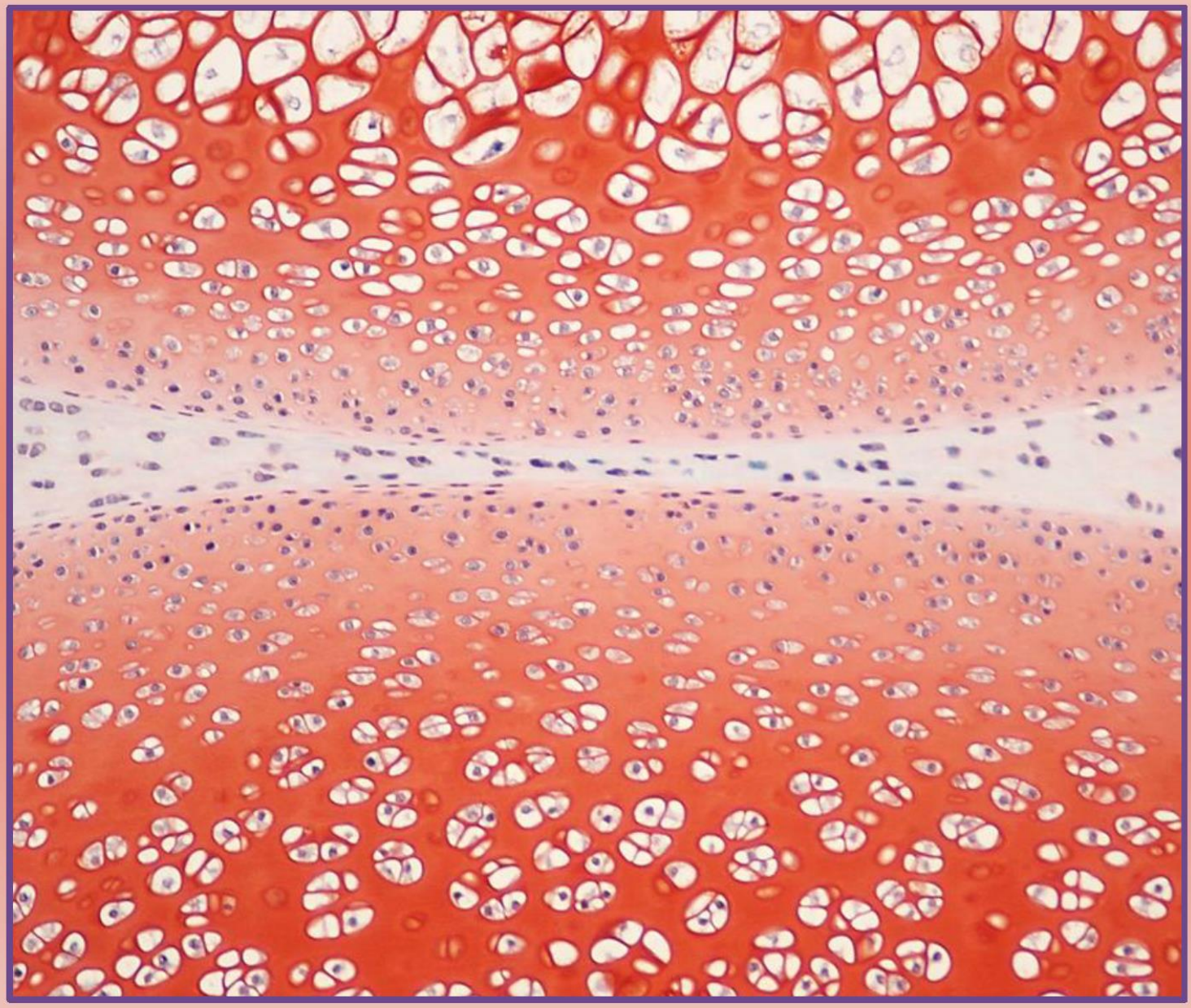
XVII Jornada Científica del Departament de Bioquímica i Biologia Molecular



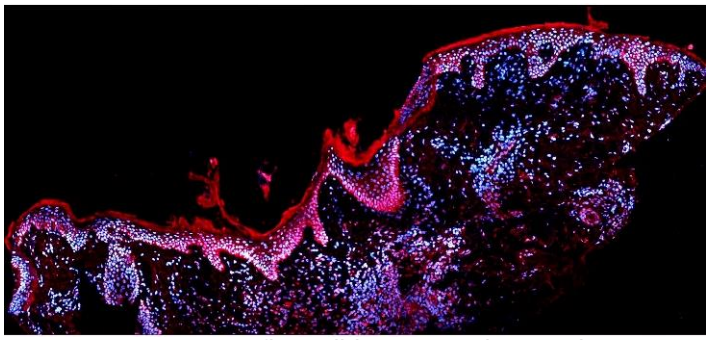
Sala d'Actes, Facultat de Biociències
17 de juny de 2021

DEPARTAMENT DE **BIOQUÍMICA I DE BIOLOGIA MOLECULAR**

UAB CEI CAMPUS D'EXCEL·LÈNCIA INTERNACIONAL

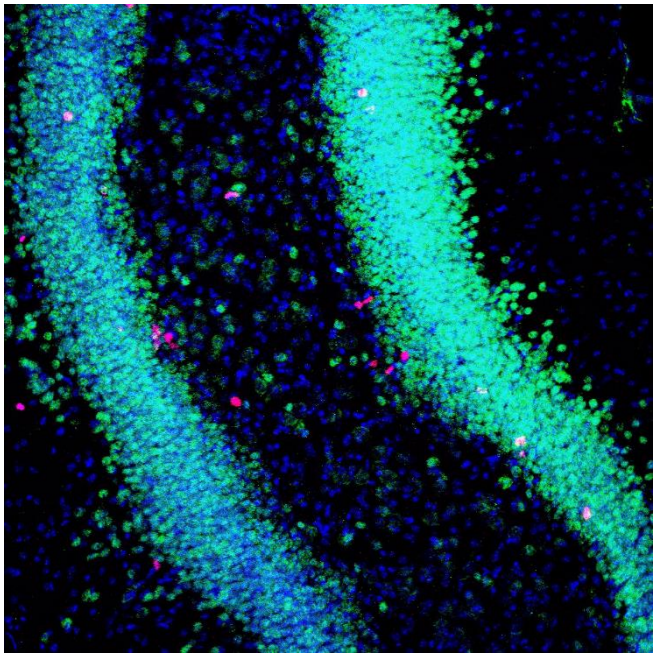
Facultat Biociències UAB

Finalista: Ratpenat en la nit.
Immunofluorescència de proteina AMPK1alpha en la pell de pacients amb Lupus Eritematos Cutàni. Realizada amb el microscopi d'alta ressolució confocal Zeiss LSM980.



Fotografia cedida per Sandra Domingo Bover
Vall d'Hebron Institut de Recerca (VHIR), Lupus Unit

Finalista: Adult neurogenesis in the mouse dentate gyrus.
Quantification of newborn neurons in the dentate gyrus of adult mice. Cellular nuclei stained with hoechst (blue). Neuronal nuclei stained with anti-NeuN antigen Ab (green). Newborn cells generated in presence of the BrdU molecule, stained with anti-BrdU Ab (magenta). These cells incorporated the molecule in the DNA, maintaining this tag during the neuronal differentiation process. Neuronal proliferation marker Ki67 (red) showing cells dividing at the sample collection moment. Double positive NeuN/BrdU cells represent newly differentiated neurons, allowing the quantification of neurons generated during the BrdU treatment period.



Fortografia cedida per Joan Roig-Soriano
Institut de Neurociències (INc), Department of Biochemistry and Molecular Biology

PORTADA: Tinció de Safranina O en cartílag articular.
Tall histològic d'un genoll de rata d'un mes d'edat tenyit amb Safranina O. El cartílag articular del fèmur (dalt) i de la tíbia (baix) apareix tenyit de color vermell i els nuclis dels condròcits de color blau.

Fotografia cedida per Joan Bertolín
Centre de Biotecnologia Animal i Teràpia Gènica (CBATEG)

Benvolgudes companyes i companys,

Des de la Direcció del Departament us donem la benvinguda a la **XVII Jornada Científica del Departament de Bioquímica i de Biologia Molecular**, tot agraint-vos la vostra presencia i contribució a l'acte en aquests moments tant especials.

Així, i després de la forçada cancel·lació del passat any, les diferents Unitats Departamentals, el personal adscrit als Instituts i Centres de Recerca, així com els doctorands del programa de Bioquímica, Biologia Molecular i Biomedicina ens tornem a retrobar en una Jornada que ja forma part de la historia del Departament. Enguany, tanquem les **Jornades de Biorecerca – UA*Bio*** que es realitzaran al llarg de la setmana a la Facultat de Biociències amb el Dept. de Genètica i Microbiologia, el Dept. de Biologia Animal, Vegetal i Ecologia, el Dept. de Biologia Cel·lular, Fisiologia i Immunologia i finalment el nostre Departament.

Volem que la Jornada, una vegada més, sigui una plataforma efectiva per a la comunicació i debat de la nostra recerca, idees i projectes. En aquest sentit, els esforços per apropar l'esdeveniment al seu format més presencial han estat ingents per part de tothom. Esperem que això serveixi per facilitar la interacció amb els companys i l'establiment de noves col·laboracions, tant amb els grups del Departament com amb altres grups de recerca de l'àmbit de Biociències i Biomedicina, tot donant una visió global del nostre potencial científic.

Desitgem que la Jornada us sigui ben profitosa!

Assumpcio Bosch, Jose Ramon Bayascas, Monica Lluch i Montserrat Godia
Directora, Secretari Acadèmic, Gestora, i Secretària del Departament

Bellaterra, 10 de Juny de 2021

Comitè Científic de la XVI Jornada

Dr. Antonio Casamayor	*Unitat de Bioquímica Facultat de Veterinària*
Dra. Verónica Jiménez	*Centre Biotecnologia Animal i Teràpia Gènica (CBATEG)*
Dra. Julia Lorenzo	*Institut de Biotecnologia i de Biomedicina (IBB)*
Dr. Alfredo Miñano	*Institut de Neurociències (INc)*
Dr. Àlex Peràlvarez	*Unitat de Biofísica. Facultat de Medicina*
Dra. Alicia Roque	*Unitat de Bioquímica. Facultat de Biociències*
Dr. Victor Yuste	*Unitat de Bioquímica. Facultat de Medicina*

Dr. Jose Ramon Bayascas	*Secretari Acadèmic de Departament*

Amb l'especial contribució de l'exdirector del Departament, Dr. Joaquin Ariño, en l'edició i producció d'aquest llibre d'abstracts.

09:00	**Inauguració i presentació**	Dr. Jaume Farrés – Degà Facultat Biociències
		Dra. Assumpció Bosch – Directora del Departament
		Dr. José Ramón Bayascas – Secretari Acadèmic del Departament
09:15	**Primera sessió d'exposicions orals**	Moderador: Dr. Jose Aguilera
09:15	LPS treatment enhances the regenerative capacity of mesenchymal stromal cells-derived exosomes	Aina Areny-Balagueró
		Institut d'Investigació I Innvovació Parc Taulí (I3PT), Sabadell
09:30	lAAV-mediated αKlotho isoforms expression in senescent mice protects from aging-associated phenotype progression	Joan Roig-Soriano
		Institut de Neurociències i Unitat de Bioquímica, Facultat de Medicina
09:45	Dysfunctional mitochondrial translation and combined oxidative phosphorylation deficiency in a mouse model of the hepatoencephalopathy due to mutations in GFM1	Miguel Molina-Berenguer
		Research Group on Neuromuscular and Mitochondrial Diseases, Vall d'Hebron Research Institute (VHIR)
10:00	Exploring the Role of GSK3 on D2 Autoreceptor Signaling Pathways	Sally Hamdon
		Institut de Neurociències i Unitat de Bioquímica, Facultat de Medicina
10:15	m6A a key player in histone H1 mRNA regulation	Daniel García-Gomis
		Unitat de Bioquímica. Facultat de Biociències
10:30	**VIRTUAL COFFEE BREAK**	
10:45	**Short communication lighting talks**	Sessió 1: Biochemistry of the Nervous System Sala d' Actes
		Sessió 2: Structure, Bioinformatics and Biotecnology Sala de Graus
12:00	**Conferència Plenària, Sala d'Actes**	Presentació: Dra. Assumpció Bosch Assistència: Vr. de Recerca i de Transferència, Dr. Armand Sánchez Bonastre
	Covid 19: Molecular pathophysiology and staging of a new disease. Biological and clinical implications	**Dr. Carlos Cordón-Cardo**
		Department of Pathology, Molecular and Cell-Based Medicine, Icahn School of Medicine, Mount Sinai, New York, USA
13.00	**FI DE LA SESSIÓ MATINAL**	

14:30	**Segona sessió d'exposicions orals**	Moderador: Dr. Emili Itarte
14:30	Unraveling the crucial role of senescence in breast cancer progression	Marta Lalinde *Vall d'Hebron Institute of Oncology (VHIO)*
14:45	Adquisición de mutaciones non-driver durante el seguimiento en pacientes con policitemia vera y trombocitemia esencial JAK2 positivos	Alicia Senín Magan *Servicio de Hematología, Hospital Universitari Germans Trias i Pujol*
15:00	In vitro and in vivo studies of novel aldehyde dehydrogenase inhibitors for the treatment of glioblastoma	Rafael Jiménez Aguilar *Institut de Biomedicina i Biotecnologia i Unitat de Bioquímica. Facultat de Biociències*
15:15	A self-stimulatory Wnt5a/Snail1 axis controls colon tumor chemo-resistance and invasion	Guillem Fuertes Marin *Centre d'estudis en Biofísica (CEB) i Unitat de Biofísica, Facultat de Medicina*
15:30	**VIRTUAL COFFEE BREAK**	
15:45	**Short communication lighting talks**	Sessió 3: Cancer and Cell Signaling Sala d' Actes Sessió 4: Metabolism, Pathology and Gene Therapy Sala de Graus
17:15	**ACTE DE CLOENDA**	

SALA D'ACTES: ORALS, PLENÀRIA I SHORT TALK SESSIONS 1 (BIOCHEMISTRY OF THE NERVOUS SYSTEM) i 3 (CANCER AND CELL SIGNALING)

SALA DE GRAUS: SHORT TALK SESSIONS 2 (STRUCTURE, BIOINFORMATICS AND BIOTECHNOLOGY) i 4 (METABOLISM, PATHOLOGY AND GENE THERAPY)

ÍNDEX

O-01

LPS treatment enhances the regenerative capacity of mesenchymal stromal cells-derived exosomes

Areny-Balagueró, A[1,2,4]; Camprubí-Rimblas, M[1,2,3]; Guillamat-Prats, R[1,3]; Roig, A[4]; Blanch, L[1,2,3,5]; Artigas, A[1,2,3,5]; Closa, D[6]

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Background: Sepsis is a complex syndrome produced by a systemic infection that is accompanied by a dysregulated host immune response, affecting principally the lungs and causing, in many cases, an acute respiratory distress syndrome (ARDS) associated with a mortality rate of 40%. Despite decades of research, there is still a lack of a specific treatment targeting the regeneration of lung tissue injury. It is well established that the administration of mesenchymal stromal cells (MSC) has a remarkable therapeutic effectiveness and that its paracrine activity, mediated by the secretion of exosomes, has a crucial role on MSCs' action (5).

Main objective: In this study we aimed to determine the effect of the exosomes from MSC *in vitro* on cell proliferation, and to observe how pre-conditioning the MSCs to a septic environment, changes their exosomes' content and consequently, their regenerative capacity.

Methodology: MSC were isolated from male Sprague–Dawley rats' femora and tibiae and cultured in non pre-stimulated and pre-stimulated (with lipopolysaccharide (LPS)) conditions. The secreted exosomes were obtained via standard ultracentrifugation protocol. Cell proliferation experiments were conducted by treating two different epithelial cell lines (BICR-18 and CAPAN-2) with exosomes from MSC and pre-stimulated MSC through an *in vitro* wound healing and a MTT assay. Exosomal protein profile was determined by a liquid chromatography-mass spectrometry analysis.

Results: The treatment of epithelial cells with exosomes derived from pre-stimulated MSC (LPS exosomes) increases a 10% its capacity to proliferate and to regenerate the wound in comparison with the epithelial cells treated with exosomes derived from non pre-stimulated MSC. The analysis of the protein content of both types of exosomes, which confirmed that pre-conditioning MSCs to a septic environment modifies the protein cargo of its exosomes, resulting in the appearance of proteins related to cell cycle regulation and cell proliferation.

Conclusion: Pre-stimulating MSC with LPS (mimicking a septic environment) enhances their paracrine activity and improves the reparative capacity of their secreted exosomes changing their protein content.

O-02

AAV-mediated αKlotho isoforms expression in senescent mice protects from aging-associated phenotype progression

Roig-Soriano, J; Griñán-Ferré, C; Espinosa-Parrilla, JF; Abraham, CR; Bosch, A; Pallàs, M; Chillon, M

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Senescence represents a stage in life associated with elevated incidence of diseases and increased risk of mortality. This is due to accumulation of metabolic damage and a decrease on the protective systems of the organism. Aging presents general characteristic hallmarks observed in most organisms which include epigenetic alterations, chronic inflammation, neuronal dysfunction, and physical status worsening. In this context, we explored in SAMP8 mouse model the effect of enhancing the expression of a well-known aging-protective factor, Klotho, testing independently its two main isoforms.

During this study, either s-KL or m-KL was over-expressed in SAMP8 animals by intraventricular administration of AAV9 vectors. After 2 months, a significant improvement in physical condition and cognitive performance of these animals was observed. Epigenetic landscape was recovered for the analyzed global markers DNA methylation (5-mC), hydroxymethylation (5-hmC), as well as restoring acetylation levels of H3 and H4. Inflammatory mediators in central nervous system like TNF-α and IL-10 recovered senescence-accelerated-mouse resistant 1 (SAMR1) healthy control levels of gene expression, similarly to Iba1 and SA β-gal histological markers.

This work presents evidence of the beneficial pleiotropic role of Klotho as an antiaging therapy as well as new specific functions of the two main KL isoforms over the phenotype of an aging mouse model.

O-03

Dysfunctional mitochondrial translation and combined oxidative phosphorylation deficiency in a mouse model of the hepatoencephalopathy due to mutations in *GFM1*.

Molina-Berenguer, M[1,2]; Vila-Julià, F[1,2]; Pérez-Ramos, S[1,2]; Salcedo-Allende, MT[3]; Cámara, Y[1,2]; Torres-Torronteras, J[1,2]; Martí, R[1,2]

1: Research Group on Neuromuscular and Mitochondrial Diseases, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain; 2: Biomedical Network Research Centre on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain; 3: Human Pathology Department, Vall d'Hebron University Hospital, Barcelona, Catalonia, Spain.

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Hepatoencephalopathy due to combined oxidative phosphorylation deficiency type 1 (COXPD1) is a mitochondrial translation disorder caused by mutations in *GFM1*, a nuclear gene encoding the mitochondrial elongation factor G1 (EFG1). Patients with COXPD1 typically present hepatoencephalopathy early after birth with rapid disease progression, and usually die in the first weeks or years of life. Currently available experimental models for this disease are based on patient-derived cell lines, but no *in vivo* models had been generated so far.

We have generated two different mouse models: a *Gfm1* knock-in (KI) harboring the p.R671C missense mutation, found in at least ten patients who survived more than one year, and a *Gfm1* knock-out (KO) model. Homozygous KO mice ($Gfm1^{-/-}$) were embryonically lethal whereas homozygous KI ($Gfm1^{R671C/R671C}$) mice were viable and showed normal growth. R671C mutation in *Gfm1* caused drastic reductions in the mitochondrial EFG1 protein content in different organs. Six- to eight-week-old $Gfm1^{R671C/R671C}$ mice showed partial reductions of *in organello* mitochondrial translation and respiratory complex IV enzyme activity in the liver. The mitochondrial translation remained reduced in the long term (50 weeks of age), but the complex IV deficiency was no longer observed.

Compound heterozygous $Gfm1^{R671C/-}$ mice showed normal survival at 80 weeks. EFG1 protein reductions observed in liver and brain mitochondrial fractions were more pronounced in $Gfm1^{R671C/-}$ than in $Gfm1^{R671C/R671C}$ mice. At 8 and 30 weeks of age, their mitochondrial translation rates were significantly reduced in both tissues and mice showed combined oxidative phosphorylation deficiency (reduced complex I and IV activities in liver and brain) due to a reduction of the assembled complexes.

We conclude that the compound heterozygous $Gfm1^{R671C/KO}$ mouse presents a clear dysfunctional molecular phenotype, showing impaired mitochondrial translation and combined respiratory chain dysfunction, making it a suitable animal model for the study of COXPD1.

O-04

Exploring the Role of GSK3 on D2 Autoreceptor Signaling Pathways

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The activation of presynaptic D2 autoreceptors in the striatum inhibits dopamine synthesis. This effect has been associated to changes in phosphorylation of tyrosine hydroxylase -the rate-limiting enzyme of brain dopamine biosynthesis-but the signaling mechanisms involved are not yet fully understood. We are currently examining the hypothesis that both the G-protein and β-arrestin signal transduction pathways could be involved. And since GSK3 is a partner in the β-arrestin pathway, we decided to further investigate glycogen synthase kinases. We have found that CHIR-99021 and SB-216763, which are both GSK3 inhibitors, significantly reduce dopamine accumulation in rat striatum ex vivo. However, CHIR displayed a higher pharmacological inhibition than SB, so moving forward we focused on CHIR in subsequent testing. We were also able to demonstrate that lithium chloride, which is known to have antipsychotic properties and inhibit GSK3, was also able to significantly reduce dopamine accumulation in rat striatum ex vivo. This shows that GSK3 plays a key role in the presynaptic D2 autoreceptor signal transduction pathway, which could be of interest as an alternative target for schizophrenia treatment.

Based on our first result, we hypothesized that CHIR affects the phosphorylation of the serine residues of TH at Ser19, Ser31, and Ser40. To test this hypothesis, we performed western blots but we could only detect a slight decrease at Ser19.

We thought that a more sensitive technique such as mass spectrometry could be better suited to search for changes in TH phosphorylation. Therefore, we immunoprecipitated TH using both a monoclonal and polyclonal antibody. Our mass spectrometry analysis of TH in control and CHIR-treated samples revealed decreases in phosphorylation in CHIR-treated samples at Ser19 and Ser31, although Ser40 could not be detected.

In order to look for alternative explanations of CHIR action, we characterized TH protein interactions by coimmunoprecipitating TH from rat brain. Subsequent analysis allowed us to identify α-synuclein and several 14-3-3 isoforms, which are known to interact with TH, as well as an abundance of microtubule and mitochondrial proteins. For this reason, we are currently testing the potential relationship between TH and mitochondria. More work is needed to determine the mechanism of action of CHIR, and perhaps a TH-mitochondria potential relationship could be a gateway to do so.

Given that DA hyperactivity seems to underly psychotic states, new classes of chemical agents that prevent DA hyperfunctioning could become beneficial in DA-related disorders.

O-05

m6A a key player in histone H1 mRNA regulation

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Linker histone H1 is the less studied member of the histone family. Binding the nucleosome near the entry/exit site of the DNA, it plays many different roles in chromatin structure and gene expression. Its regulation is very complex with seven somatic variants differentially expressed. H1 variants have been classically divided in replication-independent variants (H1X and H1.0), which have polyadenylated mRNAs and replication-dependent variants (H1.1-H1.5) that lack the polyA tail and are mostly expressed in S phase. The proportions of these variants vary depending on the cell line, during cell cycle and in cell differentiation, in a process that is not yet well understood. We have focused in N^6-Methyladenosine (m6A), a novel epigenetic mark, which is placed mostly in mRNA with different effects depending on the location of the methylation within the transcript and the reader binding it. It is involved, among others, in mRNA export from the nucleus, degradation, splicing, and translation.

Analyzing public data, we found H1 mRNAs are methylated, mainly the replication independent variants. This is due to experimental bias of polyA enrichment, which exclude replication-dependent variants. Those datasets also point to an importance of m6A location, as only replication-dependent variants have a clear localized methylation site, near the stop codon while H1X and H1.0 have m6A spread throughout their mRNA. We also confirmed that m6A is highly abundant in cell cycle associated genes as histones. We have found that H1 mRNAs are highly methylated compared to other genes, including core histones genes in methylated RNA immunoprecipitation followed by quantitative PCR (MeRIP-qPCR). Those results also showed a differential methylation patterns during cell cycle phases between the replication-dependent variants and the replication-independent variants, which could explain its differential regulation. We have also observed that methylation of RNA correlates with transcription via run-on experiments in a cell-cycle dependent manner. Finally, to study the effect of m6A methylation, we have established knock-down cell lines of the m6A machinery. The KDs have direct effects in H1 mRNA levels, with accumulation of replication dependent variants in METTL3 KD (m6A writer) and a clear loss in all H1 variants in the KD of ALKBH5 (m6A eraser). Western Blot experiments point to an accumulation of H1 histone upon METTL3 depletion and the opposite with ALKBH5 KD.

In conclusion, we propose a model that partially explains the complexity of histone H1 variants regulation. m6A promotes a decay in mRNA levels affecting directly protein levels in replication-dependent variants. Regarding H1X and H1.0 the process seems more complex, including probably effects of the m6A outside the stop codon region and other levels of regulation.

O-06

Unraveling the crucial role of senescence in breast cancer progression

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Cellular senescence is a terminal cell cycle arrest induced by a variety of stresses, including oncogenes or certain anti-tumor therapies. Mechanistically, senescence is caused by high levels of the cyclin-dependent kinases inhibitors p16 or p21, and it is characterized by profound changes in cellular function. These changes include the secretion of a wealth of inflammatory factors, the Senescence Associated Secretory Phenotype (SASP), which almost invariably includes interleukin-6 (IL-6). Recent studies, show that tumor relapses and side effects of anti-cancer therapies are in part due to the accumulation of damaged cells that stop growing but remain alive as senescent cells. In fact, the effect of cellular senescence on tumor progression remains a contentious issue. While some reports show that cellular senescence is a tumor defense barrier, others show that senescent cells may contribute to tumor progression.

Several mouse models have been developed to study cellular senescence. All of them express reporters, and/or factors that induce cell death under the control of the Cdkn2/p16(INK4a) promoter. These models have allowed to propose that clearance of senescent cells results in longer lifespan and reduces frailty caused by aging and chemotoxicities. However, a limitation of these models is that other non-dividing cells, such as terminally differentiated cells, which also overexpress p16, are also labeled or targeted.

In order to overcome this limitation, we have generated a unique transgenic mouse model (SuSe mice) that allows the labeling and clearance of senescent cells with higher precision. By coupling reporters, such as Cherry (a red fluorescent protein or GFP (Green Fluorescence Protein) and inducers of cell death to the promoters of p16 and IL-6, respectively, we will label and target cells with two fundamental features of senescence: cell cycle arrest and inflammatory abilities. By monitoring fluorescence, we can identify cells that simultaneously express GFP and Cherry and we can clear these cells by treating with AP21967, a compound that heterodimerizes FRB and FKBP domains and thus the activation of Caspase 8 and apoptosis.

We have crossed these SuSe (for Suicide of Senescent cells) mice with mouse models of breast cancer, such as MMTV-PyMT, to study the role of senescence during tumor progression and treatment, showing it is crucial to define as exactly as possible the therapeutic window of senolysis in different tumor types, as elimination of senescent cells during certain stages of tumor progression will contribute to better anti-tumor therapies by preventing tumor relapse and some deleterious side effects.

O-07

Adquisición de mutaciones *non-driver* durante el seguimiento en pacientes con policitemia vera y trombocitemia esencial *JAK2* positivos

Senín, A[1]; Fernandez-Rodríguez, C[2,4]; Bellosillo, B[2,4]; Longaron, R[2,4]; Camacho, L[2,4]; Fernández-Ibarrondo, L[4]; Besses, C[2]; Álvarez-Larrán, A[3]

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Introducción: La policitemia vera (PV) y la trombocitemia esencial (TE) son neoplasias mieloproliferativas crónicas que comparten la presencia de la mutación *driver JAK2*V617F. La adquisición de mutaciones en otros genes mieloides se ha involucrado en la progresión a mielofibrosis (MF) y leucemia aguda (LA).
Objetivo: Analizar los factores de riesgo implicados en la adquisición de mutaciones *non-driver* en pacientes con PV y TE de largo seguimiento molecular.
Métodos: Se incluyeron 100 pacientes con muestras secuenciales disponibles (PV n=63, TE n=37) en los que la enfermedad había progresado a LA (n=12), MF (n=24) o sin progresión tras seguimiento molecular de más de 10 años (n=64). Se realizó un análisis de NGS utilizando un panel de 51 genes mieloides en la última muestra disponible de su seguimiento. En los pacientes con detección de mutaciones *non-driver* se analizaron muestras del diagnóstico y del seguimiento.
Resultados: La mediana de edad al diagnóstico fue de 59 años (25-84). Setenta y siete pacientes recibieron HU durante una mediana de 4,25 años (0,1-27), 24 recibieron otros tratamientos y 24 no se trataron. El 31% de los pacientes que presentaban mutaciones adicionales en la primera muestra adquirió nuevas mutaciones durante el seguimiento. Un 40% de pacientes con mutaciones en *TP53* y un 46% de pacientes con mutaciones en genes de cromatina/splicing adquirieron nuevas mutaciones durante la evolución en comparación con el 24% y 16% de los pacientes que al diagnóstico tenían una mutación aislada de *JAK2* en estado homocigoto y heterocigoto, respectivamente (p<0.001).
La probabilidad de adquisición de mutaciones a 10 años fue del 27% en toda la cohorte, del 16,4% en los pacientes que permanecieron en fase crónica, del 34,3% en los transformados a MF y del 69% en los transformados a LA.
En el análisis univariado la presencia de mutaciones *non-driver* en el momento del diagnóstico (p=0,008), el número de mutaciones (p<0,0001) y mutaciones en *SRSF2* (p<0,0001), *IDH1/2* (p=0,045) y *RUNX1* (p<0,0001) se asociaron con una mayor probabilidad de adquisición de mutaciones. La duración de la exposición a HU no se correlacionó con la adquisición de mutaciones (p=0,794).
En el análisis multivariado, la presencia de mutaciones *non-driver* en la primera muestra (HR: 2,91, IC del 95%: 1,2-7, p=0,018), el número de mutaciones (HR: 2,53, IC del 95%: 1,45-4,4, p=0,001), las mutaciones en *SRSF2* (HR: 12,7, IC 95% 2,1-77,6, p=0,006) y en *RUNX1* (HR: 33,59; IC 95% 4,8-232,7 p<0,0001) se asociaron con mayor riesgo de adquisición de mutaciones después de la corrección por edad, tipo de diagnóstico y duración total del tratamiento con HU.
Conclusiones: La presencia y número de mutaciones *non-driver* confieren inestabilidad genética en PV y TE al favorecer la aparición de nuevos eventos genéticos. En nuestra cohorte de pacientes no se ha podido probar la genotoxicidad de HU.

O-08

In vitro and in vivo studies of novel aldehyde dehydrogenase inhibitors for the treatment of glioblastoma

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Glioblastoma (GB) is the most common and aggressive type of malignant glioma, and it is classified as a grade IV tumor by the WHO. Grade IV tumors exhibit advanced features of malignancy, and since they present resistance to radio/chemotherapy they are generally lethal within 12 months. In this regard, various isoforms of aldehyde dehydrogenase (ALDH) have been shown to contribute to therapy resistance, along with tumor relapse and progression. ALDHs, which are known to be overexpressed in the cancer stem cell (CSC) subpopulation of various tumor types, catalyze the irreversible oxidation of a wide range of aldehydes to their corresponding carboxylic acids. This contributes to cellular protection against reactive oxygen species (ROS) generated by radiation and anti-neoplastic agents. In addition, the ALDH1A subfamily may play a role in tumor progression through retinoic acid–mediated signaling pathways, which are also involved in cell proliferation and differentiation.

In the first part of this study, we tested the effect of novel ALDH inhibitors, namely DIMATE and its analogues ABD0099 and ABD0171, on a panel of different GB cell lines. Initially, the expression of several ALDHs was assessed in the various cell lines. Then, we evaluated the cytotoxicity of the inhibitors alone or in combination with temozolomide (TMZ), the standard chemotherapeutic agent currently used for the treatment of GB, in order to try to reduce TMZ resistance. In addition, experiments to determine the cellular ALDH1A activity were performed in the absence and presence of inhibitors. In the second part of the study, a therapeutic efficacy assay was carried out in a GL261 immune-competent mouse model of GB, using DIMATE prepared as a nanoliposomal formulation. During the time of treatment we monitored the animal's weight and also the tumor volume by MRI in order to evaluate the suitability of DIMATE as a novel drug for GB therapy.

In summary, inhibitors turned out to be far more cytotoxic than TMZ, and were able to reduce the ALDH1A cellular activity. Interestingly, the DIMATE formulation was shown to slow down the tumor growth rate in the mouse model of GB. Taken together, these results shed some light on our understanding of the role of ALDH in GB and could potentially lead to the development of a novel, more effective treatment against this disease.

O-09

A self-stimulatory Wnt5a/Snail1 axis controls colon tumor chemo-resistance and invasion

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Snail1 transcriptional factor is a key driver of epithelial-to-mesenchymal transition (EMT) that confers to tumor cells higher invasion and increased resistance to chemotherapeutic drugs. We demonstrate that Snail1 expression in colon tumor cells is dependent on the activity of the non-canonical Wnt pathway. Not all Wnt ligands act through the canonical signaling; some, such as Wnt5a, activate a different pathway not involving β-catenin that has been called non-canonical Wnt. Molecularly, canonical and non-canonical Wnts use a common receptor, Fz but different co-receptors: LRP5/6, the canonical and Ror2, the non-canonical. While canonical Wnts promote the stabilization of β-catenin, non-canonical Wnts induce its degradation. Besides activating specific genes, previous results of our lab have demonstrated that both, canonical and non-canonical Wnts stimulate a common set of genes associated to EMT, including Snail1.

In this work we found that non-canonical Wnt pathway is regulating Snail1 expression in colon tumor cells. Accordingly, depletion of Ror2, potently decreases Snail1, an effect due to diminished transcription and lower stability of Snail1 protein and affects tumor invasion and chemo-sensitivity. Wnt5a, Ror2 and Snail1 participate in a self-stimulatory feed-back loop since activation of the pathway amplifies its response increasing the levels of the ligand, receptor Fz and co-receptor Ror2 that initiate it. Wnt5a/Ror2 axis controls tumor invasion and chemo-resistance and also stimulates TGFβ synthesis. Therefore, tumor cells that express Snail1 are more efficient in activating cancer-associated fibroblasts than the corresponding controls.

Inhibition of the Wnt5a pathway with porcupine inhibitors decreases Snail1 expression and tumor metastasis in a murine model with primary colon tumor cells. Finally, expression of SNAIL1, ROR2 and WNT5A RNAs show a close correlation in human colon tumors. These results show that Snail1 expression in tumor cells is controlled by self-stimulated non-canonical Wnt signaling and identify inhibitors of this pathway as putative therapies to prevent colon tumor progression and chemoresistance.

P1-01

Genetically predicted telomere length and Alzheimer's disease endophenotypes: a Mendelian randomization study.

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Background: Telomere length (TL) is an objective biomarker of biological aging and aging-related outcomes. Shorter telomeres are associated with accelerated aging, consequently increasing the risk of age-related diseases such as Alzheimer's Disease (AD). However, observational studies are limited to conclude whether TL is causally associated with AD or it could be considered as a marker of an underlying pathological process.
Objective: We aimed to evaluate the association between genetically predicted TL and cognitive performance, brain vulnerability and core AD and neurodegeneration biomarkers measured in cerebrospinal fluid (CSF) through a Mendelian Randomization (MR) analysis.
Methods: Our analyses were conducted in the context of the ALFA (ALzheimer and FAmilies) study. A cognitive battery was administered to assess verbal memory, psychomotor speed, visual processing and executive function (N = 2,233). Further, episodic memory, executive function and global cognitive composites were calculated. The acquisition of neuroimaging data was performed for a subset of our participants (N = 1,134) through magnetic resonance imaging (MRI). Cortical thickness (CT) of specific brain regions were used to calculate AD and Aging brain signatures. Levels of core AD and neurodegeneration biomarkers in CSF, including amyloid-β (Aβ) 42, Aβ40, p-Tau, t-Tau, and neurofilament light (NfL), were measured using NeuroToolKit (NTK) and Elecsys® immunoassays (N = 304). Genome-wide genotyping and imputation were performed. A total of 7 single nucleotide polymorphisms (SNPs) associated with TL were used as instrumental variables to determine the predicted effect of TL on AD endophenotypes (i.e. cognitive performance, brain vulnerability signatures, and NTK CSF biomarkers). Causal effects of genetically predicted shorter TL were estimated using the MR-inverse-variance weighted method. Sensitivity analyses using the MR-Egger method were conducted to test for directional pleiotropic effects. Stratified analyses by *APOE*-ε4 allele status (the most important genetic risk factor for developing non-familial AD) were also performed.
Results: MR analysis revealed a significant association between SNPs predicting shorter TL and worse executive function performance, as well as reduced cortical thickness in age and AD-related brain signatures. Associations between SNPs predicting shorter TL and worse EF were driven by *APOE*-ε4 non-carriers, whereas significant effects on brain signatures were observed among *APOE*-ε4 carriers.
Conclusion: Our results suggest a potential causal role of telomeres on cognitive resilience and brain vulnerability in regions associated with both, accelerated aging and AD. Further observational and genetic analyses are warranted to better understand these associations.

P1-02

Biased G Protein-Independent Signaling of Dopamine D_1-D_3 Receptor Heteromers in Reserpinized Mice

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Several studies found *in vitro* evidence for heteromerization of dopamine D_1 receptors (D1R) and D_3 receptors (D3R), and it has been postulated that functional D1R-D3R heteromers that are normally present in the ventral striatum mediate synergistic locomotor-activating effects of D1R and D3R agonists in rodents. Based also on results obtained *in vitro*, with mammalian transfected cells, it has been hypothesized that those behavioral effects depend on a D1R-D3R heteromer-mediated G protein-independent signaling. Here, we demonstrate the presence on D1R-D3R heteromers in the mouse ventral striatum by using a synthetic peptide that selectively destabilizes D1R-D3R heteromers. Parallel locomotor activity and *ex vivo* experiments in reserpinized mice and *in vitro* experiments in D1R-D3R mammalian transfected cells were performed to dissect the signaling mechanisms of D1R-D3R heteromers. Co-administration of D1R and D3R agonists in reserpinized mice produced synergistic locomotor activation and a selective synergistic AKT phosphorylation in the most ventromedial region of the striatum, in the shell of the nucleus accumbens. Application of the destabilizing peptide in transfected cells and in the shell of the nucleus accumbens allowed demonstrating that, both *in vitro* and *in vivo*, co-activation of D3R induces a switch from G protein-dependent to G protein-independent D1R-mediated signaling determined by D1R-D3R heteromerization. The results therefore demonstrate that a biased G protein-independent signaling of D1R-D3R heteromers localized in the shell of the nucleus accumbens mediate the locomotor synergistic effects of D1R and D3R agonists in reserpinized mice.

P1-03

A high fat diet modifies the metabolism and the brain neurotransmitter profile in an IUGR pig model

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Intrauterine Growth Restriction (IUGR) is a pathological condition that hinders the correct growth of the foetus during pregnancy, due to oxygen or nutrient deficiency. As a consequence of this condition, the foetus adapts its metabolism and physiology to survive in such scarce environment. The major adaptation is the so called “brain sparing”, this effect gives priority to brain development to ensure the individual survival. Nevertheless, this does not warrant the normal development of the brain and the risk exists of neurological and cognitive deficits at short or long term. In turn, this adaptation leads to other systemic alterations that affect the energetic metabolism, thus inducing the emergence of a fairly characterized phenotype called “thrifty phenotype”. This phenotype is responsible for the metabolic alterations that last up until adulthood, which increase the incidence of some diseases like diabetes and metabolic syndrome.

Using a pig model of IUGR, animals are classified as normal birth weight (NBW) or low birth weight (LBW). Our hypothesis is that those animals that were affected by IUGR during their gestation, and therefore were born LBW, will present a different susceptibility to high fat diet (HFD) than NBW animals.

We have studied the long-term neurological alterations and the effect of a HFD at metabolic and neurological level. Our results suggest that IUGR neurological alterations do not persist over time, confirming the “brain sparing” effect. Nevertheless, a HFD had a significant effect on the neurotransmitter profile of some brain areas like the hippocampus, amygdala, hypothalamus, striatum and prefrontal cortex. The neurotransmitter that was most affected in most areas was serotonin (5-HT), thus affecting the indolamine pathway. Regarding the amino acids, it was observed that the animals that had been affected by IUGR, and therefore were born LBW, and also had received HFD diet, presented higher concentrations of amino acids in plasma. Finally, the biochemical serum profile of the animals was analysed. As with amino acids, the LBW animals that received the HFD diet were more sensitive at the metabolic level. These animals presented lower activity of liver and antioxidant enzymes, a decrease in cholesterol and fructosamine levels, and an increase in cortisol and MDA levels.

P1-04

TRPV2 pathophysiology in myelination disorders

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TRP channels are important pharmacological targets in pathophysiology. TRPV2 channel is widely expressed and is the closest homologue to TRPV1, by far the best studied TRP channel. TRPV2 plays distinct roles in cardiac, neuro and muscular function, immunity, and metabolism, and is associated with pathologies like muscular dystrophy and cancer. Here we perform a comparative pathology approach, characterizing the expression of TRPV2 in mouse at the cellular level in wild-type and IL-6 overexpressing strains, and at the central nervous system level in a hypomyelination mouse model (jimpy mutant).
At the cellular level, we have identified differential expression of TRPV2 in microglial cells when comparing physiological and inflammatory conditions. We analyzed the neuronal expression of TRPV2 in the jimpy hypomyelinated mutant model and we have found TRPV2 to be dysregulated. Finally, canine clinical cases of myelination disorders, of both genetic and viral origin, show that the expression of TRPV2 is affected. Altogether, our results indicate that TRPV2 plays a “key/important” role in myelination disorders and could be used as a novel therapeutic target.

P1-05

Attenuation of the Unfolded Protein Response as a mechanism of neuroprotection in Alzheimer's disease

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The Unfolding Protein Response (UPR) is a protective cellular response induced upon endoplasmic reticulum (ER) stress caused by perturbations in protein folding processes. It consists on a mechanism of adaptation and survival, which leads to the reduction of unfolded protein load and to the reestablishment of protein-folding homeostasis. Finally, if it is not enough to recover the homeostasis, cells will dead.

The ER stress is increased in AD brains, leading to a higher expression of UPR proteins in temporal cortex and hippocampus. This increment can be due to the interference of Aβ with normal functioning of the ER, which is correlated with the neurofibrillary tangles (NFTs) formation. Furthermore, the ER stress may interfere with the normal non-amyloidogenic trafficking of APP, leading to higher Aβ production. The increased production of Aβ peptides also contributes to the over-activation of the PI3K/Akt signaling pathway. This pathway plays an important role in AD, since it can reduce the TACE-mediated clearance of the Aβ peptides and increase the UPR activation, thereby exacerbating the Aβ production and the deposition of amyloid plaques and NFTs. It is known that the inhibition of PDK1 (a kinase which acts as the upstream activator of Akt) can restore the TACE α-secretase activity and reduce the UPR. Strikingly, the reduction of the levels of Akt activation by PDK1 knock-in mutation protected mice neurons against ER stress mediated toxicity.

In this study, we demonstrated that tunicamycin reduced HEK293 cell viability by inducing ER stress and activating the UPR. We showed that inhibition of Akt through the Akt1/2 inhibitor MK2206 partially recovered the viability in tunicamycin-treated cells. This beneficial effect seems to be mediated by the reduction of the UPR, and the specific mechanism by which Akt modulates the UPR is being investigated. This study suggests that inhibition of Akt could be a therapeutic approach to improve the neuronal death presented in AD brains.

P1-06

Faim knockout leads to gliosis and late-onset neurodegeneration of photoreceptors in the mouse retina

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Fas Apoptotic Inhibitory Molecule protein (FAIM) is a death-receptor antagonist and an apoptosis regulator. It encodes two isoforms that have significant neuronal functions, FAIM-S (short) and FAIM-L (long). FAIM-S, which is ubiquitously expressed, is involved in neurite outgrowth. In contrast, FAIM-L is only expressed in neurons and it protects them from cell death. Interestingly, FAIM-L is downregulated in Alzheimer's disease patients and mouse models before the onset of neurodegeneration, and *Faim* transcript levels are decreased in retinal degeneration mouse models. Nonetheless, few studies have been directed to elucidate the role of FAIM in the central nervous system, yet alone the retina. The retina is a highly specialized tissue that has proved to precede pathological mechanisms of neurodegenerative diseases. Here we describe that *Faim* depletion in mice damages the retina unrelentingly and leads to late-onset photoreceptor cell death in older mice. Immunohistochemical analyses show that *Faim* knockout (*Faim*$^{-/-}$ mice present ubiquitinated aggregates throughout the retina from early ages. Moreover, retinal cells release stress signals that can signal to Müller cells, as shown by immunofluorescence and qRT-PCR. Müller cells monitor retinal homeostasis, and hereafter trigger a gliotic response in *Faim*$^{-/-}$ mice that becomes pathogenic when is sustained over time. In this regard, we found a pronounced vascular leakage at the latter ages, which can be caused by persistent inflammation. These results suggest that FAIM is an important player in the maintenance of retinal homeostasis and support the premise that FAIM could be a plausible early marker for late photoreceptor and neuronal degeneration.

P1-07

FAIM-L as a modulator of Tau-pathology in Alzheimer's disease and other tauopathies.

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Alzheimer's disease (AD) is characterized by two main biological hallmarks: beta-amyloid aggregates and the formation of intracellular neurofibrillary tangles (NFT). NFT formation during the progression of the disease is irremediably linked to neuronal death and cognitive dysfunction. NFTs are composed of abnormally hyperphosphorylated and aggregated Tau protein. Tau is a member of the MAPT family protein. In neurons, Tau binds to microtubules (MT) and acts as a stabilizer. It has been involved in axon maintenance and outgrowth and in kinesin and dynein transport. Therefore, is essential to maintain MT dynamics in mature neurons. Recently, it has also been involved in transcriptional regulation and synaptic functionality. Tau can suffer distinct post-translational modifications, which modulates its activity and localization. The most common modification is phosphorylation. Phosphorylation of different Ser/Thr residues of Tau reduces its affinity to MT and makes Tau more prone to aggregation. Tau phosphorylation is dramatically increased in AD and other neurodegenerative diseases known as Tauopathies. This is associated with a reduction in Tau functionality and the formation of the aforementioned NFTs. Our laboratory has been focused in the study of the Fas apoptotic inhibitory molecule (FAIM-L). FAIM-L is an anti-apoptotic protein only expressed in neurons, which has also been involved in non-apoptotic functions such as neuronal pruning and axonal degeneration. We have previously reported that FAIM-L is reduced in AD patients and in the APPxPS1 mice model.

Here, we show that FAIM-L is only reduced in mice models that show Tau pathology, such as P301S mice (PS19) at 2 months of age and VLW mice at 8 months of age. Other AD models such as 5xFAD or APP23, which only present beta-amyloid pathology, do not present FAIM-L downregulation. The mechanism by which aberrant Tau could be reducing FAIM-L levels needs further investigation. In this line, we demonstrate an interaction between Tau and FAIM-L and propose, based on preliminary results, that reduction of FAIM-L levels could be dependent of Tau phosphorylation or aggregation status.

PS19 displays early progression of the disease, showing synaptic deficits by 3 months of age and extensive neurodegeneration by 9 months-old. Since we observed FAIM-L reduction in PS19 mice previous to pathological alterations, we hypothesize that FAIM-L reduction may be involved in in the progression of Tau pathology. Thus, we think that restoring FAIM-L levels could be a protective strategy against the development of the disease. Using AAVs we plan to overexpress FAIM-L in hippocampus of PS19 mice. Behavioral and biochemical analysis will be performed in order to determine the effect of restoration of FAIM-L levels in the progression of the disease. With this work would like to establish FAIM-L as a possible therapeutic target in AD and other neurodegenerative diseases.

P1-08

Proteomic study of type 2 diabetes-induced retinal neurodegeneration: Finding common molecular markers to brain neurodegeneration

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Introduction: Type 2 diabetes (T2D) is associated to cognitive impairment and a 2-fold higher risk of developing Alzheimer's disease (AD). Moreover, diabetic retinopathy, the most important microvascular complication of T2D, courses with neurodegeneration in its early stages. Besides, several authors have described retinal abnormalities in patients with neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. These alterations often precede symptoms in the brain, which indicates that retinal evaluation could offer a tool of early diagnosis. Due to the common embryological origin of retina and brain, the eye can be considered as an extension of the central nervous system. Therefore, it seems reasonable that the presence of retinal neurodegeneration may reflect brain neurodegeneration status. In the context of T2D, little is known regarding molecular mediators shared by the brain and the retina that could indicate a neuropathological progression in T2D patients.

Here, we analyze proteomic data from human diabetic and non-diabetic retinas obtained by label-free semiquantitative proteomic approach. Absence or presence of GFAP expression by Müller cells, a histological hallmark of retinal neurodegeneration, was considered to classify diabetic retinas.

Objectives: To identify pathogenic mediators of retinal neurodegeneration induced by T2D focusing on those being more relevant in AD brain neurodegeneration.

Methodology: We filtered the identified proteins obtaining different subsets related to inflammation, apoptosis, vascular function and beta-amyloid according to their Gene Ontology terms. Next, we selected the most relevant differentially expressed proteins of each subset, according to their fold change and their abundance profile in the three groups (non-diabetic controls, T2D without GFAP and T2D with GFAP).

Results: We have obtained a number of proteins that are related to neuroinflammatory and neurodegenerative processes in T2D retinal degeneration. Interestingly, some of the obtained proteins are well known risk factors for AD (APOE, CLU) and others are also altered in brain of AD donors (ANXA1, CHI3L1, NGFR).

Future perspectives: Next steps will include the validation of these candidates by molecular biology techniques in a new set of T2D post-mortem retinas. Also, we will evaluate the validated proteins on hippocampal tissue from donors with AD and/or T2D.

Identifying the common molecular alterations of retinal and brain neurodegeneration could open up new therapeutic approaches and discover new early markers for brain neurodegeneration.

P1-09

New antipsychotic Lumateperone reduces dopamine accumulation in rat striatum *ex vivo*

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Schizophrenia is a mental disorder characterized by episodes of psychosis and hallucinations, as well as anhedonia and cognitive impairments that affects around 20 million people worldwide. Psychotic symptoms are related to an increase of dopaminergic activity in the central nervous system, specifically in the mesolimbic pathway, which projects to the striatum.

Nowadays, all drugs approved to treat schizophrenia act on dopamine D2 receptors, which are Gi-protein coupled receptors found both pre and post-synaptically. The latest developed drugs such as Aripiprazole, Brexpiprazole and Cariprazine, are partial agonists to presynaptic D2 receptors, therefore inhibiting dopamine (DA) accumulation in the vesicles. We have previously shown this using an *ex vivo* protocol that permits measuring this DA accumulation in the neuron terminals over time under depolarizing and non-depolarizing conditions. This method is then valuable to study the effects of antipsychotic drugs in DA synthesis and accumulation in the brain.

At the end of 2019, a new antipsychotic drug called Lumateperone was approved by the FDA. Lumateperone is presented as a partial agonist to presynaptic D2-receptors and antagonist to postsynaptic D2-receptors, and it shows fewer secondary effects than its predecessors.

So far, our results also confirm an even more remarkable decrease in DA accumulation when samples are treated with Lumateperone. However, this decrease does not take place at concentrations in which Lumateperone acts over D2-receptors but higher. We are now studying whether a direct action of Lumateperone over Tyrosine Hydroxylase (TH), the rate-limiting enzyme of DA and other catecholamines biosynthesis, is responsible for such remarkable effect.

P1-10

Generation of a new chimeric chronokine for the treatment of aging-associated pathologies

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Chronokines are proteins that play a role in the control of aging and pathologies associated to it, primarily by modulating the metabolism, oxidative stress and inflammation. Different chronokines, such as the anti-aging factor *Klotho* among others, have been shown to present beneficial effects on aging and as potential treatments for neurodegenerative diseases. One of the diseases that could get benefitted by a treatment with chronokines would be Alzheimer's disease. Events like the accumulation of the Aβ peptide and Tau protein, cholinergic dysfunction and neuroinflammation take place along the course of this disease. Given the complexity of this pathology, a treatment through a unique factor is demonstrated to be insufficient; therefore, we propose to generate a new entity based on the union of different chronokines as a new chimeric protein, determined after an exhaustive bioinformatic analysis aimed at optimizing the stability and biological activity through several simulations.

We have tested individual chronokines *in vitro* in the mouse Microglia BV2 cell line, in order to assess the beneficial effects they have over several hallmarks of Alzheimer's disease. For instance, we observed a differential decrease of neuroinflammatory mediators, inhibition of oxidative stress and cell viability improvement. Consequently, a synergic effect is expected as a result of the union of these proteins, helping to improve the treatment for Alzheimer's targeting it from different perspectives, in contrast to conventional treatments. After the determination of the optimal form of uniting the different proteins in one entity through a bioinformatic analysis, this new chimeric chronokine will be produced and tested. Thus, the stability, efficiency and safety of the new molecule will be determined for the treatment of Alzheimer's disease using gene therapy strategies in murine animal models of the pathology.

P1-11

Understanding and treating neurodegeneration caused by Mucopolysaccharidosis type VII

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Mucopolysaccharidosis type VII is an autosomal recessive disorder caused by a β-glucuronidase deficiency, which leads to the accumulation of glycosaminoglycans within the cells. This disease is chronic and progressive affecting most organs in the body. The clinical manifestations are highly variable among patients, but always include cognitive impairment. Although some pathological processes have been well characterized in the MPS VII murine model, this is not the case for the neurodegeneration. Studies have stablished gene therapy as a great strategy to treat MPS VII, reversing different pathological features, but its effect in the neuropathology of this disease has not been analyzed in detail.

This project is focused on understanding how glycosaminoglycan accumulation in the lysosomes have an impact on the viability or functioning of the neurons. We studied neuronal loss in MPS VII mice at different ages using neuron-specific immunohistochemistry and image processing tools. We also analyzed spine density in the same brain areas of 5-month-old MPS VII mice. The labeling was performed using the Dil Stain fluorescent dye and the Gene-Gun technique or the Golgi-Cox staining method. To test if neuronal abnormalities can be restored after gene therapy, MPS VII mice were treated at 2 months of age by intrathecal injection of AAVrh10-GUSB and analyzed at the corresponding age. Finally, to characterize possible pathways involved in the neuropathology of this disease we used cellular fractionation protocols and western blot analysis.

Our results show a different affectation among brain areas, some of them showing a decrease in neuronal density or width of the neuronal layer at the last age analyzed. All areas analyzed showed significant decrease in spine density, uncovering another cause of the neurological manifestation of the disease. Finally, protein analysis has revealed several pathways to be affected in MPS VII mice, but the connection between them is not clear yet.

Although much is still unknown about the factors underlying the neuropathology of MPS VII patients, our results provide several mechanisms that may be partially responsible for this phenotype. Furthermore, our gene therapy treatment has been able to reverse many? of these alterations.

P1-12

Topical ocular administration of sitagliptin, a DPP-4 inhibitor, prevents the downregulation of neuroretinal presynaptic proteins induced by diabetes

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The progressive increase in the number of diabetic patients leads to an associated enhancement of diabetic retinopathy (DR) which is a global health concern. This is mainly due to the unsatisfactory therapeutic strategies addressed to early stages of the disease, in which the impairment of neurovascular unit plays an essential role. Neurovascular unit comprises a functional coupling between blood vessels, glia and neurons, that coordinates several physiological functions such as metabolic demand, blood delivery or synaptic activity. Neurovascular impairment leads to microvascular abnormalities and neurodegeneration, where synapse damage plays a key role. Reduced synaptic protein expression, impaired neurotransmission and alterations in neuronal morphology have been observed in retinas from several diabetic animal models. We previously reported that the topical administration of glucagon-like peptide-1 (GLP-1) using eye-drops prevented retinal neurodegeneration induced by diabetes. Dipeptidyl peptidase-4 (DPP-4) is the enzyme responsible of the cleavage of GLP-1.

The aim of this study is to evaluate if topical treatment (eye drops) with sitagliptin, a DPP-4 inhibitor (DPP-4i), is able to prevent the downregulation of presynaptic proteins in an experimental model of diabetes (db/db mouse).

For this purpose, 15 db/db mice, aged 12 weeks, received a topical administration of sitagliptin (10 mg/mL) twice per day for 2 weeks while other 15 db/db were treated with vehicle. 15 non-diabetic mice (db/+) were used as a control group. Protein levels were assessed through western blotting and immunohistochemistry (IHC), and mRNA levels were evaluated through reverse transcription polymerase chain reaction (RT-PCR).

Our results revealed a down-regulation (protein and mRNA levels) of several presynaptic proteins such as syntaxin 1A, synapsin I, synaptotagmin, synaptophysin, vesicle-associated membrane protein 2 (VAMP2) and synaptosomal-associated protein of 25 kDa (SNAP25) in diabetic mice treated with vehicle. These proteins are involved in vesicle biogenesis, mobilization and docking, membrane fusion and recycling, and synaptic neurotransmission. Sitagliptin was able to significantly prevent the down-regulation of these proteins.

We conclude that sitagliptin exerts beneficial effects in the retinas of db/db mice by preventing the downregulation of crucial presynaptic proteins. These neuroprotective effects open a new avenue for treating DR as well other retinal diseases in which neurodegeneration/synaptic abnormalities play a relevant role.

P2-01

Insights into the structure and enzymatic mechanism of the Nse2 E3 SUMO ligase

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Nse2 is a unique bone fide E3 SUMO ligase embedded in the Smc5/6 complex, a critical player during recombinational DNA repair. Sumo Interaction Motifs (SIM) has been pointed out as a key cofactor employed by E3 ligases to stabilize a “closed/active” conformation of E2~SUMO^{D} thioester and SUMO^{B} E2 backside which stimulates SUMO discharge on the substrate lysines. Here we report a unique C-terminal SIM-like motif and its role on the catalytic mechanism of budding yeast Nse2. In vitro assays with *Arm/Smc5-Nse2* show that C-terminal mutants display a clear decrease in sumoylation. Structural modelling places the Cterm SIM near the backside of E2~thioester. Indeed, the greater activity of wildtype *Arm/Smc5-Nse2* decreases in the presence of SUMO unable to bind to E2-backside. Thioester discharge assisted by this wtE3 exhibits a cooperative behavior modulated by extra SUMO and ssDNA. On the other hand, our engineered fusion constructs that carry an attached SUMO at the Cterm of Nse2 is remarkably faster compared to the non-fused Nse2, indicating the entropic benefit of having a SUMO permanently close to the E2 backside. Finally, our solved structure of *Arm/Smc5-Nse2-SUMO* in complex with E2-SUMO$_{D}$ confirms our model placing the fusion SUMO at the backside of the E2-thioester which in turn binds Nse2 through interactions stablished with its RING domain and with a large loop of Nse2 which is also a SIM motif that suffers a major conformational change in order to stabilize the SUMO^{D} into the “active” conformation.

P2-02

Deciphering the role and regulation of spatial-temporal genome architecture in B cells

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During differentiation, B cells diversify their immunoglobulin loci through mutations and translocations to create immunoglobulins that efficiently recognizes a specific pathogen, thus facilitating its neutralization and destruction. However, aberrations in differentiation can cause cancer, immunodeficiency, allergy and autoimmunity. For instance, off-target mutations and translocations are a major cause of diffuse large B cell lymphoma. Despite the clinical relevance of these processes, we do not have a complete understanding of the molecular mechanisms that regulate them. Genome architecture, or how the DNA is packed into the nucleus, broadly impacts gene expression and DNA repair, thus it may be a key mechanism regulating B cells and lymphomagenesis.

We propose a multidisciplinary approach combining state-of-the-art omics strategies, computational biology, genome engineering and mouse experimentation to provide fundamental insights into B cells and their malignant transformation from the perspective of spatio-temporal genome architecture. First, we will shed light on the regulatory factors and their underlying molecular mechanisms that spatially organize the B cell chromatin. Second, we will evaluate whether the genome architecture transcriptionally controls B cell differentiation and function, and whether it protects the genome from collateral oncogenic damage during immunoglobulin diversification. This will require the development and implementation of a novel, low-input, genome-wide method for studying promoter-centered genome architecture. Finally, we will clinically translate the mechanistic and functional insights to improve our understanding of diffuse large B cell lymphoma and its clinical management.

Collectively, we will provide unprecedented mechanistic insights into how B cells function and protect their genome from intrinsic oncogenic damage, clinically impacting regenerative medicine, immunotherapy, autoimmunity, allergy, immunodeficiencies and cancer.

P2-03

Indirect determination of biochemistry reference intervals using outpatient data

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Introduction: Currently used methods to establish reference intervals (direct) are laborious and expensive. To overcome these drawbacks, new (indirect) methodologies are promising tools. The aim of this study was to validate the indirect Dutch method (NUMBER) with an external database with routine biochemistry test results.

Methods: We used anonymized clinical results from individuals visiting general practitioners and analyzed in 2018 in Clinical Laboratories Vall d'Hebron, Barcelona. Analytical quality was checked by EQA performance and daily average. Per test, outliers were excluded using Tukey method, data were transformed to approximate a normal distribution (if necessary) and reference intervals were calculated for each test, stratified by age and sex, if necessary.

Results: After quality assessment and exclusion based on clinical criteria, we obtained 509,408 clinical requests. The normally distributed tests showed similar results between Barcelona and Dutch population. Reference intervals for creatinine (Jaffe method) and urea followed the same tendency of increasing values by increasing age. For ALT, AST, and GGT, markedly higher results for upper limits were obtained in the Dutch population, part of which can be explained by metrological differences (e.g. no use of pyridoxyl 5 phosphate in ALT/AST assays). As in the Dutch study, creatine kinase and uric acid showed higher reference intervals than traditionally used in Vall d'Hebron. The differences in LD could be explained by the use of the pyruvate to lactate method instead of lactate to pyruvate (IFCC recommended method).

Conclusions: Using an indirect approach, we determined reference intervals for 16 biochemistry tests for the Barcelona population. The reference intervals were compared with Dutch results using the same methodology (NUMBER). Although similar results were found for normally distributed tests, for kidney and liver parameters we found substantial differences which might be explained by methodological, analytical and/or population differences (e.g. lifestyle).

P2-04

RNA-binding and prion domains: the Yin and Yang of phase separation

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Proteins and RNAs assemble in membrane-less organelles that organize intracellular spaces and regulate biochemical reactions. The ability of proteins and RNAs to form condensates is encoded in their sequences, yet it is unknown which domains drive the phase separation (PS) process and what are their specific roles. Here, we systematically investigated the human and yeast proteomes to find regions promoting condensation. Using advanced computational methods to predict the PS propensity of proteins, we designed a set of experiments to investigate the contributions of Prion-Like Domains (PrLDs) and RNA-binding domains (RBDs). We found that one PrLD is sufficient to drive PS, whereas multiple RBDs are needed to modulate the dynamics of the assemblies. In the case of stress granule protein Pub1 we show that the PrLD promotes sequestration of protein partners and the RBD confers liquid-like behaviour to the condensate. Our work sheds light on the fine interplay between RBDs and PrLD to regulate formation of membrane-less organelles, opening the avenue for their manipulation.

P2-05

Structure and mechanism of the Nap adhesion complex from the human pathogen *Mycoplasma genitalium*

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Mycoplasma genitalium is an emergent human pathogen sexually transmitted causing urethritis in men and it is linked to cervicitis and pelvic inflammatory disease in women. Adhesion of *M. genitalium* to human target epithelial cells is mediated through a transmembrane complex called Nap, which is essential for infection. The Nap complex comprise the proteins P110 and P140, also known as the major adhesins of this microorganism, and it is organized forming a dimer of heterodimers. We have previously determined the crystal structure of a recombinant version of P110, revealing the binding site for the sialylated host cell receptor moiety. However, the structure and function of the Nap complex, which is an appealing target for the development of antimicrobial drugs, has yet to be determined. Here we report the crystal structures of a recombinant version of P140 and a complex between P140 and P110. Experimental binding assays demonstrated that unlike P110, adhesin P140 does not bind sialic acid compounds, though the sialic acid cell receptor binding site is located at the P110-P140 interface. The P140-P110 structure was also confirmed in solution by single-particle cryo-electron microscopy (cryo-EM) at 4.1 Å. These structures show that the sialic acid binding sites are not accessible in the P140-P110 complex. In addition, the structure of the whole Nap complex purified from *M. genitalium* was also determined in two different conformations by cryo-EM and cryo-electron tomography (cryo-ET) at ~10 Å and ~15 Å, respectively. The cryo-EM structure shows a "closed" Nap conformation, where the interactions between the P140 and P110 subunits are the same interactions that were previously detected in the P140-P110 crystal structure. However, the cryo-ET structure shows an "open" conformation with loosely coupled interfaces between the four subunits, allowing P110 to bind to sialic acid receptor moieties. Conformational changes are the result of the rearrangement of the four subunits around the central Nap two-fold axis. Structural information, in combination with functional studies show that the Nap complex alternates between "open" and "closed" conformations, suggesting a mechanism for attachment and release of *M. genitalium* cells to the host receptors. Interestingly, selected mutations introduced in the vicinity and also away from the sialic acid binding sites of P140 and P110, alter *M. genitalium* attachment and/or cell motility. All these results illustrate the architectural elements of the Nap complex and enhance our current understanding of the *Mycoplasma genitalium* infection process.

P2-06

Structure and function research of Ubiquitin and Sumo related proteins post-translational modifications

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The large family of deubiquitinating enzymes (DUBs) are involved in the regulation of a plethora of processes carried out inside the cell by protein ubiquitination. Ubiquitination is a basic pathway responsible for the correct protein homeostasis in the cell, which could regulate the fate of proteins through the ubiquitin–proteasome system (UPS). DUB proteases are responsible for cleavage and regulation of the multiple types of ubiquitin linkages that can be synthesized inside the cell, known as the ubiquitin-code, which are tightly connected to specific substrate functions. Some special USP members that have distinct specificity on the cleavage of particular Small-Ubiquitin Modifier (SUMO). For example, USPL1 neither binds nor cleaves ubiquitin, but it is a SUMO isopeptidase both *in vitro* and in cells.

P2-07

Cryptic amyloidogenic regions in Intrinsically Disordered Proteins: Function and Disease association.

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The amyloid conformation is considered a fundamental state of proteins and the propensity to populate it a generic property of the polypeptide chain. Multiple proteome-wide analyses addressed the presence of amyloidogenic regions in proteins, nurturing our understanding of their nature and biological implications. However, these analyses focused on highly aggregation-prone and hydrophobic stretches that are only marginally found in intrinsically disordered regions (IDRs) due to their intrinsic polar composition.

Here, we explore the prevalence of cryptic amyloidogenic regions (CARs) of polar nature in IDRs. We found that CARs are widespread in IDRs and associated with their innate function, with particular involvement in protein-protein interactions. Their presence is also connected to a risk of malfunction. Proteins holding CARs are more associated with diseases like Parkinson and Alzheimer than those devoid of them and they appear to play a central role in cancer related pathways.

By exploring this function/malfunction dichotomy, we speculate that ancestral CARs might have evolved into functional interacting regions playing a significant role in protein evolution at the origins of life. These regions would have been instrumental for forming the first globular complexes, establishing cooperative networks, and early coacervation. The cryptic amyloidogenicity identified in this study might well be a reminiscence of such an evolutionary pathway.

P2-08

In Vitro Selective Cleavage of human RNase 2/EDN within tRNA Anticodon Loops

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Human RNase 2, also named the Eosinophil derived Neurotoxin (EDN), is one of the main proteins secreted by the eosinophil secondary granules. This protein is endowed with a high ribonuclease catalytic activity. It is also found that EDN displays a broad antiviral activity and presents a good target to single stranded RNA viruses[1].

In previous studies, to explore EDN mechanism of action in antiviral host defense, our lab has knocked out EDN expression in the THP-1 monocyte cell line and characterized the cell response to human Respiratory Syncytial Virus (RSV). We observed that RSV infection induced EDN expression and protein secretion in THP1 macrophage-derived cells, whereas the knockout of EDN resulted in higher RSV burden in macrophage cells and cell death. By screening of a tRF&tiRNA PCR array, we identified the regulatory tRNA (tiRNA) and tRNA fragments (tRFs) associated to EDN expression and RSV infection. Further data analysis revealed that EDN selectively cleaves on particular tRNAs, which highlights a primarily preference on UU and secondarily on UC within tRNA anticodon loop. In particular, out of a total of 185 of the cleavage products related to EDN expression, we identified 3 sequences that stand out significantly different between groups (fold change >4; $p < 0.01$). So, we proceeded to confirm the potential cleavage sites of EDN in these 3 sequences *in vitro*.

The selected target sequences were synthesized by i*n vitro* transcription (IVT) based on a T7 RNA polymerase amplification method. We firstly amplified the double strand DNA containing the T7 promotor by polymerase chain reaction (PCR) and sent the product from PCR to sequence to confirm the template correctly amplified. Then, IVT had been operated by the T7 RNA polymerase, followed by further purification. Once the size of tRNA was verified by gel electrophoresis, we analyzed the tRNA degradation in the presence of recombinant EDN by 12% urea gel. From the degradation gels, we noticed that all these 3 sequences cleaved by the recombinant EDN have half-size degraded products, which indicated that the main cleavage site would be located at the anticodon loop. However, cleavage products with other sizes that may correspond to tRFs, are also produced at different levels among the 3 sequences, as a function of protein concentration.

The present results confirm the contribution of EDN in macrophage response against virus infectivity and suggest a cleavage selectivity against cellular tRNA population. Further work is in progress for a full characterization of EDN tRNA selective cleavage pattern. A better understanding of the mechanism of action of EDN targeted pattern during the antiviral host defense should provide the basis for the design of novel antiviral drugs.

1. Li J, Boix E. Host Defence RNases as Antiviral Agents against Enveloped Single Stranded RNA Viruses. Virulence. 2021 Dec;12(1):444-469. doi: 10.1080/21505594.2021.1871823.

P2-09

Novel alkaline pH-regulatable hybrid promoters for recombinant protein expression is the yeast *Saccharomyces cerevisiae*

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The production of recombinant proteins of industrial interest by become a standard method to obtain large amounts proteins that are difficult or expensive to extract from its natural origin. Whereas the yeast Saccharomyces cerevisiae is an excellent host for expression of recombinant proteins of industrial interest, its use is often limited by the lack of promoters whose expression can be regulated in an industrial scale fermentation setting.

Our previous studies on the transcriptional response of yeast genes to alkalinization of the medium allowed the identification of regulatory modules that confer sensitivity to an increase in external pH. Based on the principles of synthetic biology, we have recently isolated and combined several of such regulatory modules (CDRE, PHO, NRG) and demonstrated that, fused to a basal transcriptional element, these hybrid promoters allow pH regulatable expression of a GFP reporter, leading up to a 20-fold increase in GFP levels. We have designed a variation of the Golden Gate cloning method that should allow the generation of libraries combining one or more instances of diverse regulatory modules. These libraries could be subjected to functional screen by flow cytometry and cell sorting to recover the combination of modules displaying the best performance. The strategy is currently under development using a STRE element (5'-GGCCCCTTA-3') as a test module. Preliminary analysis of a library recently generated has allowed the identification of construct carrying up to six concatenated STRE elements, whose functional characterization is currently ongoing. Since the transcriptional response can be triggered simply by addition of KOH, a very cheap compound, the generation of alkaline pH regulatable promoters could provide a very useful and cost-effective tool for recombinant expression at the industrial scale.

P2-10

Comparison of serum hepatitis B virus RNA and DNA quasispecies variability and conservation

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BACKGROUND: Serum circulating hepatitis B virus (HBV)-RNA is potentially useful for viral quasispecies (QS) analysis, even in patients with suppressed serum HBV-DNA. The aim of this study was to compare variability and conservation these parameters between circulating HBV-RNA and DNA QS in untreated chronic hepatitis B (CHB) patients.

METHODS: A serum sample from 13 untreated CHB patients was recruited. Isolated HBV-RNA was treated with DNAse I and then a qPCR was performed. HBV-RNA was retro-transcribed into cDNA and amplified in parallel with DNA. Finally, HBV QS between nucleotides (nt) 1255-1611 [5'region of the HBV X gene (HBX)], were analyzed by next-generation sequencing (NGS, Miseq, Illumina). Conservation and variability was evaluated by the information content (IC, bits) of each nt position.

RESULTS: Conservation and variability was highly coincident between HBV-RNA and DNA QS. HBV-RNA QS showed a high degree of conservation between nts 1559-1587 and HBV-DNA between nts 1519-1543. HBV-RNA displayed more positions with any variation (IC<2 bits) than DNA [135/357 (38%) vs. 85 (24%), (p<0.01)], and slightly lower IC mean [HBV-RNA 1.84 (standard deviation (SD)±0.36) vs. 1.85 (SD±0.35) HBV-DNA, (p<0.01)].

CONCLUSION: Both HBV-DNA and RNA QS showed a high degree of conservation in the HBX 5' region, with similar conservation and variability in individual nt positions. Surprisingly, HBV-RNA QS tended to a higher variability than HBV-DNA, however
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P2-11

Conservation, variability and evolution of Hepatitis Delta virus in antigen coding region

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Hepatitis delta virus HDV affects around 5% of hepatitis B virus infected patients causing the most severe chronic viral hepatitis. HDV RNA genome encodes just for a protein that exists in two length-dependent isoforms (S- and L-HDAg) following an edition of the canonical stop codon. The HDV has a high evolutional rate resulting in a complex viral population (quasispecies, QS) that could influence viral replication and clinical evolution. Mutations in L-HDAg related to escape from adaptive immune response had been associated with more severe hepatitis. Here we analyzed by next generation sequencing (NGS) the L-HDAg quasispecies to identify highly conserved regions and mutations that potentially affect HDV evolution.

Ten patients with chronic hepatitis delta (CHD) were enrolled in the study and followed up for an average period of 1.5 years. HDV RNA was extracted from two plasma samples per patient (at inclusion and follow-up). A region in HDAg gene located between nucleotide (nt) 910 to 1270 (amino acid, aa 111-215) was analyzed by NGS (*MiSeq Illumina*, San Diego, USA). Conservation was calculated by analyzing QS information content. Amino acid substitutions were identified by aligning sample QS with its genotype sequence.

A median of 31384,5 reads/sample was obtained. Two highly conserved regions were observed between nt 1404-1383 (aa 159-172) and nt 1502-1483 (aa 191-197) of L-HDAg, whereas high variability was observed between nt 1480-1423 (aa 171-190). Of note, conservation index did not change over the time in none of the studied patients, 37 mutated aa positions were identified. Remarkably, in 5/10 patients, 7 aa changes (K113S, V144I/T, P148R, V149T/A, S159A, T180S, V188M/I) were positively selected over time and located within CD8+ T-cells epitopes. Of note, in 3/5 patients, more than 2 aa changes at epitopes were observed.

The HDAg gene seems to be relatively conserved during the time. The HDAg editing domain was highly conserved, agree with its essential role. Other hyper-conserved region was observed, which might suggest an important function in HDV replication. However, some aa changes located into CD8 immune epitopes were selected, suggesting a possible immune escape mechanism as one of the HDV evolution factor. Funding: Instituto de Salud Carlos III (grant PI17/02233), co-financed by the European Regional Development Fund (ERDF).

P2-12

Rational peptide modification in the human eosinophil cationic protein N-terminal domain retrieves a new antimicrobial peptide with enhanced serum stability

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The relentless spread of multi-drug resistant bacteria requires the discovery of new antibiotic molecules to successfully fight infections. Antimicrobial peptides (AMPs) are extensively studied molecules with broad antimicrobial action that have shown limited success. Despite its potential as antimicrobial drugs, AMPs usually display low stability in vivo, mainly due to protease degradation in serum. This issue, combined with small therapeutic windows, largely limits the potential of AMPs as leading drugs to fight infections.

With this goal in mind, we aimed to improve the stability hECP24, a potent AMP derived from the antimicrobial region of the human eosinophil cationic protein (ECP). Here, we demonstrate that the modification of functional residues in a peptide by non-natural amino acids can enhance the stability in human serum. Moreover, this strategic replacement can be used to reduce the toxic effects of hECP24, without largely affecting the antimicrobial activity. The analysis of digestion profiles obtained by cleavage in human serum enabled us to generate new peptides with even more stability (over 30-fold half-time increase) that virtually lack any toxic effects, even at high peptide concentrations in both erythrocytes (250 μM) and mammalian cells (>150 μM). Moreover, CD and NMR studies on our new peptide variants confirmed that such modifications do not affect the global structure of the peptides.

In conclusion, our results confirm that strategic non-natural amino acid replacement in AMPs can help to overcome the barrier that prevents the clinical development of these molecules.

P2-13

Role of Cerium Oxide Nanoparticles modulating the innate immune system

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Nanoceria, Cerium oxide (CeO2) nanoparticles, by buffering excess of metabolic Reactive Oxygen Species (ROS), control the immunochemical potential, allowing to regulate the immune response by administering cellular power supply. In this context, new mineral antioxidant substances may overcome actual limitations, and thus open a new era for human health management. Nanoceria, similar to other antioxidant substances, displays powerful anti-inflammatory effects. Nanoceria is indeed an antireducer and acts as heterogeneous catalytic free radical scavenger allowing both a long term activity with a single low dose, and the effective reduction of pathologic (excess of) ROS. Finally, nanoceria has shown to be safe to normal tissue and slowly biodegrade. As antioxidant substances have recurrently failed to translate promising preclinical results into clinical practise, it is also necessary to understand how antioxidant substances work. To understand how nanoceria works inside a biological system, it is essential to know how immune cells employ different metabolic pathways to sustain their energetic demands, and how ROS is a key mediator in these metabolic shifts that have been observed. In this context, macrophages have clearly shown different metabolic pathways regarding their polarization status (M0, M1, or M2), which are linked to their specific functions (quiescence, inflammation, resolution/repair). To understand that, we are investigating the modulation role of nanoceria in the THP-1 cell line. This study aims to demonstrate the capacity of nanoceria to promote M2 phenotype by scavenging oxidative stress, meanwhile, it does not suppress the innate immune system activation when Toll-like receptor (TLR) signalling pathways are induced. To understand that, we focus on the nanoceria effects on the mitochondria. Indeed, the mitochondria considered the powerhouse of the cell, recently emerged as critical mediators of immune responses. They can control the energy requirements of cells through dynamic fission and fusion events, which are linked to ROS production and concentration, a major player in immune response, inflammation, and disease.

P3-01

Development of CAR T cells against p95HER2 for the treatment of HER2+ tumors

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Advances in immunotherapy have prompted the development of novel treatments, such as chimeric antigen receptors (CARs) or T-cell bispecific antibodies (TCBs). These novel immunotherapeutic agents redirect cytotoxic T cells against tumor cells independently of the specificity of T cell receptors (TCRs). Despite the potential of these therapies, the scarcity of tumor-specific antigens has led to the generation of CARs and TCBs against tumor-associated antigens, which are also expressed in normal tissues at low levels. Toxicities caused by "on-target off-tumor" effects are considered one of the main hurdles in the development of this T cell redirecting therapies, specially against solid tumors.

In search for novel and safe tumor-specific antigens, we focused in p95HER2, a truncated form of the tyrosine-kinase receptor HER2, expressed in around 30% of HER2-amplified tumors. We have recently shown that p95HER2 is a very attractive target, since it is undetectable in normal tissues. Moreover, through the generation of a p95HER2-TCB, we have proved that the redirection of T cells against p95HER2 is a viable and an efficacious antitumor strategy against HER2-positive tumors.

We are currently developing p95HER2 CAR T cells from different murine and humanized anti-p95HER2 antibodies. We have first characterized several CAR versions *in vitro* and results showed that at least three candidates had a good antitumor activity on p95HER2 expressing cells, and no activity against normal HER2 expressing cells. These selected candidates were further tested in vivo and our best candidate achieved a complete and durable remission of p95HER2 positive cell line-derived breast tumors, while having no effect against cells with basal levels of HER2. Recent results have also shown that this CAR is active against lung and brain metastasis.

Moreover, preliminary results in a p95HER2 positive patient-derived xenograft (PDX) model, which is considered to be a better predictor of the therapeutic response in human patients, showed at least partial antitumoral response.

As second-generation CARs against solid tumors have failed in the clinic so far, our focus is now on improving the CAR design to make it more effective against PDX samples for the successful treatment of solid tumors in patients.

P3-02

ERK5 inhibition induces autophagy-mediated cancer cell death by activating ER stress

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Autophagy is a highly conserved intracellular process that preserves cellular homeostasis by mediating the lysosomal degradation of virtually any component of the cytoplasm. Autophagy is a key instrument of cellular response to several stresses, including endoplasmic reticulum (ER) stress. Cancer cells have developed high dependency on autophagy to overcome the hostile tumor microenvironment. Thus, pharmacological activation or inhibition of autophagy is emerging as a novel antitumor strategy.

ERK5 is a member of the MAP kinase family that is activated in response to growth factors and different form of stress. Recent work has pointed ERK5 as a major player controlling cancer cell proliferation and survival and, therefore small-molecule inhibitors of ERK5 have shown promising therapeutic potential in different cancer models. Here, we report for the first time ERK5 as a negative regulator of autophagy. Thus, ERK5 inhibition or silencing induces autophagy (increased LC3-II levels) in a panel of human cancer cell lines with different mutation patterns. As reported previously, ERK5 inhibitors (ERK5i) induced apoptotic cancer cell death. Importantly, we found that autophagy mediates the cytotoxic effect of ERK5i, since ATG5$^{-/-}$ autophagy-deficient cells viability were not affected by these compounds. Mechanistically, ERK5i stimulated autophagic flux independently of the canonical regulators AMPK, mTORC1 or ULK. Moreover, ERK5 inhibition resulted in ER stress and in activation of the Unfolded Protein Response (UPR) pathways. Specifically, ERK5i induced expression of the ER luminal chaperone BiP (a hallmark of ER stress) and of the UPR markers CHOP, ATF4 and the spliced form of XBP1. Pharmacological inhibition of UPR with chemical chaperone TUDC, or ATF4 silencing, impaired the UPR, autophagy and cytotoxicity exerted by ERK5 inhibition.

Overall, our results suggest that ERK5 inhibition induces autophagy-mediated cancer cell death by activating ER stress. Given the fact that ERK5 inhibition sensitizes cancer cells and tumors to chemotherapy, future work will be necessary to determine the relevance of UPR and autophagy in the combined used of chemotherapy and ERK5is to tackle cancer.

P3-03

Pap-smears allow the identification of protein biomarkers to diagnose Endometrial Cancer

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Introduction: Endometrial cancer (EC) is the most common gynecological cancer in developed countries. There are no screening tools for its early diagnosis, and the diagnostic process starts with the presence of related symptoms, mainly, abnormal vaginal bleeding (AVB). Even though 90% of EC patients will experience AVB, this symptom is not specific of the disease and only 9% of the studied patients will finally present EC. The first diagnostic step is to perform the pathological evaluation of an endometrial pipelle biopsy. However, this procedure is associated to 22% of failure and these patients will undergo additional invasive procedures such as hysteroscopy to be definitely diagnosed. Yearly, ~7M women experience AVB in Europe and begin this diagnostic process, causing morbidity to patients and a big burden to the healthcare systems. Considering that the fluid contained in the uterus drains through the cervical canal to the vagina, we aimed to identify protein biomarkers that can effectively diagnose EC in liquid cervical cytologies.

Materials and Methods: The discovery phase to identify potential biomarkers using liquid cervical cytologies was performed though a shotgun label-free proteomic approach. The study included 60 patients (20 EC patients, 20 controls suffering AVB without endometrial or cervical pathology, and 20 controls without endometrial pathology but cervical pathology). The data was analyzed using MaxQuant and R software. The levels of 110 peptides corresponding to 75 proteins identified in the discovery phase, were further measured in a verification phase of 234 (107 non-EC; 127 EC) patients by LC-PRM. Analysis was performed using SPSS and R software.

Results: The discovery study permitted to determine a total number of 2,888 proteins identified with more than a single peptide in our samples. Among those, we discovered 75 proteins that are differently expressed between EC and non-EC patients. The verification phase confirmed the potential of 58 of those proteins to become potential diagnostic biomarkers in liquid cervical cytologies (adj.p.value < 0.05, Fold-Change >2, AUC > 0.70). Specifically, 16 proteins achieved an AUC > 0.75, and 3 proteins an AUC > 0.80. Additionally, an ELISA assay of the best performance protein was tested in all the samples reproducing the results obtained by mass-spectrometry and reaching an AUC=0.93 in this dataset.

Conclusions: It is possible to identify differentially expressed proteins in liquid cervical cytologies of EC patients with a diagnostic power up to 92%. Levels of 58 proteins were shown to be significantly higher in EC patients compared to controls. The best biomarker alone showed a diagnostic power of 92% in ELISA assay. These results will allow the development of an early and non-invasive screening tool for EC, that could improve diagnostic efficacy and management of women presenting with abnormal vaginal bleeding and save great healthcare costs.

P3-04

Polymeric Micelles Co-Loaded with Paclitaxel and Anti-Cancer Stem Cell Drugs to Overcome Drug Resistance in Triple Negative Breast Cancer

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Triple negative breast cancer (TNBC) is a heterogeneous disease with higher rates of relapse and metastasis and shorter overall survival than other breast cancer types [1]. Upon the lack of targeted therapies, chemotherapy remains the primary treatment for TNBC [2,3]. Accumulating evidences indicate that cancer maintenance, metastatic dissemination and drug-resistance are sustained by a small population of cancer cells with stem cell-like properties, termed cancer stem cells (CSC) [4,5]. Interestingly, TNBC shows high numbers of CSC, a fact that has been linked to the high rate of relapse in this subtype [6]. In this regard, we hypothesized that combination of anti-CSC agents with chemotherapy could improve the treatment of aggressive TNBC. Moreover, the use of nanotechnology-based systems would allow the simultaneous delivery of synergistic ratios of both drugs, while reducing their side effects. Following this hypothesis, we found that two widely-used drugs, 8-Quinolinol (8Q) and Niclosamide (NCS), showed specific anti-CSC activity by affecting essential stemness hallmarks in TNBC cells. Subsequently, both drugs were studied in combination with Paclitaxel (PTX), reference drug for TNBC treatment, and the synergistic ratios among drugs were established in different TNBC cell lines. Interestingly, while the solely use of PTX increased the relative presence of CSC, the combination of PTX with 8Q (1:12.5) and NCS (1:2) allowed a significant reduction of CSC. Moved by these positive results, we next used Pluronic® F127 polymeric micelles (PMs) [7] to encapsulate both anti-CSC drugs, alone and co-loaded with PTX at these synergistic ratios. In vitro drug-loaded PM showed a higher efficacy in reducing tumor cell proliferation and CSC viability than free drugs, either alone or in combination. Remarkably, NCS drug-loaded PMs in combination with free PTX significantly reduced lung metastasis and circulating tumor cells in TNBC mice model. Overall, our results demonstrate that i) targeting CSC in TNBC is possible by using specific anti-CSC drugs, ii) the combination of anti-CSC drugs with PTX at established ratios leads to a synergic effect on CSC inhibition and, iii) the encapsulation of anti-CSC drugs further increases the efficacy of the drug-combination on CSC population in vitro and in vivo.

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P3-05

circRNA expression allows discrimination of NSCLC from cancer-free lung specimens using the nCounter platform

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Introduction

Lung cancer is the lead cause of cancer-related deaths. Studies on tumor profiling have soared in the last decade improving overall survival of these patients by shaping current targeted therapies; however, further investigation of novel biomarkers for early diagnosis still remains imperative. circRNAs are a class of tissue-specific stable structures controlling mammalian transcription. Their aberrant expression plays an important role in cancer emerging as valuable biomarkers; Conversely, its potential has not been fully explored in lung cancer due to several limitations of current circRNA quantification methods that prevent their clinical implementation. The nCounter technology allows for quantitative assessment of up to 800 targets providing an accurate and factual perspective of expression levels. To our knowledge, this study stands as the first on assessing circRNA differential expression with this platform for lung cancer detection both in FFPE lung tissues and cell lines providing evidence of their differential expression in lung cancer specimens.

Methods

Cells were cultured under standard conditions until harvested. RNA was isolated by using Allprep DNA/RNA/miRNA universal kit (Qiagen). RNA from FFPE lung tissue samples (n=69; 53 NSCLC,16 non-cancer) was isolated with the high Pure FFPET RNA isolation kit (Roche). Overnight hybridization and posterior nCounter processing were performed following NanoString protocol for nCounter Elements. Expression analysis was carried out based on a tailored panel of 78 circRNAs related to the biology of the disease. Machine learning (ML) algorithms were applied to generated data with RStudio (V1.3.1056).

Results

FFPE lung tissues revealed a cluster of differentially expressed circRNAs allowing distinction of lung cancer versus control. circFOXP1, circEPB41L2, circSOX13 and circBNC2 were highly downregulated, while circCHD9, circFUT8, and circACACA were the most upregulated in cancer specimens. circRNA expression of A549, H2228, H3122, PC9, H1666, HCC-827 and HOP-62 cells was compared to the AALE and HBEC3-KT epithelial cell lines. circFUT8 were also confirmed upregulated in cancer cell lines. ML results selected a 16 circRNA signature that allows discrimination of early-stage lung cancer patients from non-cancer controls.

Conclusion

This study presents for the first time the use of circRNA differential expression in FFPE tissues for lung cancer discrimination using the nCounter platform. A 16 circRNA signature has been selected which allows discrimination of early-stage lung cancer samples from non-tumor controls. Validation of the signature in liquid biopsies is warranted.

P3-06

Glioblastoma, tumour microenvironment and therapy response related changes: noninvasive MRSI-based assessment and future perspectives

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Introduction: Glioblastomas (GB) are deadly brain tumours. Magnetic resonance spectroscopic imaging (MRSI) could be a biomarker for immunoresponse in GB[1]. Glioma-associated microglia/macrophages (GAMs) are abundant immune cells in GB, polarised into anti-tumour or pro-tumour phenotypes (M1/M2). Matrix Metalloproteinases (MMPs) and 'A Disintegrin and Metalloproteinase' (ADAMs) are indicative for tumour microenvironment changes and correlate with GAM phenotypes[2]. Interaction between Programmed Death Factor 1 ligand (PD-L1) and receptor (PD-1) is another important immunosuppressive mechanism.

Methods: Tumour microenvironment changes during temozolomide (TMZ) chemotherapy were assessed in GL261 GB-bearing mice. MRI/MRSI was used to assess response extent, and qPCR analyses for GAMs M1/M2 polarisation, ADAMs 8/10/17, MMP9 and MMP14, in addition to PD-L1 expression.

Results: Responding samples identified by MRSI-based biomarker nosological images showed increased M1/GAMs and M1/M2 ratios with defined proteinase expression profiles and low MMP-9 levels. PD-L1 expression positively correlated with M1/M2 ratio, ADAMs and MMP14 expression levels.

Discussion: Higher M1/M2 ratios correlated with survival in TMZ-treated GB patients[3]. ADAM10 and ADAM17 positively correlate with M1 phenotypes and patient survival[4]. MMP9 downregulation is a biomarker for improved outcome[5] and chemotherapy may increase PD-L1 expression[6].

Conclusions: Changes in GAMs prevailing population could help to explain MRSI pattern differences, although other microenvironment components may also contribute. Protease profiles were associated with microglia/macrophage functions and could provide insight into the molecular signature while helping predict GB patients' therapy response and overall survival.

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P3-07

Does risk of malignancy algorithm (ROMA) discriminate more efficiently than HE4 or CA125 in the diagnosis of pelvic masses?

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BACKGROUND-AIM: Cancer antigen 125 (CA 125) is the most used biomarker to discriminate between benign and malignant adnexal masses. Due to his low specificity especially in benign masses in premenopausal women, new tumor markers (HE4) and algorithms (ROMA) have been included in the routine diagnostic setting. The aim of the study is to assess the discriminate value of HE4, ROMA and CA 125 in patients with pelvic masses submitted to surgical treatment.

METHODS: Serum CA125 and HE4 were measured with chemiluminescent immunoassays (Abbott) and ROMA was calculated for 199 patients (104 pre and 95 postmenopausal) who had final histological diagnosis of ovarian masses.

RESULTS: Histological diagnoses were: 137 benign, 17 borderlines and 45 malignant ovarian tumors. According to menopausal status 104 were premenopausal (PRE) and 95 postmenopausal (POST). CA 125, HE4 and ROMA showed sensibilities (SN) of 85.5 %, 75.8 % and 79 % respectively with specificities (SP) of 64.2 %, 82.5 % and 73 % using standard cut-off values of 35 U/mL (CA 125), 70 pg/mL (HE4), 7.4% (ROMA PRE) and 25.3% (ROMA POST). When using optimal cut-offs from ROC curves SP increased to expenses of SN in the case of CA 125 and ROMA but not in the case of HE4 which optimal cut-off (68.4 pg/mL) was close to the standard used. In the total group, AUC for ROMA (0.876; 95%CI: 0.816-0.936) was higher than for HE4 (0.855; 95%CI: 0.796-0.915) and for CA125 (0.826; 95%CI: 0.763-0.889) but not statistically significant (p=0.1757; p=0.0776). When women were separated based on their menopausal status, in the PRE group, AUC for HE4 (0.813; 95%CI: 0.686-0.941) was higher than for ROMA (0.805; 95%CI: 0.676-0.934) and for CA125 (0.725; 95%CI: 0.596-0.855) but not statistically significant (p=0.2568; p=0.1335). In the POST group, the highest AUC was obtained with CA125 (0.907; 95%CI: 0.846-0.968) compared with ROMA (0.890; 95%CI: 0.818-0.961) and HE4 (0.835; 95%CI: 0.754-0.916) but not statistically different (p=0.3470; p=0.0596).

CONCLUSIONS: In our experience, when all women were considered and when the menopausal status where considered, ROMA had the same discrimination capacity between benign and malignant ovarian masses than HE4 and CA125. No advantage of ROMA was observed compared with HE4 alone in premenopausal or with CA 125 alone in postmenopausal women

P3-08

Aurora Borealis (BORA) increases ovarian cancer aggressiveness by enhancing the metastatic and angiogenic potential of malignant cells

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Aurora Borealis (BORA), is the major activator of the master mitotic kinase polo-like kinase 1 (PLK1). Long ago it was demonstrated the oncogenic role of PLK1 and more recently the specific role of its activator BORA in tumorigenesis has also been explored in different tumor types. We have demonstrated that BORA has an oncogenic role in ovarian cancer (OC); an aggressive and disseminative tumor diagnosed predominantly at advanced stages, where limited therapeutic options are available. Now, we are further exploring the mechanisms by which BORA enhances OC aggressiveness, with the final goal of exploring novel therapeutic opportunities for OC management.

We carried out a transcriptome-analysis of xenograft tumors derived from OC cells overexpressing BORA (BORA_OE cells) compared to those derived from OC cells with basal levels of BORA (CTL cells). We have further validated the results in vitro and are now generating an in vivo model to mimic OC peritoneal metastasis. In addition, we are exploring for which genomic backgrounds in OC, BORA could offer a better therapeutic window.

Tumors derived from BORA-OE cells presented increased expression of genes involved in epithelial mesenchymal transition (EMT), migration and angiogenesis. In accordance to this, cells with enhanced BORA expression have higher migration, invasive and adhesive capacities, which are independent of their increased proliferation rate. These results, together with the fact that ovarian tumors are highly vascularized and that they disseminate throughout the peritoneal cavity, leading to the formation of hemorrhagic ascites, make us suggest that BORA overexpression increases tumor aggressiveness by enhancing the metastatic and angiogenic potential of OC cells. Furthermore, BORA inhibition combined with the current standard of care in advanced OC (paclitaxel and cisplatin) resulted in a greater reduction in cell viability in vitro. Together, BORA targeting could represent a promising new therapeutic strategy to manage OC.

Taken together, we have uncovered the role of BORA in human OC aggressiveness, especially in the context of metastatic dissemination, thus providing a new potential therapeutic opportunity for OC management.

P3-09

Targeting MDM2-p53 interaction in rhabdomyosarcoma: restoration of p53 and FBXW7 tumor suppressor activity

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Rhabdomyosarcoma (RMS) is the most common type of soft tissue sarcoma in children and can be divided in two main subtypes, alveolar and embryonal RMS (aRMS and eRMS), according to the presence of PAX3/7-FOXO1 fusion protein. In contrast to the majority of adult tumors, p53 mutations are extremely low in RMS accounting for 5% of RMS cases. However, a $p53^{WT}$ status is not always associated with a favorable prognosis as it occurs in PAX3/7-FOXO1 aRMS or MYOD1 mutated eRMS. Besides, other alterations like MDM2 amplification or CDKN2A deletion may affect the role of p53. In this study, we evaluated whether MDM2 inhibitors could restore p53 function in $p53^{WT}$ RMS and, in turn, activate FBXW7 tumor suppressive activity. Treatment with MDM2 inhibitors (MI-773, AMG-232, Siremadlin and Idasanutlin) reduced cell viability in RH36 and RH18 cell lines expressing wild-type p53, but not in $p53^{WT}$ RH28 characterized by PAX3-FOXO1 and CDKN2A deletion. Of importance, cell sensitivity to Siremadlin and Idasanutlin correlated with MDM2 and CDKN1A mRNA expression assessed by RT-qPCR while FBXW7 mRNA increased at 16-24h post-exposure to treatments. To further potentiate p53 and FBXW7 restoration, MDM2 inhibitors were combined with the BMI-1 inhibitor PTC-028 inducing synergistic effects *in vitro*. Combination molecular effects were assessed determining gene expression by cDNA microarrays highlighting a downregulation of GLI1/2/3 gene expression in comparison with control and treatments separately. Finally, changes in p53 and ERK phosphorylation were observed in the combination group using phospho-kinase arrays. These results support a putative new therapeutic approach to treat $p53^{WT}$ RMS.

P3-10

PRL-PRLR axis implication in chemoresistance and proliferation in Acute Myeloid Leukemia

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Current cytotoxic treatments for acute myeloid leukemia (AML) eliminate the bulk of proliferating cells but leave most quiescent leukemic stem cells (LSC) and chemoresistant cells unaffected. The last, might be able to generate a subpopulation of cells with the same characteristics, promoting chemotherapy resistance. A previous *in silico* study performed in our laboratory identified the prolactin receptor (PRLR)-signalling pathway as one of the main pathways implicated in AML progression. The main objective of the project is the identification of the role of PRL:PRLR axis in the transformation events associated with the initiation and maintenance of AML and its druggable potential.

PRLR surface expression correlated positively with a higher resistance to Cytarabine, and same results were observed when PRLRwt isoform was overexpressed. Moreover, we observed a downregulation of hENT3 and an upregulation of MRP4, transporters that internalize and externalize cytarabine, respectively. We also noticed an upregulation of NT5E, a cytarabine inactivator. On the other hand, cells transduced with natural ligand PRL did not possess such resistance.

hPRL treatment, but not the antagonist hPRL-G129R, induced proliferation only due to ectopic PRLRwt expression *in vitro* and the same results were obtained *in vivo.* Furthermore, mice transplanted with PRLmut transduced cells had a lower leukemogenesis capacity in comparison to PRLwt-expressing AML cells transplanted ones *in vivo* and a lower clonogenicity capacity *in vitro*.

Next, we used hPRL-G129R in AML patient samples to determine its cytotoxic effect *in vitro*. hPRL-G129R did not affect the viability of AML cells, but decreased the clonogenicity of treated cells. Nevertheless, this effect was not observed when healthy donor samples were treated.

Taken together, those results suggest an important role of PRLR:PRL axis in AML. First, PRLR expression, but not increased levels of its ligand, confers resistant to Cytarabine *in vitro*, and might be due to upregulation of MRP4 and NT5E and downregulation of hENT3. Second, PRL-PRLR signalling promotes proliferation signals in AML, both *in vivo* and *in vitro.* Third, an overexpression of PRLR generates a worse AML prognosis due to its effect in proliferation, while antagonizing the receptor decreases proliferation. Interestingly, the inactive form of hPRL confers a lower clonogenicity *in vitro* and leukemogenesis capacity *in vivo.* Last, hPRL-G129R has no effect in viability but impaired the clonogenicity of primary patient samples, without affection in healthy donor samples.

In summary, PRLR: PRL axis has an important role in AML proliferation and drug response, so targeting PRLR with inactive forms of its ligand may be a useful treatment against AML.

P3-11

p38α MAPK is involved in the development and progression of melanoma and modulates the anti-tumoral immune response

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The mitogen-activated protein kinase (MAPK) pathway plays a key role in melanoma development and progression, since mutations in *BRAF* and *NRAS* gens lead to increased signaling activity of the pathway promoting cell survival, proliferation, and migration. Besides this, pathways responding to external and internal stress, such as ROS response or UV damage, also play an important role in melanoma pathogenesis. Interestingly, about 25% of human cutaneous melanoma samples presented p38α mRNA upregulation, which was associated to a poor survival of the patients. Furthermore, we have observed that p38α is involved in the regulation of the proliferation and survival of the cells, but also in the regulation of the expression of several immunocheckpoint molecules in melanoma cell lines. Although the well demonstrated function of the p38 MAPK pathway in the response to UV damage and inflammatory cytokines, how p38α is involved in UV-induced melanoma development and progression has not been studied yet. In order to investigate the role of p38α in melanoma in cooperation with Braf oncogene and UV radiation *in vivo*, and its contribution to the regulation of the intratumoral immune response, we have generated the Tyr::CreER^{T2}; Braf$^{CA/CA}$; p38α$^{F/F}$ melanoma mouse model. Here, the administration of 4-hydroxytamoxifen (4OHTx) promote the expression of mutated Braf^{V600E} and the loss of p38α in melanocytes. We have observed alterations in the incidence, onset mean and tumor size and multiplicity in this animal model, which indicate that p38α has a relevant effect on the development of melanoma in cooperation with Braf^{V600E} and UVB radiation. Furthermore, analysis of tumor-infiltrating immune cells showed that p38α is involved in the modulation of the immunological profile of the tumor. Altogether, our results suggest that p38α could be also playing a crucial role in melanoma progression, promoting tumor proliferation and modifying the immune system and tumor microenvironment. Therefore, p38α could be an interesting target for the development of new therapeutic approaches for the treatment of melanoma.

P3-12

Dissecting the immune response elicited by olaparib in homologous recombination repair deficient breast cancer

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Tumours with defective DNA repair by the homologous recombination repair (HRR) pathway are exquisitely sensitive to PARP inhibitors (PARPi), which provoke the stalling of replication forks and the accumulation of double strand DNA breaks. The chromosomal instability resulting from endogenous and PARPi-induced DNA damage has been shown to activate the cyclic GMP–AMP synthase (cGAS)/stimulator of interferon genes (STING) pathway. Upon recognition of cytosolic DNA, the STING pathway arouses the anti-tumour immune response through the transcriptional activation of interferon-related genes. Accordingly, evidences that the STING pathway is suppressed in several cancer types suggest that its defects may serve as an immune escape mechanism. As a consequence, multiple STING agonists (STINGa) have been developed to enhance anti-cancer effects. However, emerging pro-tumor roles, including PD-L1 upregulation, have been described and it remains to be better understood the interplay between the DNA damage response and the STING pathway and how it impacts the efficacy of PARPi and immunotherapies targeting STING.

The antitumour activity of olaparib (PARPi) was tested in a panel of 40 HRD Patient-Derived breast cancer (BC) Xenografts (PDX) and in Mouse-Derived syngeneic Xenografts (MDXs) from the Brca1$^{f22\text{-}24\text{-}/\text{-}}$ transgenic mouse strain. Olaparib sensitivity associated with the lack of RAD51 foci, a marker of HRD, and the increase of micronuclei and γH2AX-positive micronuclei, signs of chromosomal instability. In addition, olaparib treatment resulted in the activation of cGAS, as indicated by the induction of cGAS positive micronuclei and suggesting the involvement of the STING pathway and the immune response in PARPi efficacy. Accordingly, a more profound response to olaparib was observed in PDXs engrafted in NMRI mice, than in less immunocompetent NSG mice. The PDX cohort was characterized for STING protein expression and showed generally higher levels in the stroma than in the tumor compartment, with almost 60% of PDX models suppressing STING expression in the tumor compartment. Interestingly, these PDX tumours failed to activate the IFN response upon *ex vivo* treatment with a STING agonist.

The evaluation of STING pathway activation (biochemical activation, immune-related gene/cytokine expression, IFN secretion) upon PARPi and STINGa is ongoing in our models and will allow to establish the prevalence of this effect, identify molecular alterations associated to the lack of activation and explore the rationale of combining PARPi with immunotherapies.

P3-13

Targeting protein homeostasis to impair tumor-stroma crosstalk in 3D spheroid models of aggressive B-cell lymphoma

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Proteomic profiling of patients with aggressive B-cell non-Hodgkin lymphoma (B-NHL) has not significantly improved the clinical management of these patients. Previous studies have validated the use of specific ubiquitin traps (TUBEs) associated to tandem mass spectrometry (MS/MS) to predict response to different classes of drugs targeting protein homeostasis in standard 2D co-cultures models of aggressive B-NHL. Whether this approach can be applied to the elucidation of the role of protein homeostasis in lymphomagenesis and to the discovery of new potent therapeutic targets in these entities is still to be determined. To evaluate the modulation of immune effectors by adaptive protein homeostasis in malignant B cells, we are developing a methodological strategy for the comprehensive study of ubiquitin and its interactome (ubiquitome) in two of the most aggressive subtypes of B-NHL, mantle cell lymphoma (MCL) and diffuse large B cell lymphoma (DLBCL), taking into account the main components of the lymphoid tumor microenvironment (TME). We will use the TUBEs-MS/MS to capture and identify endogenous ubiquitinated proteins in MCL and DLBCL 3D multicellular organotypic spheroid including tumor-associated macrophages (TAMs), using an innovative, electro-magnetic-based technology. We have successfully generated the first cell line based 3D models for both pathologies, combining different MCL and DLBCL cell lines (REC-1 and KARPAS 422 respectively) with different types of connective tissue cell lines: Human stromal NK.tert (bone marrow stromal cell line) and HK (follicular dendritic cell line). Using various fluorescent trackers, we are able to trace cellular interaction and migration within the spheroid by fluorescent microscopy and time-lapse filming with, including our first toxicity assays on these structures. Some spheroids are sent to the pathological anatomy department to be embedded in paraffin, cut and stained with Haematoxylin-Eosin to evaluate cell distribution, extracellular matrix formation and overall architecture. Our present results show certain degree of 3D organization, which resembles the architecture of both pathological conditions in a simplified manner. We are working on the optimization of spheroid growth medium and protocols for incorporating TAMs and other cells to the 3D structures. These protocols include increasing TAMs lifespan and M2 differentiation, which we have increased for up to 3 weeks with the correct interleukin stimuli, maintaining viability and differentiation markers stable on monocyte derived macrophages. These are the first steps towards a biologically realistic organotypic spheroid of lymphoid malignancies, which will enable us to study tumor-stroma crosstalk and have a personalized model for each patient to evaluate and generate personalized therapies.

P3-14

Evaluation of Cereblon modulators in *in vitro* models of diffuse large B-cell lymphoma (DLBCL)

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Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma (NHL) of B-cell origin, and it presents with an aggressive clinical course. Despite the efficacy of the chemoimmunotherapy regimen R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone), and the introduction of promising targeted agents into clinical practice, patients frequently relapse. Moreover, these treatments are associated with high levels of toxicity, which underlines the need for new therapeutic options. A promising approach is the targeting of E3 ubiquitin ligases, such as the $CRL4^{CRBN}$ subunit Cereblon, involved in the ubiquitin proteasome pathway (UPS) for protein degradation. CELMoDs (Cereblon E3 Ligase Modulating Drugs) are able to redirect Cereblon specificity to ubiquitinate proteins of interest, such as Ikaros and Aiolos, two transcription factors that have crucial roles in hematopoietic cell development and function, especially B and T lymphocytes. Our aim is to evaluate *in vitro* the cytotoxic effect and to characterize the molecular pathways modulated by two new Cereblon-targeted compounds that induce the degradation of Ikaros and Aiolos. Lymph node biopsy samples from patients diagnosed with DLBCL are co-cultured with either a mesenchymal stromal cell line or primary macrophages obtained from healthy donors. The cultures are exposed to each of the two compounds as single agents at doses ranging 0.1–10 μM, or to the standard-of-care (R-CHOP). After 6 hours, 3 days and 5 days of culture, immunophenotyping is performed to analyze the intracellular expression of Ikaros and Aiolos, as well as to evaluate the levels of proliferation and apoptosis in both B and T lymphocytes. Additionally, genotypic profile after 24 hours will be analyzed by RNA sequencing (RNA-seq), and cytokine production will be assessed using an ELISA-based cytokine antibody array at day 3. So far, lymph node biopsies from n=16 DLBCL patients have been collected. Both compounds have shown to induce the degradation of Ikaros and Aiolos in B and especially in T cells, with no evidence of a dose-dependent effect. Downregulating efficacy of the compounds was higher than that of R-CHOP, although it did not reach statistical significance. After 5 days of treatment, T cells showed a moderate level of apoptosis as evaluated by active caspase 3 staining, but cell proliferation assessed by histone H3 phospho-serine 10 (H3pS10) staining was dramatically reduced by both the compounds and R-CHOP. In contrast, proliferation of the B-cell compartment was maintained up to 5 days, especially when cultured with the stromal cell line, but apoptosis was particularly induced by one of the compounds at 10 μM. No correlation has been observed between the efficacy of the compounds and the DLBCL subtype, or the basal percentage of B or T cells in the biopsy sample at diagnosis. More biopsy samples are being collected to further confirm and expand these preliminary results, which may help develop new therapeutic options for DLBCL patients.

P3-15

Deciphering new biomarkers to predict recurrence in ovarian endometriosis

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Endometriosis is a common, chronic, inflammatory condition of unknown etiology and pathophysiology, characterized by the growth of ectopic endometrium outside the uterus. It affects approximately 10% of women of reproductive age and it associates with pelvic pain and infertility. Among the many unmet clinical needs of endometriosis, one worrying issue is the high percentage of recurrence after laparoscopic surgery (aprox. 30%). There is no current clinical strategy to define prognosis nor to individualize treatment for endometriosis patients. In this study, we aim to identify reliable prognosis biomarkers by transcriptomic and proteomic approaches that will be useful to estimate the risk of recurrence after laparoscopic surgery and/or for stratification of recurrently symptomatic patients.

In the present study we identified transcriptomic and proteomic signatures in formalin-paraffin embedded (FFPE) endometriosis tissue of patients that underwent a therapeutic laparoscopic surgery in Vall d'Hebron Hospital and subsequently developed a recurrence (n=11) versus patients that did not (n=12). Differentially expressed RNA transcripts ($|log2FC| > 0.5$, p-value < 0.01) were determined by microarray analysis, and differentially expressed proteins ($|log2FC| > 1$, p-value < 0.02) were discovered using non-targeted mass spectrometry using a data-independent-acquisition method.

In the transcriptomic analysis, a total of 39 promising transcripts were identified to become biomarkers of endometrioma recurrence. In the proteomic analysis, a total of 30 proteins were significant when comparing recurrent vs. non-recurrent patients. Among those, 23 proteins had an AUC value over 0.8. Interestingly, 18 significant proteins are known to be involved in tumorigenic processes and/or have been described as cancer biomarkers. We aim to validate all candidates by RT-qPCR and targeted proteomics, respectively.

This initial discovery phase will be shortly followed by a validation phase with a larger cohort of patients to confirm the presented results. Besides, for this first stage only ovarian endometriosis patients were selected, but all forms of endometriosis are intended to be included in successive steps of the project.

P3-16

Mechanism of action of Thalidomide in CLE revealed using artificial intelligence.

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Background: Cutaneous involvement in Lupus Erythematosus (CLE) is common and encompasses a wide range of dermatologic manifestations. Around 70-80% of patients develop skin lesions at some point during the course of their systemic disease. Standard therapy consists of topical steroids and antimalarials; however, up to 40% of the patients are refractory. Thalidomide has been used successfully to treat several dermatological disorders. Its efficacy in CLE is significant with 80-90% of patients achieving clinical remission; however its use is still limited due to serious side effects.

Objective: To identify mechanism of action of Thalidomide in CLE.

Methods: Skin biopsies from CLE patients before and after thalidomide treatment have been analyzed by RNA-seq (n=10). Data from clinical responder patients was evaluated using artificial neuronal networks following Therapeutic Performance Mapping System protocols to obtain two plausible mechanisms of action. Expression gene analyses, immunofluorescence in skin biopsies and in vitro experiments have been performed in order to validate the proposed models.

Results: We discover that Thalidomide acts modulating CRBN and therefore, regulating two molecular pathways: IRF4/NFKB1 and AMPK1/mTOR. Immunofluorescence showed a reduction of CRBN, IRF4, MTOR and NFKB in post-thalidomide skin. Notably, IRF4 was localized in the dermis (3-fold intensity; $p<0.01$) and MTOR in the epidermis (1.5-fold intensity; $p<0.0001$) in pre-thalidomide skin. In vitro experiments demonstrated that thalidomide modulates IRF4/NFKB1 signaling in lymphocytes and AMPK1/mTOR in keratinocytes. In addition, co-culture experiments showed a cross-linking between identified pathways: Thalidomide addition in PBMCs promoted a significant reduction of MTOR protein ($p<0.001$) and their related cytokines (IL10, TGFβ, IL-1β) in keratinocytes, showing that IRF4 targeting may mimic the dual effect of thalidomide.

Conclusion: Within this study, IRF4 has been identified as putative molecular target of thalidomide treatment in CLE. This may contribute to the development of novel analogues to treat CLE effectively avoiding thalidomide undesired adverse events.

P4-01

Revealing protective T cell immune responses against SARS-CoV-2

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T cell responses are key to protecting against viral infections and necessary for antibody development. Here, in order to inform vaccine development on the correlates of protection against SARS-CoV-2, we studied the acute infection functional profile, migration patterns and caspase-3 expression of antigen-specific T cell responses in three different cohorts of patients (n=46). In depth flow cytometry analyses indicated increased apoptosis in antigen-specific and non-specific T cells associated with disease severity.
Pattern variations associated with T cell responses against SARS-CoV-2 where based on two factors, the targeted viral protein and the cohort of patients assessed. Overall, cell stimulation with membrane (M) and nucleoprotein (N) viral peptides induced a Th1 profile exemplified by IFNɣ in $CD4^+$ T cells and degranulation in $CD8^+$ T cells respectively, whereas spike (S) peptides induced a biased Th2 profile exemplified by IL-4. Hospitalization and disease severity were associated with predominant IFNɣ and IL-4 responses, as well as increased responses against S peptides, while non-hospitalized outpatients had a dominant IL-10 response produced by CCR7 expressing cells. Importantly, antigen-specific resident memory T cells in the lung were weakly detected in an asymptomatic individual and strongly detected in a severe convalescent patient, in which contemporary blood did not reflect resident profiles. In summary, different SARS-CoV-2 viral proteins elicit unlike T cell functional profiles, which have clear implications for vaccine design. A balanced anti-inflammatory and effector antiviral response may be key to favor infection resolution without major complications during acute infection. Further, while immune responses migrate and establish in the lung as resident memory T cells, which could be determinant for future protection against reinfection, the magnitude and profile of the lung SARS-Cov-2 specific T cells strongly differ from the response detected in blood.

P4-02

Studying the immunomodulatory role of human RNase 2

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Human RNase 2, also known as Eosinophil Derived Neurotoxin, is a member of the RNase A superfamily which is expressed in eosinophils, macrophages and other blood cell types[1]. Induction of RNase 2 expression is related with viral infections and an antiviral role is attributed to this protein although the mechanisms behind are still poorly understood. An interesting hypothesis is that RNase 2 can activate TLR signaling during viral infections, by generating RNA catalytic products or by direct interaction, promoting therefore the immune system response against the infection[2]. RNase 2 expression is also related to tissue remodeling. Interestingly, human RNases 3 and 5 were recently identified as ligands for the Epithelial Growth Factor Receptor (EGFR)[3,4]. The high structural similarities between RNase 2 and those proteins of the same family makes us to consider a possible role of RNase 2 into activating EGFRs.

We are studying the possible role of RNase 2 into activating EGFR, or another Tyrosine Kinase Receptor type. For that we have performed immunoblot assays of protein extracts from HepG2 and THP-1 macrophages treated with recombinant RNase 2 in order to determine if this protein is capable of activating MAPK pathway. On the other hand, our group is trying to put light into how RNase 2 can promote antiviral responses. With that purpose, we have studied the expression of genes related to antiviral response in THP-1 macrophages. The macrophages were treated with recombinant RNase 2 and gene expression of markers related to antiviral response was studied by RT-qPCR. Macrophages were also exposed to recombinant RNase 2- H15A, a catalytic mutant of the protein, in order to elucidate the role of catalytic activity in antiviral response induction. Treatments with both WT and catalytically defective recombinant proteins were also performed in presence of the TLR8 inhibitor CU-CPT9a with the aim of confirming the role of the intracellular receptor into mediating antiviral responses induced by RNase 2.

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P4-03

Gene therapy for ALS by specifically overexpressing chronokines in skeletal muscles

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the loss of cortical and spinal motoneurons (MNs). Denervation of endplates and axonal retraction is thought to lead, in a "dying-back" pattern, to the death of MNs and subsequent muscle atrophy. ALS neuropathology is mainly associated with oxidative stress, inflammation, excitotoxicity, and mitochondrial dysfunction. Anti-aging proteins or chronokines are neuroprotective, anti-inflammatory, antioxidative and promyelinating agents. Recent studies indicate that muscle and myofiber regeneration highly depend on chronokine expression. Chronokine deficient mice have extensive fibrosis, scarring and calcification after acute muscle injury. Moreover, these mice also show altered gait and have less MNs in the spinal cord, with astrogliosis and neurofilament accumulation, resembling an ALS phenotype.

In the SOD1^{G93A} mouse model, which recapitulates most of ALS abnormalities, we have found decreased mRNA levels of the secreted and transmembrane forms of our chronokine of interest in skeletal muscles, motor cortex and lumbar spinal cord. Furthermore, we studied its mRNA expression in the brachial plexus injury model of muscle denervation. We observed a downregulation of the secreted isoform in the C1-T5 spinal cord segment at 7 and 28 days post-injury (dpi) and of both isoforms in the forearm muscles at 28 dpi, when muscle atrophy is noticeable.

Given the pleiotropic beneficial properties of chronokines we hypothesized that boosting their secretion in skeletal muscles through a one-time gene therapy treatment would protect muscles from atrophy and prevent axonal retraction and neuronal loss associated with ALS. Our initial results show that the overexpression of a selected chronokine in muscles enhanced motor function and delayed disease onset as evidenced by rotarod and grip strength tests. Improvement of the functional outcome was demonstrated by increased compound muscle action potential (CMAP) amplitude of the tibialis anterior muscle. Increased amplitude of motor evoked potentials (MEPs) also reflected an improved central connectivity between upper and lower MNs. These preliminary findings indicate that increasing the secretion of chronokines by muscles is a promising approach for promoting functional improvement in ALS.

P4-04

Gene Delivery Systems: A Nanomedicine approach for cancer treatment

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Background: In recent years, nanomedicine has evolved aiming to achieve great advances in cancer treatment and diagnosis. Natural and synthetic polymeric materials have been employed as a delivery vectors in order to overcome stability and release-related problems associated with drug molecules.
Due to its high specificity, gene therapy has appeared as a promising alternative for an effective and specific treatment of complex diseases. However, the lack of an effective vector for the delivery of the genetic material into cells has centered the efforts of the scientists in the last years.
Gelatin, as a natural and biocompatible polymer, is a suitable candidate as a gene delivery vehicle. In this work, we present gelatin nanoparticles (GNPs) as carriers for the delivery of genetic material. pDNA-GFP has been chosen as a model of genetic material to be transfected into a cancer cell line.

Materials & Methods: GNPs physicochemical characterization was performed through Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM). The entrapment efficiency of DNA was measured by Qubit Fluorometric Quantification. The cytotoxicity of the different formulations were assessed by a Tetrazolium-based assay (MTT). The transfection efficacy was evaluated by fluorescence microscopy and flow cytometry.

Results: The proposed GNPs present a mean diameter around 100nm, a proper polydispersity index (below 0.1) and a positive zeta potential to interact with negative DNA molecule structure. Moreover this increase in overall charge is essential to allow an effective DNA entrapment as was demonstrated by the obtained high entrapment efficiency (> 90%). The cytotoxicity of the formulation was evaluated for 72h and a cell viability above 50% was obtained. A transfection efficacy of 90% was quantified by flow cytometry.

Conclusions: The optimal size, surface charge, together with the biocompatibility and biodegradability of the used polymer as well as the simple preparation method of GNPs make them a suitable platform for gene therapy into cancer cells. The enormous flexibility endorsed by the proposed GNPs to formulate different types of active substances is expected to give an important contribution for the nanomedicine field applied to different diseases.

P4-05

Molecular characterization of a new family of peptides derived from intermediate filaments and study of their potential applications in biomedicine

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Intermediate filaments (IFs) are the most resistant elements of the cytoskeleton. They play an important role in providing mechanical strength of the cell and are crucial for cell motility but also participate in tissue growth and regeneration, cell survival and apoptosis,.

After the identification of a peptide derived from peripherin, called DIF-P (Dr. Joan Verdaguer, University of Lleida), it was shown that DIF-P induced a potent secretion of proinflammatory cytokines when incubated with mouse splenocytes. In addition, at micromolar concentrations (55 µM), they show a potent cytolytic activity on most of the cell types tested to date, and this effect is very fast (minutes/hours).

After the initial discovery of DIF-P, similar peptides, highly homologous to DIF-P, were found in other IFs also showing a strong cytotoxic activity. Indeed, we believe that the peptide DIF-P belongs to a family of bioactive peptides that are present in IFs (which include neurofilaments, cytokeratins, peripherin, vimentin, desmin, and nuclear lamins).

Our objective was to further characterize the functional DIF-P features. Thus, the cytolytic activity analysis demonstrated that the type of cell death caused by the peptide (apoptosis, and necroptosis/pyroptosis) highly depends on the cell type and conditions used. Another characteristic is the relative insolubility of most of these peptides in saline solutions. To this end, different compounds and nanoparticles were tested to solubilize them. The best results observed so far were obtained by conjugating the peptides with Pluronic (F127), a micelle-forming agent.

Further work will focus on exploring the potential biomedical applicability of these peptides as potential cytolytic agents, through the study of their mechanism of action, and by a more comprehensive characterization of their hemolytic, antimicrobial, cytotoxic and immunostimulatory activities.

P4-06

Efficacy and genetic safety of deoxyribonucleosides as a therapy for mitochondrial DNA replication defects caused by mutations in genes involved in mtDNA maintenance

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Mitochondrial DNA (mtDNA) depletion/deletions syndromes (MDDS) encompass a group of severe disorders characterized by defective replication of mtDNA, which results in marked decrease of mtDNA copy number (depletion) and/or presence of multiple mtDNA deletions in affected tissues. Mutations in an increasing number of nuclear genes have been identified as cause of MDDS. Some of the genes encode enzymes directly participating on dNTP anabolism, such as TK2 or DGUOK. Deoxyribonucleoside (dN) supplementation has shown to enhance mtDNA replication in former preclinical studies using models of MDDS caused by mutations in genes directly involved on dNTP metabolism, either *in vitro* (*DGUOK*) or *in vivo* (*TK2*). Furthermore, oral supplementation with dNs has been reported to have clinical efficacy in patients with mutations in *TK2*.

Based on preclinical results showing that dN supplementation helps recover mtDNA copy number in cultured cells derived from MDDS patients with mutations in *POLG*, we have proposed that treatment with dNs could be also extended to MDDS caused by mutations in *POLG* and other genes not directly related with dNTP metabolism. The rationale of this proposal relies on the hypothesis that dNTP expansion achieved by dN-mediated enhancement of the salvage pathway favours mtDNA replication, which is activated by increased substrate availability.

In order to provide more evidence supporting this hypothesis, we have tested the effect of dN supplementation on mtDNA replication in cultured primary skin fibroblasts from patients with MDDS caused by several genes, such as *MPV17*, *TWNK*, *SUCLA2*, *SUCLG1* or *OPA1*. Our results indicate that dN administration enhances mtDNA replication in samples from patients with mutations in *MPV17*, thus confirming previous observations reported elsewhere, and for the first time show that this substrate enhancement therapy also favours mtDNA copy number recovery in cells derived from patients with mutations in *SUCLG1* and in one out of five tested cell lines with four different mutations in *OPA1*.

These results support the notion that dN supplementation prevents mtDNA replication dysfunction not only when dNTP availability is limited due to mutations in genes needed for its synthesis, but also in other MDDS caused by mutations in genes not directly related with dNTP supply, such as *POLG*, *MPV17*, *SUCLG1* or *OPA1*.

P4-07

Deletion of PGC-1α and PGC-1β in adipocytes alters the adipose-pancreatic crosstalk and induces glucose intolerance

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Type 2 diabetes (T2D) is tightly associated with obesity, which is defined as the pathogenic accumulation of fat in adipose tissues. Adipose tissues (ATs) play a key role in the regulation of energy balance and glucose homeostasis. Whereas white adipose tissue (WAT) stores energy in the form of triglycerides, acts as an endocrine organ and controls glucose homeostasis, brown adipose tissue (BAT) maintains body temperature through non-shivering thermogenesis. However, the endocrine function of BAT and its role in glucose homeostasis control remains poorly understood. Interestingly, T2D has been linked to a decreased mitochondrial mass and activity in ATs, suggesting that impaired mitochondrial function contributes to T2D development. Our main aim is to study the impact that fat-specific impaired mitochondrial biogenesis has on whole body glucose homeostasis.

Mice lacking mitochondrial regulators PGC-1α and PGC-1β specifically in adipocytes (PGC-1α/β-FAT-DKO mice) were generated. PGC-1α/β-FAT-DKO mice exhibit reduced mitochondrial gene expression and function in ATs, but were not obese or insulin resistant when fed a fatty diet. However, PGC-1α/β-FAT-DKO mice showed glucose intolerance and lower insulin levels. A glucose-stimulated insulin secretion test revealed impaired insulin secretion in isolated pancreatic islets from PGC-1α/β-FAT-DKO mice, although they did not exhibit differences in β-cell mass. This suggests that lack of PGC1s in ATs modifies β-cell function by altering the adipose-pancreatic crosstalk. To identify the adipokines underlying such alteration, we analyzed ATs secretomes from Wt and PGC-1α/β-FAT-DKO mice by nano LC-MS/MS. Our results showed differences between Wt and PGC-1α/β-FAT-DKO in BAT secretome, but not in WAT, and identified approximately 130 proteins differentially secreted by dysfunctional BAT.

Our results demonstrate that disruption of BAT endocrine function results in pancreatic β-cell dysfunction that leads to glucose intolerance.

P4-08

Impact of bariatric surgery on blood levels of Sex Hormone-Binding Globulin in patients with morbid obesity

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Background-aim

Morbid obesity (MO) is one of the most important public health conditions worldwide and it is also associated with different comorbidities such as type 2 diabetes, metabolic syndrome and non-alcohol fatty liver disease among others.

It is well known that adult individuals with obesity show lower plasma sex hormone-binding globulin (SHBG) levels than normal-weight subjects. SHBG is a protein produced and secreted into the circulation by the liver that binds androgens and estrogens with high affinity. In blood, SHBG acts as a carrier of these sex steroids and regulates their bioavailability and access to target tissues and cells.

Bariatric surgery (BS) is at present the most effective therapeutic option for MO in terms of weight loss and reverse of the metabolic dysregulation. Nevertheless, little is known about the impact of BS on SHBG levels in patients with MO. On these bases, the aim of our study was to explore impact of BS in patients with MO.

Methods

Single center, prospective, observational study, including consecutive patients with MO attended at the Morbid Obesity Unit of our site, undergoing BS. All the patients underwent: complete medical history, anthropometric measurements to calculate BMI and percentage of excess weight loss (%EWL) one month before and one month and 6 months after the performance of BS. SHBG was measured before and one month later BS in serum by ELISA (Demeditec Diagnostics). For statistical analyses the MedCalc v.12 program was used.

Results

We included 33 patients (24 females and 9 males; mean age=49 ± 9) that underwent BS (Y-en-Roux gastric by-pass). A significant reduction in BMI was achieved 1 month after the BS (43.5 ± 7.2 vs 38.9 ± 6.1, $p<0.0001$) and the percentage of excess weight loss (%EWL) at 1 month was 23.5±8.0%. A significant increase in SHBG levels was seen 1 month after the BS (51.3 ± 36.2 nM vs 77.8 ± 48.4 nM, $p=0.0001$). Regarding control 6 months after BS, a significant reduction in BMI was achieved respect to 1 month after BS control (32.2±5.6, $p<0.0001$) and the %EWL was 58.8±19.3%. Significant correlation was seen between the increase in SHBG one month after BS and the %EWL at 6 months ($r=0.482$, $p=0.006$; two data excluded).

Conclusions

In our study we found a significant increase in SHBG levels at one month after the BS performance that correlated with the excess of weight loss at 6 months after BS. Our preliminary results suggest a beneficial effect of weight loss after BS on SHBG production. Further studies are needed in order to validate our results.

P4-09

Exacerbated hepatic steatosis and steatohepatitis in diabetic mice fed a high-fat diet

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Introduction & objectives
Type 2 diabetes mellitus (DM2) is a risk factor of non-alcoholic fatty liver disease (NAFLD) and might eventually progress to advanced stages of hepatic damage. Excessive hepatic fat accumulation denotes a typical feature of diabetic patients and plays an important pathogenic role. Actually, now evident is that NAFLD, may have deleterious impact for diabetic individuals increasing the risk to develop cardiovascular complications. Thus, we aimed at analyzing whether the high-fat diet impaired the hepatic phenotype of diabetic mice.

Materials & methods
Both groups of diabesic (db/db) and non-obese, db/+ mice (on a C57BKS genetic background) were fed with a high-fat (HFD) diet for 2 months. Gross parameters, plasma and hepatic biochemistry, and hepatic histology were assessed at the end of the study.

Results
Obesity and excess adiposity was exacerbated in db/db mice with a hypercaloric, HFD diet. In addition to the concomitant increase in body weight (1.6-fold, $p<0.05$) mainly due to enlarged adiposity (4.3-fold, $p<0.05$), the HFD-fed db/db mice presented an enhanced dyslipidemia, as shown by a marked increase in plasma levels of triglycerides (2.3-fold, $p<0.05$) and free-fatty acids (1.7-fold, $p<0.05$) as compared to non-obese mice. Importantly, this unhealthy metabolic phenotype was accompanied by an exacerbated ectopic accumulation of lipids in the liver and myocardium, as well as impaired insulin signaling, as revealed by increased plasma concentrations of glucose (3.4-fold, $p<0.05$) and insulin (2.5-fold, $p<0.05$), and concomitant elevations in HOMA-IR (3.6-fold, $p<0.05$) and Adipo-IR (2.8-fold, $p<0.05$) indices, common surrogates of global and adipose-specific insulin resistance. Furthermore, the histological analysis of the liver also revealed signs of steatohepatitis.

Conclusions
Caloric overfeeding in diabetic mice further aggravated insulin signaling, dyslipidemia, and worsened hepatic accumulation of lipids and steatohepatitis.

P4-10

Recovery in serum testosterone is a is an accurate predictor of survival from COVID-19 in male patients

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SARS-CoV-2 has affected over 131,020,967 confirmed cases and produced 2,850,521 deaths worldwide. Robust correlation of severe COVID-19 includes old age, poverty co-morbidities such as obesity, diabetes and cardiovascular disease, and male sex. Global Health 50/50 shows that in most countries, men are consistently dying at a higher rate compared to women. A precise knowledge is still lacking of the molecular and biological mechanisms that may explain the association of severe disease with male sex. Here, we have systematically explored the association of serum testosterone (TST) levels with routine clinical biochemical parameters and peripheral blood immune phenotypes in longitudinal analyses of 249 hospitalized male COVID-19 patients. We find that recovery of serum TST levels during hospitalization is the strongest predictor (AUC of ROC = 92.8%, p <0.0001) of survival in male patients among all biochemical parameters studied, including single-point admission serum TST values. Longitudinal determinations of serum levels of luteinizing hormone (LH) and androstenedione suggest an early inhibition of the central LH-androgen biosynthesis axis in a majority of patients, followed by full recovery in survivors together with TST reestablishment, however in lethal cases, we observed a failure to reinstate physiological TST levels despite the slight increase of LH. Finally, since this failure was associated to the decrease of lymphocytes number and percentage (r=0.312 - 0.419, respectively), p-value<0.0001, we performed extended lymphocyte phenotype in a group of 25 male and female patients, where we found evidence of association between TST decline and decrease of effectors T helper cells and augmented circulating classical monocytes. These observations are suggestive of a causal relationship between testosterone status and immune responses to COVID-19, leading to either survival or death in male patients.

P4-11

Mitochondrial network affected in McArdle mouse muscles

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Studies in PYGM50x/R50x mouse model reveals that there is a different level of affectations between different muscle fiber types. It was observed a massive glycogen accumulation in fiber type IIA and IIX, and less degeneration in pure fiber type I. Revealing a correlation between the level of affectation in muscle fiber types and their mitochondrial content.

An evaluation of electron transport chain activity in McArdle mouse model demonstrates that mitochondrial metabolism is directly affected. Decrease in complex I and citrate synthase activity were observed, as well as a decrease in SDH protein levels. This suggest an electron transport chain impaired and can imply a less mitochondrial content in the muscle fibers of McArdle mouse.

To determine whether these findings were detectable in histological images of McArdle and WT muscles, IHC staining with VDAC and VDAC/TUBULIN was performed. Images observed by Conventional Fluorescence Microscopy + Airyscan clearly show a general compromised mitochondrial network in both McArdle TA and QUAD muscles. To be more specific, we observed a reduction in the number of intermyofibrillar and subsarcolemmal mitochondria. Tubulin staining reveals a disintegrated structure in both McArdle muscles compared to WT. This deconstruction in tubulin structure also indicates that the Z-disk pattern has been lost in McArdle mouse fibers, which is consistent with that observed in muscle biopsies from McArdle patients by electron microscopy.

The importance of understanding the interaction between tubulin and VDAC is because tubulin is involved in several cell functions and plays an important role in metabolism and mitochondrial structure. The overview of these new images reveals that glycogen accumulation affects the structure of the sarcomere as well as the cytoskeleton.

P4-12

Transforming growth factor β1 downregulates hepatic sex hormone-binding globulin production by reducing P1 promoter-driven HNF4α isoforms

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Sex hormone-binding globulin (SHBG) is a glycoprotein produced by the human liver and secreted into the blood whose main function is to transport sex steroids in blood, modulating their bioavailability and accessibility at tissue and cellular level. Low plasma SHBG levels are a risk factor for developing metabolic syndrome, type 2 diabetes, cardiovascular disease and non-alcoholic fatty liver disease (NAFLD).

NAFLD represents a spectrum of diseases ranging from hepatocellular steatosis through steatohepatitis to fibrosis and irreversible cirrhosis. Transforming growth factor β1 (TGFβ1) plays an important role in the pathogenesis of liver fibrosis, being involved in activation of hepatic stellate cells, stimulation of collagen gene transcription, and modulation of matrix metalloproteinase expression.

In the last decade, several reports have identified different factors regulating hepatic SHBG production. In this regard, we have shown clear evidence that elevated plasma levels of tumor necrosis factor alpha (TNFα) or interleukin-1-β (IL1β) are factors which downregulates SHBG production. The molecular mechanism by which all these factors affects hepatic SHBG production involve the hepatic nuclear factor 4 alpha (HNF4α), one of the main transcription factors activating SHBG expression.

The aim of the present study was to evaluate the role of TGFβ1 in regulating hepatic SHBG production. For this purpose, in vitro and in vivo studies were performed using human HepG2 cells and human *SHBG* transgenic mice. The results showed that TGFβ1 treatment reduced significantly SHBG expression in HepG2 cells as well as in human *SHBG* transgenic mice. TGFβ1 downregulated P1 promoter-driven HNF4α isoforms and increased P2 promoter HNF4α isoforms via Smad3 and Stat3 pathways thorough TGFβ1 receptor I, which in turn repressed SHBG expression. Taking together, we found for the first time that TGFβ1 regulates hepatic SHBG production. These results may explain why patients suffering fatty liver disease are characterized by having low plasma SHBG levels.

P4-13

Oral administration of the new calpain inhibitor NPO2270 protects against myocardial remodeling and ventricular dysfunction in a mouse model of heart failure induced by transverse aortic constriction

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Heart failure (HF) is a chronic heart disease that represents one of the leading causes of morbidity and mortality in developed countries, especially among elderly population. Pathological myocardial remodelling caused by aortic stenosis, among other clinical situations, is strictly associated with the occurrence of ventricular dysfunction and the progression of HF. Although current pharmacological treatments aimed to prevent this adverse outcome have been shown to improve heart function and prolong life expectancy, the still high mortality rates reveal an urgent need for novel targets. Ca^{2+}-dependent proteases calpains become overactivated due to chronic cardiac stresses, and contribute to cardiac remodelling by modulating myocardial hypertrophy, fibrosis and inflammation through the proteolysis of a wide range of proteins and the regulation of several signalling pathways. Despite the experimental evidence supporting the contribution of calpains to myocardial remodelling, no clinical trials have yet tested the use of pharmacological calpain inhibitors to treat HF, mainly due to deficient pharmacological properties of current calpain inhibitors. Herein, we characterize the protective effects of a new calpain inhibitor, NPO2270, against myocardial remodelling and HF.

In this study, C57BL6/J mice were subjected to chronic pressure overload by transverse aortic constriction (TAC) for 28 days. NPO2270 was orally administered at 10mg/kg/day, starting 7 days after surgery. Markers of hypertrophy, fibrosis and echocardiographic data were measured at different time points.

TAC surgery resulted in a progressive development of hypertrophy and fibrosis that correlated with reduced ejection fraction (EF) at 4 weeks. Delayed chronic oral administration of NPO2270 prevented the progression of hypertrophy induced by TAC surgery, as reflected by measurement of heart weight/tibia length ratio (HW/TL), left ventricular wall and septum thicknesses. It also prevented interstitial fibrosis, as shown by area positive to pricosirius red and gene expression of BNP, MYH7 and collagen I and III. Moreover, NPO2270 treatment attenuated myocardial dysfunction: 27% of EF reduction in control group vs. 6% in NPO-2270 group (P=0.024).

In conclusion, these results demonstrate that chronic administration of the calpain inhibitor NPO2270 prevents the progression of hypertrophy and fibrosis, and ameliorates cardiac dysfunction. This data provides compelling evidence that the new calpain inhibitor NPO2270 is an attractive candidate to determine the potential of calpain inhibition as a strategy for treating HF in patients.

P4-14

PRESS_Slice: single slice localized ^{1}H spectroscopy

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PURPOSE: Proton (^{1}H) Magnetic Resonance Spectroscopy (MRS) is a diagnostic technique that provides metabolic information about living tissues non-invasively. Previous studies from our group suggest that the proper analysis of such metabolomic information could give hints about tumour response, by means of MRSI-based nosological images obtained for treatment monitoring, even before any changes in tumour volume. This may benefit from improved data acquisition pulse sequences with better spatial resolution and signal to noise ratio. In this work we present a localized spectroscopy method for slice selection, PRESS_Slice, which has been tested so far using phantom studies. This method is derived from the commercially available Bruker implemented method PRESS for single voxel spectroscopy. The PRESS method consists of a 90º excitation and two 180º refocusing pulses to create a volume localization. By using PRESS_Slice, a spin echo with a spatial slice (axial, sagittal or coronal) of known thickness can be acquired by applying only a 90º excitation and a 180º refocusing pulse accompanied by slice-selective gradients.

METHODS: PRESS_Slice was implemented on a 7T Bruker BioSpec 70/30 USR preclinical scanner using ParaVision 5.1. Orthogonal Hermite 90º and 180º RF pulses were applied at equal time intervals along-with slice selective gradients to create a spin echo. The acquisition parameters were set as echo time (TE) = 12 ms, repetition time (TR) = 2500 ms, dummy scans (DS) = 4, number of points (Npoints) = 2048, number of acquisitions (NA) = 1, TE calculation mode = equalise, spectral width (SW) = 10 000 Hz (33.3 ppm), outer volume saturation (OVS) = off, and receiver gain (RG) = 1. The Bruker method programming files for PRESS_Slice (parsrelations.c, baselevelrelations.c) were modified to adjust (1) the echo time intervals between the excitation and refocusing pulses to half the total echo time and (2) the offset frequency for the slice thickness. Testing experiments were performed using a two-phase phantom. The phantom was prepared in a 15 ml Falcon tube using red-coloured 2% agarose in water to form a distinguishable water layer, and colorless baby oil as fat layer. Quantitative volumetric measurements were performed to verify the slice selection. The signal from the slice can be accumulated to increase the signal-to-noise ratio (SNR).

RESULTS: The method exhibited accurate slice selection and the results were evaluated using signal-to-noise ratio (SNR) and lineshape comparisons. As expected, the SNR increases linearly with increase in slice thickness.

CONCLUSION: In order to implement sophisticated high spatial and spectral resolution MRSI sequences, a deep knowledge of the Bruker pulse programming is required. The PRESS_Slice implementation performed as part of the learning process has been shown to be a reliable and accurate method for slice localized spectroscopy, gives reproducible results and could be used for educational purposes.

P4-15

Enzymatic replacement therapy of nanoconjugated GBA as a promising therapeutic treatment for Parkinson's disease

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Parkinson's Disease (PD) is the second most common neurodegenerative disorder only after Alzheimer's Disease. PD is a multifactorial, progressive, neurodegenerative disorder characterized by the loss of dopaminergic (DA) neurons in the substantia nigra pars compacta and the accumulation α-synuclein (α-Syn) inclusions known as Lewy Bodies. Mutations in GBA1, which encodes the lysosomal enzyme β-glucocerebrosidase (GCase), are the most common known genetic risk factor for the development of PD. Little is known about the role of GCase in PD pathogenesis but, there appears to be an inverse relationship between GCase and α-Syn levels. In this scenario, the development of novel therapies for restoring the GBA levels in neurons represents an interesting goal for effective PD treatments.

The objective of this study is the restoration of lysosomal glucocerebrosidase (GCase) activity in neurons through enzyme-polymer nanoconjugation of GBA recombinant enzyme. To do so, recombinant GBA was conjugated to polypeptides polymers through a reduction sensitive disulfide bond-bearing linker to improve protein stabilization and plasma protease resistance. Nanoconjugates were validated in vitro in a new cellular model of dopaminergic-like neurons expressing GBA knock out (GBA-KO) developed by our team.

Results show that GBA nanoconjugate presented GCase activity and high stability. Efficacy in vitro was tested in our neuronal GBA-KO model where GBA nanoconjugate was properly internalized and delivered to lysosomes and was able to restore lysosomal GCase activity, reduce sphingolipids accumulation and reduce neurotoxic forms of synuclein levels.

In conclusion, the use of nanotechnological approximations to improve Enzymatic Replacement Therapy is a promising strategy to restore lysosomal GBA activity and a new therapeutic opportunity for Parkinson's disease treatment.

P4-16

Molecular chaperones increase active GCase production

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β-glucocerebrosidase (GCase) is a lysosomal enzyme whose deficiency causes Gaucher's Disease (GD) due to the accumulation of its substrate glucosylceramide inside the lysosomes. To date, there is no definitive cure for GD, but enzyme replacement therapy (ERT), consisting of periodical intravenously administration of a recombinant produced GCase, is the standard of care for patients. There are currently 3 produced GCase accepted by the Food and Drug Administration: Velaglucerase alfa, Imiglucerase and Taliglucerase. The main drawback of the ERT treatment is its high cost, even reaching 200.000€ per patient annually due to the difficulties on expressing the protein. Our aim is to define a new strategy to improve GCase production using different vectors combined with the addition of molecular chaperones which interact with GCase increasing its stability.

Since GCase requires specific post translational modifications to be active, we have used human embryonic kidney HEK 293 F cells. GCase is encoded by the GBA1 gene and contains a signal peptide that after GCase expression is cleaved. We based our strategy on cloning the GCase gene in the expression vector pTriEx-7, because it contains the IgM signal sequence to export the protein to the extracellular media facilitating its purification. Furthermore, pTriEx-7 has also been previously used in our lab to express other proteins obtaining high expression yields. Using this strategy, we observed GCase expression from 48 hours after transfection to day 9, with the maximum peak on day 6. Unfortunately, the expression levels detected were very low (1µg/mL cell culture) and almost no active protein was detected. Now, we are working on the optimization process to increase expression levels using two strategies. On the one hand, we are working on the cloning of the GBA1 gene in different expression vectors to obtain higher quantities of expressed GCase. On the other hand, we are testing the addition of molecular chaperones to cell culture media to try to increase the GCase stability and expression especially at longer times and, more importantly, its activity.

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The oxidoreductase MIA40 funnels the inefficient folding of TRIAP1, a novel small disulphide-rich protein of the intermembrane space of the mitochondria

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The MIA40 relay system is a dedicated mechanism that regulates the import of small cysteine-rich proteins into the intermembrane mitochondrial space (IMS). In this process, the mitochondrial oxidoreductase MIA40 promotes the oxidation of critical native disulfides in its substrates to facilitate their folding as they enter the subcellular compartment. MIA40 substrates are initially synthesized in a reduced state in the cytosolic ribosomes and, despite sharing little or no homology, they have all been assumed to adopt a disordered conformation before entering the mitochondria as they are depleted of structural disulfides. This unfolded state facilitates their translocation through the outer membrane of the mitochondria, being an essential trait of mitochondrial small disulfide-rich proteins. Therefore, folding of MIA40 substrates is coupled to protein translocation and functional regulation.

Here we report the biophysical characterization of a novel substrate of MIA40, TRIAP1, an IMS protein that participates in the trafficking of phospholipids between mitochondrial membranes. Despite bearing the distinctive twin CX9C canonic motif of MIA40 substrates, TRIAP1 exhibits a folding pathway that differs significantly from the archetypal small disulfide-rich proteins of the IMS. Instead of departing from a fully unfolded protein, TRIAP1 rapidly populates a molten globule in the reduced state, a feature that biases the folding reaction and makes it exceedingly slow. The folding of TRIAP1 is strongly constrained by the structural properties required to function in phospholipids traffic; however, the accumulation of a compact conformation in the cytosol could be functional on itself and connected with the p53-dependent cell survival pathway. The role of MIA40 consists in resolving the molten globule into a helix-loop-helix fold in the mitochondria, preventing the persistence of high kinetic barriers and therefore making available active TRIAP1 immediately upon its import into this organelle.