SEBBM 2021

43rd Annual Meeting of the Spanish Society of Biochemistry & Molecular Biology

BARCELONA 19-22 JULY

BOOK OF ABSTRACTS

v.2

ORGANIZES:

SEBBM

CONTENTS

WELCOME

Dear colleagues,
On behalf of the Organizing Committee, we are pleased to invite you to attend **"The 43rd Congress of the Spanish Society of Biochemical and Molecular Biology"**, to be held online on July 19-22, 2021.

The meeting includes plenary speakers with leading scientists such as Adrian Krainer (Cold Spring Harbor, New York, USA), Ana María Cuervo (Albert Einstein College of Medicine, New York, USA), Anna Akhmanova (Utrecht Univ., Utrecht, The Netherlands) and Barbara Cannon (The Wenner-Gren Institute, Stockholm, Sweden). Similar to other editions, the meeting will include keynote sessions from our counterpart societies from Chile and Argentina as well as the L'Oréal-UNESCO conference.
A central part of the Congress will be the three simultaneous Symposia about top scientific topics and state-of-the-art technologies in the field of Biomedicine. Satellite activities such as the Introduction to Research in Biochemistry and Molecular Biology Course, the Annual Meeting of Coordinators of Undergraduate and Postgraduate Degrees in Biochemistry and related subjects, the Workshop on Professional Development for Young Researchers, and multiple Biochemistry in the City activities will be held during these days. The traditional Society Prizes (Young Investigator-IBUB, Margarita Lorenzo- Lilly Foundation, Young SEBBM Member's Best Article-UCM, José Tormo Award-Bruker Española, Best Scientific Image of the Year-CerTest Biotec, Social Network Award-SEBBM and 4 Posters Prizes-FEBS Letters & SEBBM) will be also an important part of the programme.

All participants are welcome to visit the stands of the sponsoring companies, through the online link, to which we truly thank for their support.
We encourage you to join us!

Best wishes,

Manuel Serrano (Co-organizer)

Laura Herrero (Co-organizer)

Isabel Varela (President of SEBBM)

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CONGRESS PROGRAMME OVERVIEW

Monday July 19th

- 09:30 – 13:30: Introductory Course to Research in Biochemistry & Molecular Biology
- 13:30 – 16:30: Workshop on Professional Development
- 09:30 – 14:30: Undergraduate and postgraduate teaching in Biosciences
- 14:30 – 16:30: SEBBM Teaching working group meeting
- 16:30 – 17:00: Opening ceremony
- 17:00 – 17:30: BBVA Foundation opening lecture: Splicing & muscular dystrophy
- 18:30 – 19:30: Biochemistry in the city: "Ellas en ciencia" Molecular plasticity en COVID-19

Tuesday July 20th

- 09:30 – 10:30: 1.1 Epitrans criptomics & RNA editing
- 09:30 – 10:30: 1.2 Micro proteome
- 09:30 – 10:30: 1.3 Structural biology
- 10:30 – 11:30: Merck Round Table
- 10:30 – 11:30: Meet the speakers
- 10:30 – 11:30: SEBBM Coordinators
- 10:30 – 11:30: ALBA synchrotron
- 11:30 – 12:30: Niemeyer lecture: Cell biology
- 12:30 – 14:30: Sponsors Seminars
- 14:30 – 16:30: SEBBM working group meetings
- 16:30 – 17:30: L'Oréal-UNESCO lecture
- 16:30 – 17:30: Sponsors Seminars
- 17:30 – 18:30: FEBS lecture: Microtubules
- 18:30 – 19:30: Biochemistry in the city: Human genome editing Round table

Wednesday July 21st

- 09:30 – 10:30: 2.1 Cellular reprogram-ming
- 09:30 – 10:30: 2.2 3D Genomics
- 09:30 – 10:30: 2.3 Biomolecular condensation
- 10:30 – 11:30: Best article award lecture
- 11:30 – 12:30: Young investigator IBUB award lec.
- 12:30 – 13:30: Lilly-Margarita Lorenzo lecture
- 13:30 – 14:30: Sponsors Seminars
- 14:30 – 15:30: Leloir lecture: Plants biology lecture
- 15:30 – 16:30: SEBBM working group meetings
- 16:30 – 17:30: José Tormo award
- 16:30 – 17:30: SEBBM General Assembly
- 18:30 – 19:30: Biochemistry in the city: Evolution & cancer / Fasting diets

Thursday July 22nd

- 09:30 – 10:30: 3.1 Chromatin plasticity
- 09:30 – 10:30: 3.2 mTOR
- 09:30 – 10:30: 3.3 RNA/DNA transactions
- 10:30 – 11:30: SEBBM Consuls
- 10:30 – 11:30: Meet the speakers
- 10:30 – 11:30: Bio-entrepreneur
- 11:30 – 12:30: Awards ceremony
- 11:30 – 12:30: Sponsors seminary
- 12:30 – 13:30: Ramón Areces Foundation Closing lecture: Bioenergetics
- 13:30 – 14:30: IUBMB-M. Salas lecture: Autophagy
- 13:30 – 14:30: Sponsors seminary
- 14:30 – 15:30: Closing ceremony
- 18:30 – 19:30: Biochemistry in the city: Pseudotherapies

CONGRESS DETAILED PROGRAMME

MONDAY, July 19th 2021

TIME	ROOM	ACTIVITY	SESSION'S LINK
09:30 - 13:00 h	Zoom 1	Introduction to Research in Biochemistry & Molecular Biology Course	LINK
09:30 - 13:30 h	Virtual 1	Undergraduate and postgraduate teaching in Biosciences	LINK
14:00 - 16:30 h	Zoom 1	Workshop on professional Development	LINK
14:00 - 15:30 h	Virtual 1	SEBBM Education working group meeting	LINK
16:30 - 17:00 h	Virtual 1	Opening ceremony	LINK
17:00 - 17:45 h	Virtual 1	BBVA opening Lecture (Adrian Krainer)	LINK
18:00 h	Cosmo Caixa (presencial)	Biochemistry in the city: "Ellas en ciencia"	LINK
19:00 h	Cosmo Caixa (presencial)	Biochemistry in the city: Molecular plasticity in COVID-19	LINK

TUESDAY, July 20th 2021 (I)

TIME	ROOM	ACTIVITY	SESSION'S LINK
09:30 - 11:00 h	Virtual 1	Simposia 1.1: Epitranscriptomics & RNA editing	LINK
09:30 - 11:00 h	Virtual 2	Simposia 1.2: The hidden microproteome	LINK
09:30 - 11:00 h	Virtual 3	Simposia 1.3: Novel approaches in structural biology	LINK
11:00 - 12:00 h	Virtual 1	Merck Round Table: Biomedical Research in Spain	LINK
11:00 - 12:00 h	Virtual 2	Meet the speakers	LINK
11:00 - 12:00 h	Virtual 3	SEBBM Groups Coordinators Meeting	LINK
11:00 -12:00 h	Zoom 1	ALBA Synchrotron	LINK
12:00 - 12:45 h	Virtual 1	Niemeyer Lecture (Nicole Tischler)	LINK
13:00 - 13:30 h	Virtual 1	Sponsor's Seminar (Merck)	LINK
14:00 - 14:30 h	Virtual 1	Sponsor's Seminar (Bio-Rad)	LINK
14:30 - 15:00 h	Virtual 1	Sponsor's Seminar (Agilent I)	LINK
15:00 - 16:30 h	Virtual 1	Molecular Basis of Pathology	LINK
15:00 - 16:30 h	Virtual 2	Metabolic Regulation and Nutrition	LINK
15:00 - 16:30 h	Virtual 3	Developmental Biology and Genomic Modifications	LINK
15:00 - 16:30 h	Zoom 1	Omic Molecular Biology and Bioinformatics	LINK
15:00 - 16:30 h	Zoom 2	Chemical Biology	LINK
15:00 - 16:30 h	Zoom 3	Synthetic Biology and Molecular Biotechnology	LINK
15:00 - 16:30 h	Zoom 4	Biomembranes	LINK
16:30 - 17:15 h	Virtual 1	L'Oréal-UNESCO for Women in Science Lecture	LINK
16:30 - 17:00h	Virtual 2	Sponsor's Seminar (Promega)	LINK
17:00 - 18:00h	Zoom 1	1st Meeting of the Introduction to Research in Biochemistry & Molecular Biology Course	LINK

TUESDAY, July 20th 2021 (II)

TIME	ROOM	ACTIVITY	SESSION'S LINK
17:15 - 18:00 h	Virtual 1	FEBS National Lecture (Anna Akhmanova)	LINK
18:30 - 20:00 h	Virtual 1	Biochemistry in the city (2)	LINK

WEDNESDAY, July 21st 2021

TIME	ROOM	ACTIVITY	SESSION'S LINK
09:30 - 11:00 h	Virtual 1	Simposia 2.1: Cellular reprogramming beyond pluripotency	LINK
09:30 - 11:00 h	Virtual 2	Simposia 2.2: In memoriam: J. L. Gómez-Skarmeta	LINK
09:30 - 11:00 h	Virtual 3	Simposia 2.3: Biomolecular condensation	LINK
11:00 - 11:30 h	Virtual 1	Best article SEBBM award Lecture	LINK
11:30 - 12:30 h	Virtual 1	Young Investigator IBUB Award Lecture	LINK
12:30 - 13:00 h	Virtual 1	Lilly Foundation Margarita Lorenzo Lecture	LINK
13:45 - 14:15 h	Virtual 3	Sponsors' seminars (Agilent seminar II)	LINK
14:15 - 15:00 h	Virtual 1	Leloir Lecture (Raquel Chan)	LINK
15:00 - 16:30 h	Virtual 1	Cell Death and Inflammation	LINK
15:00 - 16:30 h	Virtual 2	Cell Signalling	LINK
15:00 - 16:30 h	Virtual 3	Molecular Neurobiology	LINK
15:00 - 16:30 h	Zoom 1	Gene Expression Regulation and Genome Dynamics	LINK
15:00 - 16:30 h	Zoom 2	Molecular Parasitology & Emerging Infections	LINK
15:00 - 16:30 h	Zoom 3	Free Radicals and Oxidative Stress	LINK
15:00 - 16:30 h	Zoom 4	Protein Structure and Function	LINK
16:30 - 17:00 h	Zoom 4	José Tormo Award (Rafael Ciges)	LINK
16:30 - 17:15 h	Virtual 2	SEBBM General Assembly	LINK
17:00 - 18:00 h	Zoom 1	2nd Meeting of the Introduction to Research in Biochemistry & Molecular Biology Course	LINK
18:30 - 20:00 h	Virtual 1	Biochemistry in the city (3)	LINK

THURSDAY, July 22nd 2021

TIME	ROOM	ACTIVITY	SESSION'S LINK
9:30 - 11:00 h	Virtual 1	Simposia 3.1: Chromatin plasticity and multifunctionality	LINK
09:30 - 11:00 h	Virtual 2	Simposia 3.2: The mTOR pathway in health and disease	LINK
09:30 - 11:00 h	Virtual 3	Simposia 3.3: Structural mechanisms of RNA/DNA transactions	LINK
11:00 - 12:00 h	Virtual 1	SEBBM Consuls meeting	LINK
11:00 - 12:00 h	Virtual 2	Meet the speakers	LINK
11:00 - 12:00 h	Virtual 3	Bio-entrepreneur forum	LINK
12:00 - 12:30 h	Virtual 1	**Awards ceremony**	LINK
		CerTest BIOTEC Award to the "Best Scientific Image of the Year"	
		Social Network Award	
		José Tormo Award	
		FEBS Letters Poster Prize	
		SEBBM Poster prize	
		Medals of honor	
		Golden consuls	
12:00 - 12:30 h	Virtual 2	Sponsors' seminars (PHC)	LINK
12:30 - 13:00 h	Virtual 2	Sponsors' seminars (Controltecnica)	LINK
12:30 - 13:15 h	Virtual 1	Ramón Areces Foundation Closing Lecture (Barbara Cannon)	LINK
13:00 - 13:30 h	Virtual 2	Sponsors' seminars (Waldner)	LINK
13:15 - 14:00 h	Virtual 1	IUBMB Jubilee Lecture (Ana María Cuervo)	LINK
13:30 - 14:00 h	Virtual 2	Sponsors' seminars (Eppendorf)	LINK
14:00 - 14:20 h	Virtual 1	Closing ceremony	LINK
14:00 - 14:30 h	Virtual 2	Sponsors' seminars (Biogen)	LINK
18:30 - 19:30 h	Virtual 1	Biochemistry in the city (4)	LINK

Abstracts of Oral and Poster Communications

INDEX OF SEBBM SCIENTIFIC GROUPS

COMMUNICATIONS ACCEPTED BY THE 43ND SEBBM CONGRESS, HAVE BEEN ORGANISED INTO SCIENTIC GROUPS.
EACH COMMUNICATION HAS BEEN ASSIGNED THE NEXT CODE: YYY-R/M-ZZ

- YYY: THE NUMBER OF THAT COMMUNICATION IN ITS RESPECTIVE GROUP
- ZZ: OS (ORAL SELECTED) OR OI (ORAL INVITED) OR P (POSTER))
- R,M: \R" APPLICATION FOR THE POSTER PRIZE (SPONSORED BY FEBS LETTERS AND SEBBM) OR \M" THE LILLY-MARGARITA LORENZO PRIZE.

SEBBM2021 43rd Annual Meeting of the Spanish Society of Biochemistry & Molecular Biology BARCELONA 19-22 JULY

01.- Molecular Bases of Pathology

0533-OI

Deciphering the vascular component of Alzheimer's disease

MARTA CORTES-CANTELI

CNIC Cardiovascular Imaging and Population Studies

Alzheimer's disease (AD), the leading cause of dementia in the elderly, is a multifactorial and severe neurodegenerative disorder characterized by amyloid plaques, tau tangles, and brain atrophy. Accumulating evidence also links AD with cardiovascular risk factors. Obesity, hypertension, hypercholesterolemia, hyperglycemia, and a sedentary lifestyle increase the likelihood of AD, vascular dementia, and all forms of dementia in between. When atherosclerosis, the underlying cause of most cardiovascular diseases, develops in cerebral vessels, the brain oxygen and energy supply may be compromised due to hypoperfusion, resulting in deleterious neurological consequences. Indeed, a profound cerebral hypoperfusion is present in AD together with a pro-thrombotic milieu favoring the formation and persistence of fibrin clots and contributing to disease onset and progression. This vascular component should be considered in AD preventive, diagnostic, and therapeutic approaches. Here, we present evidence that long-term anticoagulation blocks the formation of occlusive thrombi in AD, preserves cognition, cerebral perfusion, and blood-brain barrier function and ameliorates neuroinflammation and amyloid deposition in AD mice. Moreover, we also demonstrate that in asymptomatic middle-aged individuals subclinical atherosclerosis and cardiovascular risk are associated with brain hypometabolism in cerebral areas known to be affected in AD.

These results open an exciting field for future investigation on whether the use of already approved anticoagulants and the control of cardiovascular risk factors early in life could potentially reduce the brain's midlife vulnerability to future cognitive dysfunction.

Funding – Dr. Cortes-Canteli is a Miguel Servet Research Fellow (ISCIII, CP16/00174 & MS16/00174).

Cortes-Canteli et al. Subclinical Atherosclerosis & Brain Metabolism in Middle-Aged Individuals. J Am Coll Cardiol 2021;77:888-98 Cortes-Canteli et al. Alzheimer's Disease & Vascular Aging. J Am Coll Cardiol 2020;75:942-51 Cortes-Canteli et al. Long-term oral anticoagulation delays Alzheimer's disease pathogenesis. J Am Coll Cardiol 2019;74:1910-23 Cortes Canteli et al. 2015. Fibrin deposited in the Alzheimer's disease brain promotes neuronal degeneration. Neurobiol Ag 2015. 36:608-17

0534-OI

p107 at the intersection between cancer and metabolism

SULAY TOVAR

CIMUS- Universidad de Santiago de Compostela Fisiología- Diabesity group

p107 is a cell cycle regulator that belongs to Rb family formed by pRb and p130, known as pocket proteins. They have been predominantly studied in the relation of cell cycle control and tumorigenesis. Meanwhile, it is now evident that pRb and p107 are implicated in the metabolism, principally in adipocyte differentiation.

Our group has recently discovered the important role of p107 in energy homeostasis where p107 deficiency activates white and brown adipose tissue increasing energy expenditure and protects against fat steatosis. In addition, p107 manipulation specifically in liver under high fat diet produced less fat accumulation in liver, also improved the lipid profile and expressed low protein levels of fatty acid synthase, the enzyme modulating the last step in the synthesis of long-chain fatty acids. Also, mitochondrial lipid oxidation and an *de novo* lipogenesis was altered. An analysis of fibrogenic factors indicate a positive correlation with p107 levels.

In conclusion, p107 exert a clear effect in the regulation of liver metabolism and in the development of liver fibrosis establishing p107 as an important regulator of the thermogenesis and also in liver metabolism. Consequently, p107 could be considered a potential candidate in the search of new targets for the treatment of obesity or MAFLD.

0030-R/M-OS

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

MARC VELILLA[1], ROSARIO PARDO[2], NOELIA TÉLLEZ[3], JOSEP ANTONI VILLENA[2]

[1]Vall d'Hebron Research Institute (VHIR) Metabolism and Obesity, [2]Vall d'Hebron Research Institute (VHIR Metabolism and Obesity, [3]Bellvitge Biomedical Research Institute (IDIBELL) Diabetes, Nutrition and endocrinological diseases

Type 2 diabetes (T2D) has become the most common metabolic disorder worldwide, closely associated with obesity. White adipose tissue (WAT), a fat storing tissue and active endocrine organ, is known to play a central role in glucose homeostasis. Brown adipose tissue (BAT), a heat-producing tissue in response to cold and diet through non-shivering thermogenesis, also contributes to whole body energy balance and glucose homeostasis. Interestingly, T2D has been associated with decreased mitochondrial mass and activity in adipose tissues1, suggesting that impaired mitochondrial function contributes to T2D development. To study the impact that fat-specific mitochondrial dysfunction has on glucose homeostasis, mice lacking mitochondrial regulators *peroxisome proliferator-activated receptor-γ coactivators-1α* and *-1b* in adipocytes (PGC-1α/β-FAT-DKO mice) were generated2. Compared to wild type (Wt), PGC-1α/β-FAT-DKO mice do not develop obesity or insulin resistance when fed a high fat diet. However, they show glucose intolerance and low insulin levels2. Based on these results, we hypothesized that lack of mitochondrial function in adipose tissues induces β-cell dysfunction by altering the adipose-pancreatic crosstalk. Consistent with our hypothesis, *in vivo* insulin secretion in response to glucose is impaired in PGC-1α/β-FAT-DKO mice. Moreover, glucose-stimulated insulin secretion in isolated pancreatic islets is significantly reduced in PGC-1α/β-FAT-DKO mice compared to Wt, although b-cell mass, islet size and islet number are not significantly different. To identify the adipokine(s) underlying b-cell dysfunction, we have analyzed adipose tissues secretomes from PGC-1α/β-FAT-DKO and Wt mice. For this, visceral and subcutaneous WAT and BAT were incubated in serum-free media for 24h and their secretome was identified by nano-liquid chromatography coupled to mass spectrometry. We found a high variability in BAT secretomes between Wt and PGC-1α/β-FAT-DKO mice, but not in WAT. These results indicate that PGC-1α/β depletion in adipose tissues specifically alters BAT secretome, which could contribute to pancreatic β-cell dysfunction and eventually lead to glucose intolerance. Further studies will identify the specific adipokine(s) involved in BAT-pancreas crosstalk.

1. Patti ME, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc Natl Acad Sci USA 2003; 100(14): 8466-71. 2. Pardo R, et al. Calorie restriction prevents diet-induced insulin resistance independently of PGC-1-driven mitochondrial biogenesis in white adipose tissue. FASEB J 2019; 33: 2343-2358.

0093-R/M-OS

NR2E3 transcription factor and photoreceptor fate: identification of gene regulatory networks causing retinal remodeling in NR2E3-associated diseases.

IZARBE AÍSA-MARÍN[1], QUIRZE ROVIRA[2], NOELIA DÍAZ[2], JUAN M. VAQUERIZAS[2], GEMMA MARFANY[1]

[1]CIBERER, Universitat de Barcelona Genetics, Microbiology and Statistics, [2]Max-Planck-Institute for Molecular Biomedicine Regulatory Genomics

Inherited retinal dystrophies are a group of diseases associated with mutations in more than 330 genes. *NR2E3* encodes an orphan nuclear receptor with a dual function as transcriptional activator and repressor, necessary for retinal development and homeostasis1-5. Mutations in *NR2E3* cause two different retinal diseases: Enhanced S-cone Syndrome (ESCS) and Retinitis Pigmentosa (RP) 6-11. However, there is no clear phenotype-genotype correlation for most *NR2E3* mutations, which suggest different disease mechanisms. This gene produces a large protein isoform encoded in 8 exons. In addition, we found a previously unreported isoform of 7 exons generated by intron 7 retention. We dissected the *Nr2e3* function by performing CRISPR/Cas9 gene editing of the last exon and generated two different mouse models12. Allele D27 is an in-frame deletion of 27 bp that ablates the dimerization domain, whereas allele DE8 (full deletion of exon 8) lacks the dimerization and repressor domains and only expresses the short isoform. Depending on the deleted domain, these models show two different phenotypes that correspond with the two known diseases caused by mutations in *NR2E3*. Δ27 homozygotes show electrophysiological alterations in photoreceptor and synaptic activity associated to retinal development defects that resemble ESCS patient phenotype, with higher number of cones and severe alteration of rod function. The phenotype of the DE8 mutant show severe alterations in the maintenance and homeostasis of rod photoreceptors, with progressive rod cell death, which resemble the human RP phenotype. Similar to the *rd7* phenotype, a natural mouse model of *Nr2e3* of ESCS, we found rosette-like structures in the retina5. Besides, it displayed cone-rich regions and double cones that have never been described in mice before. We performed single cell RNA-seq in our two models to further investigate the gene regulatory networks guiding differentiation of rod and cone photoreceptors in the retina. Our results provide insight into the molecular mechanisms of the two rare ocular diseases caused by mutations in *NR2E3* and set the basis for further epigenetic studies on the relevance of NR2E3 transcriptional network in photoreceptor differentiation and function.

1. Kobayashi (1999). 10.1073/pnas.96.9.4814 2. Chen (2005). 10.1523/JNEUROSCI.3571-04.2005 3. Cheng (2006). 10.1093/hmg/ddl185 4. Peng (2005). 10.1093/hmg/ddi070 5. Haider (2001). 10.1093/hmg/10.16.1619 6. Bernal (2008). 10.1111/j.1399-0004.2008.00963.x 7. Coppieters (2007). 10.1086/518426 8. Escher (2009). 10.1002/humu.20858.Mutations 9. Favre (1958). 10.1159/000303360 10. Gire. Mol. Vis., 13 (2007), 1970-1975 11. Haider (2000). 10.1038/72777 12. Aísa-Marín (2020). 10.1016/j.nbd.20

0207-R/M-OS

Human antigen R (HuR) SUMOylation fine-tunes hepatocellular carcinoma (HCC) progression by modulating the expression of mitochondrial mRNAs

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CIC bioGUNE Liver Disease Lab

Liver cancer is the sixth most common and the second deadliest tumor after pancreatic cancer, worldwide. These figures are also representative of the current burden of liver cancer in Spain. As we await the results from ongoing phase III clinical trials, we sought to identify additional approaches for the management of hepatocellular carcinoma (HCC), which represents about 90% of primary liver cancers. Considering the numerous and complex molecular mechanisms underlying the malignant transformation of a healthy liver into HCC, drugs against more than one signaling route would need to be developed in order to stop the progression of the disease. In this context, the posttranslational modification (PTM) of proteins, which controls the specificity, timing, duration and amplitude of virtually all cellular processes, has emerged as a robust and multidimensional therapeutic strategy in cancer. Interestingly, PTMs are key mechanisms that regulate the function of the RNA-binding protein Human antigen R (HuR), which is known to be involved in HCC transformation, in addition to playing a role in liver physiology. Herein we described for the first time that HuR is SUMOylated in the tumor sections of HCC patients in contrast to the surrounding tissue, as well as in the *MYC-luc;sg-p53* genetically engineered mosaic mouse model of liver cancer, and in human hepatoma cell lines. SUMOylation of HuR promotes major cancer hallmarks, namely proliferation and invasion, in the human HCC HuH-7 cell line. Conversely, the absence of HuR SUMOylation results in a senescence-like phenotype with damaged mitochondrial structure and function. Regarding the mechanism of action, we propose that HuR SUMOylation might drive HCC progression by modulating mitochondrial structural integrity and functionality through the regulation of the stability and translation of mRNAs encoding key mitochondrial proteins. In conclusion, SUMOylation constitutes a novel mechanism of HuR regulation that could be potentially exploited as a therapeutic strategy for liver cancer, thus highlighting the importance of PTMs as disease targets. Furthermore, understanding the effects of HuR SUMOylation in hepatocarcinogenesis will provide new functional insights into the relatively unknown role of SUMOylation in cancer.

Vázquez–Chantada, M. et al. HuR/Methyl-HuR and AUF1 Regulate the MAT Expressed During Liver Proliferation, Differentiation, and Carcinogenesis. Gastroenterology 138, 1943-1953.e3 (2010). Seeler, J.-S. & Dejean, A. SUMO and the robustness of cancer. Nat. Rev. Cancer 17, 184–197 (2017). Da Silva-Ferrada, E. et al. Analysis of SUMOylated proteins using SUMO-traps. Sci. Rep. 3, 1690 (2013).

0409-OS

FGF15/FGF19 is involved in the control of cardiac hypertrophy and metabolism

ANA PLANAVILA, SAMANTHA MORÓN-ROS, FRANCESC VILLARROYA, ALEIX GAVALDÀ-NAVARRO

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Introduction: FGF15 (and its human orthologue, FGF19) are members of the endocrine FGF family secreted by ileal enterocytes in response to bile acids. The liver is its main target tissue1, but recent studies indicate that FGF19 also regulates skeletal muscle mass2 and adipose tissue plasticity3.

Objective: To determine the role of the enterokine FGF15/19 during the development of cardiac hypertrophy and associated metabolic alterations.

Methods: Circulating FGF19 levels were assessed in patients with heart failure and myocardial infarction. The hypertrophic response was characterized in Fgf15-null mice fed a high-fat diet for 16 weeks or infused with isoproterenol via osmotic minipumps for 7 days. Effects of experimentally increased FGF15 and FGF19 levels in vivo were determined in mice using adenoviral (Ad-Fgf15) and adeno-associated vectors (AAV8-Fgf19) 1-week or 3-weeks after injection, respectively.

Results: FGF19 levels were significantly reduced in patients with heart failure or myocardial infarction, reciprocally to FGF21 levels. Mice lacking Fgf15 do not develop cardiac hypertrophy in response to high-fat diet or isoproterenol. The heart weight/tibia length (HW/TL) ratio, the cardiomyocyte area and the hypertrophy marker gene atrial natriuretic factor (Anf) -as measures of cardiac hypertrophy development- were reduced in Fgf15-null mice subjected to hypertrophy induction compared to wild-type mice. This was associated with impaired regulation of expression of lipid metabolism-related (Pdk4, Cpt1b) and mitochondrial function-related (PGC-1a) genes in heart from Fgf15-null mice. Conversely, experimental increases in FGF15 or FGF19 induced cardiac hypertrophy *in vivo*, characterized by an increase in the HW/TL ratio, area of the cardiomyocytes and hypertrophy marker gene expression.

Conclusions: We establish a cross-talk between the intestine and the myocardium through the enterokine FGF15/19, which appears to be required for cardiac hypertrophy development.

1. Kurosu, H. et al. Tissue-specific expression of bKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. Journal of Biological Chemistry 2007, 282(37):26687e26695. 2. Benoit B et al. FGF19 regulates skeletal muscle mass and ameliorates muscle wasting in mice. Nat Med. 2017 Aug;23(8):990-996. 3. Morón-Ros S et al. FGF15/19 is required for adipose tissue plasticity in response to thermogenic adaptations. Mol Metab. 2021 Jan;43:10111

0013-R/M-P

Functional and OMICs Characterization of Fibroblasts' Sporadic Inclusion Body Myositis Model

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Introduction: Sporadic Inclusion Body Myositis (sIBM) is an inflammatory myopathy characterized by asymmetric proximal and distal muscle weakness, and mitochondrial changes, together with inflammation and degenerative aggregates of proteins in muscle biopsies. sIBM affects patients over 50 years old and the diagnosis is based in a muscle biopsy, as the disease lacks either biomarkers, treatments or validated disease models. Thus, we aim to validate fibroblasts as a disease model for sIBM.

Methodology: We examined sIBM muscle hallmarks in fibroblasts from functional to OMICs approach. In functional approach, we assessed mitochondrial, inflammatory and degenerative changes in fibroblasts from 14 sIBM patients *vs.* 12 paired controls. In OMICs approach, we analyzed the transcriptome and metabolic profile of sIBM fibroblasts, through mRNA seq and UHPLC (Ultra High-Performance Liquid Chromatography). Results were analyzed through non-parametric statistic tests.

Results: Both approaches recapitulate sIBM muscle hallmarks in fibroblasts, thus validating its usefulness as a disease model. In the functional approach, in sIBM fibroblasts we observed: 1) a lower mitochondrial basal respiration and ATP production as well as an increased anaerobic metabolism and antioxidants' concentration; 2) higher expression of some cytokines'; and 3) lower autophagosomes formation. In OMICs approach of sIBM fibroblasts, 778 DEGs in mRNA seq revealed expression changes in RNA processing, cell communication and amino acid metabolism. UHPLC showed altered amino acids and organic acids related to mitochondrial defects and Krebs cycle. Taken together, OMICs data revealed altered mISR (mitochondrial Inte-grated Stress Response) and sulfide metabolism.

Conclusion: Classical mitochondrial, inflammatory and degenerative muscle hallmarks of sIBM are also present in a fibroblasts model of the disease. An integrative combination of functional and OMICs approaches validates fibroblasts as a disease model to explore molecular targets and assay therapeutic strategies that may be eventually exported to other neuromuscular diseases.
Funding: FIS PI1800498 granted by ISCIII and FEDER.

0014-R-P

Knock-down of α2,3-sialyltransferases in pancreatic cancer cells and its effect on EGFR glycosylation

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Pancreatic ductal adenocarcinoma (PDA) is the third leading cause of cancer mortality in developed countries and presents a dreadful prognosis1, mainly due to its delayed diagnosis and its resistance to existing therapies. Epidermal growth factor receptor (EGFR) participates in the regulation of cancer cells' growth and it is a highly glycosylated protein. During the neoplastic process, the glycan structures of EGFR can undergo several modifications that can alter its activity and signalling2. We aim to analyse the glycosylation pattern of EGFR in BxPC-3 and Capan-1 PDA cells deficient in α2,3-sialyltransferases (STs) ST3GAL3 and ST3GAL4 and to determine the influence of such changes in the activation and signalling of EGFR. First, EGFR levels were evaluated by flow cytometry and western blot (WB) in the knock-down and control PDA cells. EGFR was then immunoprecipitated from cell lysates and its glycosylation for each cell line was examined by WB with lectins and antibodies against glycan structures. The activation of EGFR and its downstream signalling pathways was determined by WB analyses of the phosphorylation levels of specific residues of the EGFR intracellular tail and of MAPK and AKT proteins. EGFR expression level of STs knock-down cell lines did not change compared to control BxPC-3 and Capan-1 cells. However, a decrease in the levels of the glycan structure sialyl-Lewis x of EGFR of the knock-down cell lines vs control ones was found, in agreement with the cell surface glycosylation changes. After stimulation with EGF, specific EGFR residues, involved in the proliferation signalling and internalization of the receptor, showed higher level of phosphorylation in BxPC-3 and Capan-1 knock-down cells. Additionally, there was an increase in the phosphorylation level of AKT in the knock-down cell lines. Altogether, these results suggest that the knock-down of ST3GAL3 and ST3GAL4 in PDA cell lines modifies the glycosylation and phosphorylation pattern of EGFR, resulting in changes in the proliferation and internalization signalling of the receptor, which could alter the proliferation capacity of the tumoural cells.

Siegel, R. L., Miller, K. D. & Jemal, A. Cancer statistics, 2019. CA. Cancer J. Clin. 69, 7–34 (2019). Wee, P. & Wang, Z. Epidermal growth factor receptor cell proliferation signaling pathways. Cancers (Basel). 9, 1–45 (2017).

0017-R/M-P

Cytosine-5 methylation of ribosomal RNA in cell cycle control and migration

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5-methylcytosine (m5C) is a widespread modification in DNA and RNA. However, while the functions of m5C in DNA have been extensively studied, its role in RNA is emerging to be elucidated (1)we focus on the occurrence of 5-methylcytosine (m5C deposition is found mainly in transfer RNA (tRNA) and ribosomal RNA (rRNA) and is mediated by DNMT2 and NSUN family members (1–4). Recently it has been shown that m5C on tRNAs regulates stem cell functions and stress responses in normal epidermis and skin cancer (5–7), and its inhibition specifically eliminates cancer initiating cells (5), suggesting that RNA-methylation may regulate essential cellular and physiological processes and its dysregulation may lead to critical pathological consequences such as cancer.

By analysing cancer expression databases we have found that the cytosine-5 methylase NSUN5 is overexpressed in advanced metastatic prostate cancer (PCa), one of the most frequent form of cancer Worldwide in men. *In vitro* analysis using *NSUN5*-silenced cell lines showed that *NSUN5* depletion leads to impaired proliferation and migration capacity. Flow cytometry analysis showed that *NSUN5* silencing results in decreased cell size and arrest in G2/M phase, suggesting an impairment in cell cycle progression through G2/M phase transition. We find that NSUN5 expression fluctuates along the cell cycle, further confirming its role in regulating this process. NSUN5 is a rRNA m5C methyltransferase that methylates position C3872, located at the interphase of the small and large ribosome subunits. Whether rRNA m5C methylation is regulated along the cell cycle and whether m5C deposition at rRNA regulates protein translation in G2/M and migration capacity needs to be determined.

1. R. Garcia-Vilchez et al. Biochim Biophys Acta Gene Regul Mech 1862, 240 (2019). 2. S. Hussain et al. Genome Biol 14, 215 (2013). 3. L. Van Haute et al. Nat Commun 7, 12039 (2016). 4. Y. Motorin et al. Nucleic Acids Res 38, 1415 (2010). 5. S. Blanco et al. Nature 534, 335 (2016). 6. J. V. Flores et al. Stem Cell Reports, (2016). 7. M. C. Popis et al. Curr Opin Oncol 28, 65 (2016).

0023-R/M-P

A possible key target for blocking glioblastoma progression: chaperone-mediated autophagy in pericytes.

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The lack of knowledge of the pathogenesis and the progression mechanisms of Glioblastoma (GB), the most aggressive brain tumor, contributes to none successful therapeutic strategies. Our team has recently demonstrated a crucial new role for chaperone-mediated autophagy (CMA) in pericytes (PC)-acquired immunosuppressive function during GB progression. GB-induced CMA in PC is necessary for proteostasis that promotes interaction with GB and, therefore, for an immunosuppressive function that facilitates tumor progression. Objective: to provide knowledge about the regulation and functional consequences of GB-induced CMA in PC. Methods: studies of RNA-seq and proteomics has been done in GB-conditioned pericytes with and without CMA compared to control pericytes after 72 hours of co-culture. Results: We have found several gene expression pathways differentially enriched in LAMP2A-KO PC and affected by GB-induced CMA in PC that correlate with our previous findings. Our data show that the phagosome formation, cell senescence, focal adhesion and the effector function to promote anti-tumor immune responses are the most affected pathways, reveling some transcription factors, as positive regulators of these processes that might be degraded by GB-induced CMA in pericytes, leading to facilitate GB progression. Conclusion: our results identify gene expression signaling pathways and possible new molecular markers that drive to the consequences of an aberrant upregulation of GB-induced CMA in PC and therefore, lead to “permissive” immune niche in the progression of GB.

Valdor R, García-Bernal D, Riquelme D, Martinez CM, Moraleda JM, Cuervo AM, Macian F, Martinez S. Glioblastoma ablates pericytes antitumor immune function through aberrant up-regulation of chaperone-mediated autophagy. Proc Natl Acad Sci U S A. 2019 Oct 8;116(41):20655-20665. doi: 10.1073/pnas.1903542116. Epub 2019 Sep 23

0033-R/M-P

MAPT is not just a protein of the Nervous System: Presence of Tau in the kidney

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Tau is a cytoskeletal protein that is expressed mainly in neurons and is involved in several cellular processes such as microtubule stabilization, axonal maintenance and transport. Altered tau metabolism is related to different tauopathies being Alzheimer disease one of the most relevant, in which aberrant hyperphosphorylated and aggregated tau is found in the central nervous system. In this project, we have explored tau expression in peripheral tissues in Tau knock-out mice (B6.129S4 (Cg) -Mapttm1 (EGFP) Klt/J), which have an eGFP-coding sequence inserted into the first exon of the microtubule-associated protein tau gene. IVIS Lumina from PerkinElmer demonstrated eGFP expression mainly in the kidney. We then demonstrated by qPCR that the main tau isoform in the kidney is Tau4R. Thanks to the eGFP reporter we have been able to see that tau is found in the glomeruli of kidney cortex, and specifically in podocytes. This was further confirmed by immunohistochemistry. Tau-KO mice present a podocyte cytoskeleton more dynamic as they contain higher levels of detyrosinated tubulin than wild-type mice. In addition, transmission electron microscopy studies demonstrated glomerular damage. Our results demonstrate that tau has an important role in podocyte architecture under normal physiological conditions.

1- (Chang, Shao & Mucke, 2021) 2- (Gödel et al., 2015) 3- (Tucker, Meyer & Barde, 2001) 4- (Wang & Mandelkow, 2015) 5- (Xu et al., 2014)

0054-P

Unravelling the Implications of Two Pathogenic Mutations of the Apoptosis Inducing Factor in its NADH–oxidase Properties

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The human apoptosis inducing factor (hAIF) is a moonlighting FAD–dependent enzyme that renders an essential role in the bioenergetics and redox metabolism of mitochondria in healthy cells, but which may also trigger caspase–independent cell death upon pro–apoptotic stimuli. hAIF dimerizes after reduction of its FAD cofactor by the NADH coenzyme, prompting the formation of a remarkably stable FADH–:NAD^+ charge transfer complex (CTC) (Ferreira et al., 2014). The monomer–dimer equilibrium that is hence established can be envisaged as a sensor of the mitochondrial redox state in terms of the NADH/NAD^+ levels, being further arbitrated by the allosteric binding of a second non–catalytic NADH molecule (Ferreira et al., 2014). Defects in hAIF give rise to major dysfunctions in oxidative phosphorylation, resulting in human pathogenic disorders coursing with severe neurodegeneration amongst other considerable symptoms. In the present work, we have performed the biophysical characterization of two mutations recently identified and related to disease (Heimer et al., 2018): Met340Thr and Thr141Ile, localized in the protein NADH–dependent and FAD–dependent domains respectively. In order to elucidate their participation on the reported pathological phenotypes, we have evaluated the impact of these mutations on NADH oxidase activity, CTC stability, overall protein stability, and interaction with key biological partners (DNA and the proteins CHCHD4, H2AX and CypA).

Ferreira, P. et al. (2014). Structural insights into the coenzyme mediated monomer-dimer transition of the pro-apoptotic apoptosis inducing factor. Biochemistry, 53(25), 4204–4215. https://doi.org/10.1021/bi500343r Heimer, G. et al. (2018). Mutations in AIFM1 cause an X-linked childhood cerebellar ataxia partially responsive to riboflavin. European Journal of Paediatric Neurology, 22(1), 93–101. https://doi.org/10.1016/j.ejpn.2017.09.004

0055-P

Study of the neuroinflammatory status of new cellular models of chronic IGF-1 deficiency

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Human IGF-1 deficiency is a rare disease (OMIM608747) that causes sensorineural hearing loss (SNHL) and neurological disorders (Rodríguez-de la Rosa *et al.*, 2017). The *Igf1*-deficient mouse recapitulates this syndrome and shows impaired neuronal differentiation, along with early apoptosis of auditory neurons (Camarero *et al.*, 2001; Cediel et al., 2006). IGF-1 has pleiotropic actions, including decreasing neuroinflammation and promoting cellular senescence (Nishizawa *et al.*, 2016). To further study IGF-1 deficiency and understand the alterations linked to neuronal loss, a cellular model of the human disease was generated in the murine neuroblastoma cell line Neuro-2a using CRISPR/Cas9 technology. This model reproduces the partial deletion of exon 3 of the murine *Igf1* gene, a deletion that has been associated with human and mouse SNHL. For gene editing, the crRNA:tracrRNA:Cas9 complex was transfected as a ribonucleoprotein and the cell clones from a selected pool isolated using limiting dilution. *Igf1* gene editing was confirmed by Sanger and next-generation sequencing and two cell clones were selected to carry out the study, 4A10 and 2G3. Gene expression of IGF system components by RT-qPCR confirmed the absence of *Igf1* mRNA and revealed that *Igf1r* was downregulated in both clones. Comparative cell viability XTT assays using cisplatin, hydrogen peroxide and IGF-1 showed differences between wild type (WT) and edited clones. Neuro-2a WT cells showed the highest viability in response to FBS and IGF-1 treatment. Interestingly, both clones were more resistant to cisplatin, suggesting that the ablation of the

Igf1 gene was impairing cell cycle progression. However, the initial study of cell cycle phases by flow cytometry did not reveal significant differences between Neuro-2a WT cells and mutated clones. The neuroinflammatory state of *Igf1*-deficient clones and their response to different pro-inflammatory stimuli will be also discussed. This cell model provides an opportunity to unravel new molecular mechanisms associated with chronic IGF-1 deficiency.

Camarero et al. (2001). J. Neurosci., 21(19): 7630-7641. Cediel et al. (2006). Eur J. Neurosci., 23(2): 587-590. Nishizawa et al. (2016). Sci Rep., 6: 34605. Rodríguez-de la Rosa et al. (2017). Front Aging Neurosci., 9: 411.

0061-R-P

IGF1R acts in the tumor microenvironment as a cancer-promoting factor facilitating the implantation and progression of lung metastasis

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FUNDACIÓN RIOJA SALUD CIBIR - CIBERES-ISCIII Cáncer de Pulmón y Enfermedades Respiratorias

Lung cancer is the leading cause of cancer death worldwide. The tumor microenvironment (TME) comprising blood vessels, immune cells, fibroblasts, signaling molecules and the extracellular matrix modulates tumor implantation and progression, thus its blockade is of therapeuthical interest. IGF1R (Insulin-like Growth Factor type 1 Receptor) is an ubiquitous membrane-bound tyrosine kinase receptor with recognized protumoral activity in the lung. In order to understand the role of IGF1R in the lung TME we generated Lewis and melanoma (LLC1/B16F10) lung cancer models using Igf1r-deficient mice and their controls1: i) LLC1 cells were intratracheally administered, awaiting for lung tumors to be developed after 21 days; ii) LLC1 cells were subcutaneously inoculated, followed by primary tumor resection on day (D)14 to allow pulmonary metastasis to be triggered until D35; and iii) LLC1 and B16F10 cells were administered via tail vein injection, awaiting for lung tumors to be developed after 14 days. IGF1R deficiency diminished BALF and bone marrow inflammatory total cell counts, and reduced lung tumor burden. Additionally, lack of IGF1R lowered mRNA expression of metastasis, hypoxia, tumor associated macrophage, tumor infiltrating lymphocyte, neutrophil and dendritic cell markers. Tumors of Igf1r-depleted mice also presented reduced proliferation, vascularization and presence of activated fibroblasts, sustained by decreased expression of epithelial to mesenchymal transition markers. Our results support that IGF1R acts as a cancer-promoting factor in the TME contributing to implantation and progression of lung metastases.

1 IP López et al. Transgenic Res24:279, 2015

0064-R-P

Study of PNPT1 mutations pathogenicity

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PNPT1 is a nuclear gene which codes to polyribonucleotide nucleotidiltransferase 1, also known as polynucleotide phosphorylase (PNPase), a protein discovered by Marianne Grunberg-Manago working in Severo Ochoa's lab in 1955. Several functions and locations has been proposed to this protein, but its predominant role in humans takes place in mitochondrial matrix, where participates in mtRNA metabolism as a central enzyme by forming an homotrimeric complex which degrades RNAs transcribed from mtDNA with its 3'to 5' exoribonuclease activity (Cameron, Matz, & De Lay, 2018; Lin, Wang, Yang, Hsiao, & Yuan, 2012).

In the present work, two compound heterozygous mutations in *PNPT1* has been studied in order to establish a patient disease etiology: missense variant c.1519G>T (previous published) and nonsense variant c.1684A>T (novel). First, PNPT1 structural analysis *in silico* suggested that, on the one hand, the missense variant could affect the active site environment and/or the homotrimeric assembly and, on the other hand, the nonsense variant could remove PNPT1 interaction with RNAs. Second, *PNPT1* transcription (qPCR), PNPT1 levels (Western blot) and homotrimeric assembly (Blue Native-PAGE) has been evaluated in patient derived fibroblast and lower amount has been obtained in all analysis compared with control cell lines. Third, mitochondrial dysfunction has been evaluated by analyzing different mitochondrial transcripts (non-mature, mature and total) and mtDNA levels (qPCR), mitochondrial protein synthesis (labeled with ^{35}S) and basal oxygen consumption (O_2 electrode). In general, transcripts are increased in patient as expected, but also mtDNA levels and mitochondrial protein synthesis, whereas basal oxygen consumption is decreased. Finally, control and patient cells has been immortalized and a functional complementation assay has been applied to analyze mitochondrial function recovery, however nor mitochondrial transcript levels neither basal oxygen consumption has been recovered. Nevertheless, results of this assay has shown that mitochondrial transcripts increase in patient is not directly dependent from mtDNA levels.

All together, these results reveal that our patient's PNPT1 protein is being impaired by the mutations and, hence, causes a mitochondrial dysfunction.

Cameron, T. A., Matz, L. M., & De Lay, N. R. (2018). Polynucleotide phosphorylase : Not merely an RNase but a pivotal post-transcriptional regulator. PLoS Genetics, 14(10), 1–17. https://doi.org/10.1371/journal.pgen.1007654 Lin, C. L., Wang, Y.-T., Yang, W.-Z., Hsiao, Y.-Y., & Yuan, H. S. (2012). Crystal structure of human polynucleotide phosphorylase : insights into its domain function in RNA binding and degradation. Nucleic Acids Research, 40(9), 4146–4157. https://doi.org/10.1093/n

0065-R/M-P

ANTI-INFLAMMATORY (M2) RESPONSE IS INDUCED BY A SYNTHETIC GLYCOLIPID-TYPE MOLECULE ((1R)-1-DODECYLSULFINYL-5N,6O-OXOMETHYLIDENENOJIRIMYCIN): A NEW POSSIBLE TREATMENT IN DIABETIC RETINOPATHY

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Retinal inflammation occurs during an early stage in Diabetic Retinopathy (DR), which is believed to play a crucial role in the development and progression of DR (1,2). Retinal inflammation is mediated by microglia activation and there after leads to neuronal apoptosis (3). Various bioactive extracts from medicinal plant and algae, such as the iminosugar glycosyl hydrolase inhibitors 1-deoxynojirimycin and castanospermine or the immunoregulatory α-linked glycolipids, have shown properties against chronic diseases with an inflammatory component(2). We have recently reported the beneficial effects of molecules that combine the structural feaures of iminosugars and glycolipids, namely sp2-iminosugar glycolipids (sp2-IGLs), in reducing inflammatory parameters during DR by modulation of different signaling pathways in the immune system of the retina(4,5).

The aim of this work was to investigate the effects of the sp2-IGL (1*R*)-1-dodecylsulfinyl-5*N*,6*O*-oxomethylidenenojirimycin on inflammation associated to Diabetic Retinopathy.

Bv.2 microglial cells cultured under diabetic environment and treated with C4 reduces the pro-inflammatory markers, such as iNOS levels and IL1b, IL6 and TNFa expression. The activation of the inflammasome complex is blocked and C4 is able to induce a potent M2 response by inducing IL-10 expression and increasing the Arginase-1 levels. The C4 effect was studied in retinas from a diabetic model of Diabetes mellitus type 1, BB rat, where we detected a clear reduction of reactive gliosis, a classical feature of DR.

The C4 product exerts a beneficial effect on the inflammatory process that precedes the RD and could be an effective alternative for its treatment and / or prevention

1. Arroba AI et al. Biochim Biophys Acta (2016) 1862 (9):1663–74. 2. Gardner TW, et al. Vision Res (2017) 139:153–60. 3. Duh EJ, et al. JCI Insight (2017) 2(14):e93751. 4. Alcalde-Estevez E et al. Food Chem Toxicol (2018) 111:454–66. 5. Sanchez-Fernandez EM et al. Eur J Med Chem (2019) 182:111604.

0074-P

Genetic Characterization of Human Acral Lentiginous Melanoma

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Acral lentiginous melanoma (ALM) is a cutaneous melanoma (CM) with poor prognosis due to the diagnosis in advanced stages and poor response to conventional antitumor therapy. ALM is an uncommon clinicopathological variant of CM, which arises on palms, soles and nail bed. Most ALM (more than 50%) belong to the triple wild-type (TWT) melanoma subtype for *BRAF, NRAS*, and *NF1* genes, that are mutated in only 42-55% of ALM tumours. Furthermore, ALM with mutations in KIT (3-36%), GNAQ (11%) and TERT activating mutations (9-41%) have been identified. Copy number changes are numerous in ALM; amplifications have been described in KIT, TERT, CDK4, MDM2 and CCND1, and deletions in CDKN2A, PTEN and NF1.

In order to describe the ALM genetic profile, we obtained DNA from five ALM cell lines and conducted a retrospective review of patients newly diagnosed with ALM in the Hospital Universitari Arnau de Vilanova (Lleida) during the period 2010-2020. Paraffin-embedded biopsies from this series were reviewed and a microscopic analysis of ALM samples was performed in order to select tumoural regions to extract DNA. Then, we first performed a point mutation analysis of BRAF, NRAS and KIT genes by Sanger sequencing. Secondly, we analysed copy number variations (CNVs) of oncogenes and tumour suppressor genes by Multiplex Ligation-dependent Probe Amplification (MLPA) to identify possible biomarkers in ALM cell lines and biopsies from our patient cohort.

Our findings reveal that ALM cell lines have low point mutations and high copy number variations, which were found mainly in the MDM2/4-p53 and CDK4/6-CCND1/2 pathways.

The implication of these pathways in tumour progression and therapeutic response will be investigated to describe a characteristic molecular profile of ALM. These results will allow us to determine target molecular alterations to improve early diagnosis and personalized therapy of ALM patients.

- Yeh I, Jorgenson E, Shen L, Xu M, North JP, Shain AH, et al. Targeted genomic profiling of acral melanoma. J Natl Cancer Inst. 2019;111(10):1068–77. - Moon KR, Choi YD, Kim JM, Jin S, Shin MH, Shim HJ, et al. Genetic Alterations in Primary Acral Melanoma and Acral Melanocytic Nevus in Korea: Common Mutated Genes Show Distinct Cytomorphological Features. J Invest Dermatol. 2018;138(4):933–45.

0075-P

The R502W mutation in murine cardiac myosin-binding protein C leads to pathogenic myocardial remodeling in the absence of protein haploinsufficiency

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Centro Nacional de Investigaciones Cardiovasculares Molecular Mechanics of the Cardiovascular System

Many myocardial pathologies are caused by inherited genetic mutations that result in anatomical alterations and compromised cardiac function [1]. In humans, the missense variant R502W in cardiac myosin-binding protein C (cMyBP-C) is the most frequent mutation leading to hypertrophy cardiomyopathy (HCM) [2]. However, the molecular mechanisms sustaining pathogenicity of variant R502W remain unknown, since both cMyBP-C's mRNA and protein structure have been proposed not to be perturbed by the mutation [3]. Using CRISPR/Cas9-based genetic engineering, we have generated a knock-in mouse model that harbors the R502W mutation in murine cMyBP-C, and characterized the resulting cardiac phenotype. Using echocardiography and magnetic resonance imaging, we detect thicker trabeculae, altered ventricle geometry, reduced left ventricular systolic function and diastolic dysfunction in homozygous p.R502W mice. We also observe higher ventricular mass in older mice. Interestingly, biochemical analysis shows that cMyBP-C mRNA and protein levels are not altered by the mutation. Since we there is no evidence of cMyBP-C haploinsufficiency in the R502W mice, we propose that pathogenicity of the mutation stems from alternative mechanisms, which we are currently investigating. We expect that further investigation of R502W mice will shed light on the molecular triggers of HCM caused by the many cMyBP-C point mutations that do no lead to protein haploinsufficiency.

1. McNally, E. M., Barefield, D. Y., & Puckelwartz, M. J. The genetic landscape of cardiomyopathy and its role in heart failure. Cell metabolism. 21, 174-18 (2015). 2. Walsh, R. et al. Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. Genet. Med. 19, 192–203 (2017). 3. Suay-Corredera, C. et al. Nanomechanical phenotypes in cMyBP-C mutants that cause hypertrophic cardiomyopathy. bioRxiv (2020).

0092-R/M-P

Identification of novel substrates of Malin E3 ubiquitin ligase in Lafora disease.

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Lafora disease (LD) is a progressive neurological disorder characterized by epileptic seizures, myoclonus, cerebellar symptoms and psychic deterioration. There is no cure and patients are treated in a palliative way with anti-epileptic drugs, towards which, after some time, they become resistant. At the basis of the disease, there is a malfunction of two proteins, laforin and malin, encoded respectively by two genes: *EPM2A* and *EPM2B*. The two proteins form a complex and its incorrect functionality generates an error in the metabolism of glycogen leading to the accumulation of polyglucosan inclusions in patients. The polyglucosans, have an anomalous structure that prevents its normal degradation leading to the formation of Lafora bodies. Studies conducted on brain samples of LD mouse models show a greater accumulation of polyglucosans at the level of astrocytes compared to neurons. Considering the role of malin, known to be an E3 ubiquitin ligase, involved the ubiquitination of specific substrates, we performed a proteomic analysis of the enriched ubiquitinated fraction of proteins, in HEK293T cells expressing either wild type or an inactive form of malin carrying the pathogenic P69A mutation (the most prevalent mutation of *EPM2B* gene). In comparison to cells expressing the non-functional malin-P69A, a list of more ubiquitinated putative substrates in cells expressing wild type malin was obtained.

The aim of this study is to validate these candidates as substrates of malin and then focus on the consequences of ubiquitination on their physiological function. This information will allow the identification of putative therapeutic targets and develop new treatments that could ameliorate the pathology present in Lafora disease.

Turnbull J, et al. Lafora disease. Epileptic Disord. 2016. doi:10.1684/epd.2016.0842 Carla Rubio-Villena, et al. Astrocytes: new players in progressive myoclonus epilepsy of Lafora type, Human Molecular Genetics, 2018, https://doi.org/10.1093/hmg/ddy044 Sanchez-Martin P, et al. Regulation of the autophagic PI3KC3 complex by laforin/malin E3-ubiquitin ligase, two proteins involved in Lafora disease. Biochim Biophys Acta Mol Cell Res. 2020. doi:10.1016/j.bbamcr.2019.118613

0095-P

alpha-ENOLASE AS AN HAPTENATION TARGET OF AMOXICILLIN

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α-Enolase is a Mg2+-dependent glycolytic enzyme that occurs as homo- or hetero-dimers and that presents several proteoforms resulting from post-translational modifications (PTMs). Nevertheless, moonlighting activities have been also described for α-enolase including stimulation of immunoglobulin production (1), acting as an autoantigen or participating in food allergic responses (2). Our group previously found α-enolase among amoxicillin (AX) haptenation cellular targets (3). Here, we have carried out an *in vitro* study using recombinant α-enolase haptenation and AX or its biotinylated analog, AX-B. Under our conditions, α-enolase modification occurs at a ratio of 1:30 (AX-B haptenated:non-haptenated protein), is modulated by Mg2+ and increases in the presence of the 2-phosphoglycerate substrate. Conversely, AX-haptenation slightly decreased α-enolase activity (10-15%). Denaturation protocols did not change AX-B modification levels of α-enolase, suggesting lack of additional modification sites. 2D-electrophoresis showed the existence of 5 distinct protein spots both in native and AX-B-modified α-enolases, with higher AX-B detection in more acidic proteoforms. Putative interplay between α-enolase haptenation and acetylation was also studied using sulfo-NHS-acetate (SNA) alone or in combination with AX-B; while SNA induced a concentration-dependent increase in low pI proteoforms abundance, it decreased AX-B haptenation. Analysis of AX-modified α-enolase using a bottom-up mass spectrometry approach identified a 365 Da mass increase in the 273-251 peptide, compatible with its haptenation, that most probably occurs on K239. AX docking on α-enolase crystal structure (2PSN) provided several energy-favored modes, out of which two placed the carbonyl of the β-lactam ring close to K239. Altogether, the data indicate AX-modification of α-enolase on K239 *in vitro*, and an inverse correlation between acetylation and haptenation that could have implications in allergic reactions.

Funding: RETIC Aradyal from ISCIII/FEDER, RD16/0006/0021; RTI2018-097624-B-I00 from AEI, MICINN/FEDER

1- Sugahara, T., et al. (1992) Cytotechnology 10, 137-46. 2- Apostolovic, D., et al. (2014) Allergy. 69, 1308-15. 3- Sanchez-Gomez, F.J., et al. (2017) Allergy 72, 385-396.

0115-R-P

The Retinoid X Receptor is a key regulator of hematopoietic stem cell fate

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Hematopoietic stem cells (HSCs) self-renewal and multi-lineage differentiation capacity are opposing fate decisions required for long-term homeostatic blood formation. This binary decision relies on transcription factor (TF) programs. HSC-specific loss of certain TF signalling programs can lead to conditions such as hematopoietic aging, inflammation, myelodysplasia and/or leukemia.

Herein, we demonstrate that the Retinoid X Receptor (RXR) is a key TF for HSC homeostasis, function and identity. We showed that the deletion of RXRa and RXRb within HSCs causes loss of self-renewal and a myeloid lineage bias. RXR deficiency results in loss of symmetric cell division and quiescence as well as increased proliferation and survival of HSCs. Whole genome analysis of transcriptome, open chromatin status, RXRa binding and histone marks of regulatory regions in wild-type and/or RXRa/b deficient HSC was performed and identified specific metabolic and rRNA processing pathways regulated by RXR, controlled by Myc expression. Myc haploinsufficiency in RXR-deficient HSC restores competitive HSC self-renewal but not their myeloid differentiation skewing.

In summary, our data describe for first time RXRs as key regulators of HSC fate, opening the possibility to use RXR as a therapeutic target in hematopoietic disease.

This work was supported by grants from the Spanish Ministerio de Ciencia e Innovación (MCI) SAF2017-90604-REDT, NurCaMeIn, RTI2018-095928-BI00 and the Formación de Profesorado Universitario (FPU17/01731) programme (MCI)

0124-P

Rabphilin silencing causes dilated cardiomyopathy in a Drosophila model of heart-nephrocyte damage

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Chronic kidney disease (CKD) and Heart failure (HF) have a strong correlation. Both can be cause and consequence of the other. Many factors can lead to the appearance and development of these diseases, one of them being the genetic ones. One candidate genetic factor is *Rph*, a gene that is expressed in the excretory and nervous systems in mammals as well as in *Drosophila*. This gene encodes a Rab small GTPase family effector protein, which is related to vesicular trafficking. We have found that *Rph* is expressed in *Drodophila*'s heart. Acknowledging this, we specifically decreased Rph levels in heart and in both heart and excretory system, and we saw that lifespan was significantly compromised as compared with control flies. Moreover, diastolic and systolic diameters (EDD and ESD, respectively, both being crucial parameters for heart function) were significantly increased, being more severe in the case of reduced Rph levels in both tissues. This suggests that Rph is important in heart and excretory system, and that nephrocyte damage contributes to the development of cardiac disease in a *Drosophila* model.

1.Tuegel, C. & Bansal, N. Heart failure in patients with kidney disease. Heart (2017). doi:10.1136/heartjnl-2016-310794 2.Wu, G. et al. Increased myocardial Rab GTPase expression: A consequence and cause of cardiomyopathy. Circ. Res. (2001). doi:10.1161/hh2401.100427 3.Selma-soriano, E. et al. Rabphilin involvement in filtration and molecular uptake in Drosophila nephrocytes suggests a similar role in human podocytes. (2020). doi:10.1242/dmm.041509

0133-R-P

sTWEAK levels are associated with prostate cancer patient's metabolic status and modulates lipid metabolism in vitro

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Background and aim
Tumor necrosis factor weak inducer of apoptosis (TWEAK) is an inflammatory cytokine related to prostate cancer (PCa) progression, that exert its effect by binding to it receptor known as Fn14[1]. Its soluble form, sTWEAK, has been detected in the PCa microenvironment, and it can be used as a prognostic tool[2]. Reduced levels of sTWEAK have been associated with glucose metabolism in patients with obese related pathologies[3]. There is experimental and clinical evidence suggesting an association between insulin and PCa, however the metabolism in prostate adenocarcinoma is recognized as distinct among solid tumors because favors enhanced lipogenesis but limited glycolysis.[4] In this sense, sTWEAK has been found to modulate lipid metabolism in hepatocyte cells[5]. Our aim is to study the relation of sTWEAK with the metabolic status of PCa patients and its effect on glucose and lipid metabolism over PCa cells in vitro.

Results
Circulating serum levels of sTWEAK were significantly reduced in PCa patients when compared with healthy controls, whereas insulin, glucose and HOMA-IR were significantly higher in PCa patients. In vitro sTWEAK stimulus over PCa cells did not produced any notorious mRNA changes over glucose metabolism related genes. When analyzing expression levels of lipogenesis (ACACA, FASN); lipolysis (CPT1A, PNPLA2); lipid transporter genes (FABP4, CD36) and, lipid regulator genes (SREBP-1, PPARG) sTWEAK stimulus provoked a significantly increased gene expression levels. In vitro TWEAK receptor inhibition experiments (siFN14) confirmed previous results, indicating that sTWEAK is responsible for the observed effects.

Conclusions
TWEAK/Fn14 axis modulation can be crucial in PCa progression and pathogenesis.

1. Cheng, E. et al. Front. Immunol. 4, 1–13 (2013). 2. Ruiz-Plazas, X. et al. J. Transl. Med. 17, 1–13 (2019). 3. Vendrell, J. & Chacón, M. R. Front. Immunol. 4, 488 (2013). 4. Bader, D. A. & McGuire, S. E. Nat. Rev. Urol. 17, 214–231 (2020). 5. Lozano-Bartolomé L et al. Int J Obes 40, 1337–1345 (2016)

0137-R-P

Immunolocalization in the Golgi complex and endoplasmic reticulum of POMGNT1 and POMGNT2, two proteins involved in dystroglycanopathies, in the mammalian retina and 661W photoreceptors

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The *POMGNT1* and *POMGNT2* genes, encoding protein *O*-linked mannose β-1,2-*N*-acetylglucosaminyltransferases 1 and 2, are associated with Walker-Warburg syndrome (WWS), muscle-eye-brain disease (MEB), limb-girdle muscular dystrophy (LGMD) and other dystroglycanopathies (DGPs). These constitute a group of minority congenital neuromuscular dystrophies that course with a broad spectrum of symptoms affecting the skeletal muscle and CNS, including the brain and retina. Lack of function or expression of these proteins cause a loss of *O*-mannosyl glycosylation of α-dystroglycan (α-DG) in a complex, branched pathway where POMGNT1 catalyzes the addition of *N*-acetyl-glucosamine (GlcNAc) to synthesize the M1 and M2 core glycans on α-DG, while POMGNT2 adds GlcNAc in another subpathway leading to α-DG M3 core glycan synthesis (1,2). In the retina, α-DG *O*-glycosylation is crucial for the establishment of functional ribbon synapses between photoreceptors and bipolar cells (3).

In our group we are interested in establishing the expression pattern and function of DGP-associated proteins in the mammalian retina (3-5). In the present work we have focused on jointly analyzing the expression of proteins POMGNT1 and 2 in retinal cells. With this purpose, we have used immunofluorescence microscopy in order to characterize their distribution pattern, in mouse and monkey retinal sections and in the mouse cone photoreceptor immortalized cell line 661W, by means of their colabeling with molecular markers of the endoplasmic reticulum (ER) and the Golgi complex. Our observations revealed that POMGNT1 was located in the Golgi, and POMGNT2 in the ER, of retinal neurons in the monkey and mouse, including photoreceptors (inner segments and axon terminals) and 661W cells. In addition, in this cell line POMGNT2 was located in the Golgi, and in contrast to retinal tissue, POMGNT1 and 2 additionally accumulated in the nucleus, where they were found to colocalize. These results are indicative of a relevant role of these proteins in α-DG glycosylation in the neural retina of adult mammals. Also, they suggest that POMGNT1 and 2 could exert an additional role, yet to be defined (glycosylation of nuclear proteins?, mitogenesis?), in the nucleus of 661W photoreceptors.

Funding: Universidad de Alicante (VIGROB20-237, UADIF20-84 and UAUSTI20-14).

(1) Ragni E, Lommel M, Moro M, Crosti M, [4 authors], Lazzari L (2016) Cell Mol Life Sci 73, 445-458. (2) Halmo SM, Singh D, Patel S, Wang S, [4 authors], Wells L (2017) J Biol Chem 292, 2101-2109. (3) Rubio-Fernandez M, Uribe ML, Vicente-Tejedor J, [5 authors], Martin-Nieto J, Cruces J (2018) Sci Rep 8: 8543. (4) Uribe ML, Haro C, Ventero M, Campello L, Cruces J, Martin-Nieto J (2016) Mol Vis 22, 658-673. (5) Haro C, Uribe ML, Quereda C, Cruces J, Martin-Nieto J (2018) Mol Vis 24, 43-58.

0139-R-P

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

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Proteins and RNAs assemble in membrane-less or- ganelles 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 investi- gate 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 up the avenue for their manipulation.

0145-P

ADAM10 is a direct MITF target gene in melanoma

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Melanoma is a highly invasive type of cancer that can often lead to death. Although not all the molecular mechanisms involved in its carcinogenesis are fully understood, the Microphthalmia-Associated Transcription Factor (MITF) is considered the main regulator of the development, differentiation, function and survival of melanocytes [1]. ADAM10

is a transmembrane protein involved in several biological events, such as the cleavage of the MICA and MICB proteins, which prevents NK cells from recognizing and eliminating tumor cells [2]. Furthermore, a high percentage of ADAM10-positive melanoma cells correlates with less lymphocyte infiltration in tumors, suggesting that ADAM10 regulates the immune response in melanoma [3]. In this context, a better knowledge of the immune mechanisms associated with the establishment of melanoma could lead to the development of new strategies for the treatment of this pathology.

This experimental work focuses on evaluating the possible relationship between MITF and ADAM10 in melanoma cells. Analysis of the ADAM10 promoter showed an E-Box consensus sequence that exactly matched the MITF binding sequence. This observation was confirmed by ChIP-Seq data for MITF, showing an enrichment of MITF in the ADAM10 promoter. These results were validated by conventional ChIP experiments in IGR37 and 501mel melanoma cells, with high MITF expression. Additional luciferase assays determined that MITF was able to directly activate ADAM10 transcription. MITF silencing assays in different melanoma cell lines verified a significant reduction of ADAM10 protein. In summary, we demonstrate, for the first time, the control of ADAM10 expression mediated by MITF in melanoma and suggest that targeting ADAM10 may represent a therapeutic approach to improve tumor cell clearance by the immune system.

This work was supported by the following grants: Ramon y Cajal Programme [RYC-2016-20036/Fondo Social Europeo (FSE)/Agencia Estatal de Investigación (AEI). Título: Modulación epigenética de las células tumorales para su sensibilización a la radio e inmunoterapia] and Fundación Séneca, Región de Murcia (FS-RM) projects 20809/PI/18 and 21407/FPI/20.

0147-R-P

Exploring the link between Parkinson's disease and Diabetes Mellitus in Drosophila

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, which is caused by the selective loss of dopaminergic (DA) neurons in the substantia nigra pars compacta. However, the cause of this neurodegeneration remains unclear. To date, accumulation of misfolded proteins aggregates, mitochondrial dysfunction, oxidative stress (OS), neuroinflammation or genetic mutations have been proposed to contribute to PD onset and progression. Recently, metabolic alterations have also been shown to play an important role in PD. Previous studies carried out by our group demonstrated that fly mutants for the *DJ-1β* gene (ortholog of human *DJ-1,* involved in familial PD cases) showed enhanced glycolysis, increased carbohydrates levels and glycogen accumulation. Interestingly, metabolomic alterations displayed by those PD model flies were similar to those found in a mouse model of Diabetes Mellitus (DM). In fact, it has been recently shown that there are important links between both diseases. Therefore, we aimed to study the possible relationship between both diseases in *Drosophila*. First, we developed a *Drosophila* DM model by culturing control flies in a hypercaloric medium (HCM). They showed increased levels of carbohydrates, the most relevant DM phenotype, as well as glycogen accumulation as found in our PD model flies. Subsequently, we analyzed if DM model flies exhibited PD-related phenotypes. Our results demonstrated that those flies showed increased OS levels, reduced lifespan and motor defects, hence confirming that DM might be a potential cause of developing PD. We also found that DM model flies showed DA neurodegeneration, revealed by reduced levels of the specific DA neuron marker tyrosine hydroxylase. In addition, they displayed an enhancement of glycolysis as found in PD model flies, which strongly suggests that common metabolic alterations might be found in both diseases. Finally, we validated that viability of SH-SY5Y human neuroblastoma cells is reduced when cultured in presence of high glucose levels, as found in DM model flies, suggesting that hyperglycemia could contribute to PD-associated neurodegeneration. Therefore, results obtained in this study encourage to carry out a more in-depth study on both diseases to determine how one might influence the onset/development of the other.

Solana-Manrique, C. et al. Enhanced activity of glycolytic enzymes in Drosophila and human cell models of Parkinson's disease based on DJ-1 deficiency. Free Radic. Biol. Med. (2020). Shearer, J. et al. Metabolomic profiling of dietary-induced insulin resistance in the high fat-fed C57BL/6J mouse. Diabetes, Obes. Metab. (2008). Hassan, A. et al. Diabetes Mellitus and Parkinson's Disease: Shared Pathophysiological Links and Possible Therapeutic Implications. Cureus (2020).

0155-R-P

Transplantation of a functional adipose tissue with enhanced lipid oxidation reduces obesity and glucose intolerance

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The obesity epidemic and its associated comorbidities are increasing worldwide. The adipose tissue (AT) stores fatty acids (FAs) (white adipose tissue-WAT) or utilizes them to regulate energy expenditure by thermogenesis (brown adipose tissue-BAT). Therefore, increasing AT FA's metabolism could be a potential therapeutic approach to treat obesity. Carnitine palmitoyltransferase 1A (CPT1A) is the key enzyme in the regulation of mitochondrial FA oxidation (FAO). Our previous *in vitro* studies showed that the expression of a constitutively active CPT1A form (CPT1AM) in adipocytes is able to improve lipidic metabolism and mitochondrial activity[1,2].

Here, we aim to generate transplantable CPT1AM-expressing adipocytes able to improve the obese phenotype in mice[3]. AT-derived mesenchymal stem cells (AT-MSCs) were isolated from the inguinal WAT (iWAT) of adult lean mice, differentiated into mature adipocytes and transduced with a lentiviral vector expressing CPT1AM. Isolated AT-MSCs exhibited MSCs-specific characteristics, as they: 1) were grown as adherent cells; 2) expressed specific surface markers (CD29, CD90, Sca1); and 3) gave rise to different cell lineages (adipocytes, osteoblasts and chondrocytes). 8-weeks-old mice were subcutaneously transplanted with CPT1AM-expressing adipocytes and fed with high-fat diet (HFD) for 10 weeks. Interestingly, CPT1AM-transplanted obese mice showed lower body weight and hepatic steatosis and improved glucose tolerance, and serum insulin and cholesterol levels. In addition, HFD-induced increase in adipocyte hypertrophy, fibrosis, inflammation, ER stress and apoptosis was reduced in WAT and BAT of CPT1AM-transplanted mice. The expression of mitochondrial respiratory chain complexes was enhanced in BAT and iWAT of CPT1AM-transplanted mice.

Our results demonstrate that transplantation of CPT1AM-expressing AT-MSCs-derived adipocytes into HFD-fed mice improves the obese metabolic phenotype supporting the future clinical use of this approach.

0162-P

A Metabolic footprinting of the Short Unpredictable Variable Stress (SUVS) model in male Wistar rats

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Anxiety disorders have been increasing dramatically in recent decades, in fact they have become the most prevalent psychiatric disorder in the United States and Europe. However, anxiety disorders are complex conditions, with not fully understood pathological mechanisms. In this sense, numerous aspects, including psychological, genetic, biological, and chemical factors, are thought to be involved in their etiology. Furthermore, the diagnosis of anxiety disorders is currently based almost exclusively on symptom checklist and psychological questionnaires, making the identification of premature specific biomarkers of anxiety a valuable tool. This will allow the acquisition of better insights into patient-specific pathology mechanisms that will lead to a properly diagnostic and treatment. In order to obtain valuable potential biomarkers, metabolic profiling seems a promising approach to recognize early biochemical changes in disease which will also provide an opportunity to develop predictive metabolic footprints that can allow the initiation of earlier interventions. Thus, we studied plasma and urine metabolites in the Short Unpredictable Variable Stress (SUVS) animal model, which aims to mimic a short anxiety period. Our approach considered both predicting capability and statistical significance of individual metabolites in association with anxiety. The results showed that 23 metabolites in plasma and 3 metabolites in urine were significantly changed, and altogether they could reflect the anxiety disturbed pathways, which were mainly involved in energy and lipid metabolism. In more detail, such changes mainly consisted in modulations on tricarboxylic acid (TCA) cycle and fatty acid degradation, as well as in neurotransmitter synthesis. Specifically, 9 metabolites in plasma were considered potential biomarkers of anxiety including succinic acid, malic acid, threonic acid, alpha-ketoglutarate, pyruvic acid, cholesterol, oleic acid, 3-hydroxybutitic acid and citric acid. Interestingly, our results provide a novel metabolic fingerprint to purely determine anxiety avoiding other disorders as depression. Nevertheless, more studies profiling anxiety are recommended to further explore and validate these findings and promising biomarkers.

W.H. Organization, Depression and other common mental disorders: global health estimates, World Health Organization, 2017. R.L. Schneider, J.J. Arch, K.B. Wolitzky-Taylor, The state of personalized treatment for anxiety disorders: A systematic review of treatment moderators., Clin. Psychol. Rev. 38 (2015) 39–54. E. Humer, C. Pieh, T. Probst, Metabolomic Biomarkers in Anxiety Disorders, Int. J. Mol. Sci. 21 (2020) 4784.

0165-R-P

Therapy response in glioblastoma and tumour microenvironment participation: a film with many actors spotted by MRSI

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Introduction: Glioblastomas (GB) are deadly brain tumours. Magnetic resonance spectroscopic imaging (MRSI) could be a biomarker for immune system efficacy in GB 1 . Glioma-associated microglia/macrophages (GAMs) are abundant immune cells found within GB, polarised into anti-tumour or pro-tumour phenotypes (M1-M2). They can represent up to 30-40% of the tumour mass 2 . Matrix Metalloproteinases (MMPs) and 'A Disintegrin and Metal-

loproteinase' (ADAMs) are indicative for tumour microenvironment changes and correlate with GAM phenotypes 3 . 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 GBM-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 the MRSI-based biomarker 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 4 . *ADAM10* and *ADAM17* positively correlate with M1 phenotypes and patient survival 5 . MMP9 downregulation is a biomarker for improved outcome 6 and chemotherapy may increase PD-L1 expression 7we evaluated the frequency of PD-1/L1 expression in pre/post chemotherapy specimens and the correlation with the treatment efficacy. Methods The expression of PD-1/L1 was evaluated using immunohistochemistry in patients with TM or TC treated with chemotherapy between 2000 and 2014. Using formalin-fixed, paraffin-embedded tissue samples and a PD-L1 antibody, the expression of PD-L1 in the TM and TC specimens was reported in terms of the H-score (0–300 .

Conclusions: Changes in GAMs prevailing population could be one of the factors explaining MRSI pattern differences in our imaging biomarker, although other microenvironment components may also contribute. Protease profiles were associated with microglia/macrophage functions, could provide insight into their molecular signature and might be a predictor of GB patients' therapy response and overall survival.

0169-R/M-P

HISTONE POSTTRANSLATIONAL MODIFICATIONS IN AN ANIMAL MODEL OF SCHIZOPHRENIA

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Schizophrenia (SZ) is a severe and chronic psychiatric disorder characterized by positive (e.g. hallucinations and delusions) and negative symptoms (e.g. social withdrawal, poverty of speech and affective blunting). The etiology of SZ is multifactorial, including genetic and environmental factors. Infections during the gestational period have been associated to increased risk of SZ in the offspring. Indeed, maternal immune activation (MIA) based on the administration of polyriboinosinic-polyribocytidilic acid (Poly(I:C)) in pregnant female mice, is a validated, translational model of SZ. Infections and other environmental factors may contribute to the disorder's etiology partly through epigenetic processes, including histone posttranslational modifications (HPTM), such as trimethylation of lysine (K) 4 in histone H3 (H3K4me3) and acetylation of K9 and K27 in histone H3 (H3K9ac, H3K27ac). Own studies have shown enhanced expression of these HPTM in post-mortem human brain of subjects with SZ, possibly leading to increased gene expression.

The aim of this study was to evaluate the expression of H3K4me3, H3K9ac, H3K27ac in frontal cortex of MIA mouse model. A secondary aim of this study was to assess the possible interaction between MIA and mouse sex and strain (C57LB/6 and Swiss) on the expression of these HPTM by two-way ANOVA.

None of the HPTM were altered in the MIA model as a whole. On the other hand, among the studied HPTM, there was a statistically significant interaction between sex and MIA on H3K27ac expression ($F(1,12) = 5.625$, $p = 0.035$). The analysis showed that H3K27ac expression was increased in female mice of the MIA group ($p = 0.0413$). Similar analysis of strain effect revealed a statistically significant interaction between strain and MIA on H3K37ac ($F(1,12) = 7.094$, $p = 0.021$) and H3K4me3 expression ($F(1,12) = 5.573$, $p = 0.0360$). Specifically, H3K27ac expression was higher in female C57LB/6 than in female Swiss mice ($p = 0.0004$).

In conclusion, the MIA model did not reproduce the altered expression of H3K4me3, H3K9ac and H3K27ac found in SZ brains. However, sex and strain effect analysis has shown that H3K4me3 and H3K27ac expression was affected by mouse sex and strain. The modulation of epigenetic alterations by confounding factors must be considered in future studies.

Haddad et. al.,Neurosci. Biobehav. Rev. 113, 546-567 (2020). Martínez-Peula et. al. Schizophr. Bull. 46(1), S107, (2020). Meyer et. al. Biol. Psychiatry 75, 307-315 (2014). Reisinger et al. Pharmacol. Ther. 149, 213-226 (2015).

0170-R-P

ERASTIN-INDUCED FERROPTOSIS ENHANCES LOSS-OF FRATAXIN PHENOTYPES IN A DROSOPHILA MODEL OF FRIEDREICH´S ATAXIA

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Friedreich´s Ataxia (FRDA) is the most prevalent autosomal recessive ataxia in the European population (1:50000). The disease is caused by the reduced expression of frataxin, a mitochondrial protein involved in iron–sulfur cluster biogenesis. Deficiency of frataxin leads to a drastic reduction in the cellular energy production (1). Remarkably, the characterization of the type of cell death that affects the cells deficient for frataxin still remains unsolved. Several studies in cell culture models point towards apoptosis (2-4). However, no marker of apoptotic cell death has been observed in *in vivo* models (5). A very attractive possibility is a new type of cell death named as ferroptosis. Deregulation of iron metabolism, depletion of glutathione and accumulation of lipid peroxides are the major hallmarks of ferroptosis (6). Remarkably, these three molecular signatures have been detected in samples from FRDA patients as well as in disease models including *Drosophila melanogaster*, suggesting that loss of frataxin recapitulates ferroptotic cell death (7).

We have used 3 different known inducers of ferroptosis (buthionine sulfoximine (BSO), Erastin and Tert-Butyl Hydroperoxide) to analyse whether frataxin-deficient flies display increased sensitivity towards this stressor. Our results indicate that flies seem to react differently to all three chemicals. Erastin but not BSO and Tert-Butyl reduced locomotion of frataxin-deficient flies, boosted the production of lipoperoxides and impaired mitochondrial function (monitored as aconitase activity and ATP production) without enhancing longevity defects. Similar results were obtained when the fly ortholog of Glutathione Peroxidase 4 (GPTx1) was downregulated in frataxin-deficient flies. We are now assessing whether inhibitors of ferroptosis or upregulation of GTPx1 are able to alleviate frataxin-deficient phenotypes.

1. González-Cabo P, Palau F. 2013. doi:10.1111/jnc.12303. 2. Palomo GM, et al. 2011. doi:10.1093/hmg/ddr187. 3. Mincheva-Tasheva S, et al. 2014. doi:10.1093/hmg/ddt576. 4. Igoillo-Esteve M,et al. 2015. doi:10.1093/hmg/ddu745. 5. Simon D, et al. 2004. doi:10.1523/JNEUROSCI.4549-03.2004. 6. Xie Y, et al. 2016. doi:10.1038/cdd.2015.158. 7. Monnier V, et al. 2018. doi:10.3390/ijms19071989.

0176-P

METALLOPROTEINASE ACTIVITY AND LACTOFERRIN IN SERUM AND VAGINAL EXUDATES IN HEALTHY HOLSTEIN COWS AND COWS AFFECTED BY METRITIS

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In cows, uterine function after parturition is often compromised by bacterial contamination. Although many cows manage to eliminate the infection, around 10-17% of cattle are affected by metritis, an infection of the uterus. The inflammatory response due to infection compromises animal welfare and has negative effects on milk production and reproductive efficiency. MMPs are a proteolytic enzyme family able to degrade components of the extracellular matrix during physiological and pathological processes, and to activate cytokines and anti-microbial peptides. In the uterus, MMPs contribute to tissue remodeling but are also increased by infection and inflammation. The goal of the present study was to identify MMPs in serum and vaginal exudates, as candidate biomarkers for metritis.

Serum and vaginal exudates were obtained from healthy and metritic dairy cows at several days around parturition. An extract from the mucous exudates was prepared after grinding in liquid nitrogen. Zymographies were performed with gelatin as substrate.

In serum, the main gelatinase activity was MMP2. Bands corresponding to Pro-MMP2, MMP-9, Pro-MMP9 and the complexes MMP9/NGAL and MMP9 dimers/trimers were also visualized. Nevertheless, no differences were observed between healthy and metritic cows.

In vaginal exudates, relevant differences were observed between healthy and metritic cows. MMP2 activity remained quite constant after parturition, whereas in general there was an increase in MMP9 activity as well as in MMP9-containing complexes. The increase was higher in cows with metritis compared to healthy cows, although a high variability between animals was observed.

Finally, SDS-PAGE from vaginal exudates allowed the identification of a protein at high levels around parturition and decreasing afterwards, identified as lactoferrin (LF), a glycoprotein with antimicrobial activities. Intravaginal application of LF has been used to prevent preterm delivery and to extend pregnancy. Nevertheless, no information is available up to now about the natural presence of LF in vaginal secretions around parturition.

In conclusion, vaginal exudates allow the study of components of the inflammatory process influencing normal and pathological parturition. MMPs and LF are good example of such potential biomarkers.

I. M. Sheldon, G. S. Lewis, S. LeBlanc, and R. O. Gilbert, "Defining postpartum uterine disease in cattle," Theriogenology, vol. 65, no. 8, pp. 1516–1530, 2006.

0180-R/M-P

Identification of long non-coding RNAs with clinical relevance in ovarian cancer

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Ovarian cancer (OC) is one of the most lethal gynecological cancers globally because it is usually detected in the late stages when the disease has already spread, and in combination with the arising of treatment resistance and relapse, OC is associated with poor prognosis. Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides able to regulate gene expression at any level, playing a role in the pathogenesis of several diseases, including OC, correlating with important features for the clinical setting (Salamini-Montemurri et al., 2020)when the disease has already spread, and prognosis is poor. In this review we aim to highlight the importance of long non-coding RNAs (lncRNAs . In this regard, we aimed to identify new associations between lncRNAs and OC with prognostic, diagnostic, and presumably therapeutic value, by using RNA-seq data of ovarian serous cystadenocarcinoma from The Cancer Genome Atlas and normal ovary from Genotype-Tissue Expression, included in the database GEPIA (Tang et al., 2019)the GEPIA (Gene Expression Profiling Interactive Analysis . Our analyses show 21 non-coding transcripts previously unrelated to OC that are dysregulated in patients and affect overall survival, disease-free survival, and/or pathological stage. The levels of these transcripts were measured by RT-qPCR in SKOV3, PEO1, and A2780 OC cell lines and IOSE80 normal ovarian cell line. Co-expression and gene ontology enrichment analyses were also performed in GEPIA to gain knowledge on their possible cell functions. These results open the possibility of using these non-coding RNAs as novel biomarkers for OC.

Salamini-Montemurri, M., Lamas-Maceiras, M., Barreiro-Alonso, A., Vizoso-Vázquez, Á., Rodríguez-Belmonte, E., Quindós-Varela, M., & Esperanza Cerdán, M. (2020). The challenges and opportunities of lncRNAs in ovarian cancer research and clinical use. Cancers, 12(4), 1–25. Tang, Z., Kang, B., Li, C., Chen, T., & Zhang, Z. (2019). GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Research, 47(W1), W556–W560.

0185-R/M-P

Macrophage contribution to follicular lymphoma pathogenesis: CSF-1R as a novel prognostic factor and therapeutic target

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Microenvironment contributes to Follicular Lymphoma (FL) pathogenesis and its cell composition impacts survival, with macrophages playing a controversial role. In the present study, using primary FL samples and follicular dendritic cells (FDC) to mimic the germinal center, together with mouse models, we have analyzed the three-way crosstalk of FL-FDC-macrophages and derived therapeutic opportunities. *Ex vivo* primary FL-FDC co-cultures and *in vivo* mouse co-xenografts demonstrated that FL-FDC niche favors tumor growth and, via the secretion of CCL2 and CSF-1, promotes monocyte recruitment, differentiation and polarization towards an M2-like pro-tumor phenotype. Likewise, both monocytes and macrophages significantly increased FL primary samples viability. Moreover, using FL-M2 macrophages primary co-cultures, we have determined by gene expression profiling and functional experiments, that M2 macrophages increase angiogenesis, dissemination and immunosuppression.

Analysis of the CSF-1/CSF-1R pathway, fundamental for monocyte/macrophage differentiation and activation, uncovered that CSF-1 protein was significantly higher in serum from grade 3A FL patients compared to grade 1 and 2. In addition, high CSF-1R expression in FL biopsies correlated with grade 3A, reduced OS and risk of transformation. Furthermore, CSF-1R inhibition with pexidartinib (PLX3397) preferentially affected M2-macrophage viability and polarization program and disrupts FL-M2 positive crosstalk. *In vivo* CSF1-R inhibition caused M2 reduction and repolarization towards M1 macrophages and anti-tumor effect cooperating with anti-CD20 rituximab. In summary, these results support the role of macrophages in FL pathogenesis and suggest that therapies manipulating FL-macrophage crosstalk may be a new strategy for those patients with high macrophage infiltration, especially in combination with anti-B cell therapies.

1.Kahl, B. S. & Yang, D. T. Follicular lymphoma: evolving therapeutic strategies. Blood 127, 2055–2063 (2016). 2.Huet, S., Sujobert, P. & Salles, G. From genetics to the clinic: a translational perspective on follicular lymphoma. Nature Reviews Cancer 18, 224–239 (2018). 5. Dave, S. S. et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. The New England journal of medicine 351, 2159–69 (2004).

0187-R/M-P

Molecular targets in inflammation and autophagy deregulated pathways in Sporadic Inclusion Body Myositis

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Introduction: Sporadic Inclusion Body Myositis (sIBM) is an inflammatory myopathy that involves mitochondrial dysfunction, inflammation and accumulation of misfolded proteins. sIBM patients are frequently misdiagnosed due to lack of biomarkers and therapeutic options. One of the reasons that caveat disease breakthroughs is the lack of validated models of disease. Previous studies of the inflammatory profile of supernatant cytokines (Luminex®) and time-course study of autophagy in fibroblasts of sIBM patients revealed global deregulation in these pathways. In the present study, we aim to demonstrate the potential use of sIBM patients' fibroblasts to deepen and characterize the specific molecules deregulated in disease.

Methodology: We performed a functional characterisation of the inflammatory and autophagic alterations in 14 sIBM vs. 12 control fibroblasts. We quantified the most altered cytokines in Luminex® by using high-sensitivity immunoassays by qPCR (ProQuantum™). We further screened key autophagic molecules in fibroblasts lysates by a human autophagy array (C1 RayBio®); finally, we performed immunohistochemistry of the autophagosome marker LC3B-II to reflect the autophagosome formation. Results (means ± SEM) were analyzed through non-parametric statistic tests.

Results: The alteration in the expression of cytokines secreted by fibroblasts of sIBM patients previously observed by Luminex® (eotaxin, TNF-a, MCP-1, IL-1b) was further confirmed by ProQuantum™ immunoassays, that provided a more specific and significative detection of selected molecules. Regarding autophagy, we demonstrated that sIBM fibroblasts show an impairment of the clearance of deteriorated cell components by the alteration of specific markers of autophagy.

Conclusions: The dysregulation of inflammation in sIBM is reinforced by the autophagic imbalance. The use of fibroblasts may help to model the disease and establish therapeutic platforms to develop novel experimental drugs. Overall, better understanding of the altered molecular targets in the key processes of inflammation and autophagy may contribute to discover biomarkers and develop new therapies for earlier diagnosis and treatment for sIBM patients.

Funding: FIS PI1800498 granted by ISCIII and FEDER.

0188-P

Annexin A1 on the ocular surface: modulation by hyperosmolarity and its role in inflammation

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Introduction: Osmotic stress is a highly relevant challenge to cell function. In dry eye disorders, decreased tear secretion and/or excessive evaporation results in tear film hyperosmolarity. Although the pathogenesis of dry eye is not fully elucidated, it is recognized that elevated tear osmolarity damages the ocular surface by inducing the release of inflammatory molecules that leads to cell death [1]. Annexin A1 belongs to the annexin superfamily of calcium-dependent phospholipid-binding proteins. There is still scarce information about its presence and biological actions in the eye.

Objectives: The purpose of this study was to identify the presence of annexin A1 on the ocular surface and to detect possible changes in annexin A1 protein expression and secretion as consequence of hyperosmolar conditions. Moreover, considering the well-established contribution of inflammation in dry eye, the potential anti-inflammatory activity of a peptide analog of annexin A1, which mimics its N-terminus, was evaluated.

Methods: Annexin A1 location in human corneal and conjunctival epithelial cells was examined by confocal microscopy. Annexin A1 protein levels in conditioned media and lysates of cells exposed to hypertonic treatment were evaluated by western blot. Determination of interleukin 1β (IL-1β) by ELISA was performed to analyze the potential anti-inflammatory role of a peptide mimetic of annexin A1.

Results: Annexin A1 showed a cytosolic and membrane staining and a continuous labeling pattern in corneal and conjunctival epithelium. Western blot analysis revealed the presence of the complete native form of annexin A1 (37 kDa) together with a weaker and lower molecular weight band (33 kDa, truncated form). A significant increase in intracellular protein levels and annexin A1 secretion was detected after hyperosmotic exposure. Treatment with a peptide mimic of annexin A1 reduced IL-1β release induced by hypertonic conditions.

Conclusions: Annexin A1 was identified on the ocular surface epithelium and its expression and secretion was modified by hyperosmotic stress. Treatment with a peptide mimetic of annexin A1 ameliorated IL-1β release triggered by hyperosmolarity. These findings suggest a potential role of annexin A1 in the modulation of inflammatory events associated to dry eye pathology.

[1] Bron AJ, de Paiva CS, Chauhan SK, Bonini S, Gabison EE, Jain S, et al. TFOS DEWS II pathophysiology report. The Ocular Surface 2017;15:438-510.

0193-R/M-P

Regulation of LUZP1, a mediator in Townes-Brocks Syndrome, by the proteasome

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CIC bioGUNE Functional Genomics

The primary cilium is a conserved microtubule-based cel-

lular organelle that protrudes from the surface of cells. Its sensory and signalling function governs embryonic development and organ homeostasis. Defects in primary cilia formation and function cause a variety of genetic syndromes termed ciliophaties, whose clinical manifestations include malformations in the limbs, ears and kidneys. These anomalies are also observed in Townes-Brocks Syndrome (TBS), a rare disease caused by the expression of a dominantly acting truncated form of the transcriptional repressor Spalt-like 1 (SALL1). Based on a proximity proteomics experiment, we found that truncated SALL1 can interact with several centrosomal proteins. Among these, we identify LUZP1, a leucine zipper protein previously known to be associated with heart and neural tube defects. We found that LUZP1 localizes to actin filaments and to the centrosome, playing a negative role in ciliogenesis. Interestingly, we observed that LUZP1 is downregulated in the presence of truncated SALL1 in TBS patient-derived fibroblasts, which are characterized by abnormal cilia formation. In order to offer more clues into TBS etiology, we aim to explore how the ubiquitin proteasome system (UPS) can regulate LUZP1 homeostasis.

1. Laura Bozal-Basterra et al., Truncated SALL1 Impedes Primary Cilia Function in Townes-Brocks Syndrome, Am J Hum Genet., 2018 2. Laura Bozal-Basterra et al., LUZP1, a novel regulator of primary cilia and the actin cytoskeleton, is a contributing factor in Townes-Brocks Syndrome, Elife,2020

0194-P

TLR2 induction and IL1β release mediates LPS-induced damage in the blood-labyrinth barrier

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The blood-labyrinth barrier (BLB) separates the inner ear from blood and regulates the permeability of the stria vascularis, which is critical for the maintenance of ionic homeostasis and the prevention of the entry of deleterious substances. Understanding BLB dynamics is important because: i) its disruption or altered permeability have been associated with hearing loss in several pathologies, including inner ear infections, autoimmune inner ear disease, acoustic trauma, cisplatin-induced ototoxicity and presbycusis; and ii) controlled modification of the BLB permeability could be a tool to improve drug delivery to the inner ear.

Our purpose was to develop and characterize, at functional and molecular levels, a rat model of lipopolysaccharide (LPS)-induced BLB alteration. Hearing was assessed in vivo by auditory brainstem responses (ABR) recording. BLB permeability was evaluated by gadolinium dynamic contrast-enhanced magnetic resonance imaging (Gd-MRI) and quantification of Evans blue in the cochlea after intravenous dye injection. A time course of cochlear gene expression of LPS receptors, pro-inflammatory cytokines and injury mediators was determined by RT-qPCR.

Young (5–6-week-old) Wistar rats received an intraperitoneal injection of 5 mg/kg LPS (day 1) and 24 hours later, a second dose of 10 mg/kg. On day 4, ABR records showed a moderate increase of auditory thresholds in LPS-treated rats compared to saline controls. In addition, a higher increase in the cochlear signal enhancement in Gd-MRI, as well as a higher cochlear EB concentration was observed in LPS-treated rats compared to saline controls. LPS injections modified the expression of LPS receptors Tlr2 and Cd14, cytokines as Il1b, its receptors as Il1lr, and mediators Nfkb and iNos with specific time course patterns.

Our data indicate that LPS induced a cochlear inflammatory response, an increase in the BLB permeability and moderate hearing loss. The proposed LPS-induced BLB alteration rat model is suitable to understand the role of BLB alterations in hearing loss and to explore new strategies to improve drug delivery to the inner ear.

This work was partially funded by Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBER-ER-SPIRALTH ER17PE12) and Comunidad de Madrid ND2020/BMD-17454.

0196-R/M-P

Magnesium homeostasis by Cyclin M4: a novel therapeutic mechanism in Acetaminophen-induced liver damage

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Drug-induced liver injury (DILI) is the main cause of acute liver failure (ALF) in the Western World. Overdose of Acetaminophen (APAP), the most available pain medication in USA, accounts for 50% of ALF in USA and Europe.

N-acetylcysteine, routinely used for early stages of idiosyncratic DILI and APAP abuse, has limited effect after 20 hours of overdose. Therefore, additional therapeutic approaches are necessary in this pathology. Cyclin M4 (CNNM4) has a key role in magnesium transport across cell membranes. Here, we investigated if CNNM4 and dysregulated magnesium homeostasis could play a functional role in DILI.

Method: Hepatic CNNM4 expression and magnesium levels were assessed in human samples and in mice. Mice were treated with APAP 360 mg/kg by intraperitoneal injection and 24h thereafter *Cnnm4*siRNA or an unrelated control (siCtrl) were administered via tail vein injection and compared to control group. Mice were sacrificed 48 hours after APAP overdose. Primary hepatocytes treated with GalNAc conjugated *Cnnm4*siRNA or control siRNA were exposed to APAP and mitochondrial ROS and ER stress were evaluated.

Results: CNNM4 is a magnesium extruder across cell membranes of different organs. Patients with APAP overdose and idiosyncratic DILI presented an upregulation of hepatic CNNM4 expression and disturbances in magnesium serum levels. In the liver, we showed that CNNM4 overexpression coincides with ER stress and mitochondrial dysfunction, affecting ATP production and ROS generation.

Silencing *Cnnm4* expression in the liver with nanoparticle- or in hepatocytes with GalNAc-conjugated siRNA protects from APAP-induced liver injury and restores cellular magnesium homeostasis.

Our results suggest that CNNM4 appears as a new therapeutic approach to treat DILI with the added value of ameliorating mitochondrial dysfunction and ER stress that NAC does not provide.

1. Barbier-Torres L et al. The mitochondrial negative regulator MCJ is a therapeutic target for acetaminophen-induced liver injury. Nat. Commun. [Internet]. 2017;8:2068. Available from: https://doi.org/10.1038/s41467-017-01970-x 2. Liu M, Yang H, Mao Y. Magnesium and liver disease. Ann. Transl. Med. 2019;7:578–578. 3. Yamazaki D et al. Basolateral Mg2+ Extrusion via CNNM4 Mediates Transcellular Mg2+ Transport across Epithelia: A Mouse Model. PLoS Genet. 2013;9.

0208-R/M-P

IDENTIFICATION OF NOVEL ESSENTIAL GENES FOR PROSTATE CANCER METASTASIS BY GENOME SCALE CRISPR APPROACHES

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Background: Thanks to the availability of new biomarkers, Prostate cancer (PCa) can now be diagnosed earlier and, as a result, PCa patients have a high 5-year survival rate [1]. Nonetheless, the situation changes completely for patients who develop metastasis, for whom 5-year survival rate decreases to 29% [2]. Recent candidate-gene approaches have identified new candidates, but there are still no drugs to prevent or cure metastatic PCa. Hence, there is an urgent need to find new effective therapeutic targets.

Methodology: In this study we have conducted a high-throughput CRISPR screening in highly metastatic PCa cell lines PC3 and DU145, using the CRISPR/Cas9 knock-out (GeCKO) library [3]. To assess whether each gene loss-of-function may be critical for PCa metastasis development, invasion assays through Matrigel-coated Boyden Chamber were used followed by next generation sequencing and bioinformatic MAGeCK analysis [4]. siRNA and CRISPR/Cas9 approaches were carried out to validate and characterize some of the genes that significantly impaired invasive capacities of PC3 and DU145 cell lines.

Results: We found 29 candidates and several signaling pathways able of significatively impairing invasion of, both, PC3 and DU145 cells when knocked-out, being *PRMT7*, *SYCP3* and *TECPR1* our best candidates. *PRMT7* gene encodes a methyltransferase that can act on histones as well as on other proteins, being implicated in epithelial-mesenchymal transition, mRNA splicing, and DNA repair. SYCP3 is component of synaptonemal complexes and TECPR1 is involved in autophagosome maturation. Their upregulation correlates with metastasis appearance in different tumors [5,6]. Our results inhibiting their expression with siRNA and CRISPR validated our high-throughput CRISPR screening data and the implication of PRMT7 in PCa invasion.

Conclusions: Our high-throughput CRISPR screening uncovered promising gene candidates that in the future, could be used as therapeutical targets to prevent metastasis development in PCa patients. Furthermore, in our analysis we have also found genes previously associated to PCa metastasis in gene-by-gene candidate studies, giving a proof-of-concept for the use of our methodology for the search of PCa metastasis driver genes.

1. Bray, F., et al. (2018). CA: a cancer journal for clinicians, 68(6), 394–424. 2. Wang, G., et al. (2018). Genes & development, 32(17-18), 1105–1140. 3. Sanjana, N. E., et al. (2014). Nature methods, 11(8), 783–784. 4. Li, W., et al. (2014). Genome biology, 15(12), 554. 5. Yao, R., et al. (2014). Cancer research, 74(19), 5656–5667. 6. Chung, J. Y., et al. (2013). Human pathology, 44(4), 472–479.

0214-P

Development of in vitro and in vivo models of Acute Myeloid Leukemia. Transcriptomic differences between IDH2 R140 and R172 mutations.

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Acute Myeloid Leukemia (AML) is a complex and dynamic disease with a high molecular complexity and co ocurrence mutations patterns. The most recurrent mutations in Isocitrate Dehydrogenase gene (*IDH2*), R140 and R172, are responsible of the increase of 2-Hydroxyglutarate oncometabolite. In spite of the same effect, patients with these mutations present different co ocurrence patterns, clinics and are classified in different subgroups.

By means of BeatAML and TCGA cohorts, we performed a transcriptomic comparative analysis with gene expression data of *IDH2*R140 and *IDH2*R172 patients. This analysis identified 53 genes with significant different expression profile between both groups. The up expression of *ENO2* gene in R172 patients stands out (P = 0,02). *ENO2* encodes an enzyme related with glucose metabolism and HIF-1 path, both involved in tumoral processes.

With the objective of studying these differences we have developed cellular and *in vivo* models of these mutations. We employed CRISPR/Cas 9 gene edition technology to introduce R172 mutation in leukemic cells and both mutations in the model organism *C. elegans.* We successfully edited *IDH2 in vitro* with an alternative method to produce Cas9 single RNA guides (sgRNA). This method is based on fusion PCR system for generating constructs with the sgRNA sequences and the pU6 promoter, and optionally, the GFP reporter. Furthermore, we compare our method with ribonucleoprotein complexes. With NGS we verified that our constructs produce less indels but the edition efficiency is similar to RNPs.

Thanks to the high evolutive conservation of *IDH2* gene we could develop *C. elegans* models with each mutation and one model with both mutations. We used the co CRISPR strategy to modify our favourite gene and also edit *dpy-10* gene. This modification produces a morphology alteration that allow to identify edited animals. These *in vitro* and *in vivo* models will let us to explore the molecular process involved in *IDH2* mutations effects.

1. Papaemmanuil E, et al. N Engl J Med. 2016. 2. Pelcovits A, Niroula R. R I Med J. 2020. 3. Shih AH, et al.. Cancer Discov. 2017. 4. Liu C, et al. Cell Physiol Biochem. 2018. 5. Paix A, Folkmann A, Seydoux G. Methods. 2017.

0215-R/M-P

Recovery of mitochondrial activity by MCJ silencing improves cholestasis-induced liver injury

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Background and aims: Mitochondrial dysfunction, associated with ROS overproduction, is a key factor in the pathogenesis of cholestatic liver disease (CLD). The methylation-controlled J-protein (MCJ) is an endogenous repressor of the mitochondrial respiratory chain. The absence of this protein leads to increased complex I activity, facilitating electron transfer between complexes and thus lowers ROS production. We aimed to investigate the role of MCJ in the pathogenesis of CLD, together with evaluating the effect of MCJ silencing for the treatment of cholestasis-induced liver injury.

Methods: We studied MCJ's role in CLD patients and WT or MCJ-KO mice. Bile duct ligation (BDL) was used as an animal model of cholestasis, and for *in vitro* experiments, primary hepatocytes were treated with toxic doses of bile acids.

Results: Hepatic MCJ levels were upregulated in both CLD patients and mice after BDL. The MCJ-KO model after the BDL, showed significantly reduced inflammation and apoptosis, in addition to a recovery of the mitochondrial function. Moreover, the lack of MCJ protected mice primary hepatocytes from bile acid-induced mitochondrial ROS overproduction and ATP depletion, enabling higher cell viability. Finally, the *in vivo* inhibition of MCJ expression, reduced BDL induced liver injury and thus ameliorated cholestatic main injury.

Conclusions: These results demonstrate the involvement of MCJ in the progression of cholestatic liver injury. Therefore, we have identified MCJ as a potential therapeutic target to mitigate cholestasis-induced liver injury.

Arduini A, et al. Mitochondrial dysfunction in cholestatic liver diseases. Front Biosci (Elite Ed). 2012 Jan 1;4:2233-52. doi: 10.2741/539. PMID: 22202034. Hatle KM, et al. MCJ/DnaJC15, an endogenous mitochondrial repressor of the respiratory chain that controls metabolic alterations. Mol Cell Biol. 2013 Jun;33(11):2302-14. doi: 10.1128/MCB.00189-13. Epub 2013 Mar 25. PMID: 23530063; PMCID: PMC3648061

0217-R/M-P

Connexin43 as a therapeutic target in BRCA1 mutated triple negative breast cancer and its interacting microenvironment partners: cancer-associated fibroblasts and natural killer cells

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Triple negative (TN) is the most aggressive breast cancer subtype with no targeted therapy. The search for therapeutic targets and a proper biological understanding of the tumour-microenvironment axis are critical. Connexins are transmembrane proteins involved in gap junction (GJ)-mediated intercellular communication (GJIC) and GJ-independent roles. Connexin43 (Cx43) immunostaining in a 40-patient breast cancer cohort showed TN as the subtype with the lowest levels. Cx43 overexpression in TN primary *BRCA1*-defective and metastatic cell lines partially restored it to the plasma membrane, forming functional GJs that allowed the passage of calcein (GJIC). Cx43 recovery led to increased gene/protein levels of *CDH1* and *NOTCH3*, elevated SASP (senescence-associated secretory phenotype) production (*IL-1*, *IL-6*) and decrease of *VIM* and *SNAI2*, hinting at a potential EMT reversion. Cx43 restitution resulted in diminished 2D and 3D proliferation, migration and colony formation in both cell lines, and increased adhesion and resensitization of *BRCA1* mutated cells to Anoikis.

Patient-derived *ACTA2+FAP+ITGB1+* cancer-associated fibroblasts (CAFs) were characterized by reduction of Cx43 and GJIC when compared with paired normal fibroblasts (NAFs). Cocultures of TN cells with CAFs/NAFs displayed membranous Cx43 staining in contact regions only upon Cx43 overexpression in TN, and heterocellular CAFs/NAFs-TN GJIC was only present when Cx43 was restored in TN. Interestingly, GJ-mediated calcein transfer only happened when CAFs/NAFs were calcein donors, indicating restitution of unidirectional GJIC from CAFs/NAFs to Cx43-overexpressing TN.

Cocultures of primary natural killer (NK) cells with *BRCA1* defective TN cells showed higher antitumour cytotoxic effect of NKs against Cx43-restored TN, as opposed to wild type cells. No clear Cx43 staining was observed at the NK-tumour interface, nor heterocellular GJIC was detected, suggesting a GJ-independent role of Cx43 restitution in increasing the sensitivity of TN to NKs.

This work provides evidence supporting the tumour-suppressive role of Cx43 in both primary and metastatic TN, including *BRCA1* mutated. It also presents novel insights into Cx43 function in breast CAFs and proposes a potential strategy to improve NK-mediated TN cytotoxicity upon Cx43 restoration.

0222-R-P

The role of miR-873-5p in Alcohol-related liver disease

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Alcohol-related liver disease (ALD) is the most prevalent type of chronic liver disease and lacks effective therapies. Just cutting off alcohol intake is presented as effective therapy. Alcohol abuse promotes disbalances in lipid homeostasis inducing liver injury and inflammation. In this context, sirtuins play a key role, and SIRT1 is the most extensively studied. On the other hand, growing evidence suggests the utility of microRNAs in clinical practice. Previously, our lab demonstrated the relevance of miR-873-5p as a suitable target for liver diseases, such as NAFLD and fibrosis, by modulating among different targets the expression of the gene Glycine-N-Methyl transferase (GNMT), a pivotal modulator of the methionine cycle. In this work, miR-873-5p appears as a suitable candidate to ameliorate the damage induced by alcohol in the liver.

MiR-873-5p levels were measured in liver samples with alcoholic hepatitis vs healthy biopsy (n= 16). As a readout, Gnmt mRNA expression was studied in liver tissue from patients with different ALD stages (n=63). The expression levels of MiR-873-5p and Gnmt were further tested in different mice models (BASH and NIAAA). In vitro assays in primary hepatocytes were performed in the presence of anti-miR-873-5p, anti-miR-Control or mimic-miR-873-5p under 12 h of ethanol exposure (50 mM).

Raised levels of miR-873-5p were found in ALD patients. Accordingly, Gnmt levels were downregulated in unrelated cohorts of patients with different ALD stages. These results were consistent in primary hepatocytes and mice models. Hepatocytes transfected with anti-miR-873-5p showed minor cell death *in situ* and less ROS production compared to anti-miR-Control under ethanol exposure. The negative modulation of miR-873-5p renders higher levels of SIRT1 and CPT1 in primary hepatocytes with a concomitant reduction of phospho-S6 (Ser235/236). Furthermore, miR-873-5p inhibition modulates the inflammatory process, showing major levels of IL10 and lower TNF expression. Silencing miR-873-5p in NIAAA mice model displays major Gnmt levels and reduced levels of ALT/AST followed by less intestinal permeability measured by FitC dextran.

We suggest that targeting miR-873-5p might have relevance as a therapeutic tool for ALD mediated by SIRT/mTOR regulation. Promising results will be addressed in vivo.

1_Fernández-Ramos, David et al. "MiR-873-5p acts as an epigenetic regulator in early stages of liver fibrosis and cirrhosis." Cell death & disease vol. 9,10 958. 20 Sep. 2018, doi:10.1038/s41419-018-1014-y

0235-R/M-P

Quantification Of Pru P 3-Specific IgG1 In Lipid Transfer Protein Allergic Patients

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Food allergy affects approximately 7% of the adult population and 10% of the children. It remains unclear whether the prevalence of food allergy is increasing. Lipid transfer proteins (LTPs), present in vegetables, pollen, nuts and fruits, are true plant food allergens, i.e. they are able to induce IgE production by plasma cells. Recent studies in mice have demonstrated the existence of an alternative anaphylactic route mediated by IgG. Moreover, it has been proven that the first stages of the allergic response in mice are mediated by IgG1, while later exposures to the allergen induce the generation of IgE. In humans, it is known that most of the IgE comes from IgG1 isotype class switching. Clinical observations about the existence of a group of patients allergic to LTPs that present low levels of Pru p 3-specific IgE, and a rapid progression to severe reactions led us to hypothesize that IgG1 could be implicated in these LTP-allergic phenotypes. Thus, the main objective of this project was to elaborate a method to detect Pru p 3-specific IgG 1 in serum samples of LTP-allergic patients using the ELISA technique. Pru p 3-specific IgG 1 was found in around 70% of the samples. The results suggested that Pru p 3-specific IgG 1 levels inversely correlate with Pru p 3-specific IgE levels. However, further optimization of the current protocol could be implemented in future assays since IgG1 determinations may help identify allergic patients with a severe phenotype.

Jiménez-Saiz R, Ellenbogen Y, et al. IgG1 + B-cell immunity predates IgE responses in epicutaneous sensitization to foods. Allergy Eur J Allergy Clin Immunol. 2019. doi:10.1111/all.13481 Escribese MM, Rosace D, Chivato T, et al. Alternative anaphylactic routes: The potential role of macrophages. Front Immunol. 2017. doi:10.3389/fimmu.2017.00515 Looney TJ, Lee JY, Roskin KM, et al. Human B-cell isotype switching origins of IgE. J Allergy Clin Immunol. 2016. doi:10.1016/j.jaci.2015.07.

0250-P

Inhibición del transporte de fármacos antitumorales por bombas ABC como estrategia de quimiosensibilización en el cáncer gástrico

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Introducción: La respuesta del adenocarcinoma gástrico (ACG) al tratamiento farmacológico disponible actualmente es muy limitada. Uno de los mecanismos responsables de quimiorresistencia implicados es la expulsión de los fármacos por proteínas de la superfamilia ABC (ATP-binding cassette) situadas en la membrana plasmática de las células tumorales.

Objetivo: Evaluar el efecto de la inhibición de las proteínas ABC en la sensibilidad del ACG a la quimioterapia.

Métodos: Se determinó la expresión y la localización subcelular de las bombas ABC en biopsias de ACG así como en la línea celular AGS derivada de ACG donde además se evaluó su funcionalidad y se estudió el efecto sobre la viabilidad celular de diferentes fármacos antitumorales solos o en combinación con un inhibidor de bombas ABC. Para estudiar *in vivo* el efecto antitumoral del sorafenib en combinación con el inhibidor de bombas ABC diclofenaco, se utilizó un modelo de xenograft con células AGS en ratones inmunodeprimidos.

Resultados: Los tumores de ACG presentaron una expresión de media a alta de MRP1, MRP3, MRP4 y MRP5. Mediante IF se comprobó que solo MRP1-4 se co-localizaban con el marcador de membrana plasmática Na+/K+-ATPasa. De manera análoga, la línea celular AGS presentó una elevada expresión de estas bombas MRP, sin embargo, solo MRP1 y MRP4 se localizaron en la membrana plasmática, donde su capacidad de transporte podría ser bloqueada por inhibidores de MRPs. La incubación de las células con diclofenaco ocasionó inhibición de la función MRP, así como sensibilización selectiva a determinados fármacos como el sorafenib, el docetaxel, el etopósido y la doxorrubicina. En ratones con tumores subcutáneos derivados de células de AGS, el tratamiento con sorafenib no inhibió el crecimiento tumoral, sin embargo, la coadministración con diclofenaco sensibilizó significativamente a las células tumorales al sorafenib, que mostró en estas condiciones un marcado efecto antitumoral.

Conclusión: La combinación de inhibidores de las bombas MRP, como el diclofenaco, con fármacos antineoplásicos actualmente no considerados en el tratamiento del ACG, como el sorafenib, puede constituir una estrategia de superación de la quimiorresistencia de este tipo de cáncer.

Kothari N, et al. J. Gastrointest. Oncol 2015;6:60-74. Marin JJ, et al. Anticancer Agents Med. Chem. 2016;16:318-34. Marin JJ, et al. Curr Mol Med 2009;9:1108-29. Marin JJ, et al. Cancers 2020;12:2116.

0252-P

MAPK ERK5: A new target to tackle endometrial cancer proliferation and survival

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Endometrial cancer (EC) is the most common cancer in the female genital tract. Advanced EC is still a difficult scenario, and new and effective treatments are needed. The MAP kinase family member ERK5 drives malignancy in some cancers (breast, prostate or hepatocarcinoma cancers), by allowing sustained tumor proliferation and survival. Consequently, small-molecule inhibitors of ERK5 has shown anticancer activity. We have developed a new specific ERK5 inhibitor with excellent pharmacokinetic parameters, JWG-071, and tested in endometroid cancer (EC)

JWG-071 impaired EC proliferation and survival. Specifically, JWG-071 impaired EGF-induced proliferation of Ishikawa and AN3CA (endometroid) and HeLa (cervical) cancer cells, by reducing c-Jun expression. CRISPR/Cas9 MEK5-/- Hela or Ishikawa cells, which lack ERK5 activity, phenocopied JWG-071 and showed basal and EGF-induced proliferation. JWG-071 also impaired growth and proliferation (Ki67) of EC xenograft tumors. JWG-071 induced apoptosis in EC and cervical cancer cells but had no cytotoxic effect in apoptosis-deficient (BAX/BAK-/-) cells. Mechanistically, ERK5 inhibition impaired the tumor driver NF-kB pathway in EC and cervical cancer cells by decreasing IKKγ/NEMO expression and, consequently, IKKb/a and p65 expression levels. Impaired NF-kB activated JNK-Bim apoptotic pathway. Accordingly, overexpression of NEMO protected cells from JWG-induced apoptosis, whereas silencing of NEMO or p65 inhibition (BAY-117082 or overexpression of super-repressor of IkB) phenocopied JWG-071 cytotoxicity. JWG-071 also induced downregulation of NF-kB pathway and apoptosis in EC tumor xenografts. Of note, we found positive correlation between ERK5 and p65 protein levels in samples from EC patients. Finally, we show that ERK5 inhibition sensitizied EC cancer cells to chemotherapeutics (taxols and platins), as well as EC tumor xenografts to paclitaxel. Our preclinal development of JWG-071 supports ERK5 as a new target to tackle EC

Brooks et al. 2019. CA Cancer J Clin 694:258-79 Gomez et al. 2016. Front Cell Dev Biol. 5:80. doi: 10.3389/fcell.2017.00080. 2016 Hoang et al. 2017. Cancer Lett 392:51-59 Pallares et al. 2004. J Pathol 204:569-77 Pereira & Rodrigues 2020. Trends Mol Med 26:394-407 Wang et al. 2018. ACS Chemical Biology 13:2438-48 Yang et al. 2010. Cancer Cell 18:258-6

0253-R/M-P

RXR in podocytes: implications in renal disease associated to obesity.

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Background: Obesity is an important and independent risk factor for chronic kidney disease. Evidence suggest that renal lipid accumulation leads to glomerular damage and more specifically, podocyte dysfunction. Nuclear receptors are ligand-dependent transcription factors, which regulate the transcription of target genes in lipid metabolism and inflammation. Retinoid X Receptor (RXRs) are ligand-dependent NRs that play an important role as a heterodimeric partner for other NRs. **Aim**: To analyze the role of RXR isoforms in the integrity of the podocyte and the involvement in the maintenance of Glomerular Filtration Barrier under different nutritional conditions. **Methods:** Mice with specific deletion of RXRα and β in podocyte (pod-RXRKO) were generated and after weaning, both control (WT) and podRXRKO mice were fed with control diet (CD) or 55% Kcal high fat diet (HFD). After 12 weeks on diet, biochemical parameters in serum and urine were analyzed; kidneys were removed for histological and molecular studies. Glomerular filtration rate was measured by iohexol clearance. **Results:** No major changes were found in metabolic parameters in male or female pod-RXRKO compared to WT mice on CD. Interestingly, kidneys from female pod-RXRKO showed, at the same age, greater damage than males, concretely, fibrosis and sclerosis compared to WT mice, a reduction of the glomerulus size (1364.12±7 vs 1871 ±87.27 µm2; p≤0.01), a decrease in the urine volume and in the measured glomerular filtration ratio (mGFR) (59.85±13.31 vs 122.13±16.89 µL/min. p≤0.05) together with an increase in the urinary albumin. In addition, podocyte effacement was also observed in female pod-RXRKO compared with WT mice on CD. Under HFD, albuminuria and fibrosis were aggravated in females podRXRKO, but not the rest of parameters. Conditions of HFD also increased albuminuria (43.64±10.06 vs 21.09±4.48 µg/L. p≤0.05) and the sclerosis and fibrosis levels in male pod-RXRKO compared to WT mice. **Conclusion:** This study suggests a crucial role of RXR in the integrity of the podocyte and in the regulation of renal function particularly in females. Our data suggested that appropriate functional RXR may be a logical approach to protection against obesity-associated renal failure. Acknowledgments: BFU2016-78951-R, B2017/BMD-3684, BFU2017-90578-REDT.

0254-R-P

USP48 as a new candidate gene for retinal ciliopathies

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Ciliopathies are a broad group of heterogeneous inherited disorders associated with dysfunction of the cilium, a ubiquitous microtubule-based organelle that acts as a cellular antenna, translating extracellular stimuli into cellular responses (1). The retina is one of the most affected organs by mutations in ciliary genes due to the highly specialised neurosensory cilium that photoreceptors possess, known as outer segment, where photoreception and phototransduction occurs. To date, mutations in more than 100 ciliary genes have been associated with retinal dystrophies, accounting for almost 25% of these inherited rare disorders (2).

Cilium formation and maintenance are extremely regulated processes that highly depend on ubiquitin-proteasome system (UPS) proteins, such as E3 ligases and deubiquitinating (DUB) enzymes (3). In fact, post-translational ubiquitin and ubiquitin-like modifications play an important role during differentiation and ciliogenesis of photoreceptor cells and mutations in several genes related to the UPS in humans cause inherited retinal dystrophies (IRDs) (4–7).

We are currently focusing on the DUB *USP48* as a new potential candidate gene for retinal disease due to ciliary defects that could explain undiagnosed cases of IRDs. Our preliminary results show that *Usp48* is expressed in the mouse retina and displays 3 main isoforms. Proteomic analysis revealed that USP48 directly or indirectly interacts with proteins involved in phototransduction, protein ciliary transport, cytoskeleton organisation, synaptic transport and cell differentiation, among others. Furthermore, it is localised at the basal body of primary cilia in adult retinal pigment epithelium (ARPE-19) cells. Silencing of *USP48* in ARPE-19 cells did not yield significant results concerning ciliary length nor ciliogenesis, however, this does not rule out the possibility that USP48 acts as a regulator of ciliary formation and/or function in a retina-specific manner. Further work includes studying retina-specific *Usp48* isoforms, defining key domains of interaction with ciliary proteins and analysing the ciliary phenotype of *USP48* overexpression and knockdown in ARPE-19 cells in order to shed light on the role of *USP48* in the retina.

1. Fliegauf M, et al. (2007) doi: 10.1038/nrm2278. 2. Chen HY, et al. (2019) doi: 10.3233/trd-190038. 3. Shiromizu T, et al. (2020) doi:10.3390/ijms21175962. 4. Esquerdo-Barragán M, et al. (2019) Mol Vis. 2019;25:800–13. 5. Chiang AP, et al. (2006) doi: 10.1073/pnas.0600158103. 6. Chakarova CF, et al. (2011) doi: 10.1093/hmg/ddq543. 7. Yi Z, et al. (2019) doi: 10.1136/jmedgenet-2018-105709.

0257-R/M-P

MAP17 as a potential modulator of epithelial-mesenchymal transition in liver cancer

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The epithelial-mesenchymal transition (EMT) is important during the embryonic development but also a mechanism allows tumors to invade and colonize adjacent and distant tissues during metastasis. During the EMT, intercellular interactions are lost and cells acquire a mesenchymal phenotype that increases its migratory capacity and apoptosis resistance[1]. The modulation of EMT offers an attractive approach for the prevention of metastasis.

MAP17 is a 17kDa non-glycosylated membrane protein that is highly expressed during embryogenesis and methylated in most organs in adulthood. However, it has been identified in several types of cancers, including hepatocellular carcinoma (HCC). MAP17 is correlated with hypoxia and it is involved in inflammation, in cell apoptosis and invasion, in the Warburg effect and in tumor growth. [2], [3]

In view of the above, we have identified a potential role of MAP17 in EMT. We have evaluated *MAP17* mRNA expression in HCC, identifying its expression only in mesenchymal cells compared to epithelial. Considering a possible triggering effect of MAP17 in EMT, our target has been found overexpressed in epithelial cells treated with TGF-beta in order to induce EMT. Expressing MAP17 in epithelial cells, mesenchyme- and epithelium-associated genes reveal MAP17 as a possible EMT modulator. In further studies, we will evaluate cell proliferation, migration and 3D invasiveness, colony-forming capacity, metabolism and bioenergetics.

The characterization of MAP17 *in vitro* will allow us to evaluate its effect *in vivo* in preclinical models of metastatic HCC.

[1] David M. Gonzalez, et.al. Signaling mechanisms of the epithelial-mesenchymal transition. Sci Signal. [2] Garcia-Heredia JM, et.al. Dr. Jekyll and Mr. Hyde. MAP17's upregulation, a crosspoint in cancer and inflammatory diseases. Mol Cancer. [3] Fangyuan Dong, et.al. Hypoxia-dependent expression of MAP17 coordinates the Warburg effect to tumor growth in hepatocellular carcinoma. Journal of Exp& Clin CR.

0261-R/M-P

Hepatic transcriptional profiling in mouse models of Non-Alcoholic Fatty Liver Disease (NAFLD) reveals increased contribution of hepatic glutaminase 1 activity to ammonia production, a hallmark of advanced NASH

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In Non-Alcoholic Fatty Liver Disease (NAFLD), augmented hepatic ammonia accumulation, recognized to favor the progression of fibrosis and NAFLD disease, has been linked to its decreased elimination through reduced urea cycle. We propose to address if a direct relationship between increased glutaminase activity, the main enzyme involved in ammonia production in the liver and recently shown by our group to be induced in NAFLD, and hepatic ammonia content occurs in well-established diet-induced NAFLD mouse models. Serum and liver histological (H&E, Oil red, F4/80 immunostaining, Sirius red, Nessler staining) analysis was used to classify the different mouse models of diet-induced NAFLD from steatosis to advanced fibrosis. In agreement, principal component analysis of the hepatic transcriptional profiling with the nCounter® Metabolic Pathways Panel from nanoString® can be used to separate each experimental group. In fact, hepatic transcriptional profiling revealed decreased expression of pathways responsible for amino acid synthesis and arginine metabolism as well as pathways involved in fatty acid metabolism and mitochondrial respiration as the dietary model of NAFLD progressed. On the other hand, in later stages of NAFLD progression pathways associated with hypoxia, DNA damage repair, myc, glutamine metabolism, mTOR and glycolysis, among others, were overexpressed. In addition, immune cell profiling using cell typing signatures embedded in the Metabolic Pathways Panel revealed an enrichment of neutrophils, macrophages, dendritic cells, and CD45 cells for the most harmful dietary models. Interestingly, as NAFLD progresses hepatic Nessler staining of ammonia is increased correlating with decreased expression of the enzymes involved in urea cycle, and increased expression of the isoform 1 of glutaminase. The effect of acute silencing in advanced NAFLD pre-clinical mouse models and its effect on hepatic ammonia contents is an ongoing work. Overall, hepatic transcriptional profiling in animal models of dietary-induced NAFLD has been proven to be an important tool to assess disease progression and can be potentially used to evaluate therapeutics in preclinical studies. In particular, we have shown that impaired hepatic glutaminase 1 activity in NAFLD may contribute to hepatic ammonia accumulation.

Thomsen K. L. et al. Ammonia: A novel target for the treatment of non-alcoholic steatohepatitis. Med Hypotheses. 2018 Apr;113:91-97 De Chiara F. et al. Urea cycle dysregulation in non-alcoholic fatty liver disease. J Hepatol. 2018 Oct;69(4):905-915 Simon J. et al. Targeting Hepatic Glutaminase 1 Ameliorates Non-alcoholic Steatohepatitis by Restoring Very-Low-Density Lipoprotein Triglyceride Assembly. Cell Metab 2020;31:605-622.e10

0267-R/M-P

Understanding the role of circulating levels of adipose tissue-derived molecules in the renal dysfunction of obese patients

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Background and aim: The incidence of obesity worldwide grows in parallel with the prevalence of chronic kidney disease (CKD). Besides other comorbidities (such as diabetes and hypertension), the dysregulation of adipose tissue (AT) secretion profile in obesity, including adipokines and cytokines, together with the resulting inflammatory environment, may directly affect renal function. Within this framework, our aim is to study the differences in circulating AT-derived molecules in obese patients with and without CKD.

Methods: 12 morbid obese patients without renal injury (obese control group) and 12 morbid obese patients with CKD were included in this study. Biochemical parameters and the estimated glomerular filtration rate (eGFR) were assessed in the serum and urine of the patients, and AT biopsies were histologically studied. Circulating levels of adipokines, cytokines and growth factors were analyzed using Bioplex system.

Results and Conclusion: AT biopsies from obese patients with CKD (proteinuria: 2,63±2,98 g/24h; serum creatinine: 1,13±0,43 mg/dl; eGFR-MDRD: 71,6±30,5 ml/min/1.73m2) and the control group did not show major differences in adipocyte size within subcutaneous and visceral depots. However, increased circulating levels of the adipokines adipsin and visfatin were found in CKD patients compared to the control group ($p<0.05$). Levels of proinflammatory cytokines (IL-1b, IL1-ra, IL-6, MCP-1, TNF-α) were also augmented

in serum of CKD patients (p<0.05). Accordingly, patients with renal dysfunction showed increased serum levels of factors involved in the maturation, activation and chemotaxis of different immune cells, namely G-CSF, IL-17, IL-15 and Eotaxin. Moreover, angiogenic (VEGF, PDGF, bFGF) and profibrotic (TGFβ1, TGFβ2) factors were significantly higher in the serum of patients with CKD than in the obese controls. All these data suggest that alterations in the AT secretion pattern of adipokines, as well as altered immunologic and angiogenic factors may play a role in the physiopathology underlying obesity-related CKD. The modulation of these molecules could prevent the progression towards irreversible renal disease during obesity.

Acknowledgments: BFU2016-78951-R, B2017/BMD-3684, BFU2017-90578-REDT, Ayudas Puente URJC, 2019.

Zhu Q, Scherer PE. Immunologic and endocrine functions of adipose tissue: implications for kidney disease. Nat Rev Nephrol. 2018;14(2):105–20. Morales E, Porrini E, Martin-Taboada M, Lima SL, Vila-Bedmar R, González de Pablos I, et al. Renoprotective role of bariatric surgery in patients with established CKD. Clin Kidney J. 2020 Briffa JF, McAinch AJ, Poronnik P, Hryciw DH. Adipokines as a link between obesity and chronic kidney disease. Am J Physiol Renal Physiol. 2013;305(12):F1629-36.

0288-P

Role of Rnd3 in multiple myeloma progression

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Multiple myeloma (MM) is a hematologic neoplasm of plasmatic cells (PC) that infiltrate bone marrow and secrete monoclonal immunoglobulins. MM is preceded by a pre-malignant stage termed monoclonal gammopathy of undetermined significance (MGUS). Despite major therapeutic advances in the last decades, virtually all patients with MM experience relapse of the disease, which progressively becomes refractory to treatment. Rho proteins play an important role in the development of hematological neoplasms, since they are involved in chemotaxis and motility processes both in lymphoid and myeloid lines through ROCK-LIMK pathway. Specifically, RhoU protein has recently been related to MM progression, its expression varies depending on the evolution of the disease, decreasing as the disease progresses. Rnd3 protein is an atypical GTPase that belong to Rho family. Rnd3 is involved in cancer progression and drug resistance in different type of cancers. In our lab we are interested in the role of Rnd3 in MM progression. Therefore, we performed a bioinformatic study with software called Correlation and Rule Mining Expression Networks (CARMEN), which allowed us to analyze an Astrazeneca gene expression database with 627 different cancer cell lines (GSE57083). We confirmed the low RND3 expression in hematopoietic cell lines (already described), and identified a subgroup of 16 cell lines with RND3 overexpression, 10 of which corresponded to MM. In this line, RND3 expression obtained from RNAseq data in murine MM models and primary patient samples revealed that RND3 is expressed at higher levels in MM cells compared to bone marrow plasma cells (BMPCs) or various B cell subsets. In addition, RND3 is progressively expressed from precursor MGUS cells to clinically active MM in murine samples, suggesting that may be involved in MM progression. We also analyzed by western blot the expression of Rnd3 in different human MM cell lines, and we selected RPMI8226, KMS11 and JJN3 with higher expression to silence RND3 expression using CRISPRi technique. Finally, we carried out a phenotypic characterization of these lines respect to the wild-type cells, including analysis of cell growth, cell cycle and cell adhesion and drug sensitivity experiments in order to better understand the possible role of Rnd3 in MM progression.

Anderson, K.C. et al. Annual review of pathology, 2011. 6: p. 249-274. Azab, A.K. et al. Blood, 2009. 114(3): p. 619-629. Canovas Nunes, S. et al. Blood cancer journal, 2018. 8(2): p. 20. Chardin, P. Nature reviews. Molecular cell biology, 2006. 7(1): p. 54-62. Choi, D.S. et al. Int J Cancer, 2015. 136(5): p. E219-229. Di Marzo, L. et al. Oncotarget, 2016. 7(37): p. 60698-60711. Quigley, D.A. et al. Cell reports, 2016. 16(4): p. 1153-1165. Riou, P. et al. Bioessays, 2010. 32(11): p. 986-9

0299-P

The Search for Biomarkers: Serum Circulating MicroRNAs profiling in Idic15 Syndrome

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Idic15 Syndrome is a rare disease characterized by developmental delay and neurological symptoms, mainly autism and epileptic seizures. It is caused by duplications in the chromosomic region 15q11-q13, in the maternal allele. This region is prone to rearrangements due to the presence of repeated DNA elements, and molecular studies have shown considerable structure heterogeneity between patients, which could be the cause for the great clinical heterogeneity. In this work we approach the study of differentially expressed miRNAs in a cohort of Spanish patients diagnosed with Idic15 (N=28) compared to controls (N=17) paired by age and sex, aiming to find miRNAs which are specific to these patients. We analysed the expression levels of 179 serum miRNAs in 14 Idic15 patients and 8 controls using real-time quantitative PCR, and among the miRNAs that showed statistically significant differential expression ($P < 0.05$), 4 were selected to be individually analysed in the whole cohort due to their previously suggested connection with autism or brain damage (hsa-miR-139-5p, hsa-miR-486-5p, hsa-miR-15a-5p y hsa-miR-152-3p). One of them, hsa-miR-152-3p, showed a statistically significant increase in expression in Idic15 patients compared to controls, and the ROC analysis showed that the area under curve (AUC) was greater than 0.7, indicating its value as a biomarker. Further studies are required in order to better characterize the miRNA profile for this disease. However, this preliminary study points to the possibility of finding miRNAs that can become biomarkers of Idic15 syndrome.

Project funded via donations to the initiative "Una casa una vida" promoted by Great Chance SLU.

Battaglia, A. (2008). The inv dup (15) or idic (15) syndrome (Tetrasomy 15q). Orphanet journal of rare diseases, 3(1), 1-7. Zhang, A., Qian, Y., & Qian, J. (2019). MicroRNA-152-3p protects neurons from oxygen-glucose-deprivation/reoxygenation-induced injury through upregulation of Nrf2/ARE antioxidant signaling by targeting PSD-93. Biochemical and biophysical research communications, 517(1), 69-76.

0301-P

Transcriptome analysis in Idic15 patients reveals altered expression of developmental and immune system processes

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Idic15 Syndrome is a congenital developmental disease characterized by developmental delay and neurological symptoms, mainly autism and epileptic seizures. It is caused by duplications in the chromosomic region 15q11-q13, typically in the maternal allele. These rearrangements can occur as interstitial duplications or in the form of a supernumerary marker chromosome. The number of copies and the length of the duplication is also variable among patients, and it has been proposed that this structural heterogeneity is the cause of the great clinical heterogeneity. In this work we evaluate the relationship between phenotype and genotype through the study of the transcriptome in a cohort of Spanish patients diagnosed with Idic15 (N=28) compared to controls (N=17) paired by age and sex. This study revealed more than 1100 genes differentially expressed between patients and controls, involved in a number of biological functions, including developmental and immune system processes. Among all of them, 7 genes (*HBG2*, *CTSE, CTSK, ITGAL, CA1, OXSR1* and *UBE3A*) were selected for individual analysis via quantitative real-time PCR using TaqMan probes. 5 of these genes (*CTSE, CTSK, CA1, OXSR1* and *UBE3A*) were validated, showing statistically significant differential expression between Idic15 patients and controls. These genes have been previously associated with brain damage (*CA1*), immune system processes (*CTSE, CTSK*), seizures (*OXSR1*) and autism (*UBE3A*). This work presents and extensive *in vivo* analysis of Idic15 patients' transcriptome, and can provide help understanding the biological mechanisms involved in the correlation between the chromosomic alterations and the clinical manifestations.

Project funded via donations to the initiative "Una casa una vida" promoted by Great Chance SLU.

Battaglia, A. (2008). The inv dup (15) or idic (15) syndrome (Tetrasomy 15q). Orphanet journal of rare diseases, 3(1), 1-7. Sullivan, B. J., & Kadam, S. D. (2020). The involvement of neuronal chloride transporter deficiencies in epilepsy. In Neuronal Chloride Transporters in Health and Disease (pp. 329-366). Academic Press. Vatsa, N., & Jana, N. R. (2018). UBE3A and its link with autism. Frontiers in molecular neuroscience, 11, 448.

0302-P

How Strong is a Single-Bacterium Attachment?

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Cells have a great variety of proteins in their extracellular matrix which, in order to establish interactions, are exposed to mechanical forces. In bacteria, adhesins anchored to the cell wall are responsible for adhering to target organisms, thus initiating infection. Therefore, developing strategies that allow studying the nanomechanics of proteins anchored to the cell could be critical against bacterial infections. Heretofore, most of the research focuses on single-molecule studies; however, single-bacterium studies are crucial to understand the clinical relevance of the initial stage of infection. Here, we develop a novel technique where forces are applied in a controlled way through magnetic tweezers to a single-bacterium previously associated with magnetic nanoparticles. Furthermore, we prepare NHS functionalized surfaces with host adhesion elements that serve as target tissue mimicking the host cell's surface. Our experiments provide the first measurements of the attachment strength of a single *Staphylococcus aureus*

bacterium, which is the primary cause of infective endocarditis, an inflammatory process in the inner lining of the heart's chambers and valves. Nonetheless, the ultimate goal is to be able to apply this approach to any bacteria.

Foster, T. J. et al. Adhesion, invasion and evasion: The many functions of the surface proteins of Staphylococcus aureus. Nat. Rev. Microbiol. 12, 49–62 (2014). Grosser, M. R. & Richardson, A. R. Method for preparation and electroporation of S. Aureus and S. epidermidis. Methods Mol. Biol. 1373, 51–57 (2016). Xu, C.et al. Applications of iron oxide-based magnetic nanoparticles in the diagnosis and treatment of bacterial infections. Front. Bioeng. Biotechnol. 7, 1–15 (2019).

0308-R-P

Autophagy modulation in corneal diseases: a systematic review

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Introduction: Autophagy in mammalian eye has been studied since 1970, but few studies have described the role of autophagy in different ocular diseases (Keratitis, dry eye, and corneal dystrophy) in animal models. For the study of autophagy in corneal pathologies, it is necessary to know how it is regulated in animal models. Thus, new therapeutic targets and treatments could be studied to recover corneal tissue. For this reason, we propose a systematic review (SR) according to the PRISMA statement. **Objective**: The main objective was to collect information on animal model studies where autophagy modulation in corneal diseases has been studied, as well as to identify how the models have been performed, experimentally or genetically. In addition, we wanted to determine the different treatments and administration routes in order to modulate this process. **Materials and Method**: Preclinical trials were included in this SR by searching in three electronic databases: Web of Science, Scopus, and PubMed using as Boolean operators: TS= (autophagy AND cornea*). **Results**: Thirty-five original articles published in English were selected for this SR. The animal models which mimic corneal diseases were classified as genetically modified (13) and experimentally induced (21). Of those,twenty-two studies used pharmacological treatments that modify autophagy. **Conclusions**: Molecules and pharmacologic agents that can activate or inhibit autophagy exert a therapeutic effect. More preclinical in vivo research is needed to define the protective effect that drugs exert to maintain corneal health.

0335-P

Production of functional pulmonary surfactant is linked to glycogen synthesis in rat fetal lung

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Pulmonary surfactant, a complex of lipids and proteins, covers and stabilizes the alveolar surface, facilitating respiratory mechanics. Thus, its absence is lethal at birth, as it is critical to maintain opened the air-liquid pulmonary interface. On the other hand, mice lacking glycogenin, a protein involved in glycogen synthesis, exhibit high perinatal mortality (90%) due to respiratory failure.

To investigate the involvement of pulmonary glycogen deficiency in proper surfactant production and function, we analyzed the lungs of embryonic and neonatal glycogenin knockout mice. We found incomplete processing and decreased levels of surfactant proteins SP-B and SP-C in fetal tissue together with collapsed sacculi in non-surviving pups. Lung ultrastructure of knockout mice also displayed differences in organelles related to surfactant maturation and storage, such as multivesicular and lamellar bodies. Furthermore, surfactant obtained from several knockout animals was found to be unable to adsorb into air-liquid interfaces, a property known to be essential for alveolar stabilization and to avoid lung collapse.

Our results show that a proper glycogen production is necessary for the burst of surfactant production required to establish respiration upon birth, confirming that glycogen deficiency in lungs can cause respiratory distress syndrome, and suggesting that mutations in genes related to glycogen synthesis may underlie cases of idiopathic neonatal death.

0341-P

Preliminary characterization of a novel cysteine-sparing NOTCH3 missense mutation discovered in a patient with suspicion of CADASIL

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CADASIL (cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a neurological disease characterized by small subcortical infarcts. This pathology is recognized as the most common monogenic form of hereditary ischemic stroke, with more than 230 unique mutations reported. CADASIL is caused by mutations in the epidermal growth factor-like repeats of the extracellular domain of NOTCH3, usually affecting the number of cysteines, causing protein misfolding and receptor aggregation. However, several cysteine-sparing NOTCH3 missense mutations have been described in patients with clinical suspicion of CADASIL, although their pathogenic role is still unknown. Here, we describe the preliminary characterization of a novel cysteine-sparing missense mutation discovered in a patient with suspicion of CADASIL treated at the Service of Neurology of the University Hospital of Albacete, Spain. The only mutation found in the extracellular domain of the *NOTCH3* gene was a transversion 2749G>C within the EGF-23 domain, which produces the aminoacid change G917R in the NOTCH3 protein. As our laboratory has recently described that NOTCH3 signaling is essential for NFκB activation in TLRactivated murine macrophages, which is important for the pro-inflammatory activation of macrophages, we decided to explore if a murine NOTCH3 protein, carrying the abovementioned cysteine-sparing mutation, would alter NOTCH3 pro-inflammatory function by modulating the activation of NF-κB. We first found that the expression of NOTCH3 in LPS-activated human macrophages obtained from *Buffy Coats* of blood donors was equivalent to that of murine macrophages. Therefore, we replicated the 2749G> C mutation in the mouse *Notch3* gene using site-directed mutagenesis and studied its functional consequences in our murine models of inflammation. We focused on the effect of the mutation in the expression and activity of some NF-κB-dependent pro-inflammatory genes, such as TNFα or iNOS. We hope that our murine model allows the identification of potential functional abnormalities that could contribute to a better understanding of the molecular bases of CADASIL.

(Grant SBPLY/17/180501/000301 by Junta de Castilla-La Mancha, Spain; and PID2019-109421RB-100 by Ministerio de Ciencia e Innovación, Spain)

0353-P

Colorectal liver metastatic cells do not rely on lipids as a primary energy source to fuel proliferation

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Colorectal cancer (CRC) is the third most common cancer worldwide, and the second in associated mortality, being the liver metastasis a main cause of death. Cancer cells go through a dynamic metabolic reprogramming, being highly glycolytic in the tumorigenic process, and changing to a more lipid oxidative phenotype in the metastatic cascade. The aims were to identify changes in the rewiring of lipid metabolism in colorectal liver metastasis (CRLM), and to investigate the effect of a dietary lipid supply. A transcriptome analysis in human healthy liver (HL), CRLM and CRC was performed using the E-GEOD-14297 array. A CRLM mice model was developed by injection of MC38 tumour cells in the spleen; among them a group was fed a chow diet (CDmet) while another a high-fat diet (HFDmet). Metabolic fluxes, lipid concentration and levels of proteins of interest were analyzed. The transcriptome analysis showed that genes involved in "triglyceride (TG) biosynthesis" and "lipoprotein uptake" were upregulated in CRLM when compared with CRC while downregulated *vs* HL. In concordance, the results obtained in the animal models showed that in CDmet tumors, the hepatic TG content was decreased when compared with HL. Besides, metabolic fluxes involved in *de novo* synthesis of TG and in fatty acid (FA) esterification were downregulated while serum TG levels increased. These processes were linked to decreased levels of acetyl-CoA carboxylase and fatty acid synthase, *de novo* FA synthesis main enzymes, while no changes were observed in FA oxidation (FAO). When the exogenous lipid supply was increased by a HFD, the tumor quantity and size were the same as in the CDmet tumors. The analysis of TG and FA metabolism showed that within the tumor, the TG content and FAO rate remained as in the CDmet; however, the fluxes that regulate the *de novo* TG synthesis were decreased and the serum TG levels were even higher than in the CDmet mice. In both, CDmet and HFDmet mice, serum glucose levels were decreased when compared to non-metastatic mice. In line, the transcriptome analysis showed that genes involved in glycolysis were upregulated in CRLM when compared with HL. As a conclusion, CRLM cells do not rely on lipids as a primary energy source to fuel proliferation. The results suggest that glucose remains among the major energy substrates as in the CRC primary tumors.

0360-R/M-P

Targeting myotonic dystrophy with senolytics compounds

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Myotonic dystrophy type 1 (DM1; MIM #160900) is an autosomal dominant disorder, clinically characterized by progressive muscular weakness and multisystem de-

generation. The broad phenotypes observed in DM1 patients resemble the appearance of an accelerated aging process. However, the molecular mechanisms underlying these phenotypes remain largely unknown. Transcriptomic analysis of primary fibroblasts derived from DM1 patients and healthy individuals revealed a decrease in cell cycle activity, cell division, and DNA damage response in DM1, all of which facilitated the accumulation of cellular senescence. Serial passage studies *in vitro* confirmed the accelerated increase in senescence and the acquisition of a senescence-associated secretory phenotype in DM1 fibroblasts. Moreover, functional studies highlighted the impact of BMI1/p16INK4A pathway deregulation in DM1-associated cellular phenotypes. The data from transcriptome analyses were corroborated in human myoblasts and blood samples as well as in mouse and *Drosophila* models of the disease. Importantly, treatment with the senolytic compounds, a novel therapeutic strategy that aims to permanently remove senescent cells, reversed the accelerated aging phenotypes in both DM1 fibroblasts *in vitro* and in *Drosophila in vivo*. Our results identified the accumulation of senescence-related processes as a major driver of DM1 pathophysiology and therefore, demonstrated the efficacy of senolytic compounds in the pre-clinical setting.

García-Puga M, et al. Myotonic Dystrophy type 1 cells display impaired metabolism and mitochondrial dysfunction that are reversed by metformin. Aging (Albany NY). 2020. Mateos-Aierdi AJ, et al. Muscle wasting in myotonic dystrophies: a model of premature aging. Front Aging Neurosci. 2015. Fernández-Torrón R, García-Puga M, et al. Cancer risk in DM1 is sex-related and linked to miRNA-200/141 downregulation. Neurology. 2016.

0379-P

Stimulation of neuroendocrine differentiation in LNCaP cells by urine exosomes

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Prostate Cancer (PCa) is the second most frequent type of cancer in men around the globe and its incidence is expected to be increased by 79,8% in 2040. Most of the cases are multifocal adenocarcinomas and their differentiation and evolution shows an important heterogeneity. In particular, neuroendocrine differentiation (NED) of prostate tumour cells is associated with poor prognosis and relapse, since they produce and secrete peptide hormones and growth factors that promote cancer progression.

Exosomes are one type of extracellular vesicles, characterized by a diameter of 30-150 nm produced by all types of human cells. They are found in human fluids such as blood or urine. In PCa, exosomes contribute to cell transformation, angiogenesis and tumour progression, due to the molecular mediators that they transport. Our group has demonstrated that exosomes secreted by PC3 cells induce NED in the androgen-dependent prostate cancer cell line LNCaP. Hence, the aim of this research was to study the effect of isolated exosomes from urine on the morphology of LNCaP cells. Urine samples were provided by Urology Service of University Hospital Principe de Asturias. Exosomes were isolated by centrifugation and filtration. The presence of exosomes was confirmed by Western blotting using CD-63 antibodies.

We observed an increase in the percentage of NED of LNCaP cells at 24h and 48h after treatment with isolated exosomes from patients with PCa as compared with control (untreated) and exosomes from non-cancer patients. These results support the involvement of exosomes in the transformation of androgen-dependent prostate cancer cells into an aggressive phenotype.

This work was supported by a grant from ISC II (PI18/00526 to A.M.B.)

Muñoz-Moreno L, Carmena MJ, Schally AV, Prieto JC, Bajo AM. Stimulation of neuroendocrine differentiation in prostate cancer cells by GHRH and its blockade by GHRH antagonists. Invest New Drugs. 2020 Jun;38(3):746-754. doi: 10.1007/s10637-019-00831-2. Epub 2019 Jul 17. PMID: 31312936.

0380-R-P

ROLE OF C3G IN LIVER DEVELOPMENT AND PROGENITOR CELLS BIOLOGY

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C3G is a guanine-nucleotide exchange factor (GEF) for GTPases from Ras superfamily that regulates several cellular functions (e.g. migration, adhesion or differentiation) through GEF dependent and independent mechanisms. C3G function in the liver remains unknown, but it shows a tightly regulated expression profile, being highly expressed in oval cells and neonatal hepatocytes, downregulated in adult hepatocytes, and upregulated in hepatocarcinoma cells to promote tumor growth.

Oval cells are bipotential transit-amplifying progenitor cells able to differentiate into hepatocytes and biliary epithelial cells. They have a therapeutic potential, due to their capacity to regenerate the liver in response to chronic damage when the hepatocytes are unable to do it. However, in a context-dependent manner they can also contribute to fibrosis and hepatocarcinoma development. We have studied the role played by C3G in oval cells through gene silencing. We found that C3G knock-down increases migration and invasion, which correlates with the acquisition of a mesenchymal phenotype. In addition, C3G down-regulation increases their clonogenicity, but not their tumorigenic potential (anchorage-independent growth), leading to the upregulation of some hepatocyte lineage markers. Altogether, these data suggest that C3G down-regulation could favor, not only cell migration to places where liver repair is required, but also hepatocyte differentiation, thus enhancing oval cell regenerative potential. Nonetheless, HGF/MET signalling is defective in C3G-silenced oval cells, which might have functional consequences.

Due to the potential role played by C3G in hepatocyte differentiation and/or maturation, and its involvement in hepatocarcinoma progression, we have generated a conditional liver C3G knockout mouse bearing Cre recombinase under albumin promoter (C3GAlbKO mice). In this mouse, liver development seems unaffected and no significant changes in liver weight and histology were detected when compared to the wt. Further analysis will determine C3G involvement in liver development and function.

0383-P

A metabolomic approach to Idic15 syndrome, a rare neurodevelopmental disease

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Idic15 syndrome is a rare disease of neurodevelopment caused by duplications of the chromosomal region 15q11-q13. At the clinical and genetic level Idic15 is well defined but its low prevalence, the complexity of the genomic region involved, the variability in size and arrangement of duplications, and the weak correlation between genotype and clinical symptomatology pose a challenge in establishing its molecular bases. To contribute to this knowledge we have applied metabolomics to the evaluation of metabolic alterations in Idic15. As part of an ongoing multidisciplinary project, we have established by NMR spectroscopy the metabolomic serum and urine profiles of 28 Spanish Idic15 patients (http://www.idic15.es/) and 17 controls paired by age and gender. The spectra were acquired at 310 K on the Bruker Avance III HD 600MHz NMR spectrometer equipped with a 5 mm QXI ^{1}H probe and analyzed in the MATLAB 2017b environment. Our results indicate that the levels of several metabolites in the serum and urinary metabolomic profiles of Idic15 patients differ significantly (ANOVA) from controls. Differential metabolites related (MetaboAnalyst) to glutation metabolism, fatty acids, xenobiotics, aminoacil-tRNA biosynthesis and amino acids such as arginine. A discriminating metabolic profile (predictive model) between Idic15 patients and control was generated using a PLS-DA model. Differences in metabolomic profiles between patients with BP1-BP3 and BP1-BP5 duplications (evaluated by CGH) are notable and support the importance of the length of the genetic region in metabolic alterations in Idic15 patients. This study is the first metabolomic approach to Idic15 and its results can guide in the search for molecular biomarkers for the syndrome and the analysis of its etiopathogenic bases. Project funded by donations to the “A House a Life” Initiative promoted by Great Chance SLU.

0390-P

PLATELET C3G AS A POTENTIAL REGULATOR OF THE RESPONSE TO DAMAGE DURING LIVER DISEASE DEVELOPMENT

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C3G is a guanine nucleotide exchange factor (GEF) for GTPases from Ras superfamily, mainly Rap1. C3G regulates several cellular functions in different cell types through GEF dependent or independent mechanisms. We have previously demonstrated that C3G promotes megakaryocytic differentiation, platelet activation, adhesion and pro-angiogenic factors secretion through Rap1 activation.

Platelets can play a dual role in liver response to damage. They can accumulate at sites of liver injury, releasing pro-fibrotic factors (e.g. TGF-β). On the other hand, platelets regulate hepatic stellate cells, preventing liver fibrosis by enhancing HGF secretion. We have analysed the function of platelet C3G in liver damage induced by chronic CCl4 treatment of transgenic mice overexpressing in platelets full length C3G (tgC3G) or C3G lacking its GEF domain (tgC3GΔCat) and platelet C3G knockout (PF4-C3G-KO) mice. Our results show that collagen deposition and α-SMA expression were lower in tgC3G livers and higher in PF4-C3GKO compared to wt mice after 8 weeks CCl4 treatment. In livers from PF4-C3GKO mice, we also found a lower number of platelets as compared to wt treated mice. The liver inflammatory response was also analysed. Although we did not find significant changes in the number of liver macrophages between PF4-C3GKO and wt mice, an increase in macrophages was detected in untreated tgC3G mice. In addition, higher IL-6 mRNAs levels were found in tgC3G mice livers, while mRNA levels of IL1-b and other pro-inflammatory cytokines remained unchanged.

In hepatocarcinoma, platelets promote tumour cell prolifer-

ation and invasion, regulating immune response. We are currently analyzing the role of platelet C3G in hepatocarcinoma models induced by DEN or DEN-CCl4 treatment that induces fibrosis and adenocarcinomas. Our results indicate that platelet C3G protects from CCl4-induced liver damage, reducing fibrosis, increasing macrophage recruitment and decreasing the chronic inflammatory response.

0394-R/M-P

Study of myocardial fibrosis in a 3D human organoid with fluorescent nano-therapy

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The use of human organoids in experimentation has long proven to be a very attractive experimental model. Its versatility in allowing us to generate miniaturized and simplified versions of organs allowing us to study cell behavior under conditions similar to those native to the tissue, while maintaining a controlled environment. This work shows the use of human engineered cardiac connective tissue (hECCT) as a platform for the study of cardiac fibrosis treatments with an engineered nanocluster (CTPR390-488-Au), whose capability to inhibit fibrosis by altering the regulatory function of Hsp90 has been previously demonstrated by our group.

These hECCTs consist of a ring-shaped collagen matrix, in which primary human donor fibroblasts are embedded. This model makes it possible to maintain the cells in conditions of structural tension, contractility, elasticity similar to those of native tissue, which is not possible to achieve in traditional cell cultures or spheroids, and this may condition the cellular response to treatment.

The hECCTs were treated with TGFβ, to simulate a pro-fibrotic environment. Fluorescent CTPR390-488-Au was administered to observe the behavior of the organoid, analyzing the mechanical capabilities including its elasticity, resilience, toughness and tissue contraction.

The experimental group treated with CTPR390-488-Au under pro-fibrotic conditions was able to recover healthy mechanical characteristics, in addition to reducing the expression of key proteins in the progression of fibrosis such as collagens. Moreover, the nanocluster was localized in the cellular region of the organoid.

For the first time the anti-fibrotic effect of a new nanoformulation designed to reverse the pathological alterations generated by fibrosis has been observed in a 3D cardiac fibrosis model.

-ELSON, Elliot L.; GENIN, Guy M. Tissue constructs: platforms for basic research and drug discovery. Interface focus, 2016, vol. 6, no 1, p. 20150095. -AIRES, Antonio, et al. Engineering multifunctional metal/protein hybrid nanomaterials as tools for therapeutic intervention and high-sensitivity detection. Chemical Science, 2021, vol. 12, no 7, p. 2480-2487.

0406-R/M-P

Players in macrophage iron accumulation in LPI mouse model

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Macrophages are involved in a wide range of cellular processes. Specifically, red pulp macrophages (RPMs) orchestrate red blood cell (RBC) degradation and iron recycling, thus, ensuring body iron homeostais and metal deliverly for erythropoiesis. Herein, we uncover a novel mechanisms that links amino acid metabolism to iron homeostasis and erythropoiesis.

Slc7a7 encodes for y+LAT1, a light subunit of the heterodimeric amino acid transporter family, which is involved in cationic amino acid (CAA) transport across the basolateral membrane of epithelial cells. Mutations in Slc7a7 gene give rise to Lysinuric Protein Intolerance (LPI), a rare and severe autosomal recessive disease. Intriguingly, y+LAT1 is also involved in arginine transport in non-polarized cells such as macrophages. Here we report that complete inducible Slc7a7 ablation compromises proper erythropoiesis and that dysfunctional RBC generation leads to increased erythrophagocytosis, RPM iron overload and an altered iron metabolism. Mechanistically, the expression of the cellular iron exporter ferroportin-1 expression was compromised by increased plasma hepcidin and Lysinuric Protein Intolerance metabolic environment, ultimately all contributing to macrophage iron accumulation. Moreover, altered erythropoiesis might be compromised due to decreased erythropoietin plasma levels, influencing thus erythrocyte development and recycling. This study establishes a new crucial link between arginine metabolism and iron homeostasis in macrophage.

0418-P

The anti-inflammatory effect of xanthohumol in colon cancer cell lines depends on metastatic status.

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Xanthohumol (XN) is a hop-derived prenylflavonoid that has been reported to act both as a chemopreventive and chemotherapeutic agent [1]. XN also shows anti-inflammatory and antioxidant activity [2]. We reported that XN impairs mitochondrial functionality in a metastatic colon cancer cell line (SW620) [3]. Inflammation has been implicated in cancer progression, from the initiation process to metastasis [4].

The aim of this study was to determine the effect of XN in a high (10 μM) and in a low (10 nM) concentration on inflammation in pre-metastatic (HT29) and metastatic (SW620) colon cancer cell lines. The effect of two concentrations of XN (1nM & 10 μM) for 48h on HT29 and SW620 was determined. Cell proliferation (Hoescht), ROS production (Amplex Red), oxidative damage (4HNE), and inflammation-related gene expression (PPARg, NFκB, TNFα, R-IL6 and TGFβ) were quantified.

XN treatments produced a significant decrease in cell proliferation and an increase in ROS production in SW620 cells but had no effect on HT29 cells. XN treatments showed opposite effects on oxidative stress damage (4HNE protein adducts) at both concentrations, increasing in SW620 and decreasing in HT29. Inflammation-related gene expression was modified by XN treatment in HT29, showing a decrease in the expression of PPARg and NFkB at both high and low concentrations, a decrease in TNFα and R-IL6 only at lower doses, and no effect on TGFβ. In SW620, XN treatment produced an increase in R-IL6 only at the higher concentration and a dual effect in TGFβ expression, decreasing at the lower concentration and increasing at the higher dose. In summary, XN had an effect that depends on the metastatic status in colon cancer cell lines, producing an anti-inflammatory effect in the pre-metastatic cell line and a pro-inflammatory effect in the metastatic one.

Supported by Manuel de la Oya grant from Centro de Información Cerveza y Salud, Programa postdoctoral Margalida Comas - Comunidad Autónoma de las Islas Baleares (PD/050/2020) and ISCIII (PI14/01434) and FEDER-Unión Europea "Una manera de hacer Europa".

1. Greenwell M and Rahman PKSM. 2015. Int J Pharm Sci Res. 6: 4103–4112. 2. Liu et al. (2012) J Exp Ther Oncol 10(1):1–8. 3. Najdaghi et al. (2020) Cytokine 135:155205. 4. Sastre-Serra et al. (2019) Int J Food Sci Nutr 70(4):396–404.

0429-R/M-P

Aryl hydrocarbon receptor-interacting protein regulates tumorigenic and metastatic properties of colorectal cancer cells driving liver metastasis

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Background: Liver metastasis is the primary cause of colorectal cancer (CRC)-associated death. Aryl hydrocarbon receptor-interacting protein (AIP), a putative positive intermediary in aryl hydrocarbon receptor-mediated signaling, is overexpressed in highly metastatic human KM12SM CRC cells and present in other highly metastatic CRC cells. Methods: Meta-analysis and immunohistochemistry were used to assess the relevance of AIP in CRC. Cellular functions and signaling mechanisms mediated by AIP were assessed by gain-of-function experiments using the KM12 cell system and in vitro and in vivo experiments. Results: Using meta-analysis and tissue microarrays, we observed a significant association of high AIP expression with poor patients' survival. Gain-of-function and quantitative proteomics experiments demonstrated that AIP led to an increase in the tumorigenic and metastatic properties of KM12C (non-metastatic) and KM12SM (metastatic to liver) CRC cells. AIP overexpression caused a significant dysregulation of epithelial-to-mesenchymal (EMT) markers and induced Cadherin-17 activation and the overexpression of several transcription factors. The former induced the signaling activation of AKT, SRC, and JNK kinases to increase adhesion, migration and invasion of CRC cells as demonstrated by PCR and WB analyses. In vivo experiments showed that subcutaneous or intrasplenic injection of ectopically AIP expressing KM12 cells induced tumor growth and liver metastasis, respectively. It was especially relevant to find that KM12C (non-metastatic) cells ectopically expressing AIP became metastatic to liver. Conclusions: Our data reveal new roles for AIP regulating EMT markers, transcription factors and proteins associated with cancer and metastasis to induce tumorigenic and metastatic properties in colon cancer cells driving liver metastasis.

0432-P

β3-Adrenergic receptor plays an important role in pulmonary arterial hypertension

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Although β3-adrenergic receptor (β3-AR) has been traditionally studied in the adipose tissue, it has been demonstrated to have a role in heart and blood vessels during cardiovascular disease such as systemic hypertension, myocardial infarct and heart failure form different etiologies (1-4). In this way, we hypothesized that β3-AR could also play a relevant role in the development of pulmonary arterial hypertension (PAH). PAH is a rare disease originated by an aberrant endothelial dysfunction and vascular cell proliferation. This leads to an increase in the vascu-

lar tone and remodeling that elevates pulmonary artery pressure and subsequently induces a compensatory right ventricular hypertrophy, decompensated heart failure and premature death. To clarify the role of β3-AR in PAH, we analyzed β3-AR loss and gain of function mouse models exposed to chronic hypoxia. These experiments showed that loss of β3-AR activity aggravates the hypoxia-induced PAH phenotype while its restoration in the endothelial compartment leads to an ameliorated pathophysiology. Accordingly, pharmacological activation of β3-AR both in chronic hypoxia and monocrotaline-induced PAH models in mice and rats, respectively, also led to better hemodynamic and pathophysiological parameters in treated animals. To further assess the cellular and molecular role of β3-AR, we used human pulmonary artery endothelial and smooth muscle cells. Our results pointed out that activation of β3-AR improves pulmonary vascular function in PAH in three ways: 1) by protecting endothelium through the reduction of hypoxia-induced cellular stress, restoring mitochondrial fragmentation and preventing excessive ROS production; 2) by inducing NO-dependent vascular dilation; and 3) by reducing smooth muscle cell proliferation. These mechanisms are translated into less vascular remodeling, lower pulmonary artery pressure and improved right ventricular function. In conclusion, β3-AR stands as a potential new target for the treatment of PAH and others pulmonary vascular diseases.

1. Oliver E et al., J Pharmacol Exp Ther; 328(3):982-90, 2009. 2. García-Prieto J et al., Basic Res Cardiol; 109(4):422, 2014. 3. García-Álvarez A et al., Basic Res Cardiol; 111(4):49, 2016. 4. Dubois-Deruy E et al., ESC Heart Fail; 7(3):920-932, 2020.

0444-P

Targeting Nerve Growth Factor Receptor reduces melanoma metastasis and overcomes the resistance to immunotherapy in melanoma.

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Melanoma is a very aggressive disease with few survival expectations, mostly due to the ability of tumors to metastasize and to become refractory to current therapies. The future for melanoma treatment is based on molecular-directed cancer immunotherapies. However, little is known about how these immune cells behave influenced by tumoral cells. Our main goal is to find new targets and therapies to improve melanoma patient outcome, especially by fostering anti-tumor immunity in highly aggressive melanomas. Recent studies have shown that the nerve growth factor receptor (NGFR/p75^{NTR}/CD271) is a key player in melanoma, contributing to metastasis and therapy resistance, including immunotherapies. However, the pharmacological inhibition of NGFR in melanoma has never been exploited. Thus, in our laboratory we are analyzing the use of NGFR small molecule inhibitor (NGFRsmi) as a new strategy to overcome resistance to immunotherapy and reduce aggressiveness in melanoma. We have found that NGFR is upregulated in melanoma cells resistant to immunotherapy. Our data show that the treatment with NGFRsmi reduce metastasis in melanoma cells and melanoma cells resistant to immunotherapy. In addition, using melanoma cell lines including NGFR knock-out models, we are characterizing the molecular mechanisms underlying NGFR regulation of immunoevasive phenotype in melanoma, paying special attention to the regulation of PDL1 expression levels and antigen presentation of melanoma cells. We expect that the use of smiNGFR could be a new adjuvant of current immunotherapies for metastatic melanomas. These data suggest that combination of NGFRsmi and immunotherapy could improve melanoma therapy by targeting 1) metastatic cells and 2) serve as second hit for immunotherapy-resistant melanoma cells.

- Boiko, A. D., et al. Nature (2010). 466(7302), pp.133-7. - Boshuizen, J. et al. Nature Communications (2020). 11(1). - Restivo, G. et al. Nature Communications (2017). 8(1). - Turner, N., Ware, O. and Bosenberg, M., Clinical & Experimental Metastasis (2018). 35(5-6), pp.379-391.

0446-P

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.

Li, G., Li, Y., Liu, X. et al. ALDH1A3 induces mesenchymal differentiation and serves as a predictor for survival in glioblastoma. Cell Death Dis 9, 1190 (2018). Wu W, Wu Y, Mayer K, von Rosenstiel C, Schecker J, Baur S, Würstle S, Liesche-Starnecker F, Gempt J, Schlegel J. Lipid Peroxidation Plays an Important Role in Chemotherapeutic Effects of Temozolomide and the Development of Therapy Resistance in Human Glioblastoma. Transl Oncol. 2020 Mar;13(3):100748.

0447-R/M-P

The colorectal cancer stage influences the inflammatory status in both peritumoral and tumor tissues

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Colorectal cancer is the third-most common cancer and the second-most common cause of cancer death worldwide [1]. The inflammation status associated to the microenvironment of cancer cells participates in tumor growth, angiogenesis, epithelial-mesenchymal transition and metastasis[2]. The network of inflammatory cytokines can influence survival, growth, proliferation, differentiation, immune cell activation, and migration of stromal and tumor cells, because these cytokines can promote the communication between stromal and tumor cells and the interaction between tumor and extracellular matrix [2,3] . Our aim was to investigate changes in the inflammatory status of the peritumoral and tumor tissues according to the tumor stage (I, II, III) in patients affected of colorectal cancer.

Peritumoral and tumor tissue samples were obtained from 28 patients affected by colorectal cancer in stage I, II and III. The homogenization of about 100 mg of frozen tissue was made in a proportion 1:10 (w/v) in homogenization buffer (Tris 20 mM pH 7.4, Saccharose 250 mM, EGTA 2 mM, KCl 40 mM), and Bradford method was made to quantify protein levels. Finally, inflammation-related proteins, such as MMP9, COX-2 and IkB among others, were determined by Western blot technique.

Patients with tumors at stage II presented an increase of the inflammatory status, since both tumor and peritumoral tissues showed an increase of inflammation-related proteins expression compared to both tissues of stage I. This increment of the inflammation-related proteins is far more attenuated in both tumoral and peritumoral tissues of the stage III patients.

This increase of the inflammatory status in the peritumoral tissue of stage II patients could allow the creation of the appropriate microenvironment for the tumor cells migration and the non-tumor cells malignancy conversion. Furthermore, the rise in the expression of inflammation-related proteins in the tumor tissue of the stage II patients could enable tumoral progression of the cancer cells. Taken together, the results obtained in this work showed that these inflammation-related proteins can be used as early biomarkers for colorectal cancer.

Supported by IdISBa – Synergia (SYN18/08), Proyecto del Hospital Comarcal de Inca y la Universidad de las Islas Baleares (CINUIB), (FUEIB) and PD/050/2020).

1. Bray, F. et al. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 68, 394–424 (2018). 2. Landskron, G., Fuente, M. De, Thuwajit, P., Thuwajit, C. & Hermoso, M. A. Chronic Inflammation and Cytokines in the Tumor Microenvironment. J Immunol Res 2014, 1–19 (2014). 3. Balkwill, F. & Mantovani, A. Inflammation and cancer: Back to Virchow? Lancet 357, 539–545 (2001).

0453-P

MITOCHONDRIAL FUNCTIONALITY AND INFLAMMATION IN COLONOSPHERES. RESPONSE TO OXALIPLATIN.

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Colorectal cancer (CRC) develops following a hierarchical model of heterogeneous cell populations with subpopulations of poorly-differentiated and self-renewables cancer stem cells (CSCs)[1, 2]. Understanding this subset of cells may be crucial in CRC research, thus CSCs have been suggested as a source of cancer recurrence, drug resistance and metastasis[3]. In the last years, mitochondria have been postulated as a key organelle in cancer initiation and progression due to their role in redox status and inflammation regulation. These CSCs can form, *in vitro*, floating three-dimensional tumorospheres (colonospheres) in ultra-low attachment plates and cultured with tumorospheres specific (serum-free) media. These spheroids preserve more faithfully the features of original tumors, including gene expression and tumor biology[4,5]. We have studied the mitochondrial biogenesis-, oxidative stress- and inflammation-related genes expression in adherent SW620 colon cancer cells compared to the colonospheres. Moreover, we have evaluated the effect of increasing concentrations of oxaliplatin (1, 2.5, 5 and 10 μM) in colonospheres forming efficiency: at the seeding time or post-seeding (48 hours after seeding). Results showed an increase in mitochondrial biogenesis- and oxidative stress-related genes in colonospheres in comparison to the adherent cells. Moreover, colonospheres presented an increase in the expression of inflammation-related genes accompanied of a decrease in anti-inflammatory genes. These results suggest that the mitochondria of colonospheres are exposed to increased oxidative stress, with a severe need for the synthesis of new mitochondria. Finally, treatment with oxaliplatin produced a reduction of the colonosphere forming efficiency, especially at the highest concentration (10 μM). The treatment with oxaliplatin after 48 hours of colonosphere formation caused a reduction of the colonospheres size and an increase of the colonospheres number. These results could indicate an enrichment of CSCs in the oxaliplatin-treated colonospheres, with more well-rounded spheroids. Altogether, the results obtained in this study suggest significant changes in the mitochondria of CSCs to their adaptation to the tumor microenvironment and resistance to cytotoxic treatments.

1. Olejniczak A et al. Int J Oncol. 2018;52(2):599–612. 2. Qureshi-Baig K et al. PLoS One. 2016;11(1):1–24. 3. Zhou Y et al. Oncotarget. 2018;9(70):33403–15. 4. Riedl A et al. J Cell Sci. 2017;130(1):203–18. 5. Li YF et al. Brazilian J Med Biol Res. 2012;45(3):197–204.

0455-P

TARGETING ABERRANT SPLICEOSOME AS A NEW THERAPEUTIC STRATEGY IN CYTARABINE-RESISTANT ACUTE MYELOID LEUKEMIA

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Recently, new targeted therapies have been approved for treating AML [1]. However, 7+3 chemotherapy schemes remain as the standard of care for many patients [2], and its lack of efficacy is the main cause of death. Indeed, only 10% of relapsed/refractory patients overcome the disease [3]. Therefore, there is still an urgent need for seeking more effective treatments.

Aberrant splicing in AML has been previously described [4, 5, 6] but its relevance in resistance is unclear [6]. In this study we shed light on the role of splicing factors SR proteins in cytarabine resistance to propose new therapies.

Public data from TCGA-LAML and GTEx repository revealed differences in the expression levels of three genes encoding SR proteins: *SRRM2*, *SRSF12*, and *SRSF9*. *SRRM2* and *SRSF12* overexpression was validated by qPCR in bone marrow samples from AML patients compared to controls and other myeloid disorders (MDS and MPN). The ARN expression levels of SR proteins were then compared between cytarabine-treated patients that progressed or not, showing no significant differences. Also, *SRRM2* expression levels were evaluated in paired samples (diagnosis *vs* resistance) of cytarabine-treated patients without apparent differences between both moments.

In contrast, the phosphoproteomic profile associated to cytarabine-resistance in paired samples from 3 AML patients revealed an increase in the phosphorylation levels of SRRM2, between other SR proteins. After that, expression levels of SR proteins, or their phosphorylated forms, were evaluated by immunohistochemistry in AML patient samples observing an increase in the phospho-SR proteins at relapse, as well as in refractory patients at diagnosis.

Based on this, we evaluated the efficacy of some spliceosome inhibitors *in vitro* in cytarabine-sensitive and resistant cells. The spliceosome inhibitor H3B-8800 showed the best efficacy in both, and was then, evaluated *in vitro* in combination with cytarabine and other drugs approved for AML. The combination of H3B-8800 plus venetoclax showed strong synergistic effects in the sensitive and resistant cells.

The effectivity and safety of combining H3B-8800 plus venetoclax was then evaluated *ex vivo* in AML patients and healthy donors, suggesting that this combination appears to be a good strategy for treating AML and could not be excessive toxic.

[1] Burnett A & Stone R. Clin Lymphoma Myeloma and Leuk. 20(6), 341-350 (2020). [2] Saultz N et al. J Clin Med. 5(3), 33 (2016) [3] Bose P et al. Curr Treat Options in Oncol. 18(3), 17 (2017) [4] Bonnal SC et al. Nat Rev Clin Oncol. 17(8), 457-474 (2020). [5] Adamia S et al. Clin Cancer Res. 20(5), 1135-1145 (2014). [6] Zhou J and Chng WJ. Stem Cell Investig. 4, 6 (2017)

0457-R-P

HIV-1 Env clustering is driven by its interaction with viral membrane cholesterol

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HIV-1 entry requires the redistribution of the fusion envelope glycoprotein (Env) trimers into a cluster upon maturation, and the presence of cholesterol (chol) in the viral membrane. However, the molecular mechanisms underlying the specific role of chol in infectivity and the driving force behind Env clustering remain unknown. Our work demonstrates that the gp41 transmembrane subunit of Env directly interacts with chol in the viral membrane via residues 751-854 in the cytoplasmic tail ($CT_{751-854}$). Super-resolution stimulated emission depletion (STED) nanoscopy analysis of Env distribution further demonstrates that both truncation of gp41 $CT_{751-854}$ and depletion of chol leads to dispersion of Env clusters in the viral membrane and inhibition of virus entry. This work reveals a direct interaction of gp41 CT with chol, and indicates that this interaction is an important orchestrator of Env clustering, unraveling a key specific molecular role of molecular in HIV-1 infectivity.

Nieto-Garai JA, Arboleya A, Otaegi S, Chojnacki J, Casas J, Fabriàs G, Contreras FX, Kräusslich HG, Lorizate M. Cholesterol in the Viral Membrane is a Molecular Switch Governing HIV-1 Env Clustering. Adv Sci (Weinh). 2020 Dec 21;8(3):2003468. doi: 10.1002/advs.202003468. PMID: 33552873

0458-P

High concentrations of genistein increase oxidative stress and inflammation in two different colon cancer cell lines

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Colorectal cancer is the third-most common cancer and the second-most common cause of cancer death worldwide [1]. The phytoestrogen genistein can modulate oxidative stress [2], cell bioenergetics [3], and inflammation [4], three physiological parameters closely linked which participate in different phases of colorectal cancer progression [5]. Nevertheless, the mechanism by how genistein affects colorectal cancer cells remains unclear [6]. Our aim was to investigate the effects of genistein treatment on these three processes in two colon cancer cell lines: HT29 (primary tumor) and SW620 (metastatic tumor).

For cell viability and hydrogen peroxide production determinations, cells were treated with increasing concentrations of genistein (1, 5, 50, and 100 μM) for 48 hours to determinate the effects of several genistein concentrations. Then, cells were treated with high concentrations of genistein (100 μM) for 48 hours to determinate their effects on oxidative stress, mitochondrial biogenesis and inflammation status. Gene expression levels were determined by real-time PCR, protein expression levels were determined by Western blotting and NFκB subcellular localization was determined by immunocytofluorescence (ICF).

High concentrations of genistein decreased cell viability and detoxification of hydrogen peroxide related genes expression and increased hydrogen peroxide production, especially in SW620 cell line. In HT29 cells mitochondrial biogenesis related genes increased their expression levels but decreased in SW620 cell line. Finally, the expression of inflammation-related genes was increased in both cell lines, being more pronounced in SW620 cells. The effect of genistein in SW620 metastatic cell line can be mediated by NFκB, since ICF showed its translocation into the nucleus.

High concentrations of genistein promote an increase of oxidative stress in both colon cancer cell lines due to a decrease of antioxidant enzymes expression levels and, in SW620 cells, the accumulation of less-functional mitochondria caused by the decrease in mitochondrial biogenesis levels. The oxidative stress produced is related to an increase of the inflammation status, which would cause a decrease in cell viability of both cancer cell lines.

Supported by Instituto de Salud Carlos III. FIS: PI14/01434.

1. Bray, F. et al. CA Cancer J Clin 68, 394–424 (2018). 2. Ganai, A. A. et al. Biomed. Pharmacother. 76, 30–38 (2015). 3. de Oliveira, M. R. Mitochondrion 29, 35–44 (2016). 4. Fajardo, A. M. et al. Am. J. Physiol. - Gastrointest. Liver Physiol. 309, G59–G70 (2015). 5. Zong, W. et al. Mol. Cell 61, 667–676 (2016). 6. Shafiee, G. et al. Int J Mol Cell Med 5, 178–191 (2016).

0460-R/M-P

mTOR regulation in diabetic and hypertensive cardiomyopathies

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Introduction: Type 2 Diabetes Mellitus (T2DM) can frequently coexist with hypertension (HTN) leading to an additional cardiovascular risk. Both pathologies have been related with increased mitochondrial dysfunction in cardiac cells, but there is scarce data on specific activated pathways.

Materials and methods: Cardiac biopsies of interventricular septum were isolated from patients with T2DM+HTN or HTN, who were intervened for coronary artery bypass grafting. Then, proteomics was used to identify differentially expressed proteins. Those proteins were confirmed by Western blot, and clustered into biological pathways using Ingenuity Pathway Analysis (IPA). Also, cultured cardiomyocytes were studied under hyperglycemic, hyperlipidemic and hypertensive stimuli with high concentrations of glucose (HG), fatty acids (HF) and/or angiotensin II, respectively.

Results: T2DM+HTN and HTN exhibited 666 and 45 altered proteins in the heart (>1,5 fold-change, p<0,05), respectively. T2DM+HTN showed a reduction of proteins from glucose and fatty acid metabolism, as well as diminution in mitochondrial factors such as those of respiratory chain and ATP synthesis. Moreover, they increased inflammatory, oxidative, fibrotic, and apoptotic related factors. In contrast, HTN hearts exhibited a decrease in proteins related with carbohydrate metabolism, mitochondrial homeostasis and respiration, oxidative stress, and apoptosis. Interestingly, IPA revealed that mTORC1 and mTORC2 and subsequent mitochondrial function may be mediating these responses. Thus, in cultured cardiomyocytes mitochondrial factors such as TFAM, ACADm, MNF2 and SDHA were also altered after HG, HF or AngII. Also, HF and Ang II, but not HG, were able to enhance Phospho-p70-S6K(Thr389), as a mTORC1 downstream effector. However, they ameliorated the Phospho-Akt(Ser473), as a mTORC2 downstream factor. Interestingly, Phospho-Akt(Ser473) was increased by HG.

Conclusion: The protein alteration in human hearts with T2DM+HTN could be higher than after HTN. Mitochondrial injury may be key for cardiac failure. In particular, mitochondrial factors such as TFAM, ACADm and SDHA could be reduced by mTORC1 activation (and mTORC2 inhibition). The regulation of mTOR axis might be essential for the development of heart disease after T2DM and HTN.

Lastra, G., Syed, S., Kurukulasuriya, L.R., Manrique, C., Sowers, J.R., 2014. Type 2 diabetes mellitus and hypertension: an update. Endocrinol. Metab. Clin. North Am. 43, 103–122. https://doi.org/10.1016/j.ecl.2013.09.005 Lee, M.M.Y., McMurray, J.J.V., Lorenzo-Almorós, A., Kristensen, S.L., Sattar, N., Jhund, P.S., Petrie, M.C., 2019. Diabetic cardiomyopathy. Heart 105, 337–345. https://doi.org/10.1136/heartjnl-2016-310342

0466-P

Endothelial function response to adipose tissue secretome in an animal model of metabolic syndrome and menopause

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Visceral white adipose tissue (WAT) is a key regulator of cardiovascular health. Insulin resistance and inflammation are associated to WAT dysfunction. Dysfunctional WAT exhibits an altered secretome with an enhanced secretion of pro-inflammatory and pro-atherogenic adipokines that contributes to worsen cardiovascular function. Epidemiological studies in premenopausal women suggest that diabesity could attenuate the protective effects of estrogens on cardiovascular diseases (CVD). The aim was to investigate the effects of ovariectomy and estradiol (E2) supplementation on the secretome of gonadal WAT (gWAT) and its influence on endothelial function. *In vivo, ex vivo* and *in vitro* studies were performed as follow. Fifteen-week-old ZDF (Zucker Diabetic Fatty) female rats were used: SHAM (sham-operated), OVA (ovariectomized) and OVA+E2 (ovariectomized and treated with E2 (3 μg/day) for 5 weeks). HUVEC endothelial cells were cultured with gWAT-conditioned medium obtained from each group combined with TNFα (10ng/ml). The expression of proinflammatory adipokines and chemokines was analyzed in gWAT, as well as the levels of adiponectin in conditioned media. Markers of mitochondrial and endothelial function were assayed in HUVEC cells incubated with conditioned media. In diabetic and obese rats, ovariectomy reduced the expression of proinflammatory adipokines and chemokines and stimulated adiponectin secretion. In contrast, E2 supplementation of OVA rats increased the inflammatory profile of WAT. In addition, the treatment of HUVEC cells with OVA-conditioned medium improved mitochondrial function and reduced the expression of markers of endothelial dysfunction and inflammation. This effect was not observed when HUVEC cells were treated with OVA+E2-conditioned medium. In conclusion, in an inflammatory environment, E2 no longer exerts protective effects on endothelial function. Our results highlight hormonal status as a crucial factor to be considered in the strategies for the prevention and treatment of CVD in the context of Metabolic Syndrome.

This work has been funded by MINECO (SAF2016-80384-R). MR M-C was funded by a grant from Balearic Islands Government (FPI/1888/2016) co-financed by the European Social Fund (FSE). A G-V was funded by a contract from Garantía Juvenil SOIB co-financed by FSE.

0468-R/M-P

Translational evidence of the additive antitumor effects of metformin and statins in brain tumors

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Glioblastoma (GBM), the most aggressive glioma, remains the deadliest human brain cancer, with poor prognosis despite years of research. Current standard therapies to treat GBM are not efficient and overall survival from diagnosis is ~14 months [1]. Thus, identification of new therapeutic tools to tackle GBM is urgently needed. In this sense, metformin (MF) and statins [e.g. Simvastatin- (SVT)] have emerged as antitumor agents for several endocrine-related cancers [2-3]. Thus, our aims were to evaluate: i) the putative *in vivo* association between MF and/or SVT treatment and key clinical parameters, and ii) the direct effects of MF, SVT and their combination, on key functional endpoints and associated signaling mechanisms. An exploratory/observational retrospective cohort of patients with GBM (n=61; mean age: 63.9±5.5) was analyzed. Moreover, human GBM cells (U-87MG/U-118MG cell-lines and patient derived-GBM cells) were used to measure a set of key tumoral parameters and signaling pathways in response to MF, SVT and their combination. MF/SVT combination showed an association to longer overall survival *in vivo*. Moreover, MF and SVT exerted strong antitumor actions (i.e. inhibition of proliferation, migration, tumorsphere/colony-formation, VEGF-secretion and increase of apoptosis) on GBM cells, and that their combination further decreased, additively, these functional parameters compared with the individual treatments. These individual or combined actions were mediated through modulation of key oncogenic signaling-pathways (i.e. AKT, JAK/STAT, NFkB and TGFß pathways). Interestingly, an enrichment analysis uncovered an activation of TGFß pathway together with the AKT inactivation when MF and SVT were administered in combination, which might promote to a senescence state by a senescence-associated secretory phenotype. Altogether, our results demonstrate that MF and SVT significantly reduce tumor aggressiveness in GBM, being this effect more potent (*in vitro* and *in vivo*) when both drugs are combined. Therefore, given the demonstrated clinical safety of biguanides, such as MF, and statins (SVT), our results suggest a potential therapeutic role of these compounds, especially their combination, for the treatment of this devastating brain cancer.

1.Ostrom QT et al; 2019.CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2012–2016. Neuro-oncology. 2.Jiménez JM et al; 2020.Clinical, cellular and molecular evidence of the additive antitumor effects of biguanides and statins in prostate cancer. J Clin Endocrinol Metab. 3.Vázquez MC et al; 2019.Biguanides Exert Antitumoral Actions in Pituitary Tumor Cells Through AMPK-Dependent and -Independent Mechanisms.J Clin Endocrinol

0473-P

Role of succinate and its receptor SUCNR1 in the pathophysiology of NAFLD

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Non-alcoholic fatty liver disease (NAFLD), which is strongly associated with obesity, insulin resistance, type 2 diabetes mellitus and cardiovascular complications, is the most common cause of chronic liver disease, with a global prevalence of 25%. However, neither fully validated non-invasive biomarkers nor effective therapies are available for its diagnosis and treatment1. Succinate is a signaling metabolite, produced by both the host and the gut microbiota, that acts through the succinate receptor 1 (SUCNR1). Circulating levels of this molecule are increased in inflammatory diseases, including NAFLD, and its action through SUCNR1 has been related to liver fibrosis2. Nevertheless, the effect of succinate signaling and dynamics in hepatic steatosis, glucose homeostasis, inflammation and liver physiology has not been deeply investigated.Our hypothesis is that succinate/SUCNR1 might have a role in the spectrum of NAFLD pathophysiology. The main objective is to study the local and systemic mechanisms of action of suc-

cinate by combining clinical studies with basic research. To achieve it, several metabolic, gene, protein and staining assays in wild type, total Sucnr1 knockout mice and cell cultures were performed. Circulating levels of succinate in a well-stablished cohort of subjects with different degrees of liver disease were also evaluated. Sucnr1 expression was differentially distributed in liver, which could be related to the functional compartmentalization of this tissue. Remarkably, in response to a choline-deficient high-fat diet to promote NAFLD, Sucnr1 and plasma membrane succinate transporters were upregulated in mice liver tissue, isolated hepatocytes and Kupffer cells, which was not detected in diet-induced obesity. In this preclinical model, SUCNR1 deficiency reduced fibrosis, but increased steatosis, a phenotype associated with glucose intolerance. Besides, in vitro experiments suggested that succinate/SUCNR1 might regulate glycogen metabolism in hepatocytes. Finally, patients with steatosis and fibrosis showed higher circulating succinate levels. Overall, our results indicate that succinate dynamics and its signaling through SUCNR1 in the liver may have a key role regulating several of the alterations driving NAFLD.

1. Younossi, Z. M. et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 64, 73–84 (2016). 2. Fernández-Veledo, S. & Vendrell, J. Gut microbiota-derived succinate: Friend or foe in human metabolic diseases? Rev. Endocr. Metab. Disord. 20, 439–447 (2019).

0474-P

Estrogens increase markers of endothelial dysfunction in obese diabetic female rats

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Introduction: Males are at higher risk of cardiovascular diseases, partly because the protective effect exerted by estrogens on endothelial function. However, diabetic women are at significantly higher risk of developing cardiovascular diseases than their male counterparts, suggesting that metabolic syndrome could have an attenuating effect on the cardio metabolic protection conferred by estrogens1. Thus, our aim was to study the effect of estrogens on endothelial dysfunction using an animal model of diabesity and menopause.

Methods: Zucker Diabetic fatty rats (ZDF, fa/fa) and female lean littermates (ZDF, fa/+) were used. ZDF (fa/fa) rats were randomly divided in three groups: SHAM (sham-operated), OVA (ovariectomized) and OVA+E2 (ovariectomized and treated with 3μg/day of 17β-estradiol, E2). At week 14, oral glucose tolerance and insulin sensitivity tests were carried out and, one week later, rats were euthanized to collect blood and aorta. Circulating levels of glucose, HbA1c and adiponectin were assayed in serum samples. In aorta, gene expression of markers of endothelial function were determined by RT-PCR.

Results: In ZDF rats, ovariectomy improved glucose tolerance, insulin sensitivity, adiponectinemia and decreased both fasting glycemia and HbA1c. Moreover, aortic mRNA levels of iNOS, eNOS, VEGFA, PGC1α, PGC1β were increased, whereas those of TGFβ1, COL1A, COL3A, VCAM and PAI-1 were decreased. E2 treatment reverted these effects in all cases but insulin sensitivity, HBA1c levels and VEGFA, PGC1α and VCAM mRNA levels.

Conclusions: In the aorta of diabetic and obese rats, estrogens are associated to a higher expression of markers of fibrosis and endothelial dysfunction suggesting a greater impairment of vascular homeostasis, in accordance with the intensification of the diabetic condition observed.

This work has been funded by MINECO (SAF2016-80384-R). MR M-C was funded by a grant from Balearic Islands Government (FPI/1888/2016) co-financed by the European Social Fund (FSE). A G-V was funded by a contract from Garantía Juvenil SOIB co-financed by FSE.

1. A. Haider et al. Sex and gender in cardiovascular medicine: presentation and outcomes of acute coronary syndrome. European Health Journal (2020) 41, 1328-1336.

0486-R/M-P

Development and characterization of induced pluripotent stem cells harbouring a mitochondrial DNA deletion.

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Pearson syndrome (PS) is a rare multisystem disease caused by single large scale mitochondrial DNA deletions (SLSMDs). PS presents early in infancy and it is mainly characterized by sideroblastic anaemia treated by blood transfusions. Treatment is supportive and patients usually pass away early in childhood, but in some cases, they develop Kearns-Sayre syndrome (KSS) with multiorgan effect including neurological symptoms. Thus, development of new models for the study of these pathologies and new therapy strategies is essential.

In this communication we report the generation of induced pluripotent stem cells (iPSC) carrying a mitochondrial DNA deletion by reprogramming fibroblasts from PS patient with "common deletion". We show the validation of this new cell line by determining different parameters representative of pluripotent cells, including their capacity to differentiate into three germ layers. Our iPSCs maintain a high heteroplasmy level during culture and they show an important mitochondrial dysfunction. Therefore, the presence of the deletion does not affect the pluripotency of defective line. However, we observe that mtDNA deletion impair the correct neural differentiation of iPSC determined by morphological characterization and quantification of mRNA and protein levels of specific markers. Thus, this new model will be useful for the *in vitro* study of the pathophysiology of mtDNA deletions in specific cell types affected in PS and KSS patients and the development of new treatments.

Funding: FIS PI17/00021; Asociación de Amigos "Todos con Javier"; AEPMI.

0495-P

Lung mesenchymal stem cells from idiopathic pulmonary fibrosis patients present senescence features and express αSMA myofibroblast marker

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Introduction: Idiopathic pulmonary fibrosis (IPF) is an age-related disease characterized by progressive accumulation of extracellular matrix (ECM) proteins and lung functional decline. Mechanisms involved in IPF pathogenesis include a regenerative deficit, dysregulated repair activity and myofibroblast activation. However, the role of lung mesenchymal stem cells (LMSC) in IPF pathogenesis is still unknown. **Objective:** In this study we conducted a cellular, functional and molecular comparative characterization of IPF and non-IPF LMSC to evaluate their senescence and potential differentiation to myofibroblasts. **Results:** IPF LMSC presented signs of cellular senescence, such as an enlarged cytoplasm, a heterochromatic foci appearance and higher lipofuscin deposition. Presto Blue assay indicated that IPF LMSC present a lower metabolic activity than non-IPF LMSC (P<0,05), which could be linked to the impaired wound healing observed 48h after scratching LMSC monolayer (P<0,05). When a scratch was applied in epithelial A549 co-cultured with LMSC (1:1), only non-IPF LMSC enhanced wound closure after 48h compared to A549 alone (P<0,01). In indirect co-culture, despite differences between IPF vs non-IPF were not observed, only non-IPF LMSC induced epithelial closure after 48h compared to A549 alone (P<0,05). KEGG pathway analysis of microarray data revealed a decrease in ribosomal proteins and translation initiation factors, suggesting a limited capacity for protein synthesis. The mRNA levels of PAI-1/β2M (plasminogen activator inhibitor type-1/β-2-microglobulin), a senescence marker involved in ECM balance, were 1.1 ± 0.1 (non-IPF) and 1.8 ± 0.6 (IPF). Furthermore, the protein levels of αSMA (alpha smooth muscle actin), a myofibroblast activation marker, were 4.6-fold higher in IPF vs non-IPF LMSC. **Conclusions:** IPF LMSC present a particular phenotype characterized by the presence of cellular senescence, that could explain their limited regenerative and repair potential, and high αSMA expression, suggesting that they have acquired a myofibroblast-like phenotype and might actively contribute to IPF pathogenesis.

02.- Developmental Biology and Genomic Modification

0514-OI

Cell behaviours and cell-cell signaling involved in the development of the inner ear ganglion

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Specialized sensory systems mediate the acquisition of the external world information. Of those, the inner ear is devoted in receiving and transmitting auditory and head movement inputs to the brain. Inner ear neuronal progenitors are first specified within the otic placode and subsequently neuroblasts delaminate out of the otic epithelium where they migrate, coalesce to finally differentiate into mature neurons. Little is known of how this neuroblasts spatially organize within the ganglion, but their correct distribution and differentiation is fundamental for a proper circuitry. Through single cell labeling and in vivo imaging we have determined the migratory behaviours of the neuronal progenitors and the establishment of several neuronal lobes within the zebrafish ganglion. We have identified an early population of pioneer cells that recruit the delaminating neuroblasts and by extending pioneer axons help neuroblasts migration. High spatiotemporal imaging has also revealed that neuroblasts communicate to each other and to the adjacent endothelial cells through cytonemes. Initially, head vasculature regulates neuroblast quiescence, limiting the number of neuronal progenitors. Later, blood flow initiation activates a metabolic switch to OxPhos, together with the promotion of cranial ganglia differentiation. In contrast, no role of sensory neuroblasts in vascular development is found, suggesting unidirectional signaling from vasculature to sensory neuroblasts. Altogether, we start to understand the signals and neuronal behaviours involved in the 3D organization of the otic ganglion.

Taberner I. Bañón A, Alsina B. (2020). Sensory neuroblast quiescence depends on vascular cytoneme contacts and sensory neuronal differentiation requires initiation of blood flow. Cell Reports 32 (2), 107903

0527-M-OI

A bi-stable Turing mechanism for size-independent symmetry breaking of mouse embryoid bodies

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When cultured in suspension, mouse embryonic stem cells form spheroids called Embryoid Bodies (EBs) that spontaneously break their symmetry. This event is characterized by a polarized expression of Brachyury, which resembles the formation of anterior-posterior axis in the mouse embryo. The mechanism that underlies this symmetry breaking process however remains unknown. By using light-sheet microscopy and hight-troughput imaging, we show that EB symmetry breaking is a size-independent process that is characterized by a propagating wave of Brachyury expression. These scaling self-organizing dynamics can be recapitulated by a three-dimensional bi-stable Turing model. In agreement with model predictions, we find that although differently sized EBs form only one axis, the onset of symmetry breaking is delayed with increasing size. Finally, we show that Nodal signaling controls the expansion of the mesendodermal wavefront.

0022-R/M-OS

A new role of Cdc14 phosphatases in stem cell differentiation

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The proper progression through the different phases of the cell cycle is controlled by the sequential activation of cyclin-dependent kinases (CDKs)1. Whereas the regulatory importance of kinases in the cell cycle has been extensively studied, less focus has been given to phosphatases. In yeast, the main CDK counteracting phosphatase is Cdc14, which is essential for mitotic exit2. Although Cdc14 phosphatase family is conserved, its relevance during mitotic exit in higher organisms remains unclear due to the existence of contradictory results in different studies with cell lines3-8, and the possible redundancy between isoforms. In this work we investigate the physiological role of Cdc14 phosphatases in mammals *in vivo* by generating for the first time a Cdc14a and b double knockout mice. We show that Cdc14a/b are not essential for mitotic exit but control the differentiation of embryonic stem cells through the modification of epigenetic regulators such as Utf1. Therefore, we unravel novel functions of Cdc14 phosphatases in mammals and propose a new link between the cell cycle machinery and the differentiation of stem cells through the control of epigenetic modifications.

1. Malumbres M, Barbacid M. 2009 Mar;9(3):153-66. 2. Visintin, R. et al. Mol. Cell 2, 709–718 (1998). 3. Mailand, N. et al. Nat. Cell Biol. 4, 318–322 (2002). 4. Cho, H. P. et al. Mol. Cell. Biol. 25, 4541–4551 (2005). 5. Wu, J. et al. J. Cell Biol. 181, 475–483 (2008). 6. Dryden, S. C. et al. Mol. Cell. Biol. 23, 3173–3185 (2003). 7. Berdougo, E. et al. Cell Cycle Georget. Tex 7, 1184–1190 (2008). 8. Mocciaro, A. et al. J. Cell Biol. 189, 631–639 (2010).

0141-OS

Yap directs cell migration required for axis condensation during gastrulation.

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Gastrulation is a crucial process during development, not only for it entails the specification of the germ layers but also because it results in the assembly of the main body axis in all bilaterian animals. The condensation of this embryonic axis relies in complex morphogenetic movements including lateral cell intercalation and direct cell migration. However, little is known on how gastrulating cells interpret the morphogenetic forces and mechanical properties of their environment.

One of the more prominent mechanotransducers identified so far is YAP, which sense environmental cues and is able to transduce them into transcriptional programs. We have seen that knocking out Yap1 together with its paralog Yap1b in medaka embryos result not only in severe body flattening and eye malformations, but also in earlier defects in axis condensation. Using live quantitative imaging, we have observed that mutant cells display reduced displacement and migratory persistence. Through a RNA-seq analysis we showed that the genes affected by Yap absence are mainly controlling cytoskeleton organization and ECM-cell adhesion. To better understand the activation of Yap-dependent programs, we focused on two direct downstream candidates, Tead and Marcks. The expression dynamics of these genes indicate that Yap is acting in the migratory cells per se, rather than acting as a midline beacon to direct the migratory trajectories of gastrulation precursors. By analyzing cell shape changes, cytoskeleton organization, and cell adhesion components distribution, we concluded that mutants cells display a deficient adhesion to the substrate and lack the spreading cell shape of wild-type cells.

Our findings suggest that Yap is involve in a mechanical regulatory loop that maintains the directional migration of gastrulating cells towards the midline, where they contribute to the condensation of the embryo axis.

0277-R-OS

DIET EFFECTS ON MOUSE SPERM: A WARNING FOR RECOMBINATION STUDIES

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The observed decrease in sperm quality in healthy men worldwide has fuelled the interest for the effect of environmental exposures on the germline, as some of them have proven to compromise fertility and offspring survival. While the initial studies focused on toxicants, it has been shown that life style and diets can also have an impact on the germline. Most alterations caused by diets have been reported in reproductive organs or in the final products (ova or sperm). However, we have found a novel and unexpected effect of nutrition during mouse spermatogenesis: we observe that the frequency of recombination, or the exchange of genetic information between homologous chromosomes during meiosis, changes significantly in spermatocytes of mice fed with certain diets. This is surprising for two reasons: first, because recombination is a very tightly controlled process, as alterations in crossover number or distribution can result in chromosome missegregation and aneuploidy. And second, because while recombination rate can indeed remain unaltered after large dietary changes, it can be sensitive to small differences between common chows used in animal facilities. This sensitivity is strain-dependent, as recombination in various inbred strains responds differently to diverse nutritional exposures and, hence, it is genetically controlled. In addition, we observe that nutrition changes can affect sperm motility, though it is unclear if both diet effects are related or not. Therefore, diet constitutes a novel factor that should be taken into account in meiotic recombination studies, with potential effects on fertility.

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0476-OS

Pulmonary surfactant protein SP-B promotes intermembrane connections that facilitate endosomal escape and cytosolic siRNA delivery by SP-B-decorated nanoassemblies

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Extensive research has revealed that pulmonary surfactant protein SP-B is the key element for lung surfactant to form efficient surface-active films stabilizing the respiratory surface along breathing dynamics. It does so by assembling channels interconnecting phospholipid layers, through which surface-active phospholipids can rapidly flow to rich the air-liquid interface [1]. At the same time, SP-B makes membranes permeable to large polar polymers, introducing a highly dynamic character into surfactant membrane assemblies [2].

Interestingly, recent in vitro and in vivo work has revealed that the incorporation of SP-B into lipid-coated nanogel particles can significantly enhance cytosolic siRNA delivery in different cell lines, leading to a more efficient gene silencing [3,4]. Mechanistic studies suggest that SP-B could promote this effect by mediating membrane fusion of siRNA-bearing nanoparticles with late endosomes, facilitating the escape of RNA into the cytosol [5]. This is consistent with recent structure-function studies suggesting that the use of siRNA-loaded nanoparticles for silencing of disease-promoting genes finds a major barrier at the endosomal sequestration of nanocarriers.

These results open novel promising perspectives in the design of new therapeutic tools for gene silencing and other potential gene-based interventions through integration of pulmonary surfactant elements into inhaled nanodrugs.

[1] Liekkinen et al. (2020) J. Mol. Biol. 432, 3251. [2] Martinez-Calle et al. (2021) J. Mol. Biol. 433, 166749. [3] Merckx et al. (2018) Acta Biomaterialia 78, 236 [4] Guagliardo et al. (2019) Pharmaceutics 11, E431 [5] Guagliardo et al. (2021) ACS Nano (doi: 10.1021/acsnano.0c04489)

0041-P

BET-PROTAC, MZ1 sinergyzes with trastuzumab in HER2 positive breast cancer

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Introduction
Although that the anti-HER2 antibody trastuzumab enhances survival of patients with HER2+ breast cancer, a relevant number of patients progress with this treatment. In this context, new drug combinations are needed to improve its antitumor activity. In this work, we have evaluated the efficacy of proteolysis-targeted chimera compounds (PROTAC) based on BET inhibitors (BETi) to increase trastuzumab activity in HER2+ breast cancer models.

Material and methods
HER2+ breast cancer cell lines BT474 and SKBR3 were used. The effects of trastuzumab and BET-PROTAC MZ1, alone or in combination, were tested by MTT proliferation assays, three-dimensional invasion and adhesion cultures, flow cytometry, qPCR and Western blotting. In vivo studies were performed in a xenografted mouse model. Finally, a Clariom_S_Human transcriptomic array was applied to identify downregulated genes following treatments.

Results
MZ1 induced a stronger antiproliferative effect compared to BETi JQ1. The combination of MZ1 and trastuzumab significantly decreased cell proliferation, 3D structure formation and cell invasion compared to either drug alone. Assessment of apoptosis resulted in increased cell death after treatment with the combination, and biochemical studies showed modifications of the components of apoptosis and DNA damage. In vivo administration of the drugs, alone or in combination, to orthotopically xenografted tumors in mice resulted in a decrease in tumor volume only after MZ1-Trastuzumab combination treatment. The results of a transcriptomic array indicated a number of newly described transcription factors, including HOXB7, MEIS2, TCERG1 and DNAJC2, which were associated with poor outcome in the HER2+ breast cancer subtype and were down-regulated by the MZ1-trastuzumab combination.

Conclusion
We describe a novel active combination involving-BET-PROTAC MZ1 and trastuzumab, in HER2+ tumors. Further studies should be performed to confirm these findings and pave the route for its future clinical development.

Noblejas-López MDM, Nieto-Jiménez C, et al. MZ1 co-operates with trastuzumab in HER2 positive breast cancer. J Exp Clin Cancer Res. 2021 Mar 19;40(1):106. doi: 10.1186/s13046-021-01907-9. PMID: 33741018; PMCID: PMC7980639. Noblejas-López MDM, Nieto-Jimenez C, et al. Activity of BET-proteolysis targeting chimeric (PROTAC) compounds in triple negative breast cancer. J Exp Clin Cancer Res. 2019 Aug 30;38(1):383. doi: 10.1186/s13046-019-1387-5. PMID: 31470872; PMCID: PMC6717344.

0148-P

Scramb1 interactome: Deciphering Scramb1 role in nephrocyte slit diaphragm.

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The podocyte slit diaphragm (SD) is a specialized junction required for blood filtration. Remarkably, damage to these structures gives rise to multiple kidney diseases. A homologous structure is also found in *Drosophila* nephrocytes, allowing the use of this model organism to gain insights into SD formation, damage, maintenance, and repair (Weavers et al., 2009) . Recent work from our group found that Scramb1, a protein with phospholipid scramblase activity, is required for the correct nephrocyte SD formation, since in null mutants these structures do not form.

In this work we aimed to identify protein interactors of Scramb1 that could help us to shed light on the processes of SD formation and maintenance, and on Scramb1 contribution to these events. For that purpose, we overexpressed a tagged isoform of Scramb1 protein in *Drosophila* larvae fat body. Afterwards we obtained whole protein extracts and Immunoprecipitated (IP) Scramb1. Finally, the Co-Immunoprecipitated (Co-IP) proteins were analyzed by Mass Spectrometry (MS). With this proteomic approach we identified a subset of proteins that interact with Scramb1 in *Drosophila*. Among the identified proteins, there were members of the tetraspanin and flotillin families, both responsible for membrane microdomains definition. Proteins implicated in vesicle trafficking and other proteins related to membrane targeting were identified too. Currently we are validating these interactions through genetic approaches.

Weavers, H., Prieto-Sánchez, S., Grawe, F., Garcia-López, A., Artero, R., Wilsch-Bräuninger, M., Ruiz-Gómez, M., Skaer, H. and Denholm, B. (2009). The insect nephrocyte is a podocyte-like cell with a filtration slit diaphragm. Nature 457, 322–326.

0241-P

Ret-independent control of seminal vesicle development by a Sprouty/β-catenin pathway

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Sprouty proteins are inhibitors of receptor tyrosine kinase signalling playing key roles during embryonic development and tumour progression. Mice deficient in Sprouty1 have genitourinary development defects resulting in supernumerary kidneys and dilated ureters. We have generated knockin mice bearing a Tyrosine to Alanine mutation in residue 53 of Spry1. Analysis of Spry1Y53A/Y53A mice indicate that this Tyrosine is absolutely required for Spry1 function during genitourinary development, as knockin mice phenocopy Spry1 knockout mice. Interestingly, male Spry1Y53A/+ animals present abnormally duplicated seminal vesicles. This defect is also found in Spry1/Spry2 double heterozygous mice indicating that these family members cooperate in shaping male internal genitalia. Unlike renal defects, seminal vesicle duplication is not caused by excessive Ret signalling, as ablation of both Ret alleles does not rescue internal genital defects found in double het mice. Instead, we have found that mutant seminal vesicles present a slightly decreased activation of the Wnt/b-cat pathway. Accordingly, removal of one allele of the Wnt/b-cat pathway feedback inhibitor Axin2 partially rescues defects found in both Spry1Y53A and double het mice.

Vaquero, M., Cuesta, S., Anerillas, C., Altés, G., Ribera, J., Basson, M. A., ... & Encinas, M. (2019). Sprouty1 controls genitourinary development via its N-terminal tyrosine. Journal of the American Society of Nephrology, 30(8), 1398-1411. Murata, T., Ishitsuka, Y., Karouji, K., Kaneda, H., Toki, H., Nakai, Y., ... & Gondo, Y. (2014). β-catenin C429S mice exhibit sterility consequent to spatiotemporally sustained Wnt signalling in the internal genitalia. Scientific reports, 4(1), 1-7.

0310-P

Accelerated dissipation of emerging contaminants in different media using microbial extracts

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The presence of emerging contaminants such as pharmaceutical and personal care products (PPCPs) in the environment are raising growing concern (Wilkinson *et al.*, 2017) as, even at low concentrations, some may affect microbiota (Aguilar-Romero et al., 2020) or fauna (Farré *et al.*, 2008) in natural ecosystems. Therefore, it is necessary to develop new technologies for cleaning up contaminated water resources. Biopurification systems (BPS) based on active biomixtures composed of soil and organic wastes are an efficient method to remove organic contaminants such as pesticides (Delgado-Moreno *et al.,* 2017) and PPCPs from wastewater (Delgado-Moreno *et al.,* 2019, Aguilar-Romero *et al.*, 2020). Recently, a new bioaugmentation strategy

based on aqueous extracts from aged residual biomixtures from BPS has proven to be successful for enhancing the removal of phenylurea pesticides (Aguilar-Romero *et al.*, 2019).

The current study describes how aqueous aerated extracts from a biomixture acclimated with three PPCPs; ibuprofen, diclofenac and triclosan, can be used to accelerate the removal of these emerging contaminants in BPS and in contaminated aqueous solutions. In this manner, the application of extracts to BPS led to the dissipation of 90% of diclofenac and triclosan, 69 and 108 days faster than controls. Moreover, the amount of the metabolite methyl-triclosan in extract-amended BPS was determined to be 12 times lower than in controls. In bioaugmented solutions, ibuprofen was almost completely eliminated (99 %) within 21 days and its hydroxylated metabolites were also determined to be at lower levels than in the controls.

The bacterial community structure and composition was also studied in biomixtures and extracts. Several dominant OTUs found in the extract, such as *Flavobacterium*-, *Thermomicrobia*-, *Nonomuraea*- and *Fluviicola*-related OTUs, could be responsible for the enhanced dissipation of these contaminants. Moreover, in order to determine to what extent genes related to degradation may be carried on mobile elements within the bacterial populations in the different BPS and extracts, the plasmidomes were studied as well.

This study was supported by the Spanish Ministry of Science and Innovation (projects CTM2013-44271-R and CTM2017-86504-R) and European FEDER funds.

1.- Aguilar-Romero et al., 2019. Sci. Total Environ., 691, 749-759. 2.- Aguilar-Romero et al., 2020. Sci. Total Environ., 741, 140461. 3.- Delgado-Moreno et al., 2017. J. Environ. Manage., 204, 160-169. 4.- Delgado-Moreno et al., 2019. Sci. Total Environ. 651, 990-997. 5.- Farre et al., 2008. Trends Anal. Chem, 27(11), 991-1007. 6.- Wilkinson et al., 2017. Environ. Pollut. 231, 954-970.

0421-P

DEVELOPMENT OF AN IN VITRO MODEL OF CELL COMPETITION IN CARDIOMYOCYTES

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INTRODUCTION
Cell competition (CC) is a mechanism by which some cells can eliminate others when comparing their metabolic abilities, thus selecting those fitter for a given tissue function. This process plays a crucial role in development and adult tissue quality control, homeostasis, and repair – even in cancer. In metabolic-driven CC, cells with higher anabolism are able to induce apoptosis of their otherwise viable neighbors (1) .

Previous studies have shown that CC can be induced in the murine adult heart by inducing Myc mosaic overexpression. Consequently, these cells eliminate and substitute their viable neighbors without any deleterious phenotype (2) . This in vivo discovery opens up the possibility of promoting cell competition in heart regeneration therapeutic approaches, which are warranted after heart injury, since the adult mammalian heart cannot regenerate. The lack of in vitro models that recapitulate this process is paramount in understanding underlying mechanisms.

MATERIALS AND METHODS
In order to generate an in vitro model for CC, we extracted cardiomyocytes from P1 mouse hearts, which are disaggregated using a MACs gentle tissue disruptor followed by cell culture as per manufacturer's instructions. iMOS-Myc mice (3) , containing a genetic construct that expresses c-Myc and a reporter (EYFP) in a mosaic manner upon induction were used. This activation was carried out by the administration of a modified RNA embedded in lipofectamine, which contains the elements to express Cre recombinase. After this, we fixed the cells and performed an immunofluorescence assay to acquire and analyze confocal microscopy images of the result.

RESULTS
We obtained a 69.6% of Myc induction within our culture's cardiomyocytes, representing an estimated 68.2% of the total cell count. We also detected some signs of cell death and other cell types.

DISCUSSION
Even though our methodology requires further improvements regarding cell confluence and survival, we are obtaining a similar Myc induction percentage to that in the *in vivo* model (in which Myc is overexpressed in 3 out of 4 cells) (2) . We are currently working on alternative approaches to improve the cardiomyocyte specificity and quality in our model.

1. Clavería C, Torres M. Cell Competition: Mechanisms and Physiological Roles. Annu Rev Cell Dev Biol. 2016;32:411–39. 2. Villa del Campo C, Clavería C, Sierra R, Torres M. Cell competition promotes phenotypically silent cardiomyocyte replacement in the mammalian heart. Cell Rep. 2014;8(6):1741–51. 3. Clavería C, Giovinazzo G, Sierra R, Torres M. Myc-driven endogenous cell competition in the early mammalian embryo. Nature. 2013;500(7460):39–44.

0471-R/M-P

Differential inflammasome-mediated response against amyloid-beta (Ab) exposure from cholesterol-primed neuronal and microglial cells

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Neuroinflammation is emerging as a real cause of Alzheimer's disease (AD) progression. Mainly mediated by microglia, the inflammatory response acts to remove Ab deposits and dead cells; however, improper activation of these cells may lead to a worsening of the pathology. The factors that influence the activation status of glia cells are still unraveled. Mitochondrial oxidative stress has been remarked as an activator of inflammasomes, pathogen/damage-induced assemblies that drive the inflammatory response and gasdermin-mediated cell death (pyroptosis). In this line, previous studies from our group have shown that cholesterol-induced depletion of mitochondrial GSH levels and subsequent enhanced mitochondrial oxidative stress elicited by Aβ leads to a worsening of pathology in APP-PSEN1 mice. Bearing this into consideration, we aimed to study the role of cholesterol on Aβ-induced inflammasome activation. For this purpose, the neuroblastoma cell line SH-SY5Y and microglia cell line SIM-A9 were cholesterol-enriched before exposure to LPS+muramyl dipeptide, Aβ, or serum deprivation. In SH-SY5Y cells, the rise of intracellular cholesterol stimulated the oligomerization of the inflammasome components and a shift to pyroptosis. The cholesterol-enhanced cell death was prevented by both caspase-1 inhibitor and GSH ethyl ester treatment. In contrast, cholesterol-enriched microglia showed a neuroprotective behavior accompanied by enhanced phagocytosis after exposure to inflammasome inducers. Remarkably, the microglial phagocytic function was completely abolished when cells were incubated with conditioned media from cholesterol plus Aβ-treated SH-SY5Y cells. Overall, these results highlight the differential contribution of cholesterol to Aβ-induced inflammasome activation in neuronal and microglial cells, which may ultimately condition the inflammatory response.

0490-P

Loss of kinesin-8 improves the robustness of the acentrosomal spindle

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Chromosome segregation in female meiosis in many metazoans is mediated by acentrosomal spindles, the existence of which implies that microtubule spindles self-assemble without the participation of the centrosomes. Although it is thought that acentrosomal meiosis is not conserved in fungi, we recently reported the formation of self-assembled microtubule arrays, which were able to segregate chromosomes, in fission yeast mutants where the contribution of the spindle pole body (SPB, the centrosome equivalent in yeast) was specifically blocked during meiosis. Here, we demonstrate that this unexpected microtubule formation represents a bonafide type of acentrosomal spindle. Moreover, a comparative analysis of these self-assembled spindles and the canonical SPB-dependent spindle reveals similarities and differences: for example, both spindles have a similar polarity, but the location of the γ-tubulin complex differs. We also show that the robustness of self-assembled spindles can be reinforced by eliminating kinesin-8 family members, whereas kinesin-8 mutants have an adverse impact on SPB-dependent spindles. Hence, we consider that reinforced self-assembled spindles in yeast will help to clarify the molecular mechanisms behind acentrosomal meiosis, a crucial step towards better understanding gametogenesis.

Fernández-Álvarez, A., C. Bez, E.T. O'Toole, M. Morphew, and J.P. Cooper. 2016. Mitotic Nuclear Envelope Breakdown and Spindle Nucleation Are Controlled by Interphase Contacts between Centromeres and the Nuclear Envelope. Developmental Cell. 39:544-559 Pineda-Santaella, A., and A. Fernández-Álvarez. 2019. Spindle assembly without spindle pole body insertion into the nuclear envelope in fission yeast meiosis. Chromosoma. 128:267-277

03.- Omic Molecular Biology and Bioinformatics

0172-R-OS

A Systematic Map of the Functional Role of Protein Phosphorylation

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Phosphorylation is a critical post-translational modification involved in the regulation of almost all cellular processes. However, less than 5% of thousands of recently discovered phosphorylation sites have a known function. Here, we devised a chemical genetic approach to study the functional relevance of phosphorylation in *S. cerevisiae*. We generated 474 phospho-deficient mutants that, along with the gene deletion library, were screened for fitness in 102 conditions. Of these, 42% exhibited growth phenotypes, suggesting these phosphosites are likely functional. We inferred their function based on the similarity of their growth profiles with that of gene deletions, and validated a subset by thermal proteome profiling and lipidomics. While some phospho-mutants showed loss-of-function phenotypes, a higher fraction exhibited phenotypes not seen in the corresponding gene deletion suggestive of a gain-of-function effect. For phosphosites conserved in humans, the severity of the yeast phenotypes is indicative of their human functional relevance. This study provides a cell signaling resource and a roadmap for functionally characterizing phosphorylation in a systematic manner.

0240-OS

A community-driven roadmap to advance Ribo-seq open reading frame research

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Since the completion of the Human Genome Project twenty years ago, the number of protein-coding genes in annotation catalogs has stayed relatively constant at approximately 20,000. However, recent multiple lines of evidence have discovered a large numbers of unannotated translated open reading frames (ORFs). At the forefront of this field is the experimental data from ribosome profiling (Ribo-seq), a technique for the high throughput sequencing of ribosome protected RNA fragments (Ingolia et al. 2009) that allows the identification of small translated ORFs in UTRs, long non-coding RNAs and alternative protein-coding frames. Ribo-seq datasets would seem to herald a paradigm shift in our understanding of the translational 'vocabulary' of the human genome. Due to the uncertainty about their general importance, the major annotation projects have thus far remained circumspect in their incorporation of ORFs newly identified by Ribo-seq ('Ribo-seq ORFs'). While few of these ORFs are conserved across vertebrates (Couso and Patraquim 2017), most of these elements have limited conservation to specific species or lineages (Ruiz-Orera et al. 2018). Consequently, their possible biological roles have been largely disregarded, as these cases do not fit the canonical dogma stating that conservation of function is the ultimate evolutionary driving force. Nonetheless, it is now indisputable that at least some Ribo-seq ORFs make stable microproteins (van Heesch et al. 2019), while the translation of many others has been implicated in gene regulation. As research groups worldwide have begun to probe ORF translations in the context of normal physiology and disease states, the absence of standardized ORF annotation is hampering progress in their discovery and investigation. Here we present a new community-led effort supported by Ensembl/GENCODE and UniProt to produce a consolidated catalog of published Ribo-seq ORFs in the human genome and understand the functional relevance of these elements.

0352-OS

Deeping into intracellular signaling landscape through integrative spatial proteomics and transcriptomic

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Background
Nowadays "omics" studies integrate 2 in 2 transcriptomic-proteomic-metabolomic data to improve genome knowledge, gen encoded protein correlation and explain cell molecular mechanisms. Moreover, due to advance in high-throughput analytical techniques and size exclusion chromatography, protein-protein complexes, PTMs, proteins subcellular location, intracellular pathways… have led to discovery biomarkers, therapeutic targets and novel proteins networks associated with disease[1-6]

Method
Here, RNAseq and LC-MS/MS studies, targeting 5166 & 5674 proteins respectively, has been orthogonally integrated with affinity proteomics (targeting 162 proteins with PTMs info of 34) combined with spatial proteomics tools (subcellular localization and MW by SEC) in lymphoma cell line as model.

Results
Spatial proteomics (total & partial) are optimized for dynamic determination of protein relative abundance, interaction detection and PTMs. Here a few examples are reported:

- Non-spatial proteomics identify well-characterized protein complexes like TP53-MDM2 (and associated PTMs).

- Spatial proteomics (low & high-resolution) detect protein complexes in main cellular localizations:

  - Membrane fraction between LYN-PLCG2-ZAP70 in PTM.
  - Organelle fraction interaction BTK-CD19-BLNK-SYK.
  - Nucleus fraction detected ZAP70-BLNK-PLCG2.

In addition, this orthogonal and systematic multi-omics integration allows to detect dynamics in protein complexes and cellular localization, ie. ERK2, P21, NFKB1, BCL-2 among others.

Conclusions
This approach allows us to perform, in one step, high-throughput co-immunoprecipitation orthogonally integrated with multi-omics, protein complexes analysis, protein state/isoforms and subcellular location.

[1] J. M. Buescher, E. M. Driggers, Cancer Metab. 2016, 4, 4. [2] P. Díez et. al., Proteomics 2016, 16, 1193. [3] Y. Subbannayya et. al., Expert Rev. Proteomics 2016, 13, 297. [4] A. V. Florinskaya et. al., Biochem. Mosc. Suppl. Ser. B Biomed. Chem. 2018, 12, 241. [5]M. Heusel et. al., Mol. Syst. Biol. 2019, 15, e8438. [6] P. Díez et. al., Front. Immunol. 2021, 12, 637832.

0430-OS

Improving the scoring of protein-protein docking models by statistical analysis of residue-residue contacts

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Given that the 3D structure of the majority of protein-protein interactions remains elusive, computational molecular docking algorithms is an alternative to structurally characterize protein interactions at large scale. One of the most successful approaches for rigid-body docking and scoring is pyDock (Cheng et al 2007), with excellent results in CAPRI and CASP blind-test assessments (Lensink et al 2019). Despite the success of pyDock energy-based scoring function in rigid, globular proteins forming strong complexes, we need to improve it for challenging cases with large conformational changes or weak complexes.

We previously observed that rigid-body docking models selected by pyDock scoring function contained information about the residues that were important for the complex energy (Fernández-Recio et al. 2004), which was applied to successfully predict interface and hot-spot residues (Grosdidier & Fernández-Recio, 2008). Now we hypothesize that the frequency of residue-residue contacts in such docking subsets contains energetics information that can be extracted by statistical analysis to improve modeling. Indeed, a similar approach called CONSRANK has been used to rank docking ensembles based on the most conserved inter-residue contacts (Chermak et al. 2015).

Here we have developed a new scoring protocol, pyDockConStat, based on a statistical analysis of residue-residue contacts at the docking interfaces in different sub-sets of models from pyDock. First, the frequencies of contact pairs were computed at different levels of detail. Then, we have used these values (raw frequencies and normalized propensities) to re-score the docking models. The predictive performance has been tested in selected cases from the protein-protein docking benchmark 5.0 (Vreven et al. 2015). In most of the cases, the new contact-based scoring outperforms pyDock scoring function, often showing an excellent correlation with the quality of the docking models.

Cheng T, Blundell TL, Fernandez-Recio J (2007) Proteins 68, 503-515. Chermak E, Petta A, Serra L et al. (2015) Bioinformatics 31, 1481-1483. Fernández-Recio J, Totrov M, Abagyan R (2004) J.Mol.Biol. 335, 843-865. Grosdidier S, Fernández-Recio J (2008) BMC Bioinformatics 9, 447. Lensink MF et al. (2019) Proteins 87, 1200-1211. Vreven T, Moal IH, Vangone A et al (2015) J.Mol.Biol. 427, 3031-3041.

0008-R-P

Advances in proteome digestion for the E. coli acetylome LC-MS/MS analysis

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Liquid chromatography coupled to mass spectrometry tandem (LC-MS/MS) has become a technique of routine use in molecular biology to study differential protein expression, protein interactions and post-translational modifications (PTMs). Sample digestion is an essential step in a LC-MS/MS analysis, and the correct election of a suitable protease will determine the results obtained through peptide sequences, length and charge (1).

In this work, *Escherichia coli* (*E. coli*) proteome digestion has been evaluated combining three proteases to carry out four different digestion alternatives: trypsin, trypsin/chymotrypsin, trypsin/GluC and chymotrypsin/GluC. Results showed that the highest number of different peptides was observed with trypsin, while worse results were reached when alternative endopeptidases were employed. However, proteins detected with each digestion protocol were different, so combining different proteases will led to a higher proteome coverage.

On the other hand, *E. coli* acetylome was studied with the four digestion alternatives. Trypsin cleaves the C-terminal peptide bonds of R and K. However, when a lysine is acetylated, trypsin is not able to recognize this residue (2). The results of the presented study showed that when proteins were digested with trypsin, the number of acetylated peptides detected was lower than when trypsin was not used or was combined with another endopeptidase. Thus, bioinformatic analysis indicated that, trypsin digestion was the worst option to detect acetylated lysines, while chymotrypsin/GluC digestion led to the highest number of acetylated peptides detected.

The results presented in this work open new alternatives of proteins digestion for the study of the global proteome and lysine acetylome.

This work was supported by grants from the Ministry of Science, Innovation and Universities (MCIU), the State Research Agency (AEI) and the European Regional Development Fund (FEDER), RTI2018094393BC21MCIU/AEI/FEDER, UE, and the Seneca Foundation CARM, 20786/PI/18.

(1) Sinitcyn P, Daniel Rudolph J, Cox J. Computational Methods for Understanding Mass Spectrometry–Based Shotgun Proteomics Data. Annu Rev Biomed Data Sci. 2018;1: 207–234. (2) Verdin E, Ott M. 50 years of protein acetylation: from gene regulation to epigenetics, metabolism and beyond. Nat Rev Mol Cell Biol. 2014;16.

0042-R/M-P

Whole transcriptome analysis on lungs of house dust mite-exposed IGF1R-deficient mice provides new insights in allergic airway inflammation

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Insulin-like Growth Factor 1 Receptor (IGF1R) is a trans-membrane tyrosine kinase that belongs to the IGF signaling system. IGF activity maintains lung homeostasis and it is involved in pulmonary diseases such as cancer, ARDS, COPD, asthma and fibrosis. IGF1R deficiency attenuates house dust mice (HDM)-mediated allergic lung inflammation (PLoS One 2016, 12: e0190159). To better understand the molecular mechanisms involved in acute asthma pathogenesis and their modulation by IGF signaling, we performed RNA-seq in lungs of IGF1R-deficient and control mice after seven days of HDM or PBS intranasal administration. Transcriptomic analysis identified a large number of differentially expressed genes between HDM-treated and untreated control mice. Functional enrichment detected biological processes and signaling pathways implicated in acute asthma biopathological features such as inflammation, airway remodeling, hypoxia response and mucus secretion. Analysis of differential gene expression due to IGF1R depletion in HDM challenged mice revealed reversal of a great part of the transcriptional regulation changes triggered by HDM challenge within those functional groups. Interestingly, data mining identified significant expression changes of gene clusters with key roles in mitochondrial homeostasis, metabolism and epigenetics, providing new insights into the molecular mechanisms underlying allergic airway inflammation and the implication of IGF1R signaling in this process. These findings allow a more comprehensive view of acute asthma pathogenesis at the regulatory level and reinforce IGF1R as a potential therapeutic target in allergic asthma.

1. Girnita et al. Cell Mol Life Sci 71:2403-2427 (2014) 2. Annunziata et al. Acta Diabetol 48:1-9 (2011) 3. Osorio et al. Nat Med 22: 91–96 (2016) 4. Takasaka et al. J Immunol 192: 958-968 (2014) 5. López et al. PloS One 11: e166388 (2016) 6. Zheng et al. BioMed Res Int 2018: 6057589 (2018) 7. López et al. Transgenic Res 24: 279-294 (2015) 8. Piñeiro-Hermida et al. Allergy 72:1317 (2017) 9. Piñeiro-Hermida et al. Plos One 12: e0190159 (2017)

0059-P

A Deep Learning algorithm for the detection and analysis of caveolae in Transmission Electron Microscopy images

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Centro Nacional de Investigaciones Cardiovasculares Mechanoadaptation and Caveolae Biology lab

Due to their nanoscopic size, caveolae can only be visualized by electron microscopy, a technique that provides colourless and complicated images, difficult to classify or segment using automated methods. As a result, localization and quantification of caveolae currently involves a considerable amount of tedious and manual work.

We used the Keras library in R to train a convolutional neural network with a total of 24000 image crops obtained from adipocytes in sections of mouse epididymal depots. Each crop was manually classified previously (by a human) in one of six possible categories: three caveolae types (single-pit, rosette, cavicle), extracellular matrix, cytoplasm or lipid droplet. The resulting model can differentiate caveolae from non-caveolae regions with a 96,9% accuracy.

This classification model constitutes the core of a tool that performs automated image analysis tasks such as caveolae coordinate detection, caveolar area estimation and cytoplasm boundary delimitation and classification (plasma membrane and lipid droplet). Morphological and contextual information is used to further improve caveolae and surface classification accuracy, including object circularity and relative position to cell limits or other caveolar objects.

Analysis of a complete adipocyte section shows that algorithm and human performance are comparable, and that the magnitude of model-human discrepancies is similar to those found between different human observers. The tool uses only free and open software, and can be easily customized and adapted to user requirements.

Del Pozo MA, Lolo FN, Echarri A. Caveolae: Mechanosensing and mechanotransduction devices linking membrane trafficking to mechanoadaptation. Curr Opin Cell Biol. 2021 Feb;68:113-123. Echarri A, Del Pozo MA. Caveolae - mechanosensitive membrane invaginations linked to actin filaments. J Cell Sci. 2015 Aug 1;128(15):2747-58. Parton RG, del Pozo MA. Caveolae as plasma membrane sensors, protectors and organizers. Nat Rev Mol Cell Biol. 2013 Feb;14(2):98-112.

0082-R-P

Identification of proteins altered involved in Alzheimer's Disease by quantitative proteomics

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Introduction and objective
Alzheimer's disease (AD) is a progressive and chronic neurodegenerative disease, which is the most common form of dementia worldwide. Still, the mechanisms underlying the disease are not well known [1]. Thus, the study of proteins involved in its pathogenesis would allow getting further insights into the disease and identifying new markers for early diagnosis of AD [2]. Here our objective is to analyze protein dysregulation in AD tissues by quantitative proteomics to identify proteins, which could be used as biomarkers or therapeutic targets and might be key for the understanding of the disease [3-4].

Methods
10-Plex TMT (Tandem Mass Tags)-based quantitative proteomics experiment was performed using frozen tissue samples from the left prefrontal cortex of AD patients at Braak stages IV to VI (n=12), and healthy individuals (n=2) and Vascular (n=2) and Frontotemporal dementia (n=5) patients as controls. LC-MS/MS analyses were performed on a Q-Exactive, and proteins differentially expressed in AD patients in comparison to controls were identified with MaxQuant and Perseus.

Results and discussion
In total, 3281 proteins were identified and quantified with MaxQuant. After data analysis with Perseus, we identified 31 and 250 proteins upregulated and downregulated, respectively, in AD patients in comparisons to controls, with a fold change ≥ 1.5 or ≤ 0.67 and a p-value < 0.05. After bioinformatics analysis, we selected 10 proteins dysregulated in AD to study their role in the pathogenesis. These proteins were validated by real-time qPCR, WB, IHC and ELISA using tissue and serum samples of AD patients, and patients with other dementias and healthy individuals as controls.

Conclusion
TMT-based quantitative proteomic experiments allowed us the identification of proteins altered in AD previously and non-previously related to the disease. These proteins were further validated as altered at mRNA and protein level in AD patients' brain tissues and blood. Our results suggest these proteins should play a major role in the disease and might become biomarkers or/and therapeutic targets of intervention.

[1] Masters CL et al. Alzheimer's disease. Nat Rev Dis Primers. 2015;1:15056 [2] Sery O, et al. Molecular mechanisms of neuropathological changes in Alzheimer's disease: a review. Folia Neuropathol. 2013;51(1):1-9 [3] Aebersold R, Mann M. Mass spectrometry-based proteomics. Nature. 2003;422(6928):198-207. [4] Barderas R, et al. Colorectal cancer proteomics, molecular characterization and biomarker discovery. Proteomics Clin Appl. 2010;4(2):159-78.

0108-P

Cyanobacterial Linked Genome: generation of functional predictions based on gene arrangement

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Unknown function genes still account for a significant percentage of the cyanobacterial genomes. Their low representation in databases usually plays against the ability of bioinformatics algorithms and tools, usually biased towards better studied phylogenetic groups, to make specific predictions for cyanobacteria. Here we present the result of a specific cyanobacterial gene arrangement analysis based exclusively on gene neighborhood and co-occur-

rence weighted using average nucleotide identity between genomes. Genes are pair-wise connected based on our Linked Score indicator of functional connection, generating working hypothesis independently of any model organism. The processed information of 124 cyanobacterial genomes was used to construct a public web tool with a user-friendly interface, the Cyanobacterial Linked Genome (https://dfgm.ua.es/genetica/investigacion/cyanobacterial_genetics/dCLG/), that generates flexible gene networks at custom threshold values. A default version at conservative threshold (dCLG) is used to analyze relationships between synteny, core genes and gene function, and to validate and gain additional insights into the CLG. Finally, we compare the dCLG to other bioinformatics approaches based on guilty-by-association principles, with selected examples of networks illustrating its usefulness for genes with no experimental information available, especially for genes found exclusively in cyanobacteria or in cyanobacteria and chloroplasts. We believe that this tool will provide useful predictions that are readily testable, thus accelerating functional genomics in cyanobacteria.

0109-P

Classification and Visualization of Glioblastoma Samples using 20-gene Expression-based Model

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The development of expression-based classification models has provided further insight into the molecular mechanisms of glioblastoma. The traditional four-subtype model1 has been replaced by a three-subtype model2 (Proneural, Mesenchymal and Classical) based on the expression of 150 genes. This model excludes the original Neural subtype, which was found to be highly enriched in normal tissue. Recently, the model was further optimized to achieve classification using a 20-gene signature, with an overlap of > 90%3. Not only does this system allow for the classification of the three main subtypes, but it also provides a way of discriminating samples with high levels of normal tissue. We applied the 20-gene classification system to the development of different classification algorithms (k-nearest neighbors, Random Forest, Naïve Bayes and Support Vector Machine (SVM)) in order to automate classification of new samples. Models were trained using Affymetrix data from the TCGA cohort (n = 357) and validated with samples from the Rembrandt cohort (n = 208). Performance was also evaluated on RNA-seq cohorts, the TCGA cohort (n = 126) and the CCGA cohort (n = 183). Reference subtype and simplicity score for each sample was determined using the classification method described by Wang et. al2. For each model and subtype, accuracy, sensitivity and specificity were determined, showing accuracies of up to 99% for the training cohort and 79% for the validation cohort. However, results are strongly dependent on simplicity score. This factor was studied through non-random sampling of the TCGA cohort, using simplicity score > 0.5 as a threshold. Similar results were achieved when studying the Rembrandt cohort. Visualization of results is done through PCA graphs in which samples can be mapped and colored, thus providing a powerful tool for intuitive interpretation of sample characteristics.

1.Verhaak, R. G. W. et al. Integrated Genomic Analysis Identifies Clinically Relevant Subtypes of Glioblastoma Characterized by Abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17, 98–110 (2010) 2.Wang, Q. et al. Tumor Evolution of Glioma-Intrinsic Gene Expression Subtypes Associates with Immunological Changes in the Microenvironment. Cancer Cell 32, 42-56.e6 (2017) 3.Madurga, R. et al. Normal tissue content impact on the GBM molecular classification. Brief. Bioinform. 00, 1–11 (2

0128-R-P

Proteins altered in colorectal cancer identified in paraffin-embedded tissue samples by mass spectrometry could be key for the study of the diseas

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Introduction and objective
Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related death [1]. A better understanding of the biology of CRC would help identifying specific markers of the disease that could be used as diagnostic and/or prognostic markers or become potential targets of intervention. Our objective is to analyze the differential protein expression in CRC tissue by quantitative proteomics to identify key proteins in the disease [2-3].

Methods
Two 10-plex tandem mass tag (TMT) experiments were performed using paired paraffin embedded tissue samples of adenoma, adenocarcinoma and healthy tissue from 6 different CRC patients [4]. After protein extraction, trypsin digestion and labeling, proteins were subjected to mass spectrometry analysis using a Q-Exactive. Subsequent data analysis performed using MaxQuant and Perseus identified and quantified proteins differentially expressed in the adenoma to adenocarcinoma transition in colorectal cancer.

Results and discussion
More than 3000 proteins were identified and quantified by mass spectrometry and bioinformatics analysis. After data analysis with MaxQuant and Perseus, 156 proteins were observed to be upregulated and 150 proteins downregulated (fold change ≥ 1.5 or ≤0.67 and a p-value < 0.05) in adenoma and/or adenocarcinoma in comparison to healthy tissues. Interestingly, among the identified proteins, we found proteins that had been previously described as altered in CRC, such as CDH17, CEA or FGFR4. Following bioinformatics analysis, we selected 31 altered proteins to study their role in CRC by orthogonal techniques (real-time qPCR, western blot, immunohistochemistry and tissue microarrays, and ELISA) using tissue and serum samples from CRC patients, individuals with premalignant colorectal lesions and controls.

Conclusion
TMT experiments allowed the identification of proteins altered in CRC previously and non-previously related to the disease using paraffin-embedded tissue samples. Although further *in vitro* and *in vivo* functional assays will be performed, these proteins could play a major role in the disease and/or be therapeutic target of interventions, as CDH17 or FGFR4.

[1] Masters CL et al. Alzheimer's disease. Nat Rev Dis Primers. 2015;1:15056 [2] Sery O, et al. Molecular mechanisms of neuropathological changes in Alzheimer's disease: a review. Folia Neuropathol. 2013;51(1):1-9 [3] Aebersold R, Mann M. Mass spectrometry-based proteomics. Nature. 2003;422(6928):198-207. [4] Barderas R, et al. Colorectal cancer proteomics, molecular characterization and biomarker discovery. Proteomics Clin Appl. 2010;4(2):159-78.

0247-P

Circulating miRNAs as diagnostic biomarkers for Limb-girdle muscular dystrophies

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Limb-girdle muscular dystrophy (LGMD, ORPHA 263) or Erb's muscular dystrophy is a genetically and clinically heterogeneous group of rare muscular dystrophies. It primarily affects the proximal muscles of the hip and shoulder girdles, leading to progressive muscular weakness. LGMD has an autosomal pattern of inheritance and currently has no known cure or treatment. It can be manifested from the first decade to late adult life. Current analysis of serum CK levels, muscle biopsy, muscle imaging and, more recently genetic test cannot discriminate among other clinically overlapping myopathies.

Although each of LGMDs disorders presents a specific muscle gene deregulated that leads to the onset and progression of disease, all of them share common phenotypes and clinical features. Our main goal is to elucidate some of these common factors in LGMDs and the molecular mechanisms underlying the disease. Since most likely there are several cell types being involved (myoblasts, osteoblasts and platelets), a broad approach is needed. Therefore, epigenetic factors such as miRNAs that affect osteoblasts and myoblasts transcriptomes have been studied.

The miRnome in plasma samples from 7 patients was obtained, and its analysis revealed several miRs differentially represented in both experimental groups (control *vs*.LGMD). Hierarchical clustering analysis was able to identify both groups in the datasets. Gender was found to be irrelevant in this clustering. The functional KEGG or GO analysis showed a proliferative and differentiation pathway-enrichment for the differentially represented miRs. Some of these miRs were already described in other muscular and scoliotic pathologies, reinforcing the relevance and specificity of our findings. Furthermore, miR-206 found to be up-regulated in LGMD group, is defined as a "myomiR" and can be easily measured in serum or plasma samples of patients. Consequently, it is a potential biomarker for diagnostic, prognostic or disease stratification of LGMD patients.

Grants: Fundación R Areces (OTR-2019-19499INVES/VIÑA RIBES, J.) to JRV.

[1] M. P. Wicklund, "The Limb-Girdle Muscular Dystrophies," CONTINUUM Lifelong Learning in Neurology, 25, 6 :1599–1618, 2019. Lippincott Williams and Wilkins. [2] V. Nigro and M. Savarese, "Genetic basis of limb-girdle muscular dystrophies: the 2014 update.," Acta Myol. myopathies cardiomyopathies Off. J. Mediterr. Soc. Myol., 33, 1: 1–12, 2014. [3] G. Ma et al. Int. J. Biol. Sci., 11, 3:345–352, 2015. [4] T. C. Roberts et al. Mol. Ther. - Nucleic Acids, 1, 8: e39, 2012.

0271-R/M-P

Comparison of bioinformatics tools through the Galaxy platform

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In recent years, New Generation Sequencing (NGS) techniques have experienced an increase in both in their development and use. This boom has led to the development of analysis tools to study the sequences obtained. For this reason, the objective of the study is to combine these tools to obtain different pipelines and observe how the number of differentially expressed genes (DEGs) varies between each of the pipelines, after performing an RNA-seq assay.

Cumulus cells, which are somatic cells that surround the oocyte and are closely related to it, were used to carry out this analysis, with the aim of finding genetic markers that are potential indicators of oocyte quality. These cells come from female oocytes that underwent to an intracytoplasmic sperm injection (ICSI).

All the tools used are found on the Galaxy platform (open source)1. Three tools (Star, HiSat and TopHat) were used to map the sequences, whose quality was previously vali-

dated (FASTQC), to the reference genome (Grch38). Normalization and quantification of the expression of each gene was carried out through FeatureCounts, HTSeq and StringTie, and the statistical analysis of expression difference was performed with DESeq2.

At least 95% of the sequences are mapped with the three tools, being 99% when using the STAR tool. HiSat was the fastest tool. A lower number of DEGs is obtained through TopHat, although at least 70% of genes are common when using the three tools in each of the comparisons. In the normalization and quantification stage, we have seen that the number of total counts obtained in each gene is the same using HTSeq and FeatureCounts tools, therefore the same DEGs are obtained with DESeq2. Furthermore, the degree of coincidence with the genes obtained through StringTie is at least 50% in each comparison.

Finally, taking into account factors such as the mapping percentage, work speed, number of DEGs, etc., the pipeline that seems to be the most efficient is HiSat-StringTie-Deseq2.

1. Afgan E., Baker D., van den Beek M., Blankenberg D., Bouvier D., et al., 2016 The Galaxy platform for accessible, reproducible and collaborative biomedical analyses:2016 update. Nucleic Acids Res. 44: W3–W10

0285-P

Unravelling the molecular basis of chemical communication in the Iberian lynx

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Understanding intra-specific communication is essential for the conservation of a species as it plays an important role in mate selection, social organization, territorial maintenance and orientation (1) . In mammals, chemical communication is mediated by olfactory signals deposited in the environment. These scent marks are composed of molecules that convey information about sex, age, reproductive state and social status of the owners. Receptor animals modify their physiology and behaviour in response to such signals and initiate a learning process, which help them to recognize a territory, a social group, an individual or their relationship with the owner.

The Iberian lynx (*Lynx pardinus*) is the most endangered feline species in the world. This species has been included in the Red List of threatened species since the end of the nineties, when a population survey revealed that fewer than a hundred individuals were left. This worrying estimate led to the urgent design of an action programme directed at reversing the dramatic rate of loss of this species. Due to the genetic erosion suffered by this species (2) , major interventions included an ex-situ breeding programme to increase the number of individuals. Unfortunately, our understanding of chemical communication in the Iberian lynx, a key aspect of its physiology, is still very scarce.

The aim of our project is to unravel the molecular basis of intra-specific communication in the Iberian lynx with the objective to exploit natural chemical signals to improve conservation programmes. In the present study, we show some pheromone candidates identified by using mass spectrometry to characterize the volatile, protein and peptide composition of urinary spray and facial marks. These molecules could be used to design more successful plans for the recovery and conservation of the Iberian lynx.

1. Wyatt TD. Pheromones and animal behaviour. United kingdom: Cambridge University Press; 2014. 2. Abascal F, Corvelo A, Cruz F, Villanueva-Canas JL, Vlasova A, Marcet-Houben M, et al. Extreme genomic erosion after recurrent demographic bottlenecks in the highly endangered Iberian lynx. Genome Biol. 2016;17(1):251.

0290-R/M-P

The table salt microbiome

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Commercial table salt (or Sodium chloride) is a condiment with food preservative properties due to its inhibitory effect on the growth of living cells by decreasing water activity and increasing osmotic pressure[1]. However, salt is also considered a source of salt-loving microorganisms, halophilic bacteria and archaea[2]. In the present research, diversity of halotolerant and halophilic microorganisms was studied in six commercial table salts by culture-dependent and -independent techniques. Table salt samples were from the marine origin (Atlantic, Ibiza and Supermarket salt) and larger granulated salts supplemented with other ingredients (Himalayan pink, Hawaiian black and Viking salt). Using different media with a broad range of salinity, various nutrients, trace elements and long-term incubation allowed us to set a collection of 76 species from slightly, moderately and extremely halophilic bacteria and six species from haloarchaea. Results of 16S rRNA metagenomic reveal that Ibiza salt displayed the highest biodiversity (with 335 identified taxa) among all samples. The salts from marine origin represent a similar taxonomy (mostly archaeal population) with relevant variations. Archaeal taxa, *Halorubrum*, *Halobacterium*, *Hallobellus*, *Natronomonas, Haloplanus*, *Halonotius*, *Halomarina*, and *Haloarcula*, were prevalent in those three salts. Furthermore, the frequent archaeal genera present in all salts were: *Natronomonas*, *Halolamina*, *Halonotius*, *Halapricum*, *Halobacterium*, *Haloarcula* and Uncultured Halobacterales. Comparing the results of 16S rRNA metagenomics and culturomics revels that viable eubacteria *Acinetobacter*, *Aquibacillus*, *Bacillus*, *Brevundimonas*, *Fictibacillus*, *Gracilibacillus*, *Halobacillus*, *Micrococcus*, *Oceanobacillus*, *Salibacterium*, *Salinibacter*, *Terribacillus*, *Thalassobacillus* and also archaea *Haloarcula*, *Halobacterium*, and *Halorubrum* were identified at least in one sample by both methods. Our results show that salts from marine origin were dominated by archaeaa whereas salts from other sources or salt with added ingredients were dominated by bacteria.

1. Albarracín, W., Sánchez, I. C., Grau, R., and Barat, J. M. (2011). Salt in food processing; usage and reduction: a review. Int J Food Sci Technol 46, 1329–1336. doi:https://doi.org/10.1111/j.1365-2621.2010.02492.x. 2. DasSarma, S., and DasSarma, P. (2017). "Halophiles," in eLS (American Cancer Society), 1–13. doi:10.1002/9780470015902.a0000394.pub4.

0295-P

Structural analysis of single-stranded RNA molecules using Atomic Force Microscopy and computational methods

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The structure adopted by a long noncoding single-stranded RNA molecule is a key determinant for its function in cellular processes, such as regulation. However, the majority of RNAs remain structurally uncharacterized and the relation between the nucleotide sequence and the final folded conformation is still unclear. In this work, we used Atomic Force Microscopy (AFM) to obtain high-resolution images of single RNA molecules, which exhibited distinct structural domains. We developed a MATLAB algorithm to extract structural information of different RNA molecules imaged by AFM. Conformational variability was appreciated in the studied RNAs, and quantification of this variability is provided through an automatic classification algorithm. As a future perspective, this automatized analysis could be applied to any RNA molecule to compare between the sequence and the structure adopted.

0329-P

SEARCH FOR NEW THERMAL ENZYMES IN METAGENOMICS LIBRARIES FROM GALICIAN THERMAL SOURCES

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Cereal hemicellulose, called also arabinoxylan (AX) or pentosan, is a polysaccharide made up of a linear skeleton of xylose monomers linked by β-1,4 bonds with branches mainly due to L-arabinofuranose monomers that bind to oxygen of the C2 or C3, or even the oxygen of the C2 and C3 of the same xylose residue. Hemicellulose can be used to obtain different products depending on the degree of hydrolysis of the polysaccharide. The enzymes responsible for the hydrolysis of the xylan chain to xylose are generally called xylanases, the most important are the endo-1,4-β-xylanases, which with the β-xylosidase enzymes carry out the exhaustive hydrolysis of xylan to xylose.

The main objective of this work was to discover new thermoenzymes, mainly xylanases and β-xylosidases. The search for these new thermoenzymes was carried out by means of culture techniques and metagenomic libraries construction, from waters samples of the Burgas thermal spring with a temperature of 67º C and from the Río Caldo geothermal spring with a temperature of more than 77º C, both in the province of Ourense, using as the single carbon source two varieties of wheat straw (Caaveiro and Castilla). By means of culture techniques, positive strains for thermophilic endoxylanase and β-xylosidase enzymes have been isolated, while by functional metagenomics three positive clones have been obtained corresponding to endoxylanase and one corresponding to β-xylosidase. Parallel to the construction of the metagenomic libraries, an analysis of the biodiversity of the microbial population grown in the cultures has been carried out using 16S rRNA metabarcoding. The results show differences according to the microbiomes used as inoculum and the type of straw. All the organisms identified are bacteria and greater biodiversity is observed, at all taxonomic levels, in the culture samples from As Burgas than in those from Río Caldo.

Curr. Protein Pept. Sci., 19:48-67, 2018 Microb. Cell. Fact. 17(1):137, 2018 Biotechnol. Lett. 13: 20-5, 2008 Biotechnol. Adv. 30:920-29, 2012 Appl. Microbiol. Biotecnol. 97:6603-11, 2013

0342-R-P

EXPLORING THE TAXONOMICAL AND FUNCTIONAL PROFILE OF AS BURGAS HOT SPRING IN SEARCH OF THERMOSTABLE BETA-GALACTOSIDASES

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In the present study, we investigate the microbial community inhabiting As Burgas geothermal spring, located in Ourense (Galicia, Spain). The approximately 23 Gbp of Illumina sequences generated for each replicate revealed a complex microbial community dominated by Bacteria in which Proteobacteria and Aquificae were the two prevalent

phyla. An association between the two most abundant genera, Thermus and Hydrogenobacter, was suggested by the relationship of their metabolism. The high relative amount of sequences involved in the Calvin-Benson cycle and the reductive TCA cycle unveils the dominance of an autotrophic population. Important pathways from the nitrogen and sulfur cycle are potentially taking place in As Burgas hot spring. In the assembled reads, two complete ORFs matching GH2 β-galactosidases were found. To assess their functional characterization, the two ORFs were cloned and overexpressed in E. coli. The pTsbg enzyme had activity towards o-Nitrophenyl-β-D-galactopyranoside (ONPG) and p-Nitrophenyl-β-D-fucopyranoside, with high thermal stability and showing maximal activity at 85 °C and pH 6, nevertheless the enzyme failed to hydrolyze lactose. The other enzyme, Tsbg, was unable to hydrolyze even ONPG or lactose. This finding highlights the challenge of finding novel active enzymes based only on their sequence.

0344-P

Expresión heteróloga y caracterización bioquímica de una esterasa obtenida mediante metagenómica funcional de sedimentos procedentes de aguas termales

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Las enzimas lipolíticas, incluyendo las lipasas verdaderas (EC 3.1.1.1, hidrolasas de triacilglicerol) y esterasas (EC. 3.1.1.3, hidrolasas de ésteres carboxílicos), representan un grupo diverso de hidrolasas que catalizan la hidrólisis y formación de enlaces de tipo éster. Son enzimas ampliamente distribuidas en animales, plantas y microorganismos que presentan numerosas e importantes aplicaciones en la industria alimentaria, textil, cosmética, química, farmacéutica, de los detergentes, del papel y del biodiesel, entre otras. El mercado de las enzimas lipolíticas se estima que llegará a alcanzar los 590 millones de dólares en el 2023 (Chandra et al., 2020). El descubrimiento de nuevas enzimas lipolíticas termófilas es de interés ya que las reacciones de catálisis enzimática realizadas a temperaturas más elevadas favorecen una mayor velocidad de difusión, un aumento en la solubilidad de los lípidos y otros sustratos hidrofóbicos en agua, reduciendo el riesgo de contaminación, lo que abre nuevos horizontes para una amplia variedad de procesos biocatalizados que se realizan en condiciones extremas (Miguel-Ruano et al., 2021).

En el presente trabajo se ha aislado mediante metagenómica funcional de muestras de sedimentos presentes en las termas de Hammam Essalihine (70°C, pH 7.31), en la provincia de Khenchela (Argelia), una esterasa termoestable. Se ha procedido a su expresión heteróloga en levaduras y a una caracterización bioquímica. La esterasa presenta una temperatura y pH óptimo de 80°C y 8, respectivamente. Conserva el 85% de su actividad después de una hora de incubación a 70°C. Es estable a concentraciones de 10 mM de SDS y en presencia de diferentes detergentes comerciales lo que podría permitir su uso en la industria de los detergentes.

Chandra P, Enespa, Singh R, Arora PK. Microbial lipases and their industrial applications: a comprehensive review. Microb Cell Fact. 2020, 19(1):169. doi: 10.1186/s12934-020-01428-8 Miguel-Ruano V et al. Biochemical and Structural Characterization of a novel thermophilic esterase EstD11 provide catalytic insights for the HSL family. Comput Struct Biotechnol J. 2021, 19:1214-32. doi: 10.1016/j.csbj.2021.01.047

0362-P

Expresión y caracterización bioquímica de una celulasa obtenida mediante metagenómica basada en secuencia de aguas termales

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Las celulasas (EC. 3. 2. 1. X) son un grupo heterogéneo de enzimas que catalizan la hidrólisis de la celulosa, el principal componente de la biomasa lignocelulósica. La conversión de esta biomasa lignocelulósica en combustibles, energía o productos químicos de interés mediante biorrefinería requiere de celulasas termoestables ya que las operaciones industriales a elevadas temperaturas permiten una mayor solubilidad de los sustratos y los productos, descensos en la viscosidad y unos índices más altos de interacción sustrato-enzima lo que supone un beneficio para la hidrólisis de polímeros grandes como la celulosa (Escuder-Rodríguez et al. 2018).

En el presente trabajo se ha empleado la metagenómica basada en secuencia para el aislamiento de una celulasa a partir de muestras de aguas termales de Muiño da Veiga (con una temperatura de surgencia entre 65°C y 72°C), en la provincia de Ourense. La celulasa se ha expresado en levaduras y se ha caracterizado bioquímicamente. Se trata de una enzima termoestable, manteniendo el 60% de su actividad tras una incubación de una hora a 70°C. Presenta un pH óptimo de 5 y una temperatura óptima entre 70°C y 80°C. La presencia de MnCl2 estimula su actividad mientras que el CaCl2 no parece tener un efecto significativo en la misma. Aunque actúa preferentemente sobre el sustrato carboximetilcelulosa, presenta actividad frente a sustratos cristalinos de la celulosa (avicel y papel de filtro), almidón y algodón. Todas estas características la hacen interesante para su uso en diferentes industrias biotecnológicas.

Escuder-Rodríguez JJ, DeCastro ME, Cerdán ME, Rodríguez-Belmonte E, Becerra M, González-Siso MI. Cellulases from Thermophiles Found by Metagenomics. Microorganisms. 2018 Jul 10;6(3):66. doi: 10.3390/microorganisms6030066.

0384-R/M-P

Computational analysis of alternative splicing in peripheral blood reveals candidate novel biomarkers for the early diagnosis of Frontotemporal Dementia

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Frontotemporal Dementia (FTD) is a complex group of non-Alzheimer dementias characterized by a progressive deterioration in behaviour, personality, and language [1]. Different pathological subtypes of FTD have been identified based on the presence of specific protein inclusions in degenerating neurons [2]. FTD-TDP is the major subtype with ~45% of all FTD cases, and is characterised by nuclear depletion and cytoplasmic deposition of TAR DNA-binding protein 43 (TDP-43) [2,3]. TDP-43 is an RNA-binding protein (RBP) with a central role in RNA metabolism and processing [4,5]. It has been described that TDP-43 acts as an splicing repressor and therefore multiple altered splicing events are observed upon nuclear depletion of TDP-43 [6,7,8]. From a genetic perspective, several FTD-TDP causative genes have been identified [9,10]. In the Basque Country, a non-coding mutation (c.709-1G>A) in the *GRN* gene, represents the most common form of familial FTD [11].

As FTD patients with *GRN* mutations show TDP-43 inclusions [11], a misregulated splicing pattern is expected at transcriptional level in brain [6,8]. It is established that alterations in the primary tissue can correlate with peripheral tissues [12,13]. Therefore, we hypothesize that studying RNA-splicing alterations in peripheral blood of patients may help to diagnose this disorder.

In the present study, we have characterised splicing misregulation in the peripheral blood from FTD patients with c709-1G>A mutation and control subjects. The computational analysis of RNA-Seq data exposes a sort of splicing events and possible predictive signatures of FTD development. The identification of these specific events may serve as future biomarker of FTD, which may be relevant for early interventions that may slow down the progression of the disorder.

1. Hogan, DB et al. PMID:27307130 2. Devenney EM et al. PMID:31753137 3. Younes K & Miller BL. PMID:32439025 4. Buratti, F & Baralle, FE. PMID:20639693 5. Polymenidou M et al. PMID:21358643 6. Humphrey J et al. PMID:27009575 7. Melamed Z et al. PMID:30643298 8. Prudencio M et al. PMID:32790644 9. Pottier, C et al. PMID:27009575 10. Cruts, M et al. PMID:16862115 11. López de Munain, A et al. PMID:17950702 12. Frésard, L et al. PMID:31160820 13. Kremer, L et al. PMID:28604674

0415-P

Metabolic alterations in a Drosophila model of Parkinson's disease: identification of potential biomarkers and therapeutic targets

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DJ-1 is a causative gene for early-onset recessive Parkinson's disease (PD) form that encodes a multifunctional protein implicated, among others, in antioxidant response, mitochondrial homeostasis and central carbon metabolism. PD is an incurable neurodegenerative disorder whose underlying pathological mechanisms are still unclear. Currently, PD diagnosis is mainly based on the presence of motor symptoms that appear when neurodegeneration is highly advanced. Therefore, it is urgent to identify molecular alterations associated with the disease in order to find new biomarkers and therapeutical approaches. Accordingly, we have previously demonstrated that a *Drosophila* and neuron-like human cell PD models based on *DJ-1* deficiency exhibit an increase in the activity of several glycolytic enzymes. Indeed, compounds targeting this pathway have been shown to constitute a potential therapeutic approach. Thus, we decided to study other metabolic alterations implicated in PD physiopathology that could lead to discover new biomarkers and therapeutic targets for this disease. To do this, we performed metabolomic analyses in 1 and 15-day-old control and *DJ-1β* mutant flies (the ortholog of the human *DJ-1*) by high resolution nuclear magnetic resonance (NMR) spectroscopy. After this, we selected those pathways in which altered metabolites were identified. Next, we studied their activity by gene expression analyses and enzymatic assays of the pathway components. The metabolomic analysis led to the identification of changes in levels of several metabolites between *DJ-1β* mutant and control flies pointing to an implication of different pathways in PD physiopathology. Among them, *DJ-1β* mutant flies exhibit a decrease in the amino acid content and variations in carbohydrate levels that could be related to impaired tricarboxylic acid (TCA) cycle and the urea cycle. A more exhaustive analysis of these pathways revealed that there is a switch from TCA cycle to glycolysis and that genes involved in the urea cycle present enhanced expression in *DJ-1β* mutant flies. In conclusion, there is a link between altered metabolism and PD physiopathology involving the TCA and the urea cycle in the *Drosophila* PD model. Pro-

teins of these pathways may constitute biomarkers and potential therapeutic targets for the disease.

0504-R/M-P

Proteomics analysis in the identification of Quercus ilex allergens

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Pollen-food allergy syndrome (PFAS) is a common allergic disease caused by a cross-reaction among pollens and vegetable foods. PR10 allergens from pollen and vegetables have been well characterized as is the case of Bet v 1 from birch. Besides PR10 allergens have been described in pollen from species of *Quercus* genus. The first *Q. ilex* allergen (Que i 1), a PR10 allergen related to PFAS, has been recently identified by using a transcriptomic and proteomic approach (Pedrosa et al., 2020). Subsequent studies carried out in *Q. ilex* pollen point to the existence of more allergens. Through a targeted proteomics approach by using proteotypic peptides (specific of protein) we have identified proteins belonging to Bet v 1, thaumatin, LTP (Lipid Transfer Protein) and profilin families, containing allergenic epitopes in their sequences, and a panel of potentially allergenic peptides is proposed. This methodology has a great potential that can be exploited in this field, since these peptides could be used as molecular markers in targeted analysis techniques. The proposed proteins should be considered as putative allergens from *Q. ilex* pollen and validated by heterologous expression of the genes, to then be included in allergy diagnostic assays.

Pedrosa M., Guerrero-Sanchez V. M., Canales-Bueno N., Loli-Ausejo D., Castillejo M.A., Quirce S., Jorrin-Novo J.V. & Rodriguez-Perez R. (2020). Quercus ilex pollen allergen, Que i 1, responsible for pollen food allergy syndrome caused by fruits in Spanish allergic patients. Clinical & Experimental Allergy, 50, 815–823.

0507-R/M-P

Personalized Vaccines: Neoantigens Identification from Immunopeptidomics Characterization

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Immunopeptidomics is the science that studies the peptides assembled in the major histocompatibility complex (MHC), which are immunogenic and activate T cells immune response. This complex list of immunopeptides is playing a high relevant role in precision medicine because its direct relation with individual genetic variation and susceptibility and its critical role in many diseases such as cancer, infections, inflammatory and chronic diseases, among auto-immune and neurodegenerative pathologies.

Here we show the results of the start of the project whose main objective is the identification of potential tumour neoantigens for the future development of personalized cancer vaccines. We show the relevance of combining highly sensitive methodological approach by Data-independent acquisition (DIA) and Data-Dependent Acquisition (DDA) LC-MS/MS with computational biology which include the main databases used to identify potential peptides binding with these molecules.

We anticipate our first results obtained as a starting point for the identification of potential neoantigens from different tumour lines, as well as their in silico prediction that strengthens our study to move forward and the discussion of the next steps to be taken in their translation to the clinic.

[1] Juanes-Velasco, P., et.al. (2021). Deciphering Human Leukocyte Antigen susceptibility maps from immunopeptidomics characterization in oncology and infections. Frontiers in Cellular and Infection Microbiology, 11, 424. [2] Human Immuno-Peptidome Project. (2015). https://www.hupo.org/Human-Immuno-Peptidome-Project [HUPO-HIPP started in 2015]. [3] Purcell, A. W., et.al. (2019). Mass spectrometry–based identification of MHC-bound peptides for immunopeptidomics. Nature protocols, 14(6), 1

535-OI

Host-pathogen interaction: Proteomics-based identification of immunome biomarkers in Candida albicans infections

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Invasive candidiasis (IC) is an opportunistic fungal infection caused by Candida species (commonly Candida albicans) that remains an important public health problem worldwide despite great advances in antifungal therapy. IC is a leading infectious cause of morbidity and mortality in critically ill and severely immunocompromised patients. Clinical outcomes could be improved by timely initiation of appropriate antifungal therapy. However, IC diagnosis at an early stage is difficult because of the poor accuracy of the currently available diagnostic methods for IC [1]. Here, we examined whether molecular profiling of serum anti-C. albicans IgG-antibody responses in IC and non-IC patients could uncover immunome biomarkers for IC diagnosis and prognosis using a computational immunomic biomarker discovery-validation pipeline. Unsupervised two-way hierarchical clustering and principal-component analyses highlighted that IgG antibody-reactivity patterns to a defined set of C. albicans cell surface and intracellular immunogenic proteins discriminated between IC patients and non-IC patients as well as between IC survivors and IC non-survivors. Supervised discriminant analyses revealed that two-IgG and five-IgG antibody-reactivity signatures were accurate predictors of IC and clinical outcomes in individual IC patients at presentation, respectively. Receiver-operating characteristic curve analyses showed that these immunomic signatures outperformed clinical risk factors. Further validation of these diagnostic and prognostic signatures for IC on multiplexed immunoassay prototypes confirmed the serological proteome analysis results. We conclude that these prediction models may be useful in identifying those patients who need antifungal therapy, as well as those IC patients who may suffer poor clinical outcomes at presentation. Our work further provides new insight into the anti-Candida antibody response development in IC at the chemical and molecular level.

[1] Pitarch A, Nombela C, Gil C (2018) Diagnosis of invasive candidiasis: From gold standard methods to promising leading-edge technologies. Current Topics in Medicinal Chemistry 18: 1375-1392.

04.- Chemistry Biology

0529-OI

Supramolecular chemical biology: from molecular recognition to biomedical application

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The study of biological process from the chemical perspective allows a better understanding of life at the molecular level, as well as the specific intervention in both normal and pathological situations. However, biomolecules are not isolated entities since their function depend on their mutual interactions, thus playing a role within complex molecular networks and systems. The study of the interaction and communication between molecules represents the core of the so-called Supramolecular Chemistry, thus being fundamental to tackle biological process at the molecular level. In this lecture, I will discuss the close relationship between Supramolecular Chemistry and Chemical Biology, illustrating the idea with key recent results from our own research group. Thus, selected examples based on the design of specific receptors able to recognize molecules or ions with biological relevance will be presented. Moreover, the dynamic covalent chemistry approach will be also explained with recent studies from our research group. These molecular recognition processes can be traduced into interesting biological activities, thus illustrating the new concept of Supramolecular Chemical Biology.

Ignacio Alfonso got his PhD in Chemistry at the University of Oviedo (1999). After two post-docs at The Scripps Research Institute (2000-2002) and back to Oviedo (2002-2004), he gained the Ramón y Cajal contract (Universitat Jaume I). Since 2007 he leads the Supramolecular Chemistry Group (first Tenured Scientist, promoted to Senior Research Scientist in 2017). He is the Head of the Department of Biological Chemistry at IQAC-CSIC and the President of the Specialized Group on RMN from the RSEQ.

0531-OI

The EMT factors E2A control tumor initiating activity and metastasis potential of breast cancer cells

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Cancer stem cells (CSCs) are considered responsible for tumor-initiation, metastasis and therapeutic resistance;

however, the mechanisms governing CSC biology ant their similarities to normal stem cells are not fully understood (1, 2). EMT (Epithelial-Mesenchymal Transition) is a key process in invasion and metastatic dissemination and has also been associated with the acquisition of stem cell-like properties and therapeutic resistance (3, 4). EMT is regulated by a series of EMT-transcription factors (EMT-TFs) that seems to act in a tumor-dependent context (5, 6). Among them, Snail1 and Twist are able to confer cell stemness properties and tumor initiating activity (TIC) to mammary epithelial cells (7). Very little is known on the contribution and potential hierarchical interactions of other EMT-TFs to tumor- and metastasis-initiation properties in breast cancer. That is the case of the E2A (E47/E12) factors, two members of the bHLH (basic Helix-Loop-Helix) TFs generated by alternative splicing, originally described as potent EMT inducers and associated to human basal-like breast carcinomas (8-10). Using a combination of *in vivo* and *in vitro* analyses in a novel *PyMT-E2A* conditional KO mouse model and derived tumor cell lines we have characterized an essential novel role of E2A in TIC, metastasis competence and therapeutic resistance of breast cancer. Deletion of *E2A* in the mammary gland significantly decreases TIC ability while increased differentiation tumor potential, and severely compromises metastatic competence of PyMT-driven breast tumors. Mechanistic analyses indicate that E2A actions are mediated by transcriptional upregulation of *Snai1* and, importantly, high E2A and SNAIL1 expression levels co-occur in aggressive human basal-like breast carcinomas. These findings highlight the biological relevance of the E2A-Snail1 axis in metastatic breast cancer. Besides, E2A factors contribute to the maintenance of genomic integrity and resistance to PARP inhibitors of PyMT tumors and human basal-like breast cancer cells, supporting their potential as a novel therapeutic vulnerability in breast cancer.

1. Batlle E, Clevers H. Nat Med 2017;23:1124-34 2. Celia-Terrassa T, Kang Y. Genes Dev 2016;30:892-908 3. Nieto MA et al. Cell 2016;166:21-45 4. Gupta PB et al. Cell Stem Cell 2019;24:65-78 5. Peinado H, Olmeda D, Cano A. Nat Rev Cancer 2007;7:415-28 6. Stemmler MP et al. Nat Cell Biol 2019;21:102-12 7. Mani SA et al. Cell 2008;133:704-15 8. Perez-Moreno MA et al. J Biol Chem 2001;276:27424-31 9. Cubillo E et al. PLoS One 2013;8:e59948 10. Canesin G et al. Oncogene 2015;34:951-64

0079-R/M-OS

Develop sEV-based therapeutics to increase efficacy of BRAF/MEK inhibitors in BRAF-driven tumours

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The transmembrane protein connexin43 (Cx43) has been described as a tumor suppressor in primary melanoma, but its role in disease progression is still under debate. Cx43 is also present in small extracellular vesicles (sEVs), which allows the exchange of small molecules such as ions, metabolites or small RNAs (sRNAs) via gap junction channels between sEVs and target cells. Almost 60% of melanoma patients harbor mutations in BRAF gene which induce cell proliferation and survival by constitute activation of MAPK/ERK pathway. BRAF/MEK inhibitors (BRAF/MEKi) have become the standard therapeutic approach in patients with BRAF-mutant melanoma. However, resistance to therapy frequently develops within 12 months after treatment. The acquisition of drug resistance is still one of the greatest challenges to achieve the full effectiveness of this therapy. The reactivation of Cx43 in BRAF mutant cell lines using a vector or sEVs containing Cx43, significantly decreases cell growth and proliferation and increases cellular senescence and apoptosis mediated by Caspase3. Further, the presence of Cx43 in sEVs radically changes their function and the content of proteins and sRNAs indicating that Cx43 may participate in the recruitment of these components into the sEVs. Restoration of Cx43 in BRAF-mutant tumours using sEVs positives for Cx43 significantly increases the efficacy of the BRAF/MEKi and prevents drug resistance by reenforcing cellular senescence and enhancing cell death by apoptosis, alone and in combination with the senolytic drug navitoclax. In this study we propose a new and effective drug combinations based on sEVs containing Cx43 along with BRAF/MEKi and with navitoclax, which increases more than 80% the efficacy of these inhibitors. Also, we have demonstrated that sEVs can be used as drug carrier to transport transmembrane proteins such as Cx43. These results could impact in the manage and treatment of tumours that initially respond to BRAF/MEKi with a potential clinical benefit in patients with a metastatic disease.

0138-OS

A genome wide CRISPR/Cas9 screen for activators of dendritic cells

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Tumour immunogenicity is defined as the ability of the tumour to induce an immune response. Immunogenicity is determined by two main components: antigenicity (presence of neoantigens) and adjuvanticity (presence of signals that will activate antigen presenting cells). While genomic studies have facilitated identification of neoantigens, identification of signals that activate the immune system and facilitate initiation of immune responses remains challenging. To identify pathways that contribute to tumour cell adjuvanticity, we have developed a high-throughput co-culture assay and used it in a whole genome arrayed CRISPR/Cas9 screen.

Our co-culture assay provides an in vitro model of tumour cell - antigen presenting cell communication. We use the dendritic cell (DC) line MutuDC as our antigen presenting model as they can be expanded in an immature state and activated by pathogen-derived and endogenous signals. We selected the lung adenocarcinoma cell line A549, which did not activate MutuDC in our co-culture assay, as our cancer model.

We developed a high throughput pipeline where Cas9 inducible A549 cells are transfected with an arrayed sgRNA library, and edited cells are co-cultured with MutuDC. To monitor DC activation, we stain for membrane CD83 and CD86, and quantify the staining signal in DCs using high throughput confocal microscopy and image analysis.

Using the co-culture activation assay we have performed the first to our knowledge whole genome arrayed CRISPR/Cas9 screen for cancer cell-derived activators of dendritic cells. We have identified more than 300 candidate genes with a robust Z-score for dendritic cell activation higher than 2.5. Pathway and gene set enrichment analysis of these candidate genes reveal a potential role for arachidonic acid metabolism and eukaryotic translation initiation factor 4f complex in the regulation of tumour cell adjuvanticity.

We are currently validating the candidate genes with the aim to discover novel targets to modulate cancer cell adjuvanticity. Better understanding of the mechanisms involved in tumour cell – antigen presenting cell communication may facilitate design of new combination treatments with other immunotherapies, as well as provide tools for patient stratification or prognosis.

0233-R/M-OS

Molecular recognition and activation modulation of Toll-like receptors 2 and 4. Computational approaches.

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Toll-like receptors (TLRs), classified as pattern recognition receptors, have a primordial role in the activation of the innate immunity. TLRs are transmembrane proteins specialized in the recognition of pathogen-associated molecular patterns, such as lipopolysaccharides (LPS) of Gram-negative bacteria (TLR4), lipoteichoic acids of Gram-positive bacteria, and bacterial lipoproteins (TLR1/TLR2 and TLR2/TLR6).

Identification of TLRs has sparked great interest in the therapeutic manipulation of the innate immune system; TLRs agonists are currently under development for the treatment of cancer, allergies, and viral infections, and also as adjuvants in vaccine development and in cancer immunotherapy. As inappropriate TLR stimulation leads to inflammation and autoimmunity, significant efforts have also been directed towards the development of compounds as TLRs antagonists. For example, blocking various TLRs, such as TLR2 and TLR4, with antagonists may be useful to prevent an overactive immune response.

We have applied molecular modeling and computational techniques to characterize the molecular recognition processes of TLR1/TLR2, TLR2/TLR6, and TLR4 agonist and antagonist modulators, and to propose a mechanism for their biological activity. We have unraveled atomic details about the ligand-receptors interactions of natural and synthetic LPS-like and non LPS-like modulators, and bacterial proteins. In addition, computational studies have been undertaken, to provide the most realistic and complete 3D models of the active full TLR4 complex to date, embedded into a model membrane.

Deep structural understanding of the molecular recognition events that take place to assemble the Toll-like signaling complexes, and to recognize diverse pathogen ligands, may lead to the discovery of novel small molecules with desirable therapeutic properties.

Matamoros-Recio et al. “Full-Atom Model of the LPS-bound Toll-like Receptor 4 Dimer in a Membrane Environment.” Under revision. Facchini et al. “Structure–activity relationship in monosaccharide-based toll-like receptor 4 (TLR4) antagonists.” J Med Chem. 2018 Apr 12;61(7):2895-2909. Federico et al. “Modulation of the Innate Immune Response by Targeting Toll-like Receptors: A Perspective on Their Agonists and Antagonists.” J Med Chem. 2020 Nov 25;63(22):13466-13513.

0242-OS

Peptide-based tools: from sensors to the control of peptide function

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Peptides and proteins are natural biopolymers that perform many functions in biological systems. Among others, they play an important role as structural and signaling molecules thanks to their self-assembling and molecular recognition capabilities. Furthermore, these biopolymers offer the greatest structural and functional versatility, combined with great synthetic simplicity, intrinsic biocompatibility and high biodegradability. Therefore, taking into account these qualities, it is not surprising that these polymers have been widely used for the development of new drugs (1), biosensors (2), or even for designing a large range of nanomaterials (3).

This communication will focus on the use of different peptide tools for:

1) designing a fluorescent peptide sensor of hypoxia that

mimics the oxygen-sensing capability of HIF-1α. As such, the probe is stabilized under hypoxia, and therefore the fluorescence intensity is higher under hypoxic conditions, and readily degraded by the proteasome under normoxia, thus providing direct information of the cellular oxygen availability (4).

2) controlling the dimerization of a 4,4'-bipyridinium-peptide conjugate by the formation of a supramolecular complex with cucurbit[8]uril. This supramolecular dimer is able to specifically recognize its target dsDNA and, importantly, this binding can be reversibly switched by the application of external stimuli (5).

(1) Vinogradov, A. A.; Yin, Y.; Suga, H. J. Am. Chem. Soc. 2019, 141, 4167. (2) González-Vera, J. A. Chem. Soc. Rev. 2012, 41, 1652. (3) Ulijn, R. V.; Jerala, R. Chem. Soc. Rev. 2018, 47, 3391. (4) Iglesias, P.; Penas, C.; Barral-Cagiao, L.; Pazos, E.; Costoya, J. A. Sci. Rep. 2019, 9, 7117. (5) Novo, P.; García, M. D.; Peinador, C.; Pazos, E. Bioconjug. Chem. 2021, 32, 507.

0037-R/M-P

Modulation of the phosphodiesterase activity and lifespan in Saccharomyces cerevisiae by different stilbenes and roflumilast.

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In this contribution, the activation of the caloric restriction (CR) pathway using different three stilbenes: Resveratrol, Oxyresveratrol and Piceatannol was studied [1]. The high affinity phosphodiesterase type 2 (PDE2) of *Saccharomyces cerevisiae* was expressed in *Escherichia coli*, purified and characterized. The activity and the inhibitory activity of each stilbene was studied and the findings were compared *in vitro* and *in silico* with those obtained with Roflumilast, a human PDE4 inhibitor widely used in chronic obstructive pulmonary diseases. Finally, an *in vivo* chronological lifespan assay using WT and ΔPDE2 *S.cerevisiae* strains demonstrated that stilbenes increased the lifespan of the yeast by 18 % compared with the control. In addition, Roflumilast increased the lifespan in the WT strain. The findings as a whole would increase the range of lifespan products available, and suggest novel uses for approved drugs.

[1] Matencio, A., et al. (2020). Characterization of Resveratrol, Oxyresveratrol, Piceatannol and Roflumilast as Modulators of Phosphodiesterase Activity. Study of Yeast Lifespan. Pharmaceuticals, 13(9), 225.

0039-R/M-P

Use of bifunctional carbon dots (CDs) as fluorescent markers in vitro and in vivo bioimaging

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Although nanoparticles (particles ranging from 1 to 100 nm) were initially used in the Chemistry and Physical field, now a days have become a powerful and daily tool in the biomedicine, biochemistry or molecular biology. Among the huge variety of nanoparticles available, Carbon Dots (CDs) are an especially good choice in the field of biology due to their particular properties, such as their interesting luminescent characteristics (high quantum yields, photostability, tunable emission wavelength), their high solubility and stability in aqueous media, their biodegradability and their very low or even null cytotoxicity. Additionally, the surface of CDs is easily modifiable, and therefore it is possible to bind them compounds of interest, such as antibodies or drugs, and improve their response to changes in the microenvironment.

Since their discovery, the application of CDs has grown exponentially as antibacterial agents, fluorescent probes, drug transport, probes for the *in vitro* and *in vivo* detection of specific molecules. In this study we evaluate the analytical potential of glutathione-based CDs (Glut-CDs) derivatized with cyclodextrins as fluorescent markers in bioimaging in vitro (in U2OS and B16F10 cancer cells and in healthy mammary fibroblasts) and in vivo (mice).

0051-R-P

The encapsulation in cyclodextrins as a way to improve the solubility and susceptibility to oxidation of neochlorogenic acid

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Interest in the study of bioactive compounds and their applications in functional foods, nutraceuticals and drugs is growing due to their potential health benefits. Among these compounds, there are some of them that risk having physicochemical problems when designing a formula. This is the case of neochlorogenic acid, a caffeoylquinic acid with antioxidant, anti-inflammatory, antifungal, and anticarcinogenic activity, which also has a limited aqueous solubility and is easily oxidized by polyphenol oxidase. In this work, we developed a solution to these problems by encapsulation in cyclodextrins (CDs), cyclic molecules formed by glucose units capable of trapping hydrophobic molecules in their inner cavity increasing their solubility in water. The reaction stoichiometry and encapsulation constants were determined considering two possible complexation sites on the ligand. The fluorimetric assay revealed that α-CD and hydroxypropyl-β-CD formed the best inclusion complexes with neochlorogenic acid, followed by methyl-β-CD, β-CD and γ-CD. Molecular docking with the two best CDs gave better scores for α-CD, despite hydroxypropyl-β-CD providing stabilization through hydrogen bonds. Solubility and oxidation susceptibility were improved after complexation with CDs, while antioxidant activity was maintained. A comparison with chlorogenic acid, one of the most studied isomers of this bioactive compound, led to a similar CD order and scores, although the constants were higher for α-CD, β-CD and methyl-β-CD, lower for hydroxypropyl-β-CD, and negligible for γ-CD. These results could contribute to the design of stable formulations enriched in neochlorogenic acid for applications in the food, pharmaceutical and cosmetic industry.

Kurita, S. et al. Identification of neochlorogenic acid as the predominant antioxidant in Polygonum cuspidatum leaves. Ital. J. Food Sci. 28, 25–31 (2016). Fang, W. et al. In vitro and in vivo antitumor activity of neochlorogenic acid in human gastric carcinoma cells are complemented with ROS generation, loss of mitochondrial membrane potential and apoptosis induction. J. BUON Off. J. Balk. Union Oncol. 24, 221–226 (2019).

0084-P

Tyrosinase monophenolase activity: Its determination

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Tyrosinase is an enzyme that catalyses the first two steps in the melanins pathway: L-tyrosine to L-dopa transformation and this to L-dopaquinone. To kinetically characterize the monophenolase activity, we proposed to study both monophenolase and diphenolase activities separately. Two spectrophotometric methods have been described measuring the monophenol disappearance, L-tyrosine, in presence of borate and hydroxylamine at pH = 8.0. However, these methods have no linear dependence between fluorescence and L-tyrosine concentration from 100 μM. In this work, we described, from a kinetic point of view, that the monophenolase activity can be measured using the monochrome formation or using the adducts formation oxidated by the attack of a potent nucleophile, such as 3-methyl-2-benzothiazolinone (MBTH), to the o-quinone. In addition, it can measure the oxygen consumption when the system is in steady-state. In other hand, because of the high extinction coefficient of the adducts formed by attack of MBTH and considering the nature of the substrate, low LODM (limit of detection of the monophenolase activity) can be obtained, as it happens with the spectrofluorometric methods. The values obtained were: 4-hydroxyphenyl propionic acid (0.2490 U/ml), 4-hydroxyanisol (0.0901 U/ml), tyramine (0.6412 U/ml) and L-tyrosine (2.1023 U/ml). These results shows that the potency of the nucleophilicity of the hydroxyl oxygen at C-4, which influences the kcat value, gives rise to a different amount of product that in turn affects the spectrophotometric signal, and therefore the LOD value.

This work was supported by grants from the Ministerio de Economia y Competitividad (MINECO; Co-financing with Fondos FEDER) (SAF2016-77241-R), the Fundación Séneca, the Región de Murcia (FS-RM) (20809/PI/18) and Fondos ACI (project 372 ENZIMOLOGIA).

0085-P

Chitosan-BSA nanoparticles as vehicles for antitumour drugs. Biocompatibility studies of intravenously administered nanoparticles.

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In this study, a new alternative of ionic crosslinked nanoparticles (NPs) based on chitosan (C) and bovine serum albumin (A) was evaluated as drug delivery system for antitumour compounds (doxorubicin hydrochloride as a model). NPs revealed a nanoscale size (167–392 nm) and a positive net charge (12–26 mV), modulated by doxorubicin (DOX) loading. Drug loading capacity was higher than 5.2 ± 1.8 μgDOX/mgNP (Encapsulation efficiency=34%), and an initial burst release was followed by a sustained delivery. Cellular uptake assays confirmed the entry of NPs in three human tumor cells (MCF7, T47D and Hela), triggering antioxidant responses (superoxide dismutase, catalase, glutathione reductase and total glutathione content) in those cells. This was also consistent with the decreased in IC50 values observed after the incubation of these cells with C20/A80-DOX and C50/A50-DOX NPs (1.90–3.48 μg/mL) compared with free DOX (2.36–6.025 μg/mL). In order to evaluate their biocompatibility, NPs were intravenously administered to wistar rats. A haematology analysis and a coagulation study were conducted at different times after the injection of NPs. Haematology results demonstrated that common parameters, such as the haemoglobin level and red blood cell (RBC) count (12.48–16.03 g/dL and 6.19–8.22×1012/L, respectively) had values consist-

ent with normal values. For the coagulation assessment, three parameters were selected: fibrinogen, prothrombin time (PT) and antithrombin III (ATIII). A biochemical plasma analysis (creatinine, BUM, AST, ALT, ALP) along with antioxidant response assays in tissues (SOD activity, CAT activity, GSSG-R activity and GSSG/GSH) were carried out. In vivo results suggested that the selected proportions of chitosan-BSA created nonhemolytic and biocompatible stable NPs at the selected dose of 20 mg/kg. Despite the different formulations, this study demonstrated that these NPs could serve as safe drug carriers in further *in vivo* investigations. (Proyecto PR75/18-21575)

- Ferreira, L.M.B. et al., 2020. Design of chitosan-based particle systems: A review of the physicochemical foundations for tailored properties. Carbohydr Polym 250, 116968. - Fadeel, B. and Alexiou, C., 2020. Brave new world revisited: Focus on nanomedicine. Biochem Biophys Res Commun 533: 36-49 - Montero, N. et al., 2019. Biocompatibility studies of intravenously administered ionic-crosslinked chitosan-BSA nanoparticles as vehicles for antitumour drugs. Int J Pharmaceut 554: 337–351.

0192-R-P

Enantioselective effect of Cysteine functionalized Mesoporous Silica Nanoparticles in U87 MG and GM08680 human cells

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The use of nanoparticles for biomedical purposes has been at the pipeline of research for several decades. A lot of effort has been invested in functionalizing them with different molecules seeking to improve their selectivity or action as a consequence of a stimulus/response. It is now possible to produce complex systems capable of fulfilling very specific functions1-3. However, for accomplishing great feats, we sometimes forget to pay enough attention to small but vital details. This is the case of chirality in nanoparticles. Despite the time that nanomedicine has been studied, little attention has been paid into knowing how this phenomenon affects interaction with biological systems4-6.

The importance of chirality in biological systems is undeniable since most of the biomolecules and biological components and species are chiral and therefore recognize and respond differently depending on the enantiomer present. In order to apply nanomaterials for biomedical purposes, it is essential to understand the role that chirality of nanoparticles plays at the cellular level. Thereupon, here we report the preparation and characterization of the chiral cysteine (Cys) functionalization of mesoporous silica nanoparticles (MSN) and investigate their cell-interaction with U87 MG human glioblastoma cells in comparison with healthy human fibroblast (GM08680).

The results revealed that D-Cys MSN presents a higher internalization potential in U87 MG cells, showing a 4.3-fold increase in cell uptake in comparison with L-Cys MSN. Besides, when evaluating cell viability, cell death induced by D-Cys MSN is higher than the one produced by L-Cys MSN. These results are consistent with the internalization patterns observed, suggesting that the accumulation inside the cell is responsible for cell death.

Additionally, when the nanoparticles were evaluated in healthy fibroblasts, cell uptake was much lower than in tumour cells. And what is even more important, the internalization achieved by D-Cys MSN in healthy cells was less than L-Cys MSN, suggesting an improvement in the tumour selectivity of the treatment.

In conclusion, the selectivity and efficacy of D-Cys MSN in U87 cells, together with the ability to load antitumor drugs inside the pores, make this nanodevice a potential tool to combat neuroblastoma.

1. M. Martínez-Carmona, D. Lozano, A. Baeza, M. Colilla, M. Vallet-Regí, Nanoscale. 9 (2017). 2. L. Yuan, F. Zhang, X. Qi, Y. Yang, C. Yan, J. Jiang, J. Deng, J. Nanobiotechnology. 16 (2018). 3. Martínez-Carmona M, Lozano D, Colilla M, Vallet-Regí M, Acta Biomater. 65 (2018) 4. W. Utembe, Toxicol. Lett. 311 (2019) 5. Y. Guo, L. Wu, K. Gou, Y. Wang, B. Hu, Y. Pang, S. Li, H. Li, Microporous Mesoporous Mater. 294 (2020). 6. N.S. Kehr, J. Jose, Appl. Surf. Sci. 425 (2017).

0199-R/M-P

Site-specific localization of conjugative ATPases by optical microscopy

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Bacterial conjugation is the main mechanism for horizontal gene transfer, conferring plasticity to the genome repertoire. Unfortunately, this process is also the major instrument for the dissemination of antibiotic resistance genes. Hence, gathering primary information of the mechanism underlying this genetic transaction is of a capital interest. By using fluorescent protein fusions to the ATPases that power conjugation we have observed that the localization of these proteins suffers dramatic changes upon contact between donor and recipient cells. Moreover, we have found that more than one copy of the conjugative plasmid is transferred during mating. Altogether, these findings provide new insights into the mechanism of such an important gene transfer device.

Cabezon E, Ripoll-Rozada J, Pena A, de la Cruz F, Arechaga I. 2015. Towards an integrated model of bacterial conjugation. FEMS Microbiol Rev 39:81-95. Babic A, Lindner AB, Vulic M, Stewart EJ, Radman M. 2008. Direct visualization of horizontal gene transfer. Science 319:1533-1536

0201-R/M-P

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.

Boix, E.; Nogués, M. V. Mammalian Antimicrobial Proteins and Peptides: Overview on the RNase A Superfamily Members Involved in Innate Host Defence. Mol. Biosyst. 2007, 3 (5), 317–335. Boix, E.; Salazar, V. A.; Torrent, M.; Pulido, D.; Nogués, M. V.; Moussaoui, M. Structural Determinants of the Eosinophil Cationic Protein Antimicrobial Activity. Biol. Chem. 2012, 393 (8), 801–815.

0226-R/M-P

Combined directed chemotherapy and photothermia based on the use of biomimetic magnetic nanoparticles functionalized with a novel ChoKα1 inhibitor

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University of Granada Biochemistry and Molecular Biology I

Biomimetic magnetic nanoparticles (BMNPs) have risen as novel carrier system for chemotherapeutic agents against cancer based on their magnetic properties (superparamagnetic character and large magnetic moment per particle), their innovative surface properties determined by MamC, their biocompatibility and their ability as magnetic hyperthermia agents. In this scenario, lipid metabolism may be a starting point for designing of new anticancer drugs. In this context, phospholipid biosynthesis, specifically that of phosphocholine (PCho) and phosphatidylcholine (PC), is enhanced in tumour cells compared to healthy tissue, Furthermore, overexpression of the choline kinase α1 (ChoKα1) isoform has been found in malignant cells and tumours, arising as an excellent antitumor target. Among all the inhibitors synthetized for ChoKα1, a new compound biscationic biphenyl derivative of thienopyrimidium substituted with a cyclic amine shows an outstanding ability to inhibit ChoKα1 activity. The functionalization of BMNPs with this ChoKα1 inhibitor could provide a targeted and effective therapeutic system, with the possibility of combined treatments to fight cancer. Here we show the cytotoxicity effect of the nanoassembly BMNPs-ChoKα1 inhibitor on cancer cell line when alternating magnetic field and laser exposure are applied, serving a potential combined therapy against the cancer.

This research work is supported by Ministerio de Economía y Competitividad (PID2019-109294RB-100 project), Ramón y Cajal programme (RYC-2014-16901), Junta de Andalucía. Programa Operativo FEDER 2014-2020 (A1-FQM-341-UGR18, C-FQM-497-UGR18, A-BIO-376-UGR18). Alberto Sola-Leyva holds a Formación de Doctores 2018 grant (ref. PRE2018-085440) from the Ministerio de Ciencia, Innovación y Universidades (Spain). Ylenia Jabalera wants to acknowledge a FPU2016 grant (ref. FPU16_04580) from the Ministerio de Educación, Ciencia y Deporte y Competitividad (Spain).

0256-R-P

Effect of a cell-penetrating peptide based on Connexin43-Src interaction on the expression and localization of Connexin43 in a murine glioma model

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Gliomas are the most common and aggressive primary brain tumors. Glioblastoma, its most malignant grade, holds the worst prognosis with a median survival of 15 months in treated patients. These tumors are composed by a heterogeneous population of cells, including some with stem-cell-like properties, called glioma stem cells (GSCs). These cells are highly tumorigenic and resistant to standard therapies.

Connexin43 (Cx43) is an integral membrane and ubiquitously expressed protein, which in the brain is mainly found forming gap junctions in astrocytes. In addition, Cx43 forms hemichannels that release paracrine signals, and possesses significant channel-independent functions, including intracellular interaction with signalling molecules, such as the oncoprotein c-Src. Thus, depending on the different roles played by Cx43, it can have pro- or anti-tumorigenic effects in gliomas.

Importantly, Cx43 inhibits c-Src activity by recruiting c-Src and its inhibitors. Based on this property, we developed a cell-penetrating peptide containing the region of Cx43 that interacts with c-Src (TAT-Cx43266-283). TAT-Cx43266-283 inhibits c-Src activity, reducing the growth and invasion of glioma cells without affecting neurons and astrocytes in several preclinical glioma models, including freshly removed samples from glioblastoma patients. Consequently, we found that TAT-Cx43266-283 enhances the survival of glioma-bearing mice.

Due to the relevance of Cx43 to glioma, in the present study we addressed the effect of TAT-Cx43266-283 on Cx43 in glioma cells and the tumor microenvironment in a murine glioma model. To do so, Gl261-GSCs were intracranially injected in C57BL/6 mice. For the treated group, TAT-Cx43266-28 was coinjected with Gl261-GSCs and IP administered twice a week. Brains were processed at different times during tumor development. We first analyzed different immunohistochemical methods to find the optimal protocol to assess Cx43 in brain sections. Then, we studied the effect of TAT-Cx43266-283 on the expression and localization of Cx43 in glioma cells and astrocytes. Knowledge on Cx43 expression and localization within glioma cells and the tumor microenvironment upon TAT-Cx43266-283 treatment could encourage further research on the field of connexin dysregulation and cancer.

S. G. Pelaz et al., 2020 Jaraíz-Rodríguez et al., 2020. Aasen et al., 2019 Sánchez et al., 2020

0259-P

Dextrin-based Nanosponges as an effective approach for drug delivery applications

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The global community is faced with various health concerns, including cancers, heart disease, neurodegenerative diseases diabetes, obesity, osteoporosis, arthritis, and many others [1], considered to cause death. Therefore, the field of nanotechnology in medicine came as a new approach to develop more potent materials for the treatment of the aforementioned diseases [2]. Nano-carrier-based delivery systems have been enormously studied. One of the most promising nanocarriers, dextrin-based nanosponges, particularly cyclodextrin-based nanosponges (CD-NSs), are efficient encapsulating agents capable of delivering drugs and enhancing their bioavailability and efficacy.

Cyclodextrin Nanosponges (CD-NSs) are chemically crosslinked polymers that due to their many attractive features are found in different applications ranging from pharmacy, chemistry, gene delivery, biomedicine, and biotechnology, food, environment [3]. On the basis of numerous application studies, CD-NSs synthesized by reacting CDs with cross-linkers such as carbonyl-diimidazole, diphenyl carbonate and pyromellitic anhydride are considered the most effective delivery systems. Numerous surveys have shown that CD-NSs have emerged over years heading towards greener processes such as the CD-NSs synthesis in natural deep eutectic solvents (NADES), and solvent-free CD-NSs synthesis.

To sum up, our research serves as an introduction to the extensive literature about the synthesis and characterization of the CD-based nano delivery systems that will further meet the challenges of the twenty-first century for improving drug administration, lowering toxicity issues.

0284-P

Modelling the thermal inactivation of Candida rugose lipase free and immobilized on Immobead 150

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Lipases (EC 3.1.1.3) from *Candida rugose* are frequently used in biotransformation both in hydrolysis or synthesis approaches. The economic viability of the enzyme-based industrial processes can be improved by the enzyme immobilization, due to easier biocatalyst recovery after the reaction and the enhance of the catalytic stability. The study of the effect of the immobilization on the thermal inactivation of lipase is of great interest from an industrial standpoint (1). In this work, the inactivation of *C. rugose* lipase free and immobilized on Immobead 150 was studied at 40, 50, 60 and 70ºC. Experimental data were fitted to inactivation kinetic equation (first-order kinetics, models that suggest the existence of a mixture of enzymes, Weibull distribution, series-type and nth order decay models). The first-order model provided the best description of the inactivation of free lipase, with *k* values between 0.025 and 0.092 min-1. This enzyme showed half-lives ranging from 7.5 to 27.9 min and *D* values (decimal reduction time) from 25 to 93 min. The *z* value (derived from log D) of 35ºC. In contrast, the Weibull distribution was the best equation for the lipase immobilized. In this model, the temperature dependence of the 'rate parameter' could be described by the log logistic model, b(T)=ln {1+exp[k'(T-Tc)]}, where *Tc* is a marker of the temperature level where the inactivation occurs at a significant rate, and *k'* is the steepness for the b(T) increase once this temperature has been exceeded (2). The values of *Tc* and *k'* were 79.8ºC and 1.01ºC-1, respectively. Moreover, the reliable time (*tR)* of the enzyme, analogous to the *D* value, ranged from 44 to 26 min at 50-70ºC and the z' value (equivalent of *z* value) was 38,6ºC. Therefore, the improved on the thermal stability observed for the immobilized enzyme lipase make it potentially useful in industrial biotechnology. Additionally, thermodynamic activation parameters were determined.

(1) Rehman, S., Bhatti, H.N., Bilal, M., Asgher, M. and Wang, P. (2017). Catal Lett 147, 281-291. (2) Sant'Anna, V., Correa, A.P.F., Daroit, D. and Brandelli, A. (2013). Bioproc Biosyst Eng 36, 993-998.

0298-P

Combination of Miltefosine with polymeric micelles of poloxamines as nonocarriers for the treatment of leishmaniasis.

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Leishmaniasis is a vector-borne disease, caused by the protozoan parasite from the genus Leishmania and considered as the second most alarming parasitic disease after malaria. Different clinical forms of leishmaniasis are known, being cutaneous leishmaniasis (CL) the most common form, mainly caused by Leishmania major. Miltefosine (MF), an alkylphosphocholine drug, is currently the only recognized oral drug used for the treatment of the different types of leishmaniasis [1]. Besides its gastrointestinal side-effects, the penetration of MF through lipophilic barriers is reduced [2].

Supramolecular systems based on the self-assembly of amphiphilic block copolymers are used as a vehicle for the delivery of therapeutic agents to improve their efficacy and their bioavailability. In this context, formulations of MF with polyethylene oxide (PEO) and polypropylene oxide (PPO)-based polymeric micelles have been developed and proved to enhance the activity of MF against L. major parasites [2]. This work aims to study the combination of MF with Tetronic® T1307, an X-shaped copolymer of PEO and PPO consisting of 72 EO and 23 PO monomers per arm, capable of forming micelles and gels, depending on the temperature and concentration.

The physicochemical properties of the aggregates have been studied using dynamic light scattering (DLS), fluorescence spectroscopy, 1H 1D and 2D NMR spectroscopy, and diffusion NMR.

Our results showed the formation of mixed micelles of MF and T1307 in PBS medium with a hydrodynamic radius of six nm. The combination drug-carrier conferred temperature stability to the mixed micelles, while those of T1307 alone were demonstrated to be sensitive to the temperature. In addition, in the presence of MF, T1307 was able to form micelles at a concentration lower than the critical micelle concentration of its self-assembly. MF interacted with the polymer principally by its hydrophobic tail, in close contact with the PPO moiety of the poloxamine.

Ongoing small angle neutron scattering (SANS) experiments are been carried out to elucidate the structure of the mixed micelles, as well as gel-based formulations of MF and T1307, to be tested against L. major promastigotes and amastigotes, in order to develop a topical formulation

of MF for the treatment of CL.

[1] S. Kapil, P. K. Singh, and O. Silakari, "An update on small molecule strategies targeting leishmaniasis," Eur. J. Med. Chem., vol. 157, pp. 339–367, 2018, doi: 10.1016/j.ejmech.2018.08.012. [2] J. Puig-Rigall et al., "Structural characterization by scattering and spectroscopic methods and biological evaluation of polymeric micelles of poloxamines and TPGS as nanocarriers for miltefosine delivery," Int. J. Pharm., vol. 578, no. January, p. 119057, 2020, doi: 10.1016/j.ijpharm.2020.

0359-R/M-P

An increase of KYNA Solubility and Capacities by Cyclodextrin based-Nanosponges and Cyclodextrins Monomers

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Kynurenic acid (4-Hydroxyquinoline-2-carboxylic acid); KYNA; is an endogenous substance that is formed from tryptophan via kynurenine metabolic pathway. KYNA is an excitatory amino acid antagonist possessing neuroprotective properties. Cyclodextrin(CDs) are truncated cone-shaped oligosaccharides made up of α-(1 ➡ 4) linked glucopyranoside units with six, seven and eight glucose units,α, β and γ-CD, respectively. Cyclodextrin-based delivery systems provide a promising platform to increase drug solubility, stability, and enhance the drug release profile. Several efforts have been made to overcome drawbacks associated with native cyclodextrins and, to improve their performance, different types of cyclodextrin nanosponges were prepared. The nanosponges (NSs) are 3-dimensional hyper crosslinked polymer of native cyclodextrins prepared with a variety of crosslinking agents such as 1,1'-carbonyldiimidazole (CDI), pyromellitic dianhydride (PMDA), and diphenyl carbonate (DPC). The main purpose of the study was improve the solubility and capacities of Kynurenic acid (KYNA) as a therapeutically drug through the interaction between Kynurenic acid (KYNA) and several natural and modified cyclodextrins (CDs) and cyclodextrin nanosponges.The formation of kynurenic acid loaded Cyclodextrin(CDs) and based-nanosponge was confirmed by different characterization techniques (DLS, DSC, TGA, FTIR, XPRD,TEM) and molecular docking calculations provided different interactions and their influence in the complexation constant and the antioxidant activity of loaded KYNA was assessed. Between natural (α- and β-) CDs, the complex of KYNA with β-CD was the most efficient. The inclusion complex of KYNA with CDs showed a strong influence of pH and temperature. The solubility of kynurenic acid was significantly increased with nanosponge (111.1 µg/ml) compared to free kynurenic acid (16.4 µg/ml) and β-cyclodextrin (28.6 µg/ml), higher solubilization of kynurenic acid loaded nanosponge produced better antioxidant activity compared to free kynurenic acid, in addition molecular docking showed HydroxyPropyl-β-CD has the strongest complexation constant (KF), with a value of 270.94 ± 29.80 M- 1.

Argenziano, M., Lombardi, C., Ferrara, B., Trotta, F., Caldera, F., Blangetti, M., et al. (2018). Glutathione/pH-responsive nanosponges enhance strigolactone delivery to prostate cancer cells. Oncotarget, 9(88), 35813–35829. https://doi.org/10.18632/oncotarget.26287 Carpenedo, R., Pittaluga, A., Cozzi, A., Attucci, S., Galli, A., Raiteri, M., et al. (2001).Presynaptic kynurenate-sensitive receptors inhibit glutamate release. The European Journal of Neuroscience, 13, 2141–2147.

0377-P

Introduction of the reverse algorithm in the diagnosis of Syphilis at the Centro Hospitalar do Tâmega e Sousa

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CHTS Clinical Pathology

Introduction

Syphilis is an acute or chronic systemic infectious disease caused by *Treponema pallidum* (TP) and is diagnosed through serological tests[1,2]. In the traditional algorithm, a non-treponemal test (rapid plasma reagin [RPR] or Venereal Disease Research Laboratory test [VDRL]) is used and the reactive samples are confirmed by a treponemal test[2-4]. In the reverse algorithm, a treponemal test is used, and the reactive samples are submitted to a non-treponemal test. Discordant results are submitted to a second confirmatory treponemal test5. There is no *Gold-Standard* for the serological diagnosis of syphilis and all test results must be correlated with the clinical presentation in order to establish the diagnosis of syphilis[2,3]. This work intends to evaluate the impact of the introduction of the reverse methodology in the diagnosis of Syphilis in Centro Hospitalar do Tâmega e Sousa (CHTS).

Material and Methods

In April 2020, in the diagnosis of Syphilis, the RPR nosticon I of Biomerieux was replaced by the automated Syphilis TP Latex test. This test is used for the determination, in Beckman & Coulter - DXC 700AU systems, of the anti-TP antibody in serum and plasma by an immunoturbidimetric method, being considered positive when the result is >10U.

Results

Of the 61 samples tested, the majority were obtained by the medical consultation (57%), followed by Hospitalization (41%) and urgent care centre (2%). There was 100% agreement between both tests. There were 54 (89%) negative tests and 7 (11%) positive tests.

Discussion / Conclusion

The reverse algorithm is more sensitive in detecting primary and late syphilis, specific to syphilis and independent of the observer, allowing, through the use of automated tests, faster results[3].

At the CHTS, 5248 RPR tests were carried out in 2019. Currently, given the scarcity of generated RPR tests, the implementation of the reverse algorithm has proved to be an asset in the automation and optimization of other laboratory processes through a better human resource management. In addition, there is a moderate prevalence of HIV and other risk factors for syphilis in this region, which cannot be undervalued, increasing the likelihood of syphilis, which are not so well diagnosed by non-treponemal methods. Therefore, the implementation of this algorithm has consolidated its added value.

1.The laboratory diagnosis of syphilis.2005 2.Laboratory Diagnostic Tools for Syphilis:Current Status and Future Prospects.2021 3.The Traditional or Reverse Algorithm for Diagnosis of Syphilis:Pros and Cons.2020 4.Screening for syphilis with the treponemal immunoassay: analysis of discordant serology results and implications for clinical management.2011. 5.It is time to use treponema-specific antibody screening tests for diagnosis of syphilis.2012

0420-P

Cytolocalyzation and cytotoxicity of new luminescent cyclometalated platinum(II) complexes: use as organelle biomarkers and antitumoral drugs with potential in photodynamic therapy

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Two series of luminescent cyclometalated Pt(II) complexes were synthesized a nd their biological activity was assessed. One was based on the deprotonated donor-acceptor 2-(4-dimethylaminephenyl)benzothiazole ligand (**NMe_2-pbt**) and includes four mononuclear complexes [Pt(Me_2N-pbt)(C_6F_5)L] (L = Me_2N-pbtH) **1**, *p*-dpbH (4-diphenylphosphino)benzoic acid) **2**, *o*-dpbH (2-diphenylphosphino)benzoic acid) [Pt(Me_2N-pbt)(C_6F_5)(*o*-dpbH)] **3** (unstable)**,** and [Pt(Me_2N-pbt)(*o*-dpb)] **4**, as well as of two binuclear derivatives [{Pt(Me_2N-pbt)(C_6F_5)}2(m-PR_nP)] [PR_4P = O($CH_2CH_2OC(O)C_6H_4PPh_2$)2 **5**; $PR_{12}P$ = O{(CH_2CH_2O)3C(O)$C_6H_4PPh_2$}2 **6**]. The second includes 2,6-difluorophenylpyridine (dfppy) and phenylquinoline (pq) as chromophores and acyclic diaminocarbene (ADC) ligands as auxiliary ligands [Pt(C^N)Cl{C(NHXyl)(NHR)}] [C^N = dfppy (**a**), pq (**b**); R = Pr **7a**, **8a**, CH_2Ph **7b**, **8b**]. In the NMe_2-pbt based complexes the phosphorescent emission is lost in aerated solutions, owing to photoinduced electron transfer to $^3O_2$ and formation $^1O_2$ singlet, as confirmed in complexes **2** and **4**. Here we report some of their biological activity. Cytotoxicity studies in the human cancer cell lines A549 (lung carcinoma) and HeLa (cervix carcinoma) showed good activity for the ADC complexes **7** and **8**. To the best of our knowledge, these compounds represent the first examples of cycloplatinated complexes bearing acyclic diamino carbenes with antiproliferative properties (Ref.). Accordingly, **7a**, **7b** and **8a** altered DNA electrophoretic mobility pointing as a possible cytotoxic mechanism. NMe_2-pbt complexes **2**, **3** and **6** were also active against A549 and HeLa cancer cells, with higher efficiency in A549, in contrast to **1**, **4**, and **5**. Cytolocalization studies revealed that the no cytotoxic ligand **Me_2N-pbtH** and their derivative complexes **1-6** exhibit specific accumulation in the Golgi apparatus. Furthermore, the potential photodynamic property of this type of complexes was demonstrated with the non-cytotoxic complex **4**, which demonstrated efficient photoinduced cytotoxicity after irradiation.

Funding: ADER La Rioja Project 2017-I-IDD-0031 and MICINN Project PID2019-109742GB-I00, both co-founded by the European Regional Development and European Social Funds (ERDF/ESF). RL, MM-J and GM, and E.A-A PhD fellowships are granted by Univ. La Rioja and the Spanish Association Against Cancer (AECC), respectively.

0454-P

A chemical tool to unravel HIV-1 palmitoylome

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The major function of palmitoylation is to mediate stable membrane attachment of soluble proteins. There are multiple viral palmitoylated proteins. One of the most known is the envelope glycoprotein of HIV-1 virus, which is embedded within the viral membrane. It allows the virus to attach and fuse to target cells, starting the infectious cycle. It is composed by two subunits: the transmembrane subunit gp41 and the surface subunit gp120. Gp41 is palmitoylated in four highly conserved cysteine residues: Cys-598, Cys-604, Cys-764 and Cys-837. Cys-764 and Cys-837 are located in the Lentiviral Lytic Peptides (LLPs) of the cytoplasmic tail. Palmitate groups covalently attached to these cysteines insert into the lipid bilayer, interacting with different membrane proteins during HIV-1 budding and assembly, and anchor gp41 to the cell membrane. Furthermore, HIV-1 virions contain cellular membrane proteins, which are caught during budding from cellular membranes. Nowadays, HIV-1 proteome is described, however, a detailed HIV-1 palmitoylome remains undiscovered.

An alkyne-modified palmitoyl compound was synthetized,

which enables the natural incorporation into de proteins during palmitoylation. This analogue of palmitic acid is a clickable lipid with an alkyne group attached to its terminal moiety, which allows the analogue to suffer fluorescence detection or affinity enrichment. Therefore, the main objective of this project is trying to unravel the HIV-1 palmitoylome using as tool this palmitoyl analogue.

For this purpose, HEK 293T cells were seeded, transfected with a proviral plasmid, treated with the palmitoyl analogue, and incubated for 24 h. As a negative control, untreated viral particles were used. Palmitoylated viral particles were purified, and as a positive control, the detection of palmitoylated gp41 in treated samples was verified. Finally, palmitoylated proteins were extracted with an optimized protocol and suspected to proteomic analysis by mass spectrometry.

- Smotrys, J. E., & Linder, M. E. (2004). Annu. Rev. Biochem. 73(1), 559-587. - Veit, M. (2012). Biol. Cell. 104(9), 493-515. - Bhattacharya, J. et al. (2004). J. Virol. 78(10), 5500-5506. - Syu, W. J. et al. (1991). J. Virol. 65(11), 6349-6352. - Yang, C. et al. (1995). PNAS 92(21), 9871-9875. - Nieto-Garai, J. et al. (2021). Adv. Sci. 8(3), 2003468.

0456-P

Global profiling of protein ubiquitylation and lipid interactions crosstalk in living cells

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Post-translational modifications (PTMs) and protein-lipid interactions are critical cellular processes regulating many biological and molecular functions in a living cell. To date, an extensive body of research has been conducted to unravel the precise roles of protein ubiquitination and intramembrane protein-lipid interactions in cells. However, little is known about PTMs and protein-lipid interactions crosstalk in a living cell. In other words, how PTMs and cellular functions derived off depend on binding to specific lipids? Conversely, how post-translational protein modifications regulate the binding of lipids? These questions are challenging to investigate due to a lack of accurate technology. Here, we introduce a new methodology to decipher the precise protein-ubiquitinated-phosphatidylcholine (PC) interactome in living cells. Combining proximity-dependent Biotin Identification (BioID) with a bifunctional clickable and photoactivatable PC analogue generates a powerful tool that leads to the purification and identification by an unbiased chemoproteomic study of all the ubiquitinated proteins interacting specifically with PC in a living cell. The introduced method represents a tour de force in cell biology, facilitating to understand the crosstalk between PTMs and membrane lipids and identify the biological functions associated with these interactions.

- Franco, M., et al. (2011). Molecular & Cellular Proteomics, 10(5). - Haberkant, P., et al. (2013). Angewandte Chemie International Edition, 52(14), 4033-4038. - Li, L., & Zhang, Z. (2016). Molecules, 21(10), 1393. - Ramirez, J., et al. (2015). PloS one, 10(10). - Ramirez, J. (2016). PhD thesis. University of the Basque Country.

05.- Synthetic Biology and Molecular Biotechnology

0206-OS

Selection of high affinity RNA and DNA aptamers for the detection of hepatitis C virus core protein

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Aptamers are synthetic single-stranded nucleic acids (RNA or ssDNA) that, based on their 3D structure in solution, can bind to their targets (ranging from small molecules to cells and tissues) with high affinity and specificity (Ellington and Szostak, 1990) . They are selected by an *in vitro* process termed SELEX and constitute promising biotechnological tools for diagnostic and therapeutic applications. Hepatitis C virus (HCV) can cause chronic hepatitis, which can progress to fibrosis, cirrhosis and hepatocellular carcinoma. Current diagnostic tests are mainly based on serological assays that detect HCV-specific antibodies produced by the infected patient, as well as on molecular assays that quantify HCV RNA in plasma or serum samples. However, rapid, inexpensive and more sensitive analytical tools are still essential for viral diagnostics and treatment monitoring. HCV core is a multifunctional protein that forms the viral capsid and interacts with HCV genomic RNA. As it is the least variable of all the HCV proteins, it has been proposed as an attractive target for the development of new HCV-specific binding molecules, including aptamers. With that aim, we have designed and carried out RNA and ssDNA *in vitro* selection processes in parallel for different variants of HCV core protein belonging to genotypes 1 to 4. The individual aptamers present in the enriched populations after 10 to 14 rounds of amplification/selection (a process that included the required counter-selection steps) were analysed by either clonal sequencing or Ultra Deep Sequencing (UDS). Bioinformatics tools allowed us to identify the most abundant aptamer sequences and structures. Affinity constant (*Kd*) and maximum binding capacity (*Bmax*) of the selected aptamers were quantified by means of optimized colorimetric ELONA as well as ELONA-qPCR (ssDNA aptamers) or ELONA-RTqPCR (RNA aptamers), based on methodologies previously developed in our laboratory (Moreno et al. 2019, *Molecules* 24). The best *Kd* values of the selected aptamers were in the nano-molar range, as low as 0.4 nM, thus evidencing a very high affinity for HCV core protein. The usefulness of our RNA and ssDNA aptamers as bioaffinity probes in aptamer-based biosensors for HCV is currently being investigated.

ELLINGTON, A. D. & SZOSTAK, J. W. 1990. In vitro selection of RNA molecules that bind specific ligands. Nature, 346, 818-22. MORENO, M., FERNANDEZ-ALGAR, M., FERNANDEZ-CHAMORRO, J., RAMAJO, J., MARTINEZ-SALAS, E. & BRIONES, C. 2019. A Combined ELONA-(RT) qPCR Approach for Characterizing DNA and RNA Aptamers Selected against PCBP-2. Molecules, 24

0309-R/M-OS

A naked-eye CRISPR/Cas13a-based nucleic acid detection platform for SARS-CoV2

MARIA LÓPEZ-VALLS, CARMEN ESCALONA-NOGUERO, CIRO RODRIGUEZ-DIAZ, DEMIAN PARDO, MILAGROS CASTELLANOS, CARLOS MARTÍNEZ-GARAY, ÁLVARO SOMOZA, BEGOÑA SOT

IMDEA Nanociencia Protein engineering

Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR system is an adaptive immune system that protects prokaryotic cells against foreign nucleic acid. It has emerged as a promising tool with several applications in biomedicine, including nucleic acid detection for molecular diagnosis. Cas13 is a CRISPR associated nuclease that targets RNA and can then be programmed to specifically recognize and cleave single-stranded (ss) RNA.

The recent SARS-CoV-2 outbreak highlights the need for a fast, specific and sensitive point of care application sensing tool. Here we describe a gold-nanoparticle and CRISPR/Cas13-based naked-eye nucleic acid detection platform to detect SARS-CoV-2. The platform combines gold-nanoparticles and the specific recognition of SARS-CoV2 ssRNA by Cas13a. This recognition triggers the destabilization and aggregation of the gold nanoparticles, which can be easily followed by a change in the solution´s colour. The platform enables naked-eye detection within 30 minutes, representing a fast, cheap and highly sensitive tool.

0035-R-P

Multistable and dynamic CRISPRi-based synthetic circuits

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One of the greatest challenges of synthetic biology is how to accurately control living organisms so that they perform desired (novel) tasks. Synthetic gene circuits provide such control knobs, and constitute invaluable tools for the realization of the next generation of biological devices that will tackle health and environmental challenges of the 21st century. However, most synthetic circuits so far were based on transcription factors, which suffer from inherent constraints

that limit their applicability, such as insufficient modularity, orthogonality and programmability. Alternatively, CRISPRi has emerged as a powerful tool for creating synthetic circuits, both in prokaryotes and eukaryotes;[1] yet, its lack of cooperativity has been pointed out as a potential obstacle for dynamic or multistable synthetic circuit construction. Here we use CRISPRi to build a synthetic oscillator ("CRISPRlator"), bistable network (toggle switch) and stripe pattern-forming incoherent feed-forward loop.[2] Our circuit designs, conceived to feature high predictability and orthogonality, as well as low metabolic burden and context-dependency, allow us to achieve robust behaviors in *E. coli* populations. Mathematical modeling suggests that unspecific binding in CRISPRi is essential to establish multistability. Our work demonstrates the wide applicability of CRISPRi in synthetic circuits and paves the way for engineering more complex synthetic networks, both fundamental and applied, boosted by the advantages of CRISPR technology.[3]

1. Jusiak, B., Cleto, S., Perez-Pinera, P. & Lu, T.K. Engineering Synthetic Gene Circuits in Living Cells with CRISPR Technology. Trends in Biotechnology 34, 535-547 (2016). 2. Santos-Moreno, J., Tasiudi, E., Stelling, J. & Schaerli, Y. Multistable and dynamic CRISPRi-based synthetic circuits. Nature Communications 11, 2746 (2020). 3. Santos-Moreno, J. & Schaerli, Y. CRISPR-based gene expression control for synthetic gene circuits. Biochemical Society Transactions (2020).

0045-R-P

Engineering tools to probe titin mechanics in living cells and animals

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Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain Molecular Mechanics of the Cardiovascular System

The generation and response to mechanical forces determine cell and tissue behaviour in health and disease. The heart undergoes complex and multiscale remodelling processes in response to physiological mechanical cues. For example, sustained mechanical overload shifts the homeostasis of the cardiovascular system toward maladaptive myocyte remodelling, which may result in cardiac failure. Titin is key to the force-generating and mechanosensing properties of muscle cells and titin mutations are a major cause of dilated cardiomyopathy (DCM) and musculoskeletal disease.

Several approaches have been implemented to examine the role of protein mechanics in physiology through modulation of the levels of mechano-active proteins; however, these strategies also interfere with non-mechanical functions of the targeted proteins. Here, we propose broadly applicable methods to specifically manipulate titin mechanics in living cells and animals. Specifically, we are generating mechanical loss-of-function (mLOF) models by TEV-protease-based specific protein severing leading to specific cessation of protein force transduction. Starting from a recently generated knock-in mouse model, we have found that transduction of TEV protease in HaloTag-TEV-titin neonatal cardiomyocytes alters contractility. However, TEV appears to be expressed in vacuolar compartments in myocytes, which may reduce the efficiency of the system. We have implemented a pipeline to examine alternative TEV protease transduction using different viral vectors. Optimal vectors will be used to probe the role of titin mechanics on the resulting mechanobiochemical signaling landscape, cardiomyocyte function and differentiation, demonstrating a novel strategy to probe protein mechanics in living matter.

0063-R/M-P

Using molecular biology techniques to disentangle the origin of the exotic invasive plant Arundo donax L.

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Arundo donax L. (Poaceae) is considered to be one of the worst invasive plants in the world (Jiménez-Ruiz et al., 2021). Most of the 12 phylogenetic studies focusing on *A. donax* show low or no genetic diversity in the invaded range (including the Mediterranean) and genetically variable individuals in Asia. Based on plastid mini- and microsatellites, Hardion et al. (2014) found the nearest relative of the invasive clone of *A. donax* in a Middle East lineage distributed along the Indus Valley. They also found three other lineages along the Himalayan slopes and in China. Ahmad et al. (2008) documented the occurrence of only one genotype across the southern USA using SRAP markers, Hardion et al. (2012) documents a genotype in the Mediterranean with AFLPs, and Canavan et al. (2017) documented a genotype in South Africa with SSRs. Using AFLP, Malone et al. (2017) showed the occurrence of two lineages in Australia, which may have been the gathering of an invasive clone, and another Asian lineage expanded through Indonesia. Based on the SSRs in the maize genome, Pilu et al. (2014) found some genotypic differences in Italy, without geographical structuring. In contrast, Tarin et al. (2013) found a very high genetic diversity in the Mediterranean (129 genotypes from 203 samples and a Nei genetic diversity of 0.929) and a weak diversity in North America, using 10 SSRs specifically developed. The authors noted that sampling confusions with *Phragmites* were possible, but they screened these putative errors with control genotypes of *Phragmites*. In comparison to the results of other studies in the Mediterranean, the results of the study calls for further uses of these specific SSRs in broader sampling in the Mediterranean as well as for Asian populations using newly collected fresh material. As a preliminary result, Canavan et al. (2017) did not find genetic diversity in South Africa using these 10 SSRs. To date, new generation sequencing and large SNP datasets have not been used to estimate genetic diversity or resolve phylogenetic relationships within *Arundo*. In conclusion, in its introduced range, *A. donax* shows strong genetic uniformity and no seed production. However, in Asia, this taxon is fertile and morphologically and genetically polymorphic.

Hardion, L., R. Verlaque, A. Baumel, M. Juin, and B. Vila. 2012. "Revised systematics of Mediterranean Arundo (Poaceae) based on AFLP fingerprints and morphology." Taxon 61 (6): 1217-1226. Tarin, D., A. E. Pepper, J. A. Goolsby, P. J. Moran, A. C. Arquieta, A. E. Kirk, and J. R. Manhart. 2013. "Microsatellites uncover multiple introductions of clonal giant reed (Arundo donax)." Invasive Plant Science and Management 6 (3): 328-338.

0153-R/M-P

Nanotechnology-based strategies for non-viral delivery of CRISPR proteins

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IMDEA Nanociencia Nanobiosystems

The CRISPR/Cas technology has revolutionized the gene editing field, enabling fast and efficient manipulation of DNA and RNA sequences. CRISPR applications include knock-out and knock-in generation, base editing and transcriptional modulation. The system consists of a Cas endonuclease in complex with a guide crRNA. This crRNA mediates the recognition of complementary DNA sequences which are subsequently cleaved by the nuclease. Upon cleavage, target DNA can be repaired via non-homologous end joining or homology directed repair, less efficient but required for precise gene editing . The CRISPR system has a great potential for treatment of genetic diseases. Nonetheless, the safe and efficient delivery of Cas nucleases and their guides remains a major challenge.

So far, viral methods are the preferred strategy for CRISPR delivery. However, they present important drawbacks such as low loading capacity and immunogenicity. Nanotechnology constitutes an interesting alternative to overcome these obstacles. This project aims to design CRISPR nanostructures that allow for a safer and more efficient targeting of oncogenic mutations. In order to do so, two different kinds of biocompatible nanocomplexes have been tested.

Our first strategy is based on the attachment of Cas proteins to the surface of magnetic nanoparticles (MNPs) either through electrostatic interaction or covalent conjugation. For covalent binding, MNPs have previously been modified with a *smart* linker for the controlled intracellular release of the nuclease. In our second approach, albumin molecules have been chemically modified to form covalent interactions with Cas nucleases. The resulting nanostructures consist of a Cas nuclease molecule surrounded by albumin, which protects it from degradation and mediates cellular uptake. Albumin molecules are bound through a *smart* linker allowing for the intracellular disassembly of the nanocomplexes. Both kinds of nanostructures were successfully generated and Cas nuclease activity was preserved throughout the process as shown by *in vitro* DNA cleavage tests. Furthermore, the nanoconjugates were efficiently internalized by mammalian cells.

0361-R/M-O

Genetically engineered extracellular vesicles as fluorescent nanocarriers encapsulating a novel theragnostic nanotherapy targeting fibroblasts

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Extracellular vesicles (EVs) are small lipid bilayer vesicles that are released from cells and are involved in various biological and pathological processes. The Transforming Growth Factor b (TGFb) has been shown to be the main cytokine that promotes the production of extracellular molecules that are overexpressed in fibrosis. Since EVs function as cellular communication systems, the use of EVs as drug delivery vehicles offers advantages compared to other drug delivery systems such as liposomes and polymeric nanoparticles. EVs used as drug vehicles are not immunogenic and avoid phagocytosis and degradation by macrophages, thus prolonging the half-life of the drug in the body. There are different types of EVs regarding their intracellular origin such as: microvesicles, exosomes and apoptotic bodies. Depending on their composition.

In this work, we show the use of engineered exosomes and microvesicles as nanocarriers for the study of cardiac anti-fibrotic treatments with a theragnostic nanocluster (CTPR390-Au), whose capability to inhibit fibrosis by altering the regulatory function of Hsp90 has been previously demonstrated by our group.

EVs markers in isolated exosomes and microvesicles were verified and CTPR390-Au was encapsulated by incubation and electroporation by differential centrifugation of NIH-3T3 fibroblast cell line. Encapsulation of the CTPR390-Au nanocluster was confirmed by flow cytometry. TGFb-activated NIH-3T3 fibroblast were treated with the CTPR390-Au nanocluster encapsulated in microvesicles or exosomes, recovering healthy pro-fibrotic gene expression levels.

Here we demonstrate the anti-fibrotic effect of a novel nano-system designed to transport and reduce fibroblast-gen-

erated extracellular matrix compounds synthesis.

0370-P

Papel de la peroxirredoxina Tsa1 en el crecimiento, respuesta a estrés y acumulación de trehalosa durante la propagación de biomasa de las levaduras vínicas

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En la enología moderna se emplean inóculos comerciales puros de cepas vínicas de la levadura *Saccharomyces cerevisiae*, en forma de levadura seca activa (LSA). Para ello, la levadura atraviesa una serie de procesos industriales sujetos a múltiples condiciones de estrés. Las peroxirredoxinas son una familia de enzimas implicadas en la defensa a estrés oxidativo, protegiendo la célula de los radicales libres de oxígeno (ROS) producidos principalmente durante el metabolismo respiratorio. Para realizar su función, reciben su poder reductor de las tiorredoxinas, y estas de la tiorredoxina reductasa. La peroxirredoxina Tsa1 actúa como un interruptor redox regulando la función de enzimas metabólicas y la ruta PKA. En levaduras industriales, la tiorredoxina reductasa Trr1 regula la respuesta a nutrientes y, en condiciones de vinificación, las tiorredoxinas citosólicas (Trx1/2) controlan diversos aspectos del metabolismo y su relación con las rutas de respuesta a nutrientes. Por ello, resulta interesante estudiar si el sistema tiorredoxina, además de actuar frente al daño oxidativo, también ejerce un control sobre el metabolismo de la levadura durante su uso industrial.

El uso de mutantes de deleción en cepas vínicas ha permitido demostrar que Tsa1, Trx1/2 y Trr1 son necesarios para el crecimiento normal de la levadura en medio con glucosa y sacarosa como fuentes de carbono. Mediante simulaciones a escala de laboratorio del proceso industrial de propagación de biomasa en melazas, se ha demostrado que el mutante *tsa1Δ* en una cepa vínica industrial ve afectado su crecimiento, tanto en matraz como en biorreactor. Sorprendentemente, el mutante *tsa1Δ* además de mostrar un trastorno del estado redox celular, presenta alteraciones a nivel metabólico que permiten atribuir nuevas funciones a Tsa1. Por un lado, durante el crecimiento en melaza, Tsa1 influye en los niveles intracelulares de trehalosa, reprimiendo su acumulación temprana y manteniendo niveles altos durante la fase estacionaria. La acumulación de glucógeno también aumenta a la entrada de la fase estacionaria en el mutante *tsa1Δ*. Por otro lado, durante la fermentación del mosto de uva, la deleción de *TSA1* reduce la capacidad fermentativa de la levadura y altera los niveles de ácido acético, sin que el perfil de la vinificación se vea alterado significativamente.

Garrigós, V., Picazo, C., Matallana, E. & Aranda, A. Wine Yeast Peroxiredoxin TSA1 Plays a Role in Growth, Stress Response and Trehalose Metabolism in Biomass Propagation. Microorganisms 8, 1537 (2020).

0426-R/M-P

Hypusinated eIF5A is required for the translation of collagen

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The translation elongation factor eIF5A is an essential and highly conserved protein and the only known protein containing the post-translational modification hypusination. Hypusine formation is required for eIF5A activity and implies two sequential enzymatic steps catalyzed by a deoxyhypusine synthase and a deoxyhypusine hydroxylase [1]. Hypusinated eIF5A binds to the ribosome and interacts with the P-tRNA to promote productive positioning for peptide bond formation. eIF5A facilitates translation through polyproline sequences containing three or more consecutive prolines [2], but also alleviates ribosome stalls at tripeptide sequences combining proline with glycine and charged amino acids [3].

Collagen is the most abundant protein in vertebrates, constituting more than 25% of human body weight. Collagens are essential in the extracellular matrix and function in tissue structure, development and remodeling, cell adhesion and migration, cancer, and angiogenesis. Herein we investigated if eIF5A is involved in the synthesis of mammalian collagens as they are enriched in putative eIF5A-dependent Proline-Glycine-containing tripeptides repetitions which enable the formation of the triple-helical folding. First, we confirmed that depletion of yeast eIF5A interrupts translation at proline-glycine-proline and glutamic-proline-glycine motifs of mouse collagen fragments expressed in *Saccharomyces cerevisiae* by using a dual luciferase reporter system. Second, we showed that depletion of active eIF5A in mouse fibroblasts reduced collagen 1 content, which concentrated around the nuclei, and up-regulated endoplasmic reticulum (ER)-stress markers suggesting retention of partially synthesized collagen 1 in the ER. Third, we observed that in eIF5A-depleted human hepatic stellate cells treated with the profibrotic cytokine TGF-β1, collagen I protein was hardly detected. Altogether, our results show that eIF5A is required for collagen translation and point it as a potential target for regulating collagen production in fibrotic diseases, which account for nearly 45% of all deaths in the developed world.

[1] Park, M. H. & Wolff, E. C. 2018. Hypusine, a polyamine-derived amino acid critical for eukaryotic translation. J. Biol. Chem., 293 [2] Gutierrez, E., Shin, B. S., Woolstenhulme, C. J., Kim, J. R., Saini, P., Buskirk, A. R. & Dever, T. E. 2013. eIF5A promotes translation of polyproline motifs. Mol. Cell, 51 [3] Pelechano, V. & Alepuz, P. 2017. eIF5A facilitates translation termination globally and promotes the elongation of many non polyproline-specific tripeptides. Nucleic Acids Res., 45

06.- Biomembranes

0526-OI

Structures of human LAT2/CD98hc and bacterial BasC amino acid transporters reveal mechanisms of transport, substrate selectivity and pathology in the Heteromeric Amino acid Transporters

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The Heteromeric Amino acid Transporters (HATs) are amino acid exchangers that are composed by an ancillary protein and the transporter subunit (LAT transporter) [1]. Mutations in HATs cause or are associated with aminoacidurias, autism, age-related hearing loss and cataracts, and confer metabolic advantage to cancer cells [1]. In the last two years X-ray crystallography and cryo-EM has been used to obtain high-resolution structures of bacterial LATs and human HATs [2-4]. LATs present the APC protein fold. We used a multidisciplinary approach (structural and functional studies, and molecular dynamics) to identify key residues for transport function and substrate specificity in both the human LAT2 and bacterial BasC transporter proteins.

Structural studies of the bacterial BasC [5] identified two fully conserved residues (Tyr 236 and Lys 154 in case of BasC) within the LATs transporters that determine the asymmetric interaction of the substrates with LATs (external K_M in the μM range and internal K_M in the mM range). These two residues are located in each of the two Na^+ sides of Na^+-dependent transporters with the APC-fold. One of these residues BasC (Lys 154), is mutated in lysinuric protein intolerance and is key for the occlusion of the transporter at the intracellular side. The beauty of LATs is that residues that substitutes Na^+ ions confer an intrinsic asymmetric interaction with the substrates.

Our study of human LAT2/CD98hc identified molecular determinants of the substrate specificity of LATs for neutral amino acids (e.g., LAT2, LAT1 and Asc1) [6]. Interestingly, some of these determinants explain the molecular mechanisms that alter substrate specificity in mutations associated with disease.

[1] D Fotiadis, Y Kanai, M Palacin, Mol Aspects Med 34 (2013), p. 139-158. [2] Y Lee et al, Nat Struct Mol Biol 26 (2019), p. 510-517. [3] R Yan, X Zhao, J Lei, Q Zhou, Nature 568 (2019), p. 127-130. [4] D Wu, TN Grund, et al, PNAS 117 (2020), p. 21281-21287. [5] E Errasti-Murugarren et al, Nat Commun 10(1) (2019), 1807. [6] The authors acknowledge the received funding from "la Caixa" Foundation (LCF/PR/HR20/52400017), Ministry of Science and Innovation (RTI2018-094211-B-100) and CIBERE

0528-OI

Building a machine for secretion of bulky collagens and its application to tissue fibrosis

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Secreted collagens compose 25% of our dry protein weight and necessary for tissue organization, and skin and bone formation. But how are these bulky cargoes that are too big to fit into a conventional COPII vesicle exported from the ER? Our discovery of TANGO1 (*Bard et al., 2006; Saito et al., 2009; Santos et al., 2015*), a ubiquitously expressed, ER-exit-site-resident, transmembrane protein has made the pathway of collagen secretion amenable to molecular analysis. TANGO1 acts as a scaffold to connect collagens in the lumen to COPII coats on the cytoplasmic side of ER. However, the growth of the collagen containing mega transport carrier is not simply by accretion of a larger COPII coated patch of ER membrane, but instead by rapid addition of premade ERGIC 53 containing small vesicles and tubules. This mode of transport carrier formation is fundamentally different from that used to produce small COPII vesicles. We have seen that TANGO1 rings the ER exit site and thus organizes a sub-compartment within the ER (*Nogueira et al., 2014; Raote et al., 2017*). The transmembrane helices of TANGO1 in prevent the mixing of ERGIC53-containing membranes to the bulk of ER. This allows transport of collagen from the lumen of ER into the ERGIC 53 compartment via a tunnel. We have now mapped the components that work in concert along with the cargo to assemble TANGO1 into a ring (*Raote et al., 2018: Raote and Malhotra 2019; Raote et al., 2020; Raote and Malhotra 2021*). Mathematical modelling, biochemistry and super resolution microscopy based analyses of this process will be discussed. TANGO1 is genetically mutated in patients with collagenopathies (Lekszas et al., 2020). Our ongoing studies on TANGO1 function in exporting right quantity and quality of collagens and how this activity can be targeted to control collagen hyper secretion-dependent tissue fibrosis will be discussed.

Bard et al. Nature 439: 604-607 (2006) Saito et al. Cell 136: 891-902 (2009) Santos et al. Elife. 2015 Nov 14;4. pii: e10982 Nogueira et al. Elife. May 19;3:e02784 Santos et al. J Cell Biol. May 9;213(3):343-54 Raote

et al. J Cell Biol. (2017) Apr 3;216(4):901-909 Raote et al. Elife. Mar 7;7. pii: e32723 Raote and Malhotra. J. Cell Biol. 2019 218(3):737-739 Raote et al. Elife. 2020 May 26;9:e57822 Raote, I and Malhotra, V. Ann. Rev. Biochem. 2021 Lekszas et al. Elife. 2020 Feb 26;9. pi

0167-R-OS

ANTIBIOTIC-LOADED SOLID LIPID NANOPARTICLES TO IMPROVE ANTIBIOTIC EFFICIENCY

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The use, abuse and misuse of antibiotics during the last decades has exerted a selective pressure on bacteria that has triggered alarmingly their resistance to numerous antibiotics. As a result, therapeutic treatments that were once effective now turn out to be ineffective. This situation represents one of the greatest public health threats. If we do not act, we will run out of therapeutic resources against bacterial infections. It is therefore essential to look for alternatives to combat this problem. Nevertheless, although the search for new antibiotics is one of the most common strategies, the release of a new antibiotic on the market is expensive and it can take many years. For this reason, the possibility of improving the effectiveness of existing antibiotics is considered a more realistic possibility in the short and medium term.

Solid Lipid Nanoparticles (SLN) consists of a lipidic solid matrix surrounded by a surfactant monolayer and are promising drug delivery systems to improve the effectiveness of existing therapeutic molecules. In this regard, encapsulation of existing antibiotics into SLN is a promising strategy to treat infectious diseases.

In this work we encapsulate antibiotics that are being used regularly into SLN to treat bacterial infections. Then, physico-chemical characteristics of antibiotic-loaded SLNs are determined. Finally, the effect that encapsulated antibiotics have on bacterial growth is compared to the effect of free antibiotics.

It is expected that encapsulation of antibiotics into SLN will enhance their activity against bacteria, providing decreasing of the necessary dose of antibiotic, thus, reducing the antibiotic resistance spread among bacteria.

0337-OS

A photoswitchable protein fragment with light-controllable interface / transmembrane topology in lipidic membranes

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According to the three-step model, the spontaneous insertion and folding of helical transmembrane (TM) polypeptides into lipid bilayers is driven by three sequential equilibria: solution-to-membrane interface (MI) partition, unstructured-to-helical folding, and MI-to-TM helix insertion. Understanding these three steps with molecular detail has been challenged by the lack of suitable experimental approaches to rapidly and reversibly perturb membrane-bound hydrophobic polypeptides out of equilibrium. Here, we report on a 24-residues-long hydrophobic α-helical polypeptide, covalently coupled to an azobenzene photoswitch (KCALP-azo), which displays a light-controllable TM/MI equilibrium in hydrated lipid bilayers. FTIR spectroscopy shows that dark-adapted KCALP-azo (trans azobenzene) folds as a TM α-helix. After trans-to-cis photoisomerization of the azobenzene moiety with UV light (reversed with blue light), spectral changes by FTIR spectroscopy indicate that the helical structure of KCALP-azo is maintained but the peptide experiences a more polar environment. Polarized experiments confirmed that the membrane topology of KCALP-azo is altered by light, with its helix tilt changing reversibly from 32 ± 5° (TM topology, blue light) to 79 ± 8° (MI topology, UV light). Further analysis indicates that, while the trans isomer of KCALP-azo is 100% TM, the cis isomer exists in a ~90% TM and ~10% MI mixture.

The possibility to perturb with light the transmembrane / membrane interface equilibrium of a peptide with light, even if only partially as in the present case, will allow to use short laser pulses to study how peptides insert or move our of membranes with unprecedent temporal resolution and molecular detail. We are currently working in this direction

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0459-R/M-OS

The structural flexibility of the antimicrobial peptides LL-37 and Magainin-2 helps to understand their mechanisms of membrane disruption

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Antimicrobial peptides (AMPs) are components of the host innate defence system with activity against bacteria, fungi and viruses. The amphipathic and cationic nature of these small peptides allow them to target different molecules in the surface and inside the bacteria (LPS, LTA, phospholipids, proteins or nucleic acids). In spite of their co-evolution with bacteria during millions of years, the AMP resistance seems to evolve at much lower rate than to antibiotics, making them promising candidates for fighting the antimicrobial resistance threat.

Despite all the knowledge related to AMPs, their mechanism of membrane disruption is not fully understood. Firstly, they are attracted to the membrane by electrostatic interactions and, after a peptide/lipid threshold is reached, the membrane is disrupted by different mechanisms [1]. The difficulty to understand these mechanisms can come from the AMPs structural flexibility, which allows them to form different oligomers/complexes.

Through X-ray crystal structures, we show the conformational flexibility of the well-known human cathelicidin LL-37 and the *Xenopus laevis* magainin-2. LL-37 is monomeric but able to dimerise at high concentrations or in the presence of membrane-mimicking detergents. Together the dimeric structures revealed discrete detergent binding sites at the dimer interface indicating the presence of *in vivo* lipid binding sites. We also obtained crystals containing a LL-37 supramolecular fibril-like architecture, which can be visualised by cryoEM using nanogold-labelled LL-37 and lipid vesicles. This architecture represents a LL-37-membrane active state [2]. Finally, LL-37 was also shown to be able to form a tetrameric narrow channel with defined conductivity [3].

The magainin-2 crystals obtained in the presence of the detergent dodecylphosphocholine showed an antiparallel arrangement of monomers which is stabilized by a phenylalanine zipper motif spanning the hydrophobic side of this dimer. The trimerization of the dimer leads to a hexameric peptide channel with a positively charged pore and a hydrophobic membrane-exposed belt. By using molecular dynamic studies the flux of ions through this channel was modelled and its anion-selectivity and estimated conductivity was in agreement with previous experimental results.

1. Nguyen, L.T et al. The expanding scope of AMP structures and their modes of action. Trends Biotechnol. 29, 464–472 (2011) 2. Sancho-Vaello, E. et al. Structural remodeling and oligomerization of human cathelicidin on membranes suggest fibril-like structures as active species. Sci. Rep. 7, 15371 (2017) 3. Sancho-Vaello, E. et al. The structure of the antimicrobial human cathelicidin LL-37 shows oligomerization and channel formation in the presence of membrane mimics. Sci. Rep. 10, 17356 (2

0114-P

Exploring the insertion limits in cellular membranes

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Every cell of a living organism is delimited by biological membranes. These structures allow cells to interact with others and also act as a boundary between them and the extracellular environment. Biological membranes are composed of lipids and proteins that create a semi-permeable barrier; because the lipid bilayer halts free diffusion of most molecules and ions, membrane proteins play a major role in connecting both sides of the membrane. Membrane proteins contain hydrophobic regions on their sequences that permit them to adapt, fold and orientate within the lipid membrane, enabling them to function correctly.

The present work focuses on unraveling the limits of the insertion of polypeptide sequences into biological membranes. Therefore, we have studied systematically the capability of computationally designed amino acid sequences to insert into mammalian cell membranes. We selected from a previous computational and *in vitro* analysis1, a set of designed poorly hydrophobic polypeptide segments with naturally occurring amino acid distribution. Then, using an *in vivo* (Hek293T cell cultures) system we challenge the length and hydrophobicity threshold required for a polypeptide segment to insert efficiently into the ER membrane through the translocon. Together with known strategies to control membrane protein topology, these findings may do the groundwork for *de novo* design of membrane proteins.

1. Baeza-Delgado, C., Heijne, von, G., Marti-Renom, M. A. & Mingarro, I. Biological insertion ofcomputationally designed shorttransmembrane segments. Sci. Rep. 1–9 (2016). doi:10.1038/srep23397

0159-P

Effect of lactoferrin on intestinal physiology through the regulation of intestinal serotonergic system and innate immune receptors.

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Milk is a natural food composed of a wide variety of elements, among them lactoferrin, a bioactive protein that plays an important role in several physiological effects such as regulation of iron transport, antimicrobial activity, antioxidant function, anti-tumoral activity, immunomodulatory and anti-inflammatory effects. The aim of this work was to study the effect of bovine lactoferrin on intestinal physiology, specifically on the serotonergic system, in which serotonin regulates the whole gastrointestinal functions, and on the innate immune system, since intestinal mucosa is a key defensive component constantly exposed to potentially pathogenic foreign agents.

Caco2/TC7 cells were used as in vitro experimental model of intestinal epithelium and ileum mouse samples as in vivo model. The effect of bovine lactoferrin was assess on the serotonin transporter (SERT) activity and expression. In addition, molecular expression of serotonin receptors (5-HTR_{1A}, 5-HTR_{2A}, 5-HTR_{2B}, 5-HTR_{3}, 5-HTR_{4} and 5-HTR7), serotonin-synthesis enzymes (TPH1, TPH2) and innate immune receptors (TLR2, TLR3, TLR4, TLR5, TLR7, TLR9, TLR10, TLR11, NOD1 and NOD2) were analyzed.

Our results demonstrate that bovine lactoferrin modulates activity and expression of SERT, significantly increasing serotonin uptake and SERT expression. Moreover, lactoferrin modifies the expression of other serotonergic system components and also innate immune receptors. Similar results were also obtained in mice treated with lactoferrin, demonstrating the regulatory potential of lactoferrin on serotonergic and innate immune system and thus on the intestinal physiology. In addition, bovine lactoferrin seems to revert the effect of TLR2 activation on SERT and oxidative stress, suggesting an anti-inflammatory and antioxidant effect on intestinal epithelium.

The results of this work would support the lactoferrin potential as functional ingredient in the regulation of the intestinal serotonergic system and innate immunity in gastrointestinal disorders.

0184-P

New antimicrobial therapies against multidrug-resistant respiratory pathogens

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In recent years, the appearance of multidrug-resistant respiratory pathogens such as *Klebsiella pneumoniae* or *Pseudomonas aeruginosa* has substantially increased the need to develop new antimicrobial therapies. Attention has been focused on human host defense antimicrobial peptides (AMPs) which play a critical role in warding off invading microbial pathogens. In addition, the efficacy of conventional antimicrobials is crucial to be improved so clinical research has also focused on combining clinically used antibiotics with AMPs. Two promising host defense factors are pulmonary surfactant protein A (SP-A) and the antimicrobial peptide SP-B^N. We previously reported that both exert synergistic antimicrobial activity against the respiratory pathogen *Klebsiella pneumoniae* K2, which is resistant to either protein alone **(1)**. The aim of this work was to characterize the mechanism by which SP-A and SP-B^N act synergistically against *K. pneumoniae* and to evaluate the potential synergistic antimicrobial activity of SP-A and SP-B^N with conventional antibiotics against respiratory pathogens.

Our results indicate that SP-A/SP-B^N complex alters the bacterial ultrastructure of *K. pneumoniae* due to the ability to bind to lipopolysaccharide molecules present in the outer membrane, forming pores in the membrane that favor the translocation of both proteins to the periplasmic space. There they interact with the inner membrane by causing its permeabilization and depolarization, perhaps through the induction of toroidal pores. On the other hand, while SP-A only acts synergistically with polymyxin B, the peptide SP-B^N could act synergistically with many conventional antibiotics clinically used for respiratory infections against *K. pneumoniae* and *P. aeruginosa.* This combined therapy with SP-B^N and antibiotics did not show cytotoxicity in human alveolar epithelial cells (A549 cell line).

In conclusion, the synergistic antimicrobial activity of SP-A/SP-B^N is based on the capability to alter the integrity of bacterial membranes. SP-B^N also improves the efficacy of conventional antibiotics against *K. pneumoniae* and *P. aeruginosa*, expanding the antimicrobial use of this peptide. The characterization of mechanisms that govern this synergistic activity may provide clues to improve current therapeutic treatments.

1. Coya JM, Akinbi HT, Sáenz A, Yang L, Weaver TE, Casals C. Natural Anti-Infective Pulmonary Proteins: In Vivo Cooperative Action of Surfactant Protein SP-A and the Lung Antimicrobial Peptide SP-BN. J Immunol 195:1628-1636. 2015

0227-P

Cholesterol stimulates the lytic activity of Adenylate Cyclase Toxin on lipid membranes by promoting toxin oligomerization and formation of pores with a greater effective size

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Several toxins acting on animal cells present different, but specific, interactions with cholesterol. *Bordetella pertussis* infects the human respiratory tract and causes whooping cough, a highly contagious and resurgent disease. Its virulence factor adenylate cyclase toxin (ACT) plays an important role in the course of infection. ACT is a pore-forming cytolysin belonging to the RTX (Repeats in ToXin) family of leukotoxins/hemolysins and is capable of permeabilizing several cell types and lipid vesicles. Previously, we observed that in presence of cholesterol ACT induces greater liposome permeabilization. Similarly, recent reports also implicate cholesterol in the cytotoxicity of an increasing number of pore-forming RTX toxins. However, the mechanistic details by which this sterol promotes the lytic activity of ACT or of these other RTX toxins remain largely unexplored and poorly understood. Here, we have applied a combination of biophysical techniques to dissect the role of cholesterol in pore formation by ACT. Our results indicate that cholesterol enhances the lytic potency of ACT by promoting toxin oligomerization, indispensable step for ACT to accomplish membrane permeabilization and cell lysis. Since by our experimental design we can discard that this cholesterol effect derives from toxin accumulation due to lateral lipid phase segregation, we hypothesize that cholesterol facilitates lytic pore formation, by favouring a toxin conformation more prone for protein-protein interactions and oligomerization. Our data shed light on the complex relationship between lipid membranes and protein toxins acting on these membranes. Coupling cholesterol binding, increased oligomerization and increased lytic activity is likely pertinent for other RTX cytolysins.

0260-R/M-P

Antiproliferative effect of rational-designed ChoKα1 inhibitors and choline uptake in cancer cell lines: an enzyme-transporter paradigm

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A better understanding of cancer is one of the main objectives of both basic and applied research. For this reason, the most part of the work in this field tends to be focused on targeted therapies. Regarding these strategies, inhibitors are rationally designed to interact with proteins or enzymes that may play an important role in oncogenic and/or metabolic pathways. Choline kinase (ChoK) is a dimeric enzyme that catalyses the bioconversion of choline to phosphocholine (PCho). One of its three monomeric isoforms is alpha-1 (ChoKα1), and it has emerged as a therapeutic target against cancer because the gene that encodes for this subunit (*CHKA*) is overexpressed in tumour cell lines, resulting in an increase of the enzymatic activity of ChoK. These two facts are directly associated with alterations of oncogenic signalling pathways like RAS/MAPK or PI3K/AKT. Our research group evaluates the antiproliferative effect of rational-designed inhibitors of ChoKα1. In our latest work we also describe that the main pathway of choline uptake in tumour cells is Na+-free, and it is mostly mediated by the choline transporter-like protein 1 (CTL1). A possible correlation between the inhibition of both ChoKα1 and choline uptake by the rational-designed compounds is highlighted.

Haga T. Molecular properties of the high-affinity choline transporter CHT1. J Biochem. 2014; 156(4):181-94. Serrán-Aguilera L et al. Synthesis, biological evaluation, in silico modeling and crystallization of novel small monocationic molecules with potent antiproliferative activity by dual mechanism. Eur J Med Chem. 2020;1;207:112797. Sola-Leyva A et al. Choline kinase inhibitors EB-3D and EB-3P interferes with lipid homeostasis in HepG2 cells. Sci Rep. 2019;9(1):1–13.

0324-P

The amyloid-β peptide interacts with PMCA and potentiates its binding to methylene blue by forming a ternary complex.

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The plasma membrane Ca^{2+}-ATPase (PMCA) plays an essential role in the regulation of intracellular Ca^{2+} concentration. The toxicity of the β-amyloid peptide (Aβ) in amyloidogenic diseases such as Alzheimer's disease (AD) seems to be related to the deregulation of intracellular Ca^{2+} levels, but its mechanism of action is still unknown. We have previously reported that Aβ inhibits the PMCA activity, and this inhibition could be involved in the failure of Ca^{2+} homeostasis related to the toxicity of Aβ in AD. Therefore, the search for compounds that block this inhibition is essential for the correct transport of Ca^{2+} out of the cell and the maintenance of Ca^{2+} homeostasis. Methylene blue (MB) is a phenothiazine that has revealed to have protective effects against several neurodegenerative disorders, including AD. Previous results of the group show that MB up-modulates PMCA and prevents the inhibitory effect of Aβ through its interaction with the pump and the peptide. Here, we have used fluorescence approaches to show that MB interacts with PMCA with high-affinity (Kd=23 μM). Besides, Aβ also interacts with PMCA and potentiates the binding of MB to form a MB-(Aβ-PMCA) ternary complex (Kd=2 μM). These results allow us to deep in the mechanism of neurotoxicity produced by Aβ and show MB as a potential agent to be considered in the development of future therapeutic approaches.

This work has been supported by Grants from Ministerio de Economía y Competitividad (MINECO, BFU201785723P) and Junta de Extremadura (GR18118), cofinanced by FEDER funds.

0345-R-P

Topological studies of the sodium leak channel NACLN

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The sodium leak channel non-selective (NALCN) is essential for survival in mammals and there is multiple evidence of its physiological significance. For example, it is implicated in respiratory rhythm, motor function, pain sensitivity and circadian rhythm. NALCN is mainly expressed in the central nervous systems and plays a key role in regulating the resting membrane potential and controlling neuronal excitability. Therefore, mutations in NALCN are implicated in neurodevelopmental disorders and lead to complex neurodevelopmental syndromes, including infantile hypotonia with psychomotor retardation and characteristic facies (IHPRF) and congenital contractures of limbs and face, hypotonia and developmental delay (CLIFAHDD).

The present work focuses on studying the membrane topology and folding of domain I of NALCN. First of all, we used an *in vitro* transcription/translation system to study the insertion capacity of the isolated predicted hydrophobic regions of domain I of NALCN protein into microsomal membranes and an *in vivo* glycosylation-based reporter system in HEK-293T cells. Then, we studied the mechanism of membrane integration by characterizing the insertion of different truncates *in vitro* in microsomal membranes and *in vivo* in bacterial membranes fusing the truncates to a dual reporter system phoA-lacZ. Experimental data reveal that NALCN domain I has six transmembrane (TM) segments with N-terminus and C-terminus exposed to the cytoplasmic side. These results are in agreement with the structures recently solved by CryoEM and allow us to envision the folding at the early stages of membrane integration.

Furthermore, the mutations associated with CLIFAHDD syndrome found in the sixth TM segment of domain I of NALCN were evaluated *in vitro*. The results show that these mutations do not affect the insertion into the membrane. However, additional studies are in progress to understand how these mutations alter NALCN function.

Kschonsak, M. et al. Structure of the human sodium leak channel NALCN. Nature 587, 313–318 (2020). https://doi.org/10.1038/s41586-020-2570-8 Xie, J. et al. Structure of the human sodium leak channel NALCN in complex with FAM155A. Nat Commun 11, 5831 (2020). https://doi.org/10.1038/s41467-020-19667-z Kang, Y., Wu, JX. & Chen, L. (2020) Structure of voltage-modulated sodium-selective NALCN-FAM155A channel complex. Nat Commun 11, 6199 (2020). https://doi.org/10.1038/s41467-020-20002-9

0404-R/M-P

Interfacial Delivery: Using Pulmonary Surfactant to Deliver Inhaled Therapeutics

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Pulmonary surfactant (PS), a membrane-based lipid-protein material essential for the process of breathing, offers novel opportunities for pulmonary drug delivery. The current paradigm in pulmonary drug delivery relies on aerosols that deposit, depending on their aerodynamic properties, directly in the place of action or adsorption. However, these strategies are not very efficient to reach the distal airways. In this respect, we propose a novel perspective that may change the current respiratory drug delivery paradigm. A scenario where, after deposition in the upper airways, PS-based formulations may continue to distal areas by "surfing" over the respiratory air-liquid interface.

Combining *in vitro* and pre-clinical *in vivo* studies, we demonstrated that PS-assisted interfacial delivery of pharmaceuticals may constitute a novel way for efficiently solubilizing and distributing poorly water-soluble therapeutics over the respiratory air-liquid interface, reaching the distal airways more efficiently. The *in vitro* studies were performed in a special setup consisting of two different aqueous wells, acting as donor and recipient compartments, connected by an interfacial bridge. By measuring the appearance of cargo molecules in the recipient compartment, we observed that PS-based formulations may carry and distribute different therapeutics (e.g. corticoids [1], tacrolimus (TAC) [2] or proteins [3]) by spreading over air-liquid interfaces, with compression/expansion breathing-like dynamics enhancing interfacial spreading and drug release. The pre-clinical studies, conducted in a mouse model of LPS-induced acute lung injury, showed that PS-based formulations promoted TAC uptake by alveolar macrophages, exerting synergistic anti-inflammatory effects in comparison with PS-free formulations.

0407-P

Oxidized cardiolipin recognition by the autophagy proteins LC3A and LC3B: A comparative study.

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Cardiolipin (CL) is a phospholipid that plays an important role in the structural organization and function of mitochondrial membranes. Although CL is found mostly in the inner mitochondrial membrane, upon mitochondrial injury a significant portion of CL becomes exposed on the mitochondrial surface, where it serves as either a pro-mitophagic or a pro-apoptotic signal. It has been proposed that, when CL participates in apoptosis, it is previously oxidized due to the peroxidase activity of cytochrome C, while the CL fraction that is recognized by the autophagy machinery is not. Considering that the interplay between autophagy and apoptosis implies a high level of complexity, understanding how the role of CL is affected by its oxidation state could be helpful to understand how those two important processes are regulated. It has been shown that the mitophagy machinery element that recognizes CL is the LC3B protein. This protein belongs to the LC3/GABARAP family, that plays different roles in autophagy, including cargo recognition, autophagosome elongation and fusion with lysosomes. Apart from LC3B, we have studied other members of this family, showing that LC3A is also able to interact with CL and therefore, could also participate in CL-mediated mitophagy. In this work, we assessed and compared the ability of LC3B and LC3A to recognise oxidized cardiolipin. CL-containing liposomes were oxidized and the amount of protein bound to non-oxidized and oxidized vesicles was measured by a flotation assay. Although LC3B binding decreased with CL oxidation, LC3A binding did not. Our results suggest that LC3A and LC3B could play different roles in the intteraction between autophagy and apoptosis.

(This work was supported in part by the Spanish Ministerio de Ciencia e Innovación (MCI), Agencia Estatal de Investigación (AEI) and Fondo Europeo de Desarrollo Regional (FEDER) (grant No. PGC2018-099857-B-I00), by the Basque Government (Grants No. IT1264-19, IT1196-19 and IT1270-19), by the Fundación Biofísica Bizkaia and by the Basque Excellence Research Centre (BERC) program of the Basque Government).

0465-P

CHO/LY-B cell growth under limiting sphingolipid supply: correlation between lipid composition and biophysical properties of sphingolipid-restricted cell membranes

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Sphingolipids (SL) are ubiquitous in mammalian cell membranes, yet there is little data on the behavior of cells under SL-restriction conditions. LY-B cells derive from a CHO line in which serine palmitoyl transferase (SPT), thus *de novo* SL synthesis, is suppressed, while maintaining the capacity of taking up and metabolizing exogenous sphingoid bases from the culture medium. In the present study LY-B cells were adapted to grow in a fetal bovine serum (FBS)-deficient medium to avoid external uptake of lipids. The lowest FBS concentration that allowed LY-B cell growth, though at a slow rate, under our conditions was 0.04%. Cells grown under limiting SL concentrations remained viable for at least 72 h. Enriching with sphingomyelin the SL-deficient medium allowed the recovery of growth rates analogous to those of control LY-B cells. Studies including whole cells, plasma membrane preparations, and derived lipid vesicles were carried out. Laurdan fluorescence was recorded to measure membrane molecular order, showing a significant decrease in the rigidity of LY-B cells, not only in plasma membrane but also in whole cell lipid extract, as a result of SL limitation in the growth medium. Atomic force microscopy mediated force measurements demonstrated that lower

breakthrough forces were required to penetrate samples obtained from SL-poor LY-B cells than those obtained from control cells. Mass-spectroscopic analysis was also a helpful tool to understand the rearrangement undergone by the LY-B cell lipid metabolism. The most abundant SL in LY-B cells, sphingomyelin, decreased by about 85% as a result of SL limitation in the medium, the bioactive lipid ceramide and the ganglioside precursor hexosylceramide decreased similarly, together with cholesterol. Quantitative SL analysis showed that a 250-fold reduction in sphingolipid supply to LY-B cells led only to a 6-fold decrease in membrane sphingolipids, underlining the resistance to changes in composition of these cells. Plasma membrane compositions exhibited similar changes, at least qualitatively, as the whole cells with SL restriction. A linear correlation was observed between the sphingomyelin concentration in the membranes, the degree of lipid order as measured by laurdan fluorescence, and membrane breakthrough forces assessed by atomic force microscopy.

Monasterio, B. G. et al. (2020) Patches and blebs: A comparative study of the composition and biophysical properties of two plasma membrane preparations from CHO cells. Int. J. Mol. Sci. 21, 2643 Monasterio, B. G. et al. (2021) CHO/LY-B cell growth under limiting sphingolipid supply: correlation between lipid composition and biophysical properties of sphingolipid-restricted cell membranes. Faseb 0, e21657

07.- Education

0087-OI

Molecular Games: una App para la docencia de Biología Molecular

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La experiencia docente que aquí se presenta se ha aplicado en las asignaturas de Biología Molecular del primer curso de los grados de Medicina y de Ingeniería Biomédica. Hace varios años evidenciamos la necesidad de ofrecer en estas asignaturas actividades de aprendizaje autónomo que potenciasen el trabajo continuado. Nos pareció que incluir elementos de gamificación sería una buena manera de generar interés y motivación en el alumnado. Con estas premisas diseñamos una actividad que denominamos Molecular Games que hemos venido incluyendo en estas asignaturas desde el curso 2016-17. Consiste en una serie de pruebas que se van abriendo en el campus virtual a ritmo de una prueba por semana. En los Molecular Games los estudiantes han de resolver preguntas aplicando conocimientos trabajados en la asignatura. Las pruebas se encadenan y contienen elementos de gamificación. Cada curso creamos una nueva edición de los Molecular Games.

A partir de la primera edición de los primeros Molecular Games hemos creado una App de acceso libre y gratuito. Si bien las pruebas de la App son prácticamente idénticas a las de la versión original para campus virtual, la dinámica de resolución en la App es diferente pues en la App los estudiantes resuelven todas las pruebas generalmente en una sola sesión. Hemos utilizado la App en seminarios presenciales y a partir de nuestra experiencia creemos que la dinámica ideal es que sea resuelta en grupos de 2-3 estudiantes, en una sesión de 45-60 minutos seguida de preguntas de discusión con todo el grupo.

En esta presentación mostraremos datos sobre la consecución de los objetivos planteados con esta herramienta, mejoras en el aprendizaje y satisfacción de los estudiantes. Igualmente discutiremos diferencias, pros y contras del uso de esta herramienta en el campus virtual respecto al formato App.

Como conclusión, nuestra experiencia con los Molecular Games en el campus virtual indica que esta actividad es una herramienta útil para potenciar en aprendizaje autónomo y continuado. En formato App puede utilizarse como actividad no presencial, autónoma y complementaria, si bien creemos que su uso en forma presencial y colaborativa resulta en un aprendizaje superior.

0105-P

Use of social networks for the development of a business project in the field of nutrigenomics and personalized nutrition

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The current global health emergency has further encouraged the use of social media and ICT technologies, not only on a personal level, but also significantly in the labor sphere. In this context, our aim was the implementation of the business use of social networks inside one of the evaluable items of the subject 10285-Seminars of Development and Innovation of the Master of Nutrigenomics and Personalized Nutrition at the University of the Balearic Islands. Therefore, students have to create a business project in the field of nutrigenomics and personalized nutrition, also preparing a business plan, integrating it into a social network profile. Thus, students can know and be able to handle social networks within the framework of business possibilities, which represents an important competence to potentiate their employability. Likewise, a second objective was to increase student motivation, teamwork and promote creativity. At the end of the activities, we will evaluate the degree of satisfaction of the students, and three opinion reports will be prepared by external teachers of the subject and who have participated in the project. Although activities are currently in progress, the first impressions are very pleasing, and we are convinced that the proposed tasks are being very useful and motivating for students. In short, the teachers' planning is to continue applying this methodology to the new editions of the subject, accommodating any possible imbalances that may arise.

0216-R/M-P

PERDIDOS EN EL NUCLEO: UNA INICIATIVA DE GAMIFICACION PARA ENSEÑAR EXPRESION GENICA

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La asignatura "Bioquímica y Biofísica" se imparte en el primer semestre del grado de Podología. Los estudiantes de nuevo ingreso presentan una gran heterogeneidad debido a las diversas vías de acceso al Grado, así como una falta de motivación por las asignaturas básicas. En consecuencia, la asignatura de Bioquímica y Biofísica presenta dificultades para muchos estudiantes. Por ello decidimos implementar técnicas de gamificación para consolidar la adquisición de los conocimientos impartidos en las clases teóricas, incrementar el interés por la asignatura y estimular el intercambio de conocimientos entre los estudiantes con diferentes "backgrounds".

La Gamificación es una técnica de aprendizaje que traslada la mecánica de los juegos al ámbito educativo-profesional, generando una experiencia positiva en el usuario. En 2019 adaptamos un juego de pruebas tipo "escape room" a los contenidos básicos de Biología Molecular. De esta manera, los estudiantes necesitaban aplicar conocimientos de este bloque para salir de la célula, escenario de la actividad, donde por un error experimental habían quedado atrapados. Las pruebas o misiones incluían la identificación de un gen, síntesis de un fragmento de RNA, su traducción a la secuencia de la proteína y el plegamiento de esta. Después de cada prueba se incluía un "checkpoint", que debían superar para poder acceder al contenido de la siguiente misión. Los profesores podrían incluir preguntas o elementos sorpresa relacionados con los contenidos de la asignatura en cualquier momento del juego. Debido a la situación sanitaria, en 2020 adaptamos el formato del juego cambiando la metodología de "escape room" por un juego de pistas, donde pequeños grupos de alumnos debían sintetizar correctamente una proteína a partir de los contenidos que recibían en una caja. Para mantener la motivación de los estudiantes por el juego, la actividad se realizó en grupos que competían entre sí. Se elaboró un ranking y un sistema de recompensas en función de su posición.

La experiencia ha sido muy bien acogida tanto por parte de los estudiantes como del profesorado participante. Más del 80% de los estudiantes afirmó que repetiría la experiencia y más del 89% la encontró útil para entender los contenidos de la materia.

0220-R/M-P

APRENDIZAJE BASADO EN PROBLEMAS: INNOVACIÓN EN LAS CIENCIAS DE LA SALUD

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Introducción: En los últimos años el profesorado del Campus de Bellvitge de la *Facultat de Medicina i Ciències de la Salut – Universitat de Barcelona* ha realizado innovaciones en la docencia que han incluido el aprendizaje basado en problemas (ABP) como metodología docente.

El ABP es una estrategia didáctica innovadora centrada en el estudiante, que facilita la adquisición de habilidades y competencias profesionales indispensables. Asimismo,

partiendo de un problema real a resolver, proporciona el contexto adecuado para favorecer el trabajo en grupo y el aprendizaje significativo.

Metodología: Profesores de nuestro Campus han asesorado, diseñado y adaptado de forma innovadora esta metodología docente para varias materias de los grados de Medicina, Odontología, Podología e Enfermería. Entre ellas hay tanto asignaturas troncales como optativas de distintos cursos.

El número de alumnos participantes ha sido variable. Se ha aplicado el ABP de forma generalizada a todos los estudiantes o de forma particular a modo de prueba piloto. Se ha utilizado mayoritariamente en actividades prácticas o seminarios que permiten trabajar en grupos reducidos.

Resultados: Después de varios años de aplicación de esta metodología activa, los resultados obtenidos desde diversas asignaturas y grados, ha permitido demostrar su gran versatilidad y validez tanto para asignaturas troncales con gran número de alumnos como para asignaturas optativas con menor número de participantes. El número de alumnos en los equipos de trabajo se muestra como el factor más limitante cuando se comparan los resultados de satisfacción obtenidos entre las diferentes experiencias. No obstante, en general es una experiencia muy bien valorada por los alumnos y el profesorado

Conclusión: De los resultados analizados se puede concluir que la dinámica ABP permitió conseguir los objetivos de aprendizaje propuestos, incrementando la motivación de los estudiantes por las asignaturas, favoreciendo el aprendizaje significativo, el trabajo en equipo y mejorando los resultados académicos y la satisfacción de los estudiantes.

0223-P

Desarrollo y aplicación de herramientas de enseñanza on line para estudiantes de Máster y de los Grados de Fisioterapia y Farmacia

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Durante el curso académico 2019/2020, debido al estado de alarma y subsecuente confinamiento, la modalidad de docencia virtual cobró de manera inesperada un papel relevante en el desarrollo de las actividades lectivas. Debido a la incertidumbre de que la presencialidad del actual curso 2020/2021 tuviese que ser interrumpida decidimos desarrollar una serie de herramientas virtuales para la docencia on-line, con potencial aplicación también como apoyo a la docencia presencial o para un modelo híbrido de enseñanza. Para ello, se elaboraron lecciones virtuales basadas en la presentación guiada por el profesor en la plataforma "Campus Virtual Studium" de la Universidad de Salamanca, materiales audiovisuales para sustituir o complementar las sesiones prácticas de laboratorio, así como cuestionarios de autoevaluación en la plataforma Kahoot por bloques docentes que estaban a disposición de los alumnos para su visualización a demanda. La actividad fue financiada mediante un proyecto de innovación docente concedido por la Universidad de Salamanca (Ref. ID2020/039). Hasta el momento, en lo que va de curso se ha mantenido la presencialidad segura, pero los recursos virtuales generados están siendo de utilidad para el refuerzo de los conceptos impartidos en el aula, adaptar las prácticas al número de alumnos necesariamente reducido para cumplir las normas de seguridad, así como para el seguimiento de la materia por los alumnos que no pueden asistir de modo presencial por confinamientos puntuales. Se han realizado encuestas de satisfacción a los alumnos y profesores implicados sobre la utilidad de estas herramientas y se ha detectado una gran aceptación y una valoración general muy positiva. Por lo que consideramos que se alcanzó el objetivo planteado de proporcionar a los alumnos un abanico de recursos para el aprendizaje, así como la elaboración de material para la aplicación a la docencia virtual.

0269-R/M-P

Use of electronic portfolio as a digital tool for monitoring and evaluating learning in laboratory practices

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Electronic portfolio (e-portfolio) has numerous uses and purposes, among which we highlight that of application in the educational field as a learning and evaluation strategy. Through its use, students can collect their experiences, reflect what they are learning and how they are learning it, and enhance their learning through reflection. Additionally, the use of the e-portfolio offers the possibility that the acquisition of knowledge is not generated exclusively within the classroom and that the professor can monitor the evolution of the student through a virtual space and follow the learning progress. We aim to incorporate this methodology for the monitoring and evaluation of learning in the laboratory practices of the subject 21508- Integrated Laboratory I of the Biochemistry degree of the University of the Balearic Islands. Specifically, it is intended that students, through digital elements made by themselves (videos, images, texts ...), capture the work done during the practices, review and update the data obtained in the determinations made, and show a critical vision for the analysis and discussion of the results, if they are physiologically possible and coherent, and their biological significance. It is important to note that the e-portfolio is a complementary training activity to the laboratory notebook, providing added value by encouraging reflection on the results, and at the same time it is an evaluation tool that allows the professor to assess whether the student has achieved the knowledge and/or lab skills.

Acknowledgement: Innovation projects and improvement of the teaching quality of the IRIE (PID202119)

0273-R/M-P

Role-playing in bioethics: a tool for discussing ethical issues in the world of science.

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Already in ancient Greece, humans were constantly debating "what is right" and "what is wrong" from an ethical-moral point of view. The ethics and morality of human actions have been a hot topic in any social field, and the world of science is no exception. Gene therapy in humans, transgenic organisms' generation, cloning, animal experimentation or the use of embryo stem cells are clear examples. The subject "Bioethics and Quality in the Laboratories of Biosciences" of the degree of Biochemistry of the University of the Balearic Islands deals with these topics and aims to reach complete formative learning of the students, promoting their critical spirit and the capacity to defend situations that they believe are most ethical, based on science.

In this context, the "Role-Playing" allows students to conduct mock sessions where ethical-moral conflicts inherent in the world of biosciences are discussed by themselves, assuming specific roles. We have organized a serial of face-to-face sessions about the ethical viability of potential conflicting research. The group-class is divided into defenders, opponents (who have to argue if the proposed investigation must be carried out or not, supporting their argument with documental materials), and the rest of the class, acting as the jury and responsible for the final decision of approving or not the simulated research proposal. We collect data on the performance of the sessions and from questionnaires for the alumni.

All in all, Role-Playing proposes a complimentary training activity to carry out learning active methodologies in a simulated environment of professional situations where intellectual skills are put into practice, including the ability to manage information, problem-solving and the organization and public presentation of arguments, preparing them for the professional research life.

Acknowledgments: Innovation projects and improvement of the teaching quality of the IRIE (PID202156).

0366-R-P

Nuevas metodologías activas para el aprendizaje crítico en el campo de la bioquímica de la nutrición humana

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La impartición de la asignatura "Bases Bioquímicas de la Nutrición Humana" del Grado en Bioquímica se basa en la premisa de que los alumnos apliquen los conocimientos adquiridos en cursos anteriores relativos a la bioquímica y el metabolismo. Sin embargo, para muchos temas tratados en esta asignatura, no se ha detectado una aplicación rigurosa de estos conocimientos, existiendo influencias derivadas de la información no experta disponible en los medios de comunicación. En gran medida, este problema radica en que la nutrición es un tema ampliamente tratado en los medios de comunicación, aunque a menudo de forma generalizada, incompleta y poco rigurosa.

Para solucionar este problema, en este proyecto proponemos a los alumnos aplicar una visión crítica sobre la información relacionada con la nutrición disponible en los medios de comunicación, haciendo especial hincapié en los temas más candentes (*hot-topics*), como los alimentos transgénicos, la dieta mediterránea o las dietas veganas. Para ello, hemos diseñado dos estrategias: (1) una actividad de *miniworkshop* en relación con la asignatura "Biotecnología Alimentaria" centrada en el uso de alimentos transgénicos; (2) implicar a los alumnos en la creación y gestión de una página web (*La Web Natural*, www.lawebnatural.com) destinada a tratar temas relacionados con la nutrición, trabajada desde dos enfoques (informativo y científico). De este modo, pretendemos implicar a los alumnos en actividades reales de búsqueda y criba de información por parte de expertos, para comunicarla en diferentes entornos.

Esta comunicación se deriva del Proyecto de Innovación Educativa PIE19-068 (Universidad de Málaga), destinado a mejorar la enseñanza de la Bioquímica de la Nutrición Humana a los estudiantes de grado.

Financiación: fondos del Proyecto de Innovación Educativa PIE19-068 (Universidad de Málaga); fondos de My Scientific Company.

0389-R-P

Use of a Problem-Based Learning Approach to Help in the Collaborative Learning of the Respiratory and Photosynthetic Chemiosmotic Coupling

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Many students of biology, biochemistry and biomedical science grades consider metabolism a remarkably difficult subject. The very extensive contents of metabolism as a subject and the need of integrate them in a biologically meaningful manner are primary causes of this difficulty [1]. Collaborative learning strategies could contribute to make it easier for students to study metabolism [2]. We are developing in the academic curses 2019-20 and 2020-21 an Educative Innovation Project (EIP), entitled *"Collaborative learning of Biochemistry based on projects and case and problem solving"* as a natural continuation of two other previous EIPs. For these EIPs, we have designed and used problem-based learning (PBL) cases to help our students to study metabolism and tis regulation. One of these PBL cases used in the present EIP was focused in the chemiosmotic coupling theory and its implementation in the relevant cases of cellular respiration and photophosphorylation. In two subjects dedicated to the study of metabolism regulation, one from the Biology Degree and the other from the Biochemistry Degree, we recruited volunteers to work in groups and collaboratively to solve the PBL cases. Students who voluntarily signed up for this activity obtained in the final global evaluation of the subject grades notably better than those who did not. In the present communication, this experience will be described, analyzed and discussed. This work is supported by an Educative Innovation Project (PIE19-057, funded by University of Málaga).

[1] García-Ponce AL, Martínez-Poveda B, Blanco-López A, Quesada AR, Alonso-Carrión FJ, Medina MA. A PBL case on glycogen as an evaluable task for students studying metabolism. Education and New Developments 2019. [2] Medina MA, García-Ponce AL, Blanco-López A, Quesada AR, Suárez F, Alonso-Carrión FJ. Advances in the use of the model of flipped classroom with collaborative learning as a helpful tool to study metabolism. In: Future of Education 2020, SCI4727. Filodiritto Editore, Florence,

0395-R/M-P

Implementation of ICT in cooperative learning by projects: remote modality of a joint and coordinated activity between subjects of the Master in Nutrigenomics and Personalized Nutrition

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In the disciplines of science areas, where specialized knowledge grows exponentially, in addition to transmitting the scientific legacy, the university professor must promote the development of personal qualities and values, such as curiosity, logical reasoning, critical spirit, teamwork, etc. These tasks have been hampered by the prevention measures that have been adopted in the face of the current health crisis, and the need arises to have non-face-to-face teaching systems in place. In this sense, and to promote these qualities in the current pandemic situation, we have developed a project implementing information and communication technologies (ICT) in a joint and coordinated activity between 4 courses of the Master of Nutrigenomics and Personalized Nutrition.

In this project, active and motivating learning methodologies have been used, in order to promote the student's autonomous work and guide him towards a professional environment within the non-face-to-face modality. Specifically, we have made it easier for students to carry out the activities of the different courses together in an organized way without the need to generate face-to-face social contact between students and teachers. A key aspect for this project has been the use of tools such as Zoom, or Collaborative Office, among others.

This innovative implementation has allowed greater coordination to carry out group work, and has facilitated the integration into the working groups of those students who have followed the non-face-to-face itinerary. In this way, the acquisition of competences has been favored within an innovative and cohesive activity, which combines the knowledge acquired by the students in more than one subject. This contributes to an improvement in teaching quality and to a more holistic understanding of the Master contents. Moreover, the use of remote working activities is helpful to deal with the situation of social isolation promoted by the current health situation, caused by COVID-19. In conclusion, the project has managed to relate and involve 4 courses, giving a more general and inclusive vision of the Master degree, all promoting a safe working environment.

Acknowledgment: Teaching Innovation Project (UIB-IRIE): PID202114

0405-P

AULA INVERTIDA: CREACIÓN DE VÍDEOS INTERACTIVOS EN MOODLE CON H5P

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En el modelo de Aula Invertida o *Flipped Classroom*, antes de comenzar a trabajar un tema en clase, los estudiantes aprenden el contenido básico de forma autónoma con materiales proporcionados por el profesor, utilizándose principalmente el tiempo en el aula para que los alumnos realicen actividades en las que aplican lo aprendido. De esta forma, se fomenta un aprendizaje dinámico y más profundo de los estudiantes.

Los vídeos docentes son uno de los materiales más utilizados, siendo más eficaces si son vídeos docentes interactivos, con preguntas incrustadas relativas a los contenidos del vídeo que deberán ser contestadas por los estudiantes. Con ello, se consigue aumentar la atención y el aprendizaje de los alumnos, además de permitir al profesor llevar un control de los estudiantes que han visto el vídeo y de los contenidos que les resultan más complejos en base a sus respuestas.

Existen diferentes herramientas gratuitas para crear vídeos interactivos como EdPuzzle, las cuales son sencillas de utilizar, aunque requieren que el profesor y los estudiantes se registren en la web para acceder, por lo que la información y contenido suministrados quedan fuera del espacio docente online protegido que poseen las universidades. Para evitar esto, dentro del Campus Virtual de la Universidad de Castilla-La Mancha (UCLM), soportado sobre la plataforma Moodle, se incluye el módulo de actividad H5P. Entre otras cosas, H5P permite crear vídeos docentes interactivos de forma sencilla y segura, con las ventajas añadidas de que las respuestas y calificaciones de los estudiantes quedan recogidas en el Libro de Calificaciones de Moodle y de que se puede restringir el acceso a los vídeos hasta que el estudiante no haya finalizado alguna actividad anterior.

Santiago, R. y Bergman J., (2018) Aprender al revés, Paidós Educación, Barcelona. MOODLE. https://docs.moodle.org/all/es/H5P Fecha de consulta: 25 de abril de 2021

0469-P

25 years of

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Transformative education involves envisaging the future of learning and attempting to find practical ways to develop aspects of this future. Twenty-five years ago, schools and universities operated in entirely different spheres, each being unaware of the other. In that context, the Department of Biochemistry and Molecular Biology at the University of Barcelona, in collaboration with SEBBM (The Spanish Society for Biochemistry and Molecular Biology), channelled effort into designing a course called "I love Biochemistry, *¿Y tú? Yo, Bioquímica*", with the aim to bridge the gap in biochemistry between the secondary school and university.

This summer course was planned with lectures and practical classes for 24 students interested in biochemistry, molecular and cellular biology, in their last year of secondary school. "I love Biochemistry" is a new strategy for introducing these students to biochemistry, and it is crucial if we are to increase interest in and vocation for biochemistry among young people.

The topics covered include "The DNA book of life", "ATP and energy balance", "Protein structure and synthesis", "Transgenic plants", "Cancer, diabetes and AIDS", "Gene Therapy: Is the future here?", "Bread, wine, beer and biochemistry", "Cell culture" and "Bioinformatics tools". Laboratory experiments encourage students to observe, ask questions, and perform and interpret experiments. The course seeks to promote creative thought and encourage students to really think about the work they are doing.

After 25 years, and after following the students' careers, their feedback reveals that:

Most of the participants considered the course a valuable experience for their training and they greatly appreciated personal interaction with the university professors and scientists at lectures.

The participants also greatly appreciated the opportunity to have practical classes in a "real" laboratory, and the majority emphasized the opportunity to increase their knowledge of science.

Finally, the impact of the course has been considerable, and it is now a reference model for other disciplines. Consequently, secondary school teachers have requested similar courses adapted to their own knowledge and the university has responded. The bridge between secondary education and the university has been built.

1. Fernández-Novell, J.M., Gomis, R., Cid, E., Barberà, A. and Guinovart, J.J. (2002). Bridging the gap in biochemistry between secondary school and university. Biochemistry and Molecular Biology Education 30,172-

174. 2. Fernández-Novell, J.M., Cid, E., Gomis, R., Barberà, A. and Guinovart, J.J. (2004). A Biochemistry and Molecular Biology Course for Secondary School Teachers. Biochemistry and Molecular Biology Education 32 (2004)378-380.

0508-P

"NI DEMASIADO FÁCIL NI DEMASIADO DIFÍCIL": COEVALUACIÓN EN PRÁCTICAS DE LABORATORIO

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En tercer curso del grado de bioquímica de la Universidad de las Islas Baleares (UIB) se imparte la asignatura *Métodos y Técnicas en Biología Molecular*, realizándose 45 horas de prácticas de laboratorio con el objetivo de adquirir habilidad y destreza en el manejo de técnicas de biología molecular y cultivos celulares, así como complementar las 30 horas de teoría que tiene la asignatura.

La realización de unas prácticas de laboratorio en una asignatura basada en técnicas experimentales es imprescindible, y, a pesar de que la parte práctica de esta asignatura es evaluada mediante un sistema de exposición de resultados y preguntas como si de una reunión de un equipo investigador se tratara, es necesaria la mayor implicación del alumnado en la adquisición de los conocimientos que se desprende de las prácticas.

Para llevar a cabo este propósito, consideramos que la coevaluación entre iguales y con el profesorado es la herramienta que mejor puede adecuarse. Así pues, el alumnado formulará y responderá preguntas tipo V/F de 2 de prácticas asignadas al azar, enviándolas a compañeros/as para que las categoricen. El profesorado de las prácticas también recibirá y categorizará esas preguntas, generando así una coevaluación de las preguntas del alumnado.

El sistema de categorización está basado en 4 colores, siendo Rojo la categoría difícil, Verde es la fácil y Amarillo o Naranja son consideradas como "Ni demasiado fácil ni demasiado difícil". El investigador principal del proyecto recibe todas las preguntas categorizadas por el alumnado y el profesorado, a partir de las cuales genera un banco de preguntas eliminando aquellas que se hayan marcado como verdes o rojas en alguna categorización

Finalmente, el alumnado realiza una prueba objetiva con 20 preguntas de las categorizadas como "Ni muy fácil ni muy difícil", suponiendo un 15% de la nota final de la asignatura.

La participación del alumnado ha sido excelente, comprometida y responsable. El resultado de la prueba objetiva ha sido excelente, así como las opiniones generadas en la encuesta de satisfacción del proyecto de innovación docente por parte del alumnado.

08.- Protein Structure and Function

0510-OI

Targeting microtubules beyond cancer

MARIAN OLIVA

Centro de Investigaciones Biológicas Margarita Salas - CSIC Structural and Chemical Biology

Microtubules are a well-known target for anticancer treatment, essentially because of the central function of the mitotic spindle on DNA segregation during cell division, and tubulin role in angiogenesis. However, these cellular fibers are key players in many pivotal biological processes, including scaffolding, intracellular transport and cell motion. These functions rely either on a static behavior or on the movement triggered by GTP hydrolysis, which is finely tuned by several partner proteins in cells. In addition, there is an arsenal of natural and synthetic microtubule targeting agents that allows the pharmacological modulation of microtubules function by preventing tubulin conformational changes necessary for filament formation or by hampering disassembly.

Our recent contributions include a new structural model of the microtubule's end cap, where punctual axial expansions with sudden changes on the twist of protofilaments breaks the lattice upon Pi release. This sharply contrast with subtle changes found due to GTP hydrolysis that lightly affect lateral interactions. Interestingly, some drugs and chemicals are able to replicate lattice expansion along the whole fiber, promoting highly stable fibers with a structurally distorted lattice unable to produce mechanical forces. These discoveries grounded a new hypothesis about the effect of drugs on microtubule's function and seeded the analysis of how drugs modulate protein-protein interactions on the intracellular trafficking. We have found that clinically used microtubule targeting drugs directly affect the motion of cellular motor proteins along the microtubules at doses below the IC50, which has further consequences. For instance, the microtubular network is commonly used by viruses during the infection cycle and, we found that the perturbation of these cellular roads directly reflects in the inhibition of the viral replication in five unrelated RNA (HCoV, SARS-CoV-2 and VSV) and DNA (VACV and ASFV) viruses. These results open the window of opportunity for using these drugs as wide spectrum antivirals.

Estévez-Gallego J et al. 2020. Structural model for differential cap maturation at growing microtubule ends. eLife. DOI: 10.7554/eLife.50155

0519-OI

Architecture of the Mycobacterial Type VII Secretion System

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Bacterial type VII secretion systems (T7SSs) have a key role in the secretion of effector proteins in both, pathogenic an non-pathogenic bacteria. *Mycobacterium tuberculosis* is the main causative agent of tuberculosis, the infectious disease that has killed most people throughout history, and relies on T7SSs for host infection [1]. A molecular understanding of the type VII secretion mechanism has been missing due to the lack of three-dimensional structures of the fully assembled secretion machinery.

We recently solved the cryo-electron microscopy structure of a detergent-solubilized core complex of the ESX-3/T7SS from *Mycobacterium smegmatis* at 3.7 Å resolution, revealing for the first time the overall topology of the building block of T7SSs [2].

The core of the ESX-3 secretion machine consists of four protein components—EccB3, EccC3, EccD3 and EccE3, in a 1:1:2:1 stoichiometry—which assemble into a protomer. The EccC3 coupling protein interacts with the substrate before translocation, and consists of a flexible array of four ATPase domains linked to the membrane through a stalk domain. EccB3 is the main periplasmic component and the N-terminal region crosses the cytoplasmic membrane and contacts the stalk domain. We therefore propose a secretion mechanism in which conformational changes in the stalk domain—triggered by substrate binding at the distal end of EccC3 and subsequent ATP hydrolysis in the ATPase domain next to the stalk domain—could be coupled to substrate secretion to the periplasm.

In our structure two protomers assemble as a stable dimer thanks to the dimerization of their EccB3 periplasmic domains. We therefore generated an atomic model for the the fully-assembled membrane-embedded T7SS consisting of a trimer of our dimeric structure. This atomic model has been confirmed by the recent atomic structures of the hexameric T7SSs from *M. tuberculosis* and *M. xenopi* [3, 4].

In summary our results revealed for the first time the architecture of the building blocks that constitute T7SSs, evidencing that this secretion system differs markedly from other known bacterial secretion machines. More importantly our structure provides a first structural understanding of these systems that will be extremely valuable for the design of antimicrobial strategies that target bacterial secretion.

[1] Rivera-Calzada, A. et al. Nat Rev Microbiol (2021). [2] Famelis, N. et al. Nature 576, 321–325 (2019). [3] Bunduc, C.M. et al. Nature 593, 445–448 (2021). [4] Beckham, K. S. H. et al. Preprint at https://www.biorxiv.org/content/10.1101/2020.11.17.387225v1 (2020).

0058-OS

Structural basis for the activation of the Vip3Aa toxin.

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CIB MARGARITA SALAS (CSIC) Structural and Chemical Biology

Bacillus thuringiensis (Bt) synthetizes potent insecticidal proteins commonly used in biotech and conventional agriculture. The members of the Vip3 family are synthesized in an inactive state, as protoxins, and they require to be activated by proteases present in the midgut tract of the insect. In particular, trypsin-like enzymes cleave the molecule at residue 198, generating two fragments of around 19 and 65 kDa that remain strongly associated. The activated toxin then recognizes specific receptors in the brush border of columnar cells and triggers cell death.

Notably, although the first member of the Vip3 family was discovered more than 20 years ago, numerous questions persist regarding the three-dimensional organization, activation and mode of action of these proteins at the molecular level. To shed light on some of these questions, we have determined the 2.9 Å resolution structures of Vip3Aa in the protoxin and activated states by cryo-electron microscopy. The reconstruction of the uncleaved protein shows that it assembles into a highly stable tetrameric dimer of dimers that contains three putative sugar-binding domains poised for receptor engagement. Interestingly, moreover, the trypsin-activated structure reveals that, upon protease digestion, the N-terminal region of the toxin undergoes a large conformational change that leads to the formation of an extended four-helix coiled coil. Collectively, these results provide a high-resolution view of a Vip3 member in the protoxin and toxin conformation that serve as a strong foundation for the development of more efficient insecticidal proteins, and allows us to propose an activation mechanism for this family of entomopathogenic proteins akin to that of other toxins and viral fusion proteins.

0151-R-OS

Human HELB is a processive motor protein which catalyses RPA clearance from single-stranded DNA

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Human HELB is a poorly characterized helicase with roles in DNA repair and replication. Its precise function is still a matter of discussion, with different studies pointing towards both positive and negative roles in homologous recombination and replication restart pathways. We have combined bulk and single molecule approaches to characterise the biochemical activities of purified recombinant HELB protein, focusing on its interactions with RPA and RPA-ssDNA filaments. We found that HELB is a monomeric protein which binds tightly to ssDNA (K_d ~5 nM) with a footprint of 20-25 nucleotides. It turns over ATP at a maximal rate of ~45 s^{-1}, an activity that is efficiently transduced into 5' to 3' translocation along ssDNA at ~40 nt s^{-1} and accompanied by the formation of DNA loops, as revealed in Magnetic Tweezers experiments. HELB can also act as a DNA helicase, but in contrast to the ssDNA translocase activity, duplex DNA unwinding is modest in the absence of an applied assisting force. HELB binds specifically to its cognate ssDNA binding protein (human RPA) in the presence of ssDNA, and this enhances the ATPase and translocase activity, whereas yeast RPA strongly inhibits both activities under the same conditions. Finally, direct observation of HELB on RPA nucleoprotein filaments with an instrument that combines Optical Tweezers and Confocal Microscopy, reveals that HELB translocation concomitantly clears RPA from single-stranded DNA. The important implications of these observations for our understanding of the roles played by HELB in DNA replication and damage response are discussed.

0225-R/M-OS

Falling into the TRAPP: A cryo-EM structure of TRAPPIII, the multisubunit complex that activates the GTPase Rab1

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MRC Laboratory of Molecular Biology Cell Biology Division

The TRAPP complexes are nucleotide exchange factors that have essential roles in membrane traffic and autophagy. TRAPPII activates Rab11, and TRAPPIII activates Rab1. The two complexes share a core of small subunits that affect nucleotide exchange, but are distinguished by specific large subunits essential for activity in vivo. Crystal structures of core subunits have revealed the mechanism of Rab activation, but how the core and the large subunits assemble to form the complexes was unknown. We report a cryo-EM structure of the entire TRAPPIII complex from Drosophila. The TRAPPIII-specific subunits TRAPPC8 and TRAPPC11 hold the catalytic core like a pair of tongs, with TRAPPC12 and TRAPPC13 positioned at the joint between them. TRAPPC2 and TRAPPC2L link the core to the two large arms, with the interfaces containing residues affected by disease-causing mutations. The TRAPPC8 arm is positioned such that it would contact Rab1 that is bound to the core, indicating how the arm could determine the specificity of the complex. A lower resolution structure of TRAPPII shows a similar architecture and suggests that the TRAPP complexes evolved from a single ur-TRAPP.

Antonio Galindo, Vicente J. Planelles-Herrero, Gianluca Degliesposti & Sean Munro A cryo-EM structure of metazoan TRAPPIII, the multisubunit complex that activates the GTPase Rab1. bioRxiv 2020.12.17.423307; doi: https://doi.org/10.1101/2020.12.17.423307 Falko Riedel, Antonio Galindo, Nadine Muschalik & Sean Munro. The two TRAPP complexes of metazoans have distinct roles and act on different Rab GTPases J. Cell. Biol. Volume: 217 Issue: 2 Pages: 601-617 2018. DOI: 10.1083/jcb.201705068

0248-OS

High-resolution snapshots of human N-myristoyltransferase revealing a unique enzymatic mechanism

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N-myristoyltransferase (NMT) is a eukaryotic enzyme mediating lipidation of hundreds of proteins in all eukaryotic cells[1-3]. This lipidation is a co/post-translational modification consisting of the transference of a myristoyl moiety from myristoyl coenzyme A (Myr-CoA) to the N-terminal glycine of substrate proteins[1-3]. NMTs are thought to be essential in all organisms and thus, they have attracted interest as a drug target in multiple diseases such as cancer and infection and they are in fact validated drug targets for the treatment of parasite infections such as malaria[4-8]. However, progress in the field of human NMT biology has been undermined by non-selective and now invalidated compounds, raising questions about previous studies using these drugs. In this sense, a complete understanding of NMT catalysis and substrate selectivity would greatly benefit therapeutic development. But while a catalytic mechanism has been proposed for NMT enzymes a definitive proof is still missing. Here we present unique high-resolution human NMT1 structures co-crystallised with Myr-CoA and peptide substrates, which provide high-resolution snapshots of the entire catalytic mechanism from the initial to final reaction states[9]. Our structural studies together with biochemical analysis provide unforeseen details about how NMT1 reaches a catalytically competent conformation in which the reactive groups are brought into close proximity to enable catalysis[9]. Moreover, close examination of our structures offers inestimable valuable information for the future improvement of existing NMT inhibitors of pharmacological interest, and particularly for improved selectivity[9].

1. Bhatnagar et al., In: The enzymes (eds Tamanoi F, Sigman DS). Academic Press (2001) 2. Giglione et al., Biochimie 114, 134-146 (2015) 3. Thinon et al., Nat. Commun. 5, 4919 (2014) 4. Mousnier et al., Nat. Chem. 10, 599-606 (2018) 5. Tate EW et al., Parasitology 141, 37-49 (2014) 6. Wright et al., Nat. Chem. 6, 112-121 (2014) 7. Wen et al. Nat. Immunol. 20, 313-325 (2019) 8. Corpas-Lopez et al., ACS Infect. Dis. (2018) 9. Dian and Pérez-Dorado et al., Nat. Commun. 28, 1132 (2020)

0532-OS

The selection process of licensing a DNA mismatch for repair

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CNIO Structural Biology Program

DNA mismatch repair detects and removes mismatches from DNA by a conserved mechanism, reducing the error rate of DNA replication by 100- to 1,000-fold. In this process, MutS homologs scan DNA, recognize mismatches and initiate repair. How the MutS homologs selectively license repair of a mismatch among millions of matched base pairs is not understood. Here we present four cryo-EM structures of Escherichia coli MutS that provide snapshots from scanning homoduplex DNA, to mismatch binding and MutL activation via an intermediate state. During scanning, the homoduplex DNA forms a steric block that pre- vents MutS from transitioning into the MutL-bound clamp state, which can only be overcome through kinking of the DNA at a mismatch. Structural asymmetry in all four structures indicates a division of labor between the two MutS monomers. Together, these structures reveal how a small conformational change from the homoduplex- to heteroduplex-bound MutS acts as a licens- ing step that triggers a dramatic conformational change that enables MutL binding and initiation of the repair cascade.

Fernandez-Leiro, R.; Bhairosing-Kok, D.; Kunetsky, V.; Laffeber, C.; Winterwerp, H. H.; Groothuizen, F.; Fish, A.; Lebbink, J. H. G.; Friedhoff, P.; Sixma, T. K.; Lamers, M. H. The Selection Process of Licensing a DNA Mismatch for Repair. Nat. Struct. Mol. Biol. 2021, 28 (4), 373–381. https://doi.org/10.1038/s41594-021-00577-7.

0015-R/M-P

Identification of prostate specific antigen glycoforms in aggressive prostate cancer

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Prostate Cancer (PCa) is the most common cancer and the second cause of cancer death in men (1). The biomarker used nowadays is prostate-specific antigen (PSA), a glycoprotein synthetized by the prostate. However, blood serum PSA levels are not specific enough to distinguish between benign prostate pathologies and PCa, or between low and high-risk PCa. Therefore, the development of non-invasive biomarkers is required to reduce PCa overdiagnosis and overtreatment. Study of PSA glycosylation has been emerged as a very promising field. In particular, an increase in the percentage of α2,3-linked sialic acid (SA) of PSA glycoforms are indicative of aggressive PCa (2). However, specific PSA glycoforms that are differently expressed either increased or decreased in aggressive PCa have not been characterized yet. Thus, we aim to identify the main PSA glycan structures from high-risk PCa patients that can be very useful for the diagnosis of clinical significance PCa cancers. For that, 6 serum samples from aggressive PCa patients with high levels of PSA (>300 ng/ml) were obtained from the Hospital Dr. J. Trueta and PSA purified from healthy individuals' seminal plasma was used as a control. PSA was immunoprecipitated and α2,3/α2,6-SA glycoforms were separated by SNA-lectin affinity chromatography. The PSA collected fractions were immunoprecipitated and resolved on SDS-PAGE. *N*-glycan sequencing was performed and PSA bands were excised and digested with PNGaseF. The obtained *N*-glycans were analyzed by Hydrophilic Liquid Chromatography combined with exoglycosidase digestions and glycan differences between healthy and aggressive PCa patients were determined. The results showed that PSA sialylalated glycoforms containing a GalNAc residues were increased in aggressive PCa, whereas the disialylated core fucosylated biantennary structures with α2,6-SA, which are the major PSA glycoforms form healthy individuals, were significantly reduced. To conclude, changes in the proportions of the PSA glycoform structures in aggressive PCa may be used as templates to develop new methodologies for their detection and improve PCa risk stratification.

1. R. L. Siegel et al., (2019) "Cancer statistics, 2019". CA. Cancer J. Clin. no. 69, p. 7–34. 2. E. Llop et al., (2016) "Improvement of Prostate Cancer Diagnosis by Detecting PSA Glycosylation-Specific Changes". Theranostics. no. 6, p. 1190–1204.

0036-R/M-P

Small is Beautiful

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Exceptionally brilliant, femtosecond-pulsed X-ray sources, the X-ray free-electron lasers (XFELs), have brought a new way of conducting crystallography by probing nano/micrometre-sized crystals in a serial fashion. Since the first XFEL, the Linac Coherent Light Source (LCLS), started operation in 2009, the serial femtosecond crystallography (SFX) technique has opened up a new era in structural biology with new and exciting opportunities for the determination of static structures as well as the structural dynamics of macromolecules. In addition to XFELs, the serial crystallography approach can also be done at synchrotrons sources and have gained popularity in the past few years up to the point that it can now be a viable alternative to scarce X-ray free electron laser sources. Monochromatic and pink beam experiments have demonstrated the feasibility of serial data collection using micro-crystals at numerous microfocus beamlines at the most powerful synchrotron radiation sources in the world. Upcoming developments in beamline optics, detector technology and synchrotron sources by itself will enable the use of even smaller micro-crystals, the use of larger macromolecules as well as the possibility of conducting mix-and-inject time-resolved studies.

0046-P

New Insights Into the Regulatory Mechanism of RcsB

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Enteric bacteria possess a phosphorelay signal transduction system named Rcs, which senses stresses in the cell envelope and elicits responses to adapt (1). RcsB is the response regulator of the system, exerting its function by altering the expression of many genes. RcsB accomplishes this alone or with other transcription factors, and independent or dependently of phosphorylation (2, 3). To understand the mechanisms underlying the function of gene regulation by *Salmonella enterica serovar Typhimurium* RcsB we have performed structure-based studies that has allowed us to solve the structures of RcsB bound to the promoter region of the small RNA gene *rprA* and flagellar gene *flhDC* .

Structural data has revealed that homodimeric *S. Typhimurium* RcsB docks into the major groove of DNA in an active conformation. Moreover, comparative structural analysis showed that DNA could allow the stabillization of an active conformation in unphosphorylated RcsB, revealing a bi-directional activation mechanism. We also delimited the box for homodimeric binding of DNA, as the pseudopalindromic sequence TN(G/A)GAN$_4$TC(T/C)NA. This sequence was found in promoter, intergenic and intragenic regions and was cross-matched with RNAseq data obtained from a *S.* typhimurium MD4822 strain expressing wild-type RcsB or mutant variants. This revealed that intergenic or promotor sequences are related to increased gene expression, while intragenic sequences correlate to gene repression, probably involving a “roadblocking mechanism”. To validate RcsB binding sites found in regulatory regions of genes regulated by RcsB, we performed EMSA experiments with promoter or intergenic, and intragenic regions as well as performed qPCR analysis with mRNA samples used for RNAseq. Interestingly, our studies have uncovered a new relation between RcsB and iron regulation, by means of altering the expression of iron-related genes, and finding a RcsB binding site in the intergenic region between *entF-fepE*. Overall, this data expands the RcsB regulon, proposes a box for the homodimeric RcsB and reveals interesting links to iron regulation (4).

1. Wall,E. et al. 2018 Annu. Rev. Microbiol. 72. 2. Casino,P. et al. 2018 Nucleic Acids Res. 46. 3. Pannen,D. et al. 2016 J. Biol. Chem. 291. 4. Huesa, J., et al. 2021. Nucleic Acids Res. 49

0049-P

Exploring the molecular basis of the selective activation of β1 and β2-containing AMPK complexes by small molecules

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Up to 30% of all human proteins could be modified by protein kinases, which are known to regulate most of cellular pathways.1Mammalian adenosine monophosphate-activated protein kinase (AMPK) is a Ser/Thr protein kinase that has an important role in cellular energy homeostasis. AMPK is a heterotrimeric complex which consists of a catalytic α subunit and two regulatory subunits, β and γ.The evolutionary adaptation of AMPK to different tissues is accomplished through the expression of distinct isoforms that can form up to 12 complexes, which exhibit notable differences in the sensitivity to allosteric activators. AMPK activity is regulated by several mechanisms,including AMP binding to the CBS domains in the γ subunit and phosphorylation of Thr172 in the activation loop of the α subunit by upstream kinases that causes allosteric activation.2There are also indirect AMPK activators that act by increasing the cellular AMP concentration. Furthermore, in 2006, a novel mechanism of action that involves the first direct activation of AMPK by the thienopyridone drug A-769662 was reported. A-769662 does not bind to the CBS motifs in the γ-subunit but to a binding site located at the interface between α and β subunits, which is called Allosteric Drug and Metabolite binding site. In the recent years, different kinds of direct activators of AMPK were reported. Parts of these activators are specific for certain isoforms of AMPK; some of them can activate both β1 and β2 isoforms while the others can only trigger the activation in one specific isoform.To shed light into the molecular determinants of the allosteric regulation of AMPK, we have examined the structural and dynamical properties of β1- and β2-containing AMPK complexes formed with A-769662 and SC4, and dissected the mechanical response leading to active-like enzyme conformations through the analysis of interaction networks between structural domains.The results show the mechanical sensitivity of α2β1 complexes in contrast to the large resilience of α2β2.The binding of the activator to α2β1 promotes the pre-organization of the ATP-binding site3 favoring the adoption of the activated form of the enzyme. Moreover,we hypothesize that the change of β1Asn111 to β2Asp111 could be the key factor in modulating the mechanical sensitivity of β1 and β2 containing AMPK complexes.

1.Roskoski R. A historical overview of protein kinases and their targeted small molecule inhibitors. Pharmacol Res [Internet]. 2015;100:1–23. Available from: http://dx.doi.org/10.1016/j.phrs.2015.07.010 2.Carling D, et al. AMP-activated protein kinase: Nature’s energy sensor. Nat Chem Biol. 2011;7(8):512–8. 3.Aledavood E, et al. Understanding the Mechanism of Direct Activation of AMP-Kinase: Toward a Fine Allosteric Tuning of the Kinase Activity. J Chem Inf Model. 2019;59(6):2859–70

0060-R-P

Confirmation that ATAD3C is a mitochondrial protein

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The *ATAD3*, ATPase family AAA domain-containing protein 3, gene family in humans includes three paralogs (*ATAD3A*, *ATAD3B*, and *ATAD3C*) positioned in tandem on chromosome 1p36.33. Pathogenic genetic variants in the mitochondrial *ATAD3* gene family were recently associated with a diversity of neurological syndromes [MIM 612316, 612317]. *ATAD3C* is normally expressed at low or negligible levels across tissues and the absence of 4 exons with respect to *ATAD3A*, has suggested that it could be a pseudogene[1]. However, using quantitative TaqMan real-time PCR, we could detect *ATAD3C* expression in RPMI 8226 cells. In addition, the ATAD3C protein was detected (one unique peptide) by mass spectrometric analysis in mitochondria derived from HEK293T cells, indicating the presence of the protein in a human cell line.

It has been demonstrated that a central transmembrane segment of ATAD3A anchors the protein in the inner membrane and positions the C-terminal AAA ATPase domain in the matrix and the N- terminal part of ATAD3A outside the inner membrane. Moreover, it has been showed that ATAD3A regulates dynamic interactions between the mitochondrial OM and IM sensed by the cell fission machinery[2]. However, ATAD3C has not been studied to the date. Using mitochondria of HEK293T cells expressing a plasmid encoding *ATAD3C* with the epitope HA included either in the N-terminal or in the C- terminal domain, we demonstrated that ATAD3C is a mitochondrial protein that resides in the inner mitochondrial membrane. The treatment of isolated mitochondria with proteinase K and trypsin lead us to conclude that ATAD3C C-terminal AAA ATPase domain is facing the matrix and its N-terminal domain is exposed in the intermembrane space of mitochondria.

Although our results shed some light on ATAD3C subcellular location, more work will be required to determine the exact role of ATAD3C in mitochondria.

1. Harel, T. et al. (2016) ‘Recurrent De Novo and Biallelic Variation of ATAD3A, Encoding a Mitochondrial Membrane Protein, Results in Distinct Neurological Syndromes’, American journal of human genetics. 2016/09/15. Elsevier, 99(4), pp. 831–845. 2. Gilquin, B. et al. (2010) ‘The AAA+ ATPase ATAD3A controls mitochondrial dynamics at the interface of the inner and outer membranes’, Molecular and cellular biology. 2010/02/12. American Society for Microbiology (ASM), 30(8), pp. 1984–19

0077-P

The Eosinophil Cationic Protein (human RNase3) modulates the macrophage response during infection by catalytic –dependent and -independent modes.

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The Eosinophil Cationic Protein, also called human RNase3, is a member of the RNaseA superfamily involved in host immunity. RNase3 is expressed by leukocytes and show broad-spectrum antipathogen activities[1,2]. Togeth-

er with a direct antimicrobial and antiviral action, RNase3 exhibits immunomodulatory properties. Here, we have analysed the transcriptome of macrophages exposed to the wild-type protein and a catalytic-defective mutant (RNase3-H15A). The analysis of differently expressed genes (DEGs) in treated THP1-derived macrophages highlighted a common pro-inflammatory "core-response" independent of the protein ribonucleolytic activity. Network analysis identified the epidermal growth factor receptor (EGFR) as the main central regulatory protein. Structural analysis suggested that RNase3 can activate the EGFR pathway by direct interaction with the receptor. In addition, we identified a subset of DEGs specifically related to the protein ribonucleolytic activity, characteristic of virus infection response and interferon signalling. Transcriptome analysis revealed an early pro-inflammatory response (4h), not dependent on the protein catalytic activity, followed by a late activation (12h) in a ribonucleolytic dependent manner. Next, we demonstrated that overexpression of the macrophage endogenous RNase3 can protect the cells against intracellular infection by *Mycobacterium aurum* and the human Respiratory Syncytial Virus (RSV). Eventually, comparison of cell infection profiles in the presence of Erlotinib, an EGFR inhibitor, revealed that the receptor activation is linked to the antibacterial but not to the antiviral protein action. Moreover, the differentially expressed genes linked to the protein catalytic activity are associated to the immune response to viral infection. We conclude that RNase3 modulates the macrophage defence against infection in both catalytic-dependent and independent manners.

1) Lu L, Li J, Moussaoui M and Boix E Immune modulation by human secreted RNases at the extracellular space. Front Immunol (2018) 8:1499. 1-17 https://doi.org/10.3389/fimmu.2018.01012 2) Li J. and Boix E. Host Defense RNases as Antiviral agents against enveloped single-stranded RNA Viruses. Virulence (2021) 12(1):444-469. https://doi.org/10.1080/21505594.2021.1871823

0078-P

Heterologous production and characterization of thermostable proteins involved in eukaryotic large ribosomal subunit maturation

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Thermophilic proteins are known to be more stable than their mesophilic counterparts and therefore thermophilic genes from bacteria and archaea are commonly used in Structural Biology. Since the sequencing of the fungus *Chaetomium thermophilum* in 2011, more than 360 entries in the PDB correspond to this source organism, including large particles with many proteins. This model organism has been particularly useful in the study of the eukaryotic ribosome biogenesis process. Therefore, we have aimed to structurally characterize different assembly factors involved in eukaryotic large ribosome subunit (60S) biogenesis using *C. thermophilum* as a model organism. Using the Ligase Independent Cloning (LIC) approach we have cloned, expressed and purified different assembly factors involved in this biological process, such as CIC1, TIF6, the GTPase NOG1 and the RNA helicases MAK5 and HAS1. Some of these proteins have been cloned in a truncated form in order to eliminate disordered regions that represent a challenge for protein expression and purification. Resulting constructs have been overexpressed in *Escherichia coli* and purified using FPLC techniques. As already described (1, 2), the specific interactions established among the different assembly factors are crucial for the correct maturation of the eukaryotic 60S subunit. In our study, we have focused on two particular protein-protein interactions: NOG1-TIF6 and HAS1-CIC1. Interactions between these pairs of proteins have already been reported in *Saccharomyces cerevisiae* employing an approach of affinity co-purification coupled to mass spectrometry and western blott. We have used the biolayer interferometry (BLI) technique and we have performed in parallel a series of pull-down experiments. Preliminary results show a possible interaction between HAS1 and CIC1, but we have not been able to detect any interaction between NOG1 and TIF6. This lack of interaction could be due to the fact that these proteins can only interact in the presence of ribosomal RNA, or that their interaction depends on particular post-translational modifications. However, alternative methods such as microcalorimetry should be used to confirm these preliminary results and to test other possible protein-protein interactions that might be relevant for 60S subunit biogenesis.

1. Saveanu C et al.Mol Cell Biol, 2003 Jul;23(13):4449-60. 2. McCann KL et al. Genes Dev, 2015 Apr 15;29(8):862-75

0090-P

Inhibition of the PP2A activity by the histone chaperone ANP32B is long-range allosterically regulated by respiratory cytochrome c

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Repair of injured DNA relies on nucleosome dismantling by histone chaperones and de-phosphorylation events carried out by Protein Phosphatase 2A (PP2A). Typical histone chaperones are the Acidic leucine-rich Nuclear Phosphoprotein 32 family (ANP32) members, *e.g.* ANP32A, which is also a well-known PP2A inhibitor (a.k.a. I1PP2A). Here we report the novel interaction between the endogenous family member B—so-called ANP32B—and endogenous cytochrome *c* in cells undergoing camptothecin-induced DNA damage. Soon after DNA lesions but prior to caspase cascade activation, the hemeprotein translocates to the nucleus to target the Low Complexity Acidic Region (LCAR) of ANP32B; in a similar way, our group recently reported that the hemeprotein targets the acidic domain of SET/Template Activating Factor-Iβ (SET/TAF-Iβ), which is another histone chaperone and PP2A inhibitor (a.k.a. I_2PP2A). The nucleosome assembly activity of ANP32B is indeed unaffected by cytochrome *c* binding. Like ANP32A, ANP32B inhibits PP2A activity and is thus herein referred to as I_3PP2A. Our data demonstrates that ANP32B-dependent inhibition of PP2A is regulated by respiratory cytochrome *c*, which induces long-distance allosteric changes in the structured N-terminal domain of ANP32B upon binding to the C-terminal LCAR. In agreement with the reported role of PP2A in the DNA damage response, we propose a model wherein cytochrome *c* is translocated from the mitochondria into the nucleus upon DNA damage to modulate PP2A activity via its interaction with ANP32B.

0107-P

The N-terminal Region of Yeast Protein Phosphatase Ppz1 Is a Determinant for its Toxicity and Subcellular Localization

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Ppz phosphatases are Ser/Thr protein phosphatases only found in fungi. They have a catalytic carboxyl-terminal half related to PP1c phosphatases and an NH_2-terminal unstructured extension, absent in PP1c, which contains a myristoylable Gly-2. In the yeast *Saccharomyces cerevisiae*, Ppz phosphatases are encoded by the *PPZ1* and *PPZ2* genes. When comparing the phosphatases, their C-terminal catalytic moieties are very close related (85.9% identity, 89.9% similarity), whereas their N-terminal regions are more divergent (32.2% identity, 45.9% similarity). Both Ppz are involved in several cell processes as the regulation of salt tolerance, the maintenance of cell wall integrity, and control of the cell cycle at the G_1/S transition. In all cases Ppz1 is more relevant than Ppz2.

Ppz1 is the most toxic protein when overexpressed in budding yeast, and this effect depends on its phosphatase activity on multiple cellular targets. Even though the catalytical moieties of Ppz1 and Ppz2 are similar, we have shown that the overexpression of Ppz2 is not toxic. By using hybrid versions combining N- and C-terminal regions of Ppz1 and Ppz2, we demonstrate that the version carrying the N-terminal half of Ppz1 and the catalytic moiety of Ppz2 (Ppz1:2) is as toxic as native Ppz1, but the Ppz2:1 hybrid is not. These results point out that the N-terminal half of Ppz1 is a determinant for its toxicity.

We also show that Ppz1 subcellular localization depends on the integrity of its N-terminus. While native Ppz1 mainly localizes to peripheral and internal membranes, mutation of Gly-2 to Ala results in a cytosolic phosphatase which is concomitant with a slight attenuation of its toxicity. Previous work from our laboratory had shown that the expression of the C-terminal half of Ppz1 alone also results in cellular toxicity. We show here that the C-terminal catalytical region localizes in the nucleus, suggesting that the nature of its toxicity is different from that of the native protein. Therefore, the unstructured amino-terminal moiety would be a key determinant for Ppz1 toxicity as well as for its subcellular localization.

Calafí, C., López-Malo, M., Albacar, M., Casamayor, A., & Ariño, J. (2020). The N-Terminal Region of Yeast Protein Phosphatase Ppz1 Is a Determinant for Its Toxicity. International Journal of Molecular Sciences, 21(20), 1–16. https://doi.org/10.3390/ijms21207733

0110-R-P

The N-terminal extension of Hal3 contributes to the regulation of Ppz1 function

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The *Saccharomyces cerevisiae SIS2*/*HAL3* gene was identified, at the same time, as a suppressor of the *sit4* mutation and a regulator of salt tolerance. Subsequently it was shown that these two functions were the result of the inhibition of Ppz1 Ser/Thr protein phosphatase. Ppz1 has two differentiated domains: an unstructured N-terminal region, comprising the first 344 residues and a structured C-terminal catalytic domain. Hal3 binds to the catalytic, C-terminal domain ($Ppz1_{\Delta 1\text{-}344}$) of the phosphatase with a 1:1 stoichiometry. Remarkably, Hal3 is a moonlighting protein, contributing to the formation of an unusual heterotrimeric PPC decarboxylase involved in CoA biosynthesis.

Hal3 has 3 differentiated regions: an unstructured N-terminus, which contributes to, but is not essential for Ppz1-Hal3 interaction; a central domain, called PD, which has double function: it is required for binding and inhibition of Ppz1, and contributes to the PPCDC trimer; and a short C-terminus rich in acidic residues, not necessary for interaction but required for inhibition. Besides this, little is known about the structural determinants required for interaction of Ppz1 and Hal3.

Using bioinformatics tools and cross-linking proteomics we hypothesized that Hal3's regions 39-51, 67-110, 118-148, 209-227 and 248-256, all of them located at the N-terminal region, could be important in the interaction and/or inhibition of Ppz1. Therefore, we deleted these regions, and

characterized the effect on these Hal3 versions as Ppz1 inhibitors. Our results indicated that only deletions in the region 67-110 abolished the function of Hal3 as Ppz1 regulator. Further *In vivo* analysis narrowed this effect to specific mutations in the 90-98 region. *In vitro* inhibition assays proved that most of these versions displayed decreased inhibitory capacity on full-length Ppz1, but not on the $Ppz1_{\Delta 1\text{-}344}$ catalytic domain. Therefore, our results suggest that the region 90-98 must be important in the Ppz1-Hal3 functional interaction, in a way that also involves the N-terminal extension of Ppz1.

0118-P

Post-translational Control of RNA-Binding Proteins and Disease-Related Dysregulation

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Cell signaling mechanisms modulate gene expression in response to internal and external stimuli. Cellular adaptation requires a precise and coordinated regulation of the transcription and translation processes. The post-transcriptional control of mRNA metabolism is mediated by the so-called RNA-binding proteins (RBPs), which assemble with specific transcripts forming messenger ribonucleoprotein particles of highly dynamic composition. RBPs constitute a class of trans-acting regulatory proteins with affinity for certain consensus elements present in mRNA molecules. However, these regulators are subjected to post-translational modifications (PTMs) that constantly adjust their activity to maintain cell homeostasis. PTMs can dramatically change the subcellular localization, the binding affinity for RNA and protein partners, and the turnover rate of RBPs. Moreover, the ability of many RBPs to undergo phase transition and/or their recruitment to previously formed membrane-less organelles, such as stress granules, is also regulated by specific PTMs. Interestingly, the dysregulation of PTMs in RBPs has been associated with the pathophysiology of many different diseases. Abnormal PTM patterns can lead to the distortion of the physiological role of RBPs due to mislocalization, loss or gain of function, and/or accelerated or disrupted degradation. This poster offers a broad overview of the post-translational regulation of RBPs and the involvement of their dysregulation in neurodegenerative disorders, cancer and other pathologies.

0129-R-P

Molecular recognition of Histone H3K4me3 by Tumor Suppressor ING3

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The Inhibitors of growth (ING) family of tumor suppressors bind to the N-terminal disordered tail of histone H3 trimethylated at lysine 4 (H3K4me3) through their C-terminal plant homeodomain (PHD). They recruit histoneacetylation and deacetylation complexes to the chromatin affecting DNAreplication and transcription [1]. We have studied the PHD of ING3 and its

binding to histone H3 peptides with different methylation states at K4 by NMR, calorimetry, and crystallography. The structure of the free domain is very similar to those of the other ING proteins, and it binds the H3K4me3 peptide through the same region, with a dissociation constant of 2.5 ± 0.3 μM. The affinity for the unmethylated peptide is 50 times smaller. The binding is enthalpically driven, and it reduces the backbone dynamics of the PHD region interacting with the peptide N-terminus.

[1] A. Dantas et al., Cancers 11 (2019) 1817.

0164-P

Cytidine triphosphate promotes efficient ParB-dependent DNA condensation by facilitating one-dimensional spreading from parS

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The ParABS partition system is formed by the palindromic centromere-like DNA sequence *parS,* the *parS* binding protein ParB-CTPase and the ParA-ATPase. This partition system in combination with the SMC complex allow the faithful segregation of bacterial plasmidic and chromosomic DNA. The effect of cyditine triphosphate in the initial nucleation of ParB at the *parS* and the subsequent spreading of the protein has not been fully described yet. In this work, we use single molecule techniques to investigates the role of the CTP in the ParB function. By mean of optical tweezers and confocal microscopy, we observed an increase in the binding of ParB to *parS* in the presence of CTP. Under these conditions, ParB spreads from the parS region to distal non specific region of DNA. We show that the spreading of ParB requires *parS* loading sites, is prevented by EcoRI roadblocks and is enhanced by CTP binding. We also observed by Magnetic tweezers that in this condition ParB can induce the condensation of *parS*- containing DNA molecules at low nanomolar concentrations. We present here a model in which ParB nucleates at parS in the presence of CTP-Mg^{2+} and moves along DNA inducing the DNA condensation by protein:protein interactions.

1. Osorio-Valeriano M, Altegoer F, Steinchen W et al (2019). ParB-type DNA Segregation Proteins Are CTP-Dependent Molecular Switches. Cell 179:1512–1524. 2. Soh YM, Davidson IF, Zamuner S et al.(2019). Self-organization of parS centromeres by the ParB CTP hydrolase. Science 366:1129–1133. 3. Taylor JA, Pastrana CL, Butterer A et al (2015). Specific and non-specific interactions of ParB with DNA: Implications for chromosome segregation. Nucleic Acids Res 43:719–731.

0198-P

Physical contact between cytochrome c1 and cytochrome c increases the driving force for electron transfer

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In oxidative phosphorylation, the transfer of electrons from reduced cofactors to molecular oxygen via the electron transport chain (ETC) sustains the electrochemical transmembrane potential needed for ATP synthesis. A key component of the ETC is complex III (CIII, cytochrome bc_1), which transfers electrons from reduced ubiquinone to soluble cytochrome *c* (C*c*) coupled to proton translocation into the mitochondrial intermembrane space. One electron from every two donated by hydroquinone at site P is transferred to C*c* via the Rieske-cytochrome c_1 (Cc_1) pathway. According to recent structural analyses of CIII and its transitory complex with C*c*, the interaction between the Rieske subunit and C*c*1 switches intermittently during CIII activity. However, the electrochemical properties of C*c*1 and their function as a wire between Rieske and C*c* are rather unexplored. Here, temperature variable cyclic voltammetry provides novel data on the thermodynamics and kinetics of interfacial electron transfer of immobilized C*c*1. Findings reveal that C*c*1 displays two channels for electron exchange, with a remarkably fast heterogeneous electron transfer rate. Furthermore, the electrochemical properties are strongly modulated by the binding mode of the protein. Additionally, we show that electron transfer from Cc_1 to C*c* is thermodynamically favored in the immobilized Cc_1-C*c* complex. Nuclear Magnetic Resonance, HADDOCK, and Surface Plasmon Resonance experiments provide further structural and functional data of the Cc_1-C*c* complex. Our data supports the Rieske-C*c*1-C*c* pathway acting as a unilateral switch thyristor in which redox potential modulation through protein-protein contacts are complemented with the relay-like Rieske behavior.

Pérez-Mejías G, Olloqui-Sariego JL, Guerra-Castellano A, Díaz-Quintana A, Calvente JJ, Andreu R, De la Rosa MA, Díaz-Moreno I. Physical contact between cytochrome c1 and cytochrome c increases the driving force for electron transfer. Biochim Biophys Acta Bioenerg. 2020 1861: 148277.

0221-P

Molecular characterization of the G288S mutant in the Na+/I- symporter (NIS) of a patient with Congenital hypothyroidism

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Mutation in the SLC5A5 gene, which encodes the sodium/iodide symporter (NIS) protein, causes congenital hypothyroidism (CH) due to iodide transport defects (ITD) that may cause developmental deficiencies [1]. NIS is a plasma membrane glycoprotein that mediates active iodide (I-), together with 2 Na^+, transport in epithelial cells of the thyroid gland and other tissues [2]. To date only 15 NIS mutations causing ITD have been described, causing alterations in maturation/glycosylation, plasma membrane trafficking or I- accumulation, at different levels. We analysed a SLC5A5 heterozygosis variant in exon 7 (c.862G>A.p.G288S) of a patient with congenital hypothyroidism. NIS-G288S is located in the transmembrane segment (TMS) 8 [3]. Using a NIS homology model and molecular dynamic simulations we identified that G288 amino acid is in close proximity to critical residues implicated in the second Na^+ transport. In vitro studies, both in NIS expressing transiently (COS-7) and stably (MDCK) transfected cells, by western-blot, immunofluorescence, and flow cytometry assays, determined that NIS-G288S protein expression, maturation and plasma membrane trafficking is not altered. Radioiodide transport steady-state assays indicated that NIS-G288S is active, however time-course and initial-rate assays show slow I- accumulation. Additionally, substitution of G288 by large or charged side chain amino acids yields inactive NIS protein that fails to traffic to the plasma membrane. In conclusion, G288S NIS mutation leads to a partial ITD effect in patients, and the presence of a neutral or short hydrophobic side-change amino acid at 288 position in NIS protein is essential.

[1] De la Vieja A, Santisteban P. Role of iodide metabolism in physiology and cancer. Endocr Relat Cancer. 2018;25(4):R225-R45. [2] De La Vieja A, et al. Molecular analysis of the sodium/iodide symporter: impact on thyroid and extrathyroid pathophysiology. Physiol Rev. 2000;80(3):1083-105. [3] Ravera S, Reyna-Neyra A, Ferrandino G, Amzel LM, Carrasco N. The Sodium/Iodide Symporter (NIS): Molecular Physiology and Preclinical and Clinical Applications. Annu Rev Physiol. 2017;79:261-89.

0243-P

Structural and Molecular Insights into IS21 transposition

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Transposons are ubiquitous, mobile DNAs that rely on dynamic nucleoprotein complexes to mediate transposition reactions. These elements can modify gene expression, promote organismal evolution, and disseminate antibiotic resistance and virulence factors. In humans, their activity has been associated to multiple diseases and, in addition, some elements have shown a significant potential as biomedical or biotechnological tools. However, the molecular mechanisms that control DNA transposition remain poorly understood. Here, we have used cryo-electron microscopy in combination with biochemical assays to analyse the regulation a member of the particularly widespread family of transposons IS21. Notably, these streamlined mobile elements encode only two proteins: a canonical DDE transposase and, in a similar way to some classic transposons such as Mu or Tn7, an essential regulatory ATPase that belongs to the AAA+ super-family of proteins. Our results provide fundamental insights into the molecular interplay between these factors, how they recognize the client DNA substrates and use the energy of nucleotide binding and hydrolysis to promote efficient DNA transposition.

Arias-Palomo, E. and Berger, JM. (2015) An atypical AAA+ ATPase assembly controls efficient transposition through DNA remodeling and transposase recruitment. Cell. 2015 Aug 13; 162(4): 860–871. doi: 10.1016/j.cell.2015.07.037

0245-R/M-P

Polar-to-Hydrophobic Core Mutations Have Strongly Stabilizing Effects in Coiled-Coil Mimetic Proteins of HIV-1 gp41 and Produce Diverse Effects on Target Binding and Inhibitory Activity

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A promising strategy to neutralize HIV-1 is to target the gp41 spike subunit to block membrane fusion with the cell. We previously designed a series of single-chain proteins (named covNHR) that mimic the trimeric coiled-coil structure of the gp41 N-terminal heptad repeat (NHR) region and potently inhibit HIV-1 cell infection by avidly binding the complementary C-terminal heptad repeat region (CHR). These proteins constitute excellent tools to understand the structural and thermodynamic features of this therapeutically important interaction. Gp41, as many coiled-coil proteins, contains in core positions of the NHR trimer several highly conserved, buried polar residues, whose role in gp41 structure and function is unclear. Here we produced three covNHR mutants by substituting each triad of polar residues for the canonical isoleucine. The mutants preserve their helical structure and show an extremely increased thermal stability. However, increased hydrophobicity enhances their self-association. Calorimetric analyses show a marked influence of mutations on the binding thermodynamics of CHR-derived peptides. The mutations do not affect however the *in vitro* HIV-1 inhibitory activity of the proteins. The results support a role of buried core polar residues in maintaining structural uniqueness and promoting an energetic coupling between conformational stability and NHR-CHR binding.

0270-R/M-P

Modulation of the redox status of the Ferric Uptake regulator FurA in Anabaena sp. PCC 7120

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Fur (ferric uptake regulator) is a master transcriptional regulator in prokaryotes which modulates a wide set of genes not only related to iron homeostasis, but also involved in other cellular processes. Unlike most Fur proteins, FurA from *Anabaena* sp. PCC7120 does not contain zinc, which allows the occurrence of intra and intermolecular thiol-disulfide exchanges. Consequently, FurA acts as a redox regulator able to integrate iron homeostasis with the redox status of its five cysteines. Our previous work showed that four out of these five cysteines are involved in the formation of two intramolecular disulfide bridges, namely C104-C133 and C141-C144, and unveiled that FurA could be in several redox states in the cyanobacterial cytosol. However, the precise mechanism underlying the reduction of FurA, as well as its functional electron donor, remain still unknown. As thioredoxins are essential players in thiol-based redox regulation and are involved, among many other processes, in the regulation of the activity of many redox-sensitive transcription factors, we sought to investigate the potential role of thioredoxin-m1 (named TrxA) in the redox modulation of FurA. Although photosynthetic organisms contain at least 19 types of thioredoxins, TrxA is the most abundant among the major types of thioredoxins present in chloroplasts and cyanobacteria and one of the major hubs in their redox networks.

In this study, we present a novel functional relationship between the global regulator FurA and TrxA in cyanobacteria. Our results demonstrate that TrxA is able to interact both *in vitro* and *in vivo* with FurA from *Anabaena* sp. PCC7120 as assessed using cross-linking and bacterial two-hybrid assays. Besides, we found that TrxA is able to reduce FurA and, in basis of our previous knowledge on the dynamics of thiol-disulfide exchanges in FurA and the reduction patterns of FurA site-directed mutants by TrxA, a plausible mechanism for the reduction of this transcriptional regulator has been proposed.

Jorge Guío is a PhD student in the Biochemistry and Molecular Biology program at the University of Zaragoza (Spain). He holds a BSc in Biotechnology and a Master's degree in Molecular and Cell Biology. His research focuses on understanding transcriptional networks in cyanobacteria, in particular those that control nitrogen metabolism and abiotic stresses.

0275-R/M-P

Cryo-EM structure of the reconstituted human γ-Tubulin Ring Complex

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Microtubules (MTs) are essential cytoskeletal polymers made up of αβ-tubulin heterodimers that provide structural support for the cell and play important roles in key cell processes such as division, motility and intracellular transport. The γ-tubulin ring complex (γTuRC) is the major MT nucleator in animal cells. γTuRC not only promotes the kinetically unfavorable assembly of MTs but also appears to determine their polarity.

The molecular mechanism by which the γTuRC promotes MT nucleation remains poorly understood although a template-based mechanism, based on pioneering structural studies of Drosophila γTuRC and related complexes in yeast[1], remains the most widely accepted. According to this model γTuRC, a 2 MDa multi-subunit protein complex composed of γ-tubulin, members of the γ-tubulin complex protein (GCP) family, and additional proteins, forms a lock washer-like structure, in which the ring-shaped arrangement of γ-tubulin molecules resembles the symmetry of a MT in cross-section and thus serves as a template for the assembly of αβ-tubulin heterodimers.

We have demonstrated that RUVBL1-RUVBL2 AAA-ATPase complex (RUVBL) controls assembly and composition of γTuRC in human cells. Likewise, RUVBL assembles γTuRC from a minimal set of core subunits in a heterologous co-expression system. Purified, reconstituted γTuRC has nucleation activity and resembles native γTuRC[2] as revealed by its cryo-EM structure at ~4.0 Å resolution (Figure 1). We further use cryo-EM to identify novel features that determine the intricate, higher-order γTuRC architecture3.

1.Moritz M, Braunfeld MB, Guénebaut V, Heuser J, Agard DA (2000). Nat Cell Biol 2:365–370. doi: 10.1038/35014058 2.Zupa E, Liu P, Würtz M, Schiebel E, Pfeffer S (2021). Curr Opin Struct Biol 11(2):200325. doi: 10,1098/rsob,200325. 3.Zimmermann F*, Serna M*, Ezquerra A, Fernandez-Leiro R, Llorca O, Luders J (2020). Sci Adv 6(15):eabe0894. *Both authors contributed equally. doi: 10.1126/sciadv.abe0894

0283-P

Single-molecule characterization of CST complex dynamic interactions with ssDNA

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CST (Cdc13 in yeast/Ctc1 in higher eukaryotes - Stn1 - Ten1) is a telomere capping protein complex which has a key role in telomere protection and replication. A large protein sequence divergence between the biggest subunits of the complex (Cdc13/Ctc1) brings into question the conservation of the CST complex functions between species. Our research aims to describe interactions of the *Candida glabrata* CST with single-stranded DNA at the single-molecule level. To this end, we employ the magnetic tweezers technique, which enables to follow ssDNA-protein interplay in real time. At low forces, the CST complex, Stn1-Ten1 complex and Cdc13 protein bind ssDNA and unfold its secondary structures, leading to an increase in ssDNA extension. Consistent with this, the CST complex, Stn1-Ten1 complex and Cdc13 protein impede ssDNA secondary structures formation, when bound to ssDNA at a high force. Cdc13 binds ssDNA with a higher affinity than Stn1-Ten1 complex, but with a lower affinity than RPA. Finally, we show that Cdc13 binds preferentially to G-rich telomere sequences. This research shall contribute to the understanding of molecular processes governing telomere maintenance.

0286-P

Revealing the mechanical response corresponding to the isoform-sensitivity of AMPK

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Mammalian AMP-kinase (AMPK) is a Ser/Thr kinase, present in almost all eukaryotic cells, playing a vital role as a master regulator of cellular energy homeostasis [1] . Due to its critical function in cell metabolism, AMPK is a potential therapeutic target for the treatment of chronic metabolic diseases such as type 2 diabetes, obesity but also neurodegenerative diseases and cancer [2] . AMPK is a~145 kDa heterotrimer αβγ complex consisting of a catalytic α-subunit and regulatory β- and γ- subunits. In total, there are 12 AMPK isoform systems which exhibit different sensitivity to molecular activators [1] . The allosteric activation by binding AMP in the γ subunit needs from the phosphorylation of Thr172 located in the activation loop of the α-subunit by upstream kinases [3]cells require mechanisms to balance energy demand with supply. In eukaryotic cells the AMP-activated protein kinase (AMPK . Also, the indirect AMPK activators, like metformin, act by increasing the cellular AMP concentration [4] . Finally, direct activation mechanism was observed by small molecules. This novel mechanism of AMPK action by the thienopyridone drug A769662 [5] , binds the ligand at the interface of α and β subunits which is called Allosteric Drug and Metabolite binding (ADaM) site [6] . In last years, several direct activators of AMPK were reported. To shed light into the molecular factors of the direct regulation of AMPK [7], we have already examined the structural and dynamical properties of β1- and β2- AMPK complexes formed with A769662 and SC4 activators trying to dissect the mechanical response leading to active-like conformations. The results of these analyses revealed the mechanical sensitivity of α2β1 complexes in contrast to the large resilience of α2β2, which favors the pre-organization of the ATP-binding. Moreover, we realized that the change of β1Asn111 to β2Asp111 could be the key factor in modulating the mechanical sensitivity of β1 and β2 containing AMPK complexes. To confirm our hypothesis, I will show the results of Molecular Dynamics simulations of the α2β1 in absence and presence of A769662, considering the most important changes observed between β1 and β2 isoforms related to the ADaM site. Additionally, we will extend our study to the different effect of MT47-100 ligand, which could activate α2β1, but inhibit α2β2 complex. Performing these simulations, we will gain a better comprehension of the mechanism of direct activation/inhibition of AMPK.

[1] R. G. Kurumbail et al. Exp. Suppl., 107, 3–22, 2016. [2] D. Neumann et al. AMPK: Methods and Protocols. Springer New York, 2018. [3] D. Carling et al. Nat. Chem. Biol., 7, 512–518, 2011. [4] M. F. Calabrese et al. Structure, 22, 1161–1172, 2014. [5] B. Cool et al. Cell Metab., 3, 403–416, 2006. [6] X. Gu et al. J. Biol. Chem., 293, 16994–17007, 2018. [7] E. Aledavood et al. J. Chem. Inf. Model., 59, 2859–2870, 2019.

0292-R-P

Zika and Dengue: Identification of new inhibitors combining computational and experimental strategies

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Zika (ZIKV) and Dengue (DENV) are *flavivirus*es that are transmitted to humans through the bite of infected mosquitoes of the genus *Aedes (Ae. Aegypti* and *Ae. Albopictus*), highly prevalent in tropical and subtropical regions. DENV has symptoms very similar to those of the flu, becoming fatal when taking serious haemorrhages. WHO estimates that DENV produces about 390 million infections per year [1]. In 2018, CCAES reported six cases of native Dengue in Spain, attributed to *Ae. Albopictus* (tiger mosquito) widespread in the Iberian Peninsula [2]. ZIKV, unlike DENV, causes congenital brain abnormalities during pregnancy, including microcephaly, and is a trigger for Guillain-Barré syndrome [3]. ZIKV continues to expand geographically, due to the propagation of the vector. In the Spanish Mediterranean area and after recent passages of cold droplets, it has been warned of the high risk of DENV and ZIKA infections due to the flooding after heavy rains and high temperatures that have happened after them. This zone constitutes the best and largest breeding ground for the proliferation of the tiger mosquito, whose expansion has been being meteoric throughout the area. Currently no medications or antiviral vaccines have been developed against ZIKV or DENV, therefore becoming a global public health problem with a very high economic cost. We have identified natural compounds and organic molecules, such as new inhibitors of NS3 ZIKA / DENV enzyme, selected from several chemical libraries through analysis of docking *in silico*, against binding sites recognized in their 3D crystallographic structures. We have also designed a peptide (PepE) with its sequence derived from the membrane interaction regions of the envelope protein (E) of DENV, ZIKV, TBEV and JEV viruses identified by molecular dynamics simulations [4] and have studied its interaction with different biomembrane model systems, using *in vitro* biophysical techniques. The results obtained could establish a first step to develop new antiviral agents against ZIKV and DENV.

[1] Bhatt S et al. (2013). Nature 496:504-507. [2] Informe evaluación rápida de riesgo. (31/05/2019). Dengue. CCAES, Gob. de España. [3] Esposito S et al. (2016). Autoimmun. 16(1):96-101. [4] Carpio L. et al. J Biomol Struct Dyn. (2020) Oct;38(17):5136-5147.

0303-P

Structure of the cytoplasmic domain of the injectisomal SctV and implications for a pH sensing mechanism

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Bacterial type III secretion systems assemble the axial structures of both injectisomes and flagella. Injectisome type III secretion systems subsequently secrete effector proteins through their hollow needle into a host, requiring co-ordination. In the Salmonella enterica serovar Typhimurium SPI-2 injectisome, this switch is triggered by sensing the neutral pH of the host cytoplasm. Central to specificity switching is a nonameric SctV protein with an N-terminal transmembrane domain and a toroidal C-terminal cytoplasmic domain. A 'gatekeeper' complex interacts with the SctV cytoplasmic domain in a pH dependent manner, facilitating translocon secretion while repressing effector secretion through a poorly understood mechanism.

To better understand the role of SctV in SPI-2 translocon-effector specificity switching, we purified full-length SctV and determined its toroidal cytoplasmic region's structure using cryo-EM. Structural comparisons and molecular dynamics simulations revealed that the cytoplasmic torus is stabilized by its core subdomain 3, about which subdomains 2 and 4 hinge, varying the flexible outside cleft implicated in gatekeeper and substrate binding. In light of patterns of surface conservation, deprotonation, and structural motion, the location of previously identified critical residues suggest that gatekeeper binds a cleft buried between neighboring subdomain 4s. Simulations suggest that a local pH change from 5 to 7.2 stabilizes the subdomain 3 hinge and narrows the central aperture of the nonameric torus. Our results are consistent with a model of local pH sensing at SctV, where pH-dependent dynamics of SctV cytoplasmic domain affect binding of gatekeeper complex.

0307-P

Functional characterization of a Sinorhizobium meliloti thioesterase involved in the synthesis of the infochemical 2-tridecanone

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CSIC Estacion Experimental del Zaidin

Sinorhizobium meliloti is a soil bacterium that can establish beneficial symbiosis with legume plants. Recently, it was shown that this microbe releases several volatile methylketones (MKs), which are overproduced in a *fadD* mutant. One of them, 2-tridecanone (2-TDC), was found to act as an infochemical that affects important bacterial traits and hampers plant–bacteria interactions (López-Lara et al. 2018). The aim of this work was to identify the enzymatic activities responsible for 2-TDC production in *S. meliloti*.

In wild tomato species, MK synthesis requires intermediates of fatty acid biosynthesis and the activity of the methylketone synthase 2 (ShMKS2), a thioesterase belonging to the hot-dog fold family (Yu et al. 2010). A search for ShMKS2 orthologs in the *S. meliloti* proteome database retrieved SMc03960, a conserved hypothetical protein with homology to bacterial thioesterases YbgC. Heterologous expression of SMc03960 in *Escherichia coli* led to the formation of several MKs including 2-TDC, and caused the accumulation of free fatty acids. Accumulation of MKs and fatty acids was abolished in cells expressing a SMc03960 version mutated in the conserved Asp27 residue of the active site. Thioesterase activity of purified His-SMc03960 was assayed against a wide range of acyl groups linked either to acyl carrier protein (ACP) or to coenzyme A (CoA). Results revealed that SMc03960 is a thioesterase that shows broad substrate specificity with preference for C14 substrates. Moreover, formation of 2-TDC *in vitro* was achieved by using His-SMc03960 and 3-oxo-myristoyl-ACP.

To test whether SMc03960 plays a role in the generation of MKs in *S. meliloti*, *smc03960* was either inactivated or overexpressed in both wild-type and *fadD* mutant genetic backgrounds. Analyses of volatiles emitted by the different strains indicate that SMc03960 contributes to 2-TDC formation in *S. meliloti* but additional as yet unidentified activities must also be involved.

Work supported by BIO2013-42801-P and PGC2018-096477-B-I00 (MCIU/AEI/FEDER, UE) and by UNAM-PAPIIT (IN200819)-Mexico.

Lopez-Lara et al. 2018. 2-Tridecanone impacts surface-associated bacterial behaviours and hinders plant-bacteria interactions. Environ Microbiol 2018, 20, 2049-2065 Yu et al. Enzymatic functions of wild tomato methylketone synthases 1 and 2. Plant Physiol 2010, 154, 67-77.

0315-R/M-P

FUNCTIONAL AND BIOPHYSICAL CHARACTERIZATION OF RELEVANT HMGB-lncRNAs INTERACTIONS IN THE DIAGNOSIS OF OVARY CANCER

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CICA - UNIVERSITY OF A CORUÑA BIOLOGY

Ovarian cancer is one of the most lethal gynecological malignancies worldwide because it tends to be detected late, when metastasis has taken place. Early diagnosis, when the tumor is still localized in the ovaries, is a clear advantage, since this rate then increases up to 92% (1). Long non-coding RNAs (lncRNAs) are non-protein-encoding transcripts longer than 200 nucleotides with the ability to regulate gene expression at many different levels into the cell. Additionally, lncRNAs are frequently tissue-specific and deregulated in tumor cells, making them potential biomarkers to be detected by liquid biopsy (2). It is well established that RNA stability is improved by formation of higher molecular complexes with proteins, a phenomena that becomes crucial for detection of these biomarkers. HMGB1 has been repeatedly proposed as a diagnostic and prognostic biomarker for human ovarian cancer (3). Despite being a very well established and characterised DNA-binding protein, very little is known about its RNA binding capacities. In the present work, we characterize the HMGB1 ability to form complexes with lncRNAs related to OC in terms of structural and biophysical properties by optical and calorimetric assays.

(1) Brett, M.R. et al. (2017) Epidemiology of ovarian cancer: A review. Cancer Biol. Med. (2) Salamini-Montemurri M et al. (2020) The Challenges and Opportunities of LncRNAs in Ovarian Cancer Research and Clinical Use. Cancers. doi: 10.3390/cancers12041020. (3) Cámara-Quílez M et al. (2020) The HMGB1-2 Ovarian Cancer Interactome. The Role of HMGB Proteins and Their Interacting Partners MIEN1 and NOP53 in Ovary Cancer and Drug-Response. Cancers. doi: 10.3390/cancers12092435.

0367-P

Native mass spectrometry for the study of phospholipid-protein complexes

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Native mass spectrometry (native MS) is a robust, versatile and reliable tool to screen and characterize compound interactions of non-covalent complexes. This label-free analytical technique also referred to as non-covalent, non-denaturing or supramolecular MS, utilizes electrospray to ionize and transfer non-covalent assemblies from solution to gas phase. Native MS provides an accurate measurement of the molecular masses of intact complexes which enables valuable insights into biomolecule interactions including subunit stoichiometry, site-specificity, affinity and folding. Native-MS is widely applicable to analyse a variety of biomolecular assemblies, not only multi-protein and nucleic acid systems, but also small molecules complexed to proteins or nucleic acids.

We have explored conditions for preserving phospholipid-protein interactions during the transference into the gas phase and to gradually release phospholipids from the complex. This enables us to characterize lipid binding in bacterial phospholipid transporter proteins. In particular, we have applied this methodology to determine the nature of the phospholipid binding to the soluble periplasmic component of the ABC transport system Ttg2/Mla in Pseudomonas aeruginosa.

0392-R-P

High-Throughput search of mechano-active small-molecules for controlling the mechanical stability of proteins

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CIC nanoGUNE NANOBIOTECHNOLOGY

Protein mechanical stability determines the function of a myriad of proteins, especially proteins from the extracellular matrix. Failure to maintain protein mechanical stability may results in diseases and disorders such as cancer, cardiomyopathies or muscular dystrophy. Thus, developing a mutation-free approach to enhance and control the mechanical stability of proteins using pharmacology-based methods, may have important implications in drug development and discovery. Here, we present the first approach that employs computational High-Throughput Virtual Screening (HTVS) and Molecular Docking to search for small-molecules in chemical libraries that function as mechano-regulators of the stability of human CD4. Using single-molecule force spectroscopy we probe that these small-molecules can increase the mechanical stability of CD4 over 60% also modifying the mechanical function of the molecule. Our experiments demonstrate that chemical libraries are a source of mechanoactive molecules and that drug discovery approaches provide the foundation of a new type of molecular function, opening up the way towards mechanopharmacology.

Hu, X. & Li, H. Force spectroscopy studies on protein–ligand interactions: A single protein mechanics perspective. FEBS Letters 588, 3613-3620 (2014). Friesner, R.A. et al. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem 47, 1739-1749 (2004).

0413-P

Exploring regulatory conformational rearrangements in the DNA repair protein APE1 by fluorescence resonance energy transfer (FRET).

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Apurinic / apyrimidinic endonuclease 1 (APE1) is a key enzyme of the BER (base excision repair) pathway of DNA damage repair, recognizing abasic sites and cleaving the phosphodiester backbone to allow for the correction of mostly oxidative lesions. While APE1 activity has been deeply characterized at catalytic and structural levels, less is known about the regulation mechanisms modulating its function. Human APE1 consists of a globular domain responsible for its endonuclease activity, preceded by a conformationally flexible N-terminal extension, missing from crystal structures. The N-terminal tail of APE1 seems to play a key role in the modulation of APE1, which however remains to be elucidated. Thus, it could contribute to the DNA recognition event, and facilitate release of the enzyme from the reaction product in coordination with the BER machinery. Moreover, binding of the nuclear chaperone nucleophosmin (NPM1) to this region probably modulates such APE1 dynamics.

APE1 regulatory mechanisms likely imply dynamic variations in the relative positions of the N-terminal tail with respect to the globular domain and of both domains with respect to the DNA. We are using Förster resonance energy transfer (FRET), a fluorescence spectroscopy technique sensitive to the distances between two fluorophores (donor and acceptor of the transference) to evaluate intra- and inter-molecular conformational rearrangements that may take place upon DNA binding, incision, and interaction with NPM1. Our results suggest that the N-terminal tail closes onto the globular domain when APE1 binds its cognate DNA lesion. Furthermore, spectroscopic methods such as FRET represent useful probes to reveal molecular mechanisms involving an intrinsically disordered region that underpins APE1 function and regulation.

0438-R/M-P

Integrative Modelling to explore the structure and functionality of cellular complexes

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The exocyst is an hetero-octamer responsible for tethering secretory vesicles to the plasma membrane during exocytosis. The exocyst and its interplay with the rest of the exocytic machinery (SNAREs, GTPases, etc) is essential for all eukaryotic cells. However, the complexity and dynamism of this protein machinery has maintained the molecular mechanism mediating the exocyst function unknown. Recently, the development of integrative approaches combining in vitro and in situ structural information opened up the possibility to study the molecular bases of cellular functions. Integrative modelling offers a unique opportunity to study complex and dynamic molecular systems, allowing high-resolution observations in a near-physiological context. We are now developing a method to integrate data derived from in vitro (high-resolution structures obtained by x-ray and cryo-EM) and in situ (functional structural data by live-cell imaging) experiments. We use the Integrative Modelling Platform (IMP) to set the representation of the system, transform the input data into spatial restraints and sample the configurational space of solutions using the Monte Carlo method. We have modelled the functional architecture of the exocyst complex bound to a secretory vesicle. Our analysis uncovers the structural dynamics that mediate the activity of exocyst during exocytosis and it benchmarks the approach for the analysis in situ of large protein assemblies.

1. Irastorza-Azcarate, Ibai, et al. “Live-cell structural biology to solve biological mechanisms: the case of the exocyst.” Structure 27.6 (2019):886-892. 2. Mei,K. et al. (2018) Cryo-EM structure of the exocyst complex. Nat. Struct.Mol. Biol. 3. Picco, Andrea, et al. “The in vivo architecture of the exocyst provides structural basis for exocytosis.” Cell 168.3 (2017): 400-412.

0439-P

The Bateman domain of IMP dehydrogenase is a binding target for dinucleoside polyphosphates

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IMP dehydrogenase (IMPDH) is an essential enzyme that catalyzes the rate-limiting step in the *de novo* guanine nucleotide biosynthetic pathway. Consequently, IMPDH is involved in the control of cell division and proliferation and represents a therapeutic target for managing several diseases, including microbial infections and cancer. Despite the fact that IMPDH is a widely studied therapeutic target, the molecular mechanisms responsible for its physiological regulation remain largely unknown. To this end, we reported an important role of adenine and guanine mononucleotides that bind to the regulatory Bateman domain to allosterically modulate the catalytic activity of eukaryotic IMPDHs (1,2,3).

Dinucleoside polyphosphates are ubiquitous molecules in which two nucleosides are linked by a chain of two to seven phosphate moieties. Recently, we have demonstrated that adenine/guanine dinucleoside polyphosphates bind with submicromolar affinities to eukaryotic Bateman domains occupying two nucleotide binding sites simultaneously. These dinucleoside polyphosphates modulate the catalytic activity of IMPDHs *in vitro* by efficiently competing with the adenine/guanine mononucleotides for the allosteric sites (4). These results suggest that dinucleoside polyphosphates play important physiological roles in the allosteric regulation of IMPDHs by adding an additional mechanism for fine-tuning the activities of these enzymes. Furthermore, the reported data might have important implications for the design of novel therapeutic strategies to inhibit IMPDHs.

1. Buey RM et al. Nat Commun. 2015;6:8923. doi: 10.1038/ncomms9923 2. Buey RM et al. Sci Rep. 2017;7(1):2648. doi: 10.1038/s41598-017-02805-x 3. Fernández-Justel et al. J Mol Biol. 2019;431(5):956-969. doi: 10.1016/j.jmb.2019.01.020 4. Fernández-Justel et al. J Biol Chem. 2019;4;294(40):14768-14775. doi: 10.1074/jbc.AC119.010055

0445-P

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.

0451-P

Structure and Evolution of Zn2+-Dependent Histone Deacetylases in Plants

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Zn2+-dependent histone deacetylases are widely distributed in archaea, bacteria, and eukaryotes. Through deacetylation of histones and other biomolecules, these enzymes regulate mammalian gene expression, microtubule stability, and polyamine metabolism. In plants, they play essential roles in development and stress response, but the molecular mechanisms of action remain largely unknown, in part owing to the lack of structural and biochemical studies. We provide here a holistic revision of plant histone deacetylase (HDA) structure evolution and translate recent lessons from other organisms. HDA evolution correlates with a gain of structural ductility/disorder, as observed for other proteins. We also highlight two additional and functionally distinct *Brassicaceae*-specific HDAs, as well as unprecedented key mutations that would affect the catalytic activity of individual HDAs. This work will contextualize future studies and illuminate research on plant development and adaptation.

1. Maolanon, A.R. et al. (2016) Cell Chem. Biol. 23, 759–768. 2. Porter, N.J. and Christianson, D.W. (2019) Curr. Opin. Struct. Biol. 59, 9–18. 3. Chen, C.Y. et al. (2020) Plant Physiol. 184, 1585–1600. 4. Yruela I, Moreno-Yruela C, Olsen CA. (2021) Trends in Plant Science, Epub ahead of print, DOI:10.1016/j.tplants.2020.12.011.

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0472-R-P

Biochemical and functional characterization of rhodopsin mutants associated with retinitis pigmentosa and effect of sodium valproate on their conformational stability

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Retinitis pigmentosa is a group of inherited retinal degenerative diseases genetically and clinically heterogeneous for which no effective treatment has been developed to date. One of the leading causes of retinal degeneration in humans is the presence of mutations in retinal proteins particularly in the photoreceptor rhodopsin [1]. Rhodopsin is the G protein-coupled receptor of rod photoreceptor cells that is responsible for vertebrate scotopic vision at low light intensities. Two rhodopsin mutations, Y102H and I307N, which have been obtained in chemically mutagenized mice, have been proposed as good models for the study of retinal degeneration in humans [2]. Different therapeutic approaches have been proposed for the treatment of these diseases, including the use of pharmacological chaperones that can bind to mutated rhodopsin and stabilize its proper folded conformation. We functionally and biochemically characterized the structural and functional alterations of Y102H and I307N rhodopsin mutants *in vitro* for a better understanding of *in vivo* studies aimed at developing novel therapeutic approaches for retinitis pigmentosa. We show that these mutations affect the inactive-active equilibrium of rhodopsin by reducing the stability of the inactive conformation and increasing that of the active conformation. Furthermore, the results regarding I307N mutant suggest a significant effect on its signal transduction profile. In view of this behavior, we also analyzed the effect of sodium valproate, which has been tested as a potential therapeutic agent for the treatment of retinal degeneration associated with retinitis pigmentosa [3], on the conformational stability of wild-type rhodopsin and the I307N mutant. We found that the active conformation of the mutated receptor appears to be destabilized by sodium valproate as shown by a faster decay of its active conformation [4]. Therefore, our results would provide molecular support for recent clinical studies reporting negative effects of sodium valproate on the visual function of retinitis pigmentosa patients.

Athanasiou et al. The molecular and cellular basis of rhodopsin retinitis pigmentosa reveals potential strategies for therapy. 2018 Budzynski et al. Mutations of the opsin gene (Y102H and I307N) lead to light-induced degeneration of photoreceptors and constitutive activation of phototransduction in mice. 2010 Totan et al. The adverse effects of valproic acid on visual functions in the treatment of retinitis pigmentosa.2017 Razzaghi et al.2021. Accepted for publication.

0477-P

On the use of Direct-Coupling Analysis with a reduced alphabet of amino acids combined with super-secondary structure motifs for protein fold prediction.

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Direct-coupling analysis (DCA) for studying the coevolution of residues in proteins has been widely used to predict the three-dimensional structure of a protein from its sequence. We present RADI/raDIMod, a variation of the original DCA algorithm that groups chemically equivalent residues combined with super-secondary structure motifs to model protein structures. Interestingly, the simplification produced by grouping amino acids into only two groups (polar and non-polar) is still representative of the physicochemical nature that characterizes the protein structure and it is in line with the role of hydrophobic forces in protein-folding funneling. As a result of a compressed alphabet, the number of sequences required for the multiple sequence alignment is reduced. The number of long-range contacts predicted is limited, therefore our approach requires the use of neighboring sequence-positions. We use the prediction of secondary structure and motifs of super-secondary structures to predict local contacts. We use RADI and raDIMod, a fragment-based protein structure modelling package, to achieve near native conformations when the number of super-secondary motifs covers more than 30-50% of the sequence. Interestingly, although different contacts are predicted with different alphabets, they produce similar structures.

Availability: RADI is available at https://github.com/structuralbioinformatics/RADI and raDIMod at https://github.com/structuralbioinformatics/raDIMod

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On the use of direct-coupling analysis with a reduced alphabet of amino acids combined with super-secondary structure motifs for protein fold prediction. NAR genomics and bioinformatics 2021. 3(2) lqab027

0481-R-P

IgE-epitope analysis and characterization of Pru p 9: the first peach aeroallergen

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Background: Peach tree pollen (PTP) is responsible of the induction of respiratory and ocular symptoms in a population near to peach orchards, being the third most prevalent in sensitization. In its allergenic profile, Pru p 9 is the first relevant allergen identified in peach pollen, belonging to the Pathogenesis-Related protein family (PR-1a). According to protein properties, Pru p 9 is a 21 kDa glycoprotein with a tertiary structure that is stabilized by 3 disulfide bounds. In order to deepen our understanding of this allergen, the main objective of this study was the purification of natural Pru p 9, the expression as a recombinant non-glycosylated allergen and the characterization of the immunological properties of its glycosylated region.

Methods: A total of 60 subjects (18-65 yrs) with positive SPT to PTP were included in the study. Natural Pru p 9 was purified from the extract and recombinant allergen was produced in *P.pastoris* KM71H cells and E.coli T7 cells. The impact of glycosylation and conformational epitopes on IgE reactivity was evaluated by means of enzymatic deglycosylation assay, measurement of IgE to CCD/MUXF, production of glycosylated recombinant allergen variants, and reduction-alkylation experiments.

Results: The purification yield of the natural allergen obtained from PTP was 25%. Of the 60 subjects studied, 25 (41%) displayed IgE reactivity to nPru p 9, but only one was MUXF positive. *P. pastoris* rPru p 9 was produced as glycoallergen, being recognized by IgE in a similar way as its natural counterpart. In contrast, *E.coli* rPru p 9 was produced as non-glycosylated protein, corroborated by mass spectrometry analysis. The reduction alkylation assay resulted in a non-IgE reactive protein. Deglycosylation of nPru p 9 caused a drop in molecular mass from 21 kDa to around 17 kDa. Both the natural deglycosylated form and the *E.coli* rPru p 9 exhibited diminished but still substantial IgE-reactivity with a pool of sera.

Conclusion: Pru p 9 is the most relevant peach aeroallergen described to date. The presence of disulfide bonds, and therefore a proper 3D structure, is essential to immunological reactivity. The glycan moiety of Pru p 9 could participate in a conformational epitope, as described for other PR-1 allergens, but being responsible in only a small part for the IgE recognition.

Graduated in Biology and Master in Biochemistry, Cell Biology and Biomedicine. In July 2019 I finished my International PhD in Biochemistry, Molecular Biology and Biomedicine at UCM. I have attended more than 10 national and international congresses and I have published 11 scientific articles/reviews. Since October 2019 Postdoctoral researcher in H. U. Infanta Leonor and since October 2020 postdoctoral fellow at Paul-Ehrlich-Institut, Langen (Germany).

0489-P

New Possibilities at ALBA for the Structural Biology Community

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ALBA SYNCHROTRON LIFE SCIENCE SECTION

Since December 2020, we entered a new and exciting phase of transitioning ALBA to a 4th generation light source with a strong focus on microscopy and high throughput setups, linked with state-of-the-art data-analytics programs and a broad supporting infrastructure. The life science section of ALBA is developing a strategy to provide system solutions in the area of structural molecular biology as well as structural cellular and tissue biology by specializing and optimizing existing instruments, adding new beamlines, combining these instruments with non-X-ray base characterization tools in a multi-modal approach, providing missing sample preparation infrastructure and developing the necessary computing services.

ALBA II has to answer to demands of the Life Sciences community and provide the necessary tools. These tools ideally cover all length scales that are relevant for understanding biological processes on the molecular, organelle and cellular scale. The current beamline portfolio offers high resolution structure determination (MX), low resolution shape determination (SAXS) and the imaging of cells and cellular components (X-ray microscopy) as well as a range of biophysical characterization techniques. However, gaps exist between the future requirements of the portfolio and the current possibilities at ALBA. Some of these gaps have been identified, but there may be others that need pinpointing in close collaboration with the ALBA structural biology users.

This contribution will summarize the plans to develop ALBA's tools to a platform which can support our mission. It will also introduce the opportunities and new approaches which ALBA II will bring for the structural biology community. I will discuss our current view on the technical possibilities and new methodologies that are necessary to implement these capabilities and highlight the synergies between them.

0505-R/M-P

Molecular characterization of retriever complex assembly

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The composition of integral membrane proteins localized on the cell surface is essential for regulating the physiology complexities at the cell, tissue and organism levels. Endosomes play a central role in regulating the abundance of plasma membrane proteins. Endosomally localized multi-protein complexes sort integral membrane proteins internalized by endocytosis, called cargo, either to a degradation pathway to lysosomes or to a recycling pathway where cargo is transported back to the plasma membrane in vesicular carriers. Defects in this recycling process are associated with various human pathologies including neurodegenerative disorders. A recently identified new player in the recycling pathway is retriever, a multiprotein complex that recycles integral membrane proteins from endosomes to the plasma membrane. A wide range of cargoes of this carrier has been identified as cell adhesion proteins, signaling receptors and solute transporters. Here, we present the molecular characterization of retriever complex assembly. A recombinant form of the retriever complex was expressed in insect cells, purified to homogeneity and analyzed by mass spectroscopy. We employed small angle x-ray scattering (SAXS) to characterize the solution structure of the purified retriever complex. In addition, by expressing truncation mutants of retriever subunits we could identify the interaction domains between subunits. These results have allowed us to depict a model of retriever complex assembly and architecture.

0506-P

Redox response of CP12 in organisms without an iron-sulfur thioredoxin reductase

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The 12 kDa Chloroplast Protein (CP12) is a conditionally disorder small protein present in oxygenic photosynthetic organisms that participates in the regulation of the Calvin-Benson cycle (CBC) in response to light/dark cycles. In most organisms, CP12 contain two redox-active disulfide bonds each of which interacts with phosphoribulokinase (PRK) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) enzymes, respectively, two CBC enzymes that play an essential role in regulating the amount of substrate available for the enzyme Rubisco (McFarlane et al., 2019). During the night and oxidizing conditions, the formation of the ternary complex results in the inhibition of the activity of enzymes. Upon disulfide reduction in CP12 by the thioredoxin (Trx) system in the light, the complex dissociates and PRK/GADPH activities are restored (López-Calcagno et al., 2014; Gurrieri et al., 2021). Further, the activity of PRK and GADPH enzymes have an additional level of regulation that responds to redox and metabolic state of the cell. Typically, the Trx system in cyanobacteria and chloroplasts is composed of an Fe-S ferredoxin:thioredoxin reductase (FTR) that catalyzes the transfer of electrons from photosynthetically-reduced ferredoxin (Fdx) to a thioredoxin protein, that itself reduces the target proteins, such as CP12 (Balsera et al., 2019; Buey et al., 2021). In this work we have investigated the redox properties of CP12 in oxygenic photosynthetic organisms that do not contain either FTR or an NADPH-dependent thioredoxin reductase (NTR) enzyme, the most common type present in most type of cells. For this purpose, we have used the CP12 protein of the cyanobacterium *Gloeobacter violaceus* as model system. In this presentation, we will describe the experiments we have performed aimed to determine the molecular mechanisms underlying its putative role in the activity of essential enzymes functional in the Calvin-Benson cycle, experiments including the recombinant expression of *Gloeobacter violaceus* CP12, protein isolation and purification, and protein analysis. Our preliminary results suggest that the purified CP12 undergoes control response to redox conditions.

Balsera, M., & Buchanan, B. B. (2019). Evolution of the thioredoxin system as a step enabling adaptation to oxidative stress. Free Radical Biology and Medicine, 140, 28-35. Buey, R. M., Fernández-Justel, D., González-Holgado, G., Martínez-Júlvez, M., González-López, A., Velázquez-Campoy, A., ... & Balsera, M. (2021). Unexpected diversity of ferredoxin-dependent thioredoxin reductases in cyanobacteria. Plant Physiology.

09.- Nitrogen Metabolism and Biochemistry of Plants and Microorganisms

0113-P

Functional connections between nodes of the PipX synteny network

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PipX, a protein identified by its ability to bind to cyanobacterial nitrogen regulators PII and NtcA, provides a mechanistic link between signaling the nitrogen/carbon and energy status by the widely distributed protein PII and the control of gene expression by NtcA, a global transcriptional regulator involved in nitrogen assimilation in cyanobacteria. PipX uses the same surface of its N-terminal Tudor domain, to bind to 2-OG-free PII or, alternatively, to 2-OG-bound NtcA in order to increase its transcriptional activity. ADP favors PII-PipX complex formation and these complexes interact with PlmA, a yet poorly characterized transcriptional regulator also restricted to cyanobacteria. Genetic analyses in *S. elongatus* emphasized the importance of PII to counteract PipX activity, particularly deleterious under certain environmental conditions, and further revealed functional connections between PipX and the co-expressed factor PipY, an intriguing and conserved pyridoxal-phosphate binding protein (PLPBP) involved in vitamin B6 and amino acid homeostasis and whose loss-of-function mutations cause B6-dependent epilepsy in humans.

To get additional insights into the multiple functions of PipX we are now focussing on the Cyanobacterial Synteny Network, particularly on the inferred functional connections between PipX, EngA and EcfTC proteins. Since *engA* and *ecfTC* are two out of the 5 high-confidence nodes (with default parameters) that are not linked to *pipX* in *S. elongatus* and both show conserved and unique features in the cyanobacteria-chloroplast lineage, they are being used as a proof of concept for the Cyanobacterial Synteny Network. We will present and discuss data from different experimental approaches supporting their functional connections as well as highlighting the complexity of the PipX signalling network.

0228-P

Factors affecting biofilm formation in the filamentous cyanobacterium Anabaena sp. PCC7120

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Cyanobacterial biotechnology is considered promising in several areas, with different potential applications being studied, with examples in the case of cyanobacterial biofilms such as their use as chelating agents for biosorption and removal of metal-contaminated waters, hydrogen production or aquaculture.

The structural integrity of biofilms and protection against environmental stresses relies on its extracellular matrix, with a particularly relevant role of its expolysaccharide (EPS) composition. Cyanobacterial EPSs have been shown to act as nutrient sink, as well as sequester metal cations due to their highly anionic nature.

While biofilm formation in heterotrophic biofilms has been thoroughly studied and described, there is still much left to understand about cyanobacterial biofilms, including the factors that affect their development and their formation process, including EPSs assembly and export pathways.

We have observed in our laboratory that biofilm formation and EPS release in the filamentous, nitrogen-fixing cyanobacteria *Anabaena* sp PCC7120 are affected by different stresses, such as salt stress or nitrogen deficiency.

Our results also suggest that the transcriptional regulators FurA, FurB and FurC, which control several aspects of *Anabaena* sp PCC7120 physiology, are involved in these processes. By combining genome-mining bioinformatic approaches to identify key proteins in biofilm formation in *Anabaena* with comparative transcriptomic analysis of the wild-type strain with variants affected in the synthesis of the FUR transcriptional regulators, along with electrophoretic mobility shift assays (EMSA), we have defined novel players in the formation of phototrophic biofilms and gained new insights into their regulation.

Costerton, J.W., Stewart, P.S., Greenberg, E.P. 1999. Science, 284:1318-1322. Mitra, A. and Mukhopadhyay, S. 2016. AIMS Bioengineering, 3:44-59. Micheletti E., Pereira S., Mannelli F., Moradas-Ferreira P., Tamagnini P., de Philippis R. 2008. Appl. Environ. Microbiol., 74:2797–2804. De Philippis R., Colica G., Micheletti E. Appl. Microbiol. Biotechnol., 2011. 92:697–708. Fillat, M.F. 2014. Arch Biochem Biophys. 546:41-52.

0428-P

Domains and residues involved in the K+ transport activity of the Arabidopsis HAK5 transporter

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Potassium (K+) is an essential nutrient for plants that needs to be taken up by roots from the soil solution [1]. Roots are equipped with specialized transport systems in cell membranes that allow K+ acquisition under a wide range of environmental conditions [1]. In Arabidopsis, the AtHAK5 transporter mediates root K+ uptake at low external concentrations, ensuring plant growth when this nutrient is limiting [1]. This transporter is activated by an AtCIPK23/AtCBL1-9 complex by phosphorylation [2]. AtHAK5 belongs to the HAK/KUP/KT family of K+ transporters present, besides plants, in bacteria, viruses, and fungi [3]. However, functional domains and residues involved in K+ transport have not yet been identified in plant HAK/KUP/KT transporters. We conducted a structure-function study of the AtHAK5 transporter using yeast as an expression system [4]. The role of selected sites in AtHAK5 function was studied by characterizing high-affinity K+ uptake in yeast expressing mutated versions of AtHAK5. Based on the recent crystal structure of the homologous bacterial transporter KimA [5], we performed a homology modeling of the AtHAK5 transmembrane domains to identify putative K+ binding residues. Mutation of these residues into alanine abolished AtHAK5-mediated K+(Rb+) transport. Moreover, Ser35, positioned in the N-terminal cytoplasmic domain, was critical for phosphorylation-dependent AtCIPK23/AtCBL1-9 activation. In addition, the role of the C-terminus of AtHAK5 on its regulation was also studied. Deletions in the AtHAK5 C-terminal cytoplasmic tail showed that an autoinhibitory domain and a AtCIPK23-activation domain were present in the C-terminal tail. Our results indicated that phosphorylation of Ser35 prevents autoinhibition by the C-terminal tail and serves as a molecular model for K+-mediated transport by HAK/KUP/KT transporters in plants.

1. Nieves-Cordones, M., et al. J. Plant Physiol. 171, 688–695 (2014).doi: 10.1016/j.jplph.2013.09.021 2. Ragel, P. et al. Plant Physiol. 169, 2863–2873 (2015). doi: 10.1104/pp.15.01401 3. Nieves-Cordones, M. et al. Front. Plant Sci. 7, 1–7 (2016). doi: 10.3389/fpls.2016.00127 4. Ródenas, R. et al. Plant Physiol. 1860–1874 (2021) doi:10.1093/plphys/kiab028. 5. Tascón, I. et al. Nat. Commun. 11, 1–10 (2020). doi: 10.1038/s41467-020-14441-7

10.- Cell Death and Inflammation

0517-OI

Linking Extracellular Factors to Epigenetic Control in Inflammation

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Monocytes are extremely plastic as they terminally differentiate into a wide diversity of functional types, including different macrophages subtypes and dendritic cells, in the blood or tissues, in response to a variety of growth factors, cytokines and pathogenic molecules. Monocytes, like other myeloid cell types, express high levels of TET2 and DNMT3A, two key enzymes for the occurrence of DNA methylation changes in the absence of DNA replication. Our studies have shown, the occurrence of specific DNA methylation changes that take place in response to a number of inflammatory cytokines and other compounds that involve various signaling pathways and downstream factors, including additional epigenetic enzymes. In this presentation, I will present some mechanistic insights on the interplay between different transcription factors, epigenetic enzymes and phenotype of monocyte-derived effector cells. These studies are relevant to both understand the role of these factors to confer immunogenic or tolerogenic properties to these cells as well as to modulate them as potential targets for pharmacological uses.

0518-OI

INTERPLAY BETWEEN BAX AND MITOCHONDRIAL DYNAMICS DURING APOPTOSIS

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The proteins of the BCL-2 family are key regulators of apoptosis. The pro-apoptotic protein BAX plays a key role in mediating the key event of mitochondrial permeabilization, which defines the point of no-return in the cell's commitment to death. In previous work, we have investigated the mechanism how BAX forms pores at mitochondria during apoptosis. Now, we focus on the regulation of BAX by the dynamin like protein DRP1, involved in mitochondrial fission, which co-localize at mitochondria during apoptosis to mediate mitochondrial permeabilization and fragmentation. We show that BAX and DRP1 can physically interact and that their association is enhanced during apoptosis.

We found that complex formation between BAX and DRP1 takes place exclusively in the membrane environment and involves several surfaces in BAX, of which the N-terminal region is required. Furthermore, the interaction of BAX with DRP1 reported here is functionally relevant, as it modulates the activity of both proteins. Remarkably, when forced to dimerize, both BAX and DRP1 become activated and translocate to mitochondria, where they induce mitochondrial remodeling and permeabilization, resulting in apoptosis even in the absence of apoptotic triggers. Based on this, we propose that DRP1 can promote apoptosis by acting as non-canonical direct activator of BAX through physical contacts with its N-terminal region.

0099-R/M-OS

Impact of p53 on the microglial response in an in vivo model of amyloid-beta-induced neurodegeneration.

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Alzheimer's disease (AD) is a neurodegenerative disorder characterised by the presence of amyloid-β (Aβ) and Tau protein aggregates. Although research has classically focused on neuronal alterations, neuroinflammation has emerged as a key factor in AD pathophysiology. The main cellular components involved in the brain inflammatory process are glial cells. Aβ aggregates bind to pattern recognition receptors on microglia and astrocytes, which triggers an immune response that contributes to disease progression[1,2]. Functionally, microglia can be classified into M1 proinflammatory/neurotoxic or M2 anti-inflammatory/neuroprotective phenotypes[2]. Previously, we described a key role for p53 in Aβ-induced neurodegeneration *in vivo*[3]. Recently, p53 has emerged as a modulator of the immune response in microglia4. Here, we evaluated the possible role of p53 as a modulator of the microglial response to Aβ25-35, leading to neurodegeneration.

Oligomerized $A\beta_{25-35}$ (9 nmol) was intracerebroventricularly injected into wild-type (WT) and p53 knockout (p53KO) mice. Some animals were treated intraperitoneally with the p53 transcriptional activity inhibitor, pifithrin-alpha (PFT-α; 2 mg/kg). Neuroinflammation and neurodegeneration were assessed up to 5 days after injection by Western blot and immunofluorescence. Mice cognitive status was assessed 5 days post-injection using the AnyMaze™ system.

We found that $A\beta_{25-35}$ oligomers caused p53 accumulation, leading to rapid microglial activation. We found a M1 profile (high iNOS / low Arg1) that evolved to an M2 profile (low iNOS / high Arg1) in a p53-dependent manner, at 3 and 5 days after injection, respectively. Moreover, $A\beta_{25-35}$ injection also induced reactive astrogliosis. Together, these events led to neurodegeneration and memory impairment, at 5 days after injection, which were prevented by genetic and pharmacological inhibition of p53.

Our results demonstrate a key role of p53 in the Aβ-induced inflammatory response, which may contribute to neurodegeneration and cognitive impairment in AD.

Funds: Instituto de Salud Carlos III (PI18/00265; RD16/0019/0018); FEDER; European Union's Horizon 2020 Research and Innovation Programme (GA 686009); Junta de Castilla y León (PhD grant co-financed by Fondo Social Europeo. EDU/601/2020).

1. Scheltens P, et al. Alzheimer's disease. Lancet. 2021; S0140-6736(20)32205-4 2. Heneka MT, et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 2015;14(4):388-405 3. Lapresa R, et al. Amyloid-beta promotes neurotoxicity by Cdk5-induced p53 stabilization. Neuropharmacology. 2019;146:19-27 4. Jayadev S, et al. Transcription factor p53 influences microglial activation phenotype. Glia. 2011;59(10):1402-13

0422-OS

USE OF NANOPARTICLES IN THE TREATMENT OF RETINITIS PIGMENTOSA

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CIPF-IIS La Fe Pathophysiology and Therapies for Vision Disorders

Retinitis pigmentosa (RP) is a group of inherited retinal dystrophies characterized by the progressive and irreversible loss of vision due to photoreceptor cell death. More than 100 genes involved in its pathogenesis are currently described. Photoreceptor death begins with degeneration of the rods and eventually cones degenerate. (1-2)

It is likely that the death of cones, responsible for central vision, occurs as a consequence of progressive oxidative damage, inflammation, metabolic imbalance, etc. Several studies suggest an important inflammatory component in the progression of RP. Activation of microglia, a common event present in retinal degenerations, or increased inflammatory mediators such as TNFα have been described in patients and murine models of RP. In models of RP, microglial activation precedes or coincides with the peak of photoreceptor death and with elevated levels of TNFα. (3-5). We previously found that intraperitoneal or intravitreal administration of Adalimumab (ADA), a monoclonal anti-TNFα antibody, slowed down retinal degeneration in the murine model of RP, the rd10 mice. (6,7)

Drug Delivery Systems (DDS) are having a huge impact on medical technology by improving the mode of drug delivery. DDS protect drugs from degradation, improve penetration across biological barriers, increase circulating half-life and stability, and allow sustained and controlled drug release. In recent years, DDS have emerged as an alternative to conventional dosage forms and have proven to be promising candidates for drug encapsulation and application in ocular diseases (8).

The aim of this study were to achieve a most effective, specific and economic therapy for RP. We performed the following objectives: i) to characterize different DDS formulations loaded with ADA; ii) to evaluate the cell internalization and citotoxicity of these DDS formulations in retinal cells; iIi) to assess the efficiency of these DDS formulations to ameliorate retinal degeneration in rd10 mice.

The results suggest that the DDS formulations loaded with ADA could be useful to treat RP in preclinical models.

Ayuso C, et al.Genome Med 2010, 2:34 Yoshida N et al.Ophthalmology 2013, 120(1):e5-e12 Sasahara M, et al.Am J Pathol 2008, 172:1693-1703 Ebert S, et al.J Neurochem 2009, 110:1863-1875 Sheets KG, et al.Mol Vis 2013, 19:1747-1759 Jiang S. et al.Int J Ophthalmol. 2018;11(6):1038-1044 Martínez-Fernández de la Cámara C et al.Sci Rep.2015;5:11764 Olivares-González L. et al.The FASEB Journal.2020;00:1–23 Puglia C et al.Nanomaterials. 2020 10(2).pii:E287 Platania CBM et al.Drug Deliv.201

0487-R-OS

TRAIL receptor signaling mediates tonic secretion of the angiogenic chemokine IL-8 by non-small cell lung carcinoma.

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Interleukin 8 (IL-8) is a proangiogenic and inflammatory chemokine. IL-8 reflects tumor burden and it corelates with poor survival of non-small cell lung cancer (NSCLC) patients treated with immunotherapy. In our study we investigated the signaling pathways regulating the tonic IL-8 secretion in NSCLC. IL-8 was secreted by multiple cell lines regardless of their mutational pattern. A panel of inhibitors led to identify the NFkB and MAPK pathways to be implicated in the secretion of IL-8. Since chemokine secretion, NFkB and MAPK pathways are known to be regulated by TRAIL receptors, we hypothesized the possible involvement of TRAIL-R1 (DR4) and TRAIL-R2 (DR5). We showed that the silencing of DR4 and DR5 reduces IL-8 production and secretion in several lung cancer cell lines under basal conditions and nutritional stress. TRAIL, however, was not involved in this phenomenon. We then studied the possible involvement of the TRAIL receptor cytoplasmic complex (FADDosome/DISC) and demonstrated that TRADD and RIPK1 mediate IL-8 secretion. To sum up, our results show that lung cancer cells maintain tonic secretion of the chemokine IL-8 via TRAIL receptors 4 and 5 that in turn activate the NFkB and MAPK pathways.

0027-P

Personalized Combination Therapies for Advanced Prostate Cancer with PARP Inhibitors

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Prostate cancer (PCa) is the second cause of cancer worldwide in males [1]. The high impact of prostate cancer mainly derives from its highly heterogeneous nature, which impedes the design of effective therapeutic approaches for patients [2]. However, around 20% of patients with advanced PCa harbor DNA repair pathway aberrations, with tumor cells relying on poly (ADP-ribose) polymerase (PARP) to fix any DNA damage [3]. Treatment with combination drug therapies and PARP inhibitors (PARPi) have therefore emerged as promising therapeutic approaches in these patients, with good outcomes observed in clinical trials [4, 5, 6].

Our aim was to discover synergistic combinations between PARPi and other conventional PCa therapeutics to treat advanced PCa in a personalized manner. We first evaluated levels of DNA repair proteins (BRCA1/2, ATM, FANCD2, and Rad 51) in various PCa cell lines (androgen-sensitive VCaP and LNCaP and androgen-insensitive PC3 cell lines) under basal conditions and then studied PARPi treatment in combination with two common PCa treatment approaches, an androgen biosynthesis inhibitor or a chemotherapeutic agent, via cell viabillity (MTS) assays.

We discovered that LNCaP cells expressed significantly lower levels of BRCA1/2 and ATM in comparison with VCaP and PC3, and PARPi treatment prompted a significant reduction in cell viability in LNCaP only. However, we failed to observe synergism between PARPi and other PCa treatments in LNCaP cells, given the significant effect of PARPi treatment. We did observe a synergistic effect in VCaP and PC3 cells, although the VCaP cells showed synergistic effects at different range of concentrations (but same drug ratio) when compared to PC3 cells. We hypothesize that the different outcomes derive from differing BRCA1/2 (lower in VCaP) and FANCD2/Rad51 (lower in PC3) protein levels. We are currently performing further studies to decipher this possible different mechanism of action in VCaP and PC3 cells.

In this communication, we corroborate that DNA repair pathway aberrations represent biomarkers for PARPi treatment in PCa, a fining that may allow patient stratification. Furthermore, we establish combination therapies involving PARPi as a promising approach for PCa treatment, regardless of the DNA repair protein status and androgen sensitivity.

[1] https://gco.iarc.fr/today/home [2] Frame FM et al. (2017). J Cancer Metastasis Treat 2017;3:302-14. [3] Robinson et al., (2015). Cell, 161(5),

pp.1215-1228. [4] Mateo et al. (2015). New England Journal of Medicine, 373(18), pp.1697-1708. [5] Pichler, Horninger and Heidegger (2018). Magazine of European Medical Oncology, 11(4), pp.284-290. [6] Asim et al. (2017). Nature Communications, 8(1).

0040-R-P

cFLIP down-regulation is an early event required for endoplasmic reticulum stress-induced apoptosis in tumor cells

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Protein misfolding or unfolding and the resulting endoplasmic reticulum (ER) stress frequently occur in highly proliferative tumor (Chen and Cubillos-Ruiz, 2021) . How tumor cells escape cell death by apoptosis after chronic ER stress remains poorly understood. We have investigated in both two-dimensional (2D) cultures and multicellular tumor spheroids (MCTSs) the role of caspase-8 inhibitor cFLIP (Smyth et al., 2020) as regulator of the balance between apoptosis and survival in colon cancer cells undergoing ER stress. We report that down-regulation of cFLIP proteins levels is an early event upon treatment of 2D cultures of colon cancer cells with ER stress inducers, preceding TNF-Related Apoptosis-Inducing Ligand Receptor 2 (TRAIL-R2) up-regulation, caspase-8 activation and apoptosis (Stöhr et al., 2020) . Maintaining high cFLIP levels during ER stress by ectopic expression of cFLIP markedly inhibits ER stress-induced caspase-8 activation and apoptosis. Conversely, cFLIP knockdown by RNA interference significantly accelerates caspase-8 activation and apoptosis upon ER stress. Despite activation of the proapoptotic PERK branch of the unfolded protein response (UPR) and up-regulation of TRAIL-R2, MCTSs are markedly more resistant to ER stress than 2D cultures of tumor cells. Interestingly, resistance of MCTSs correlates with sustained cFLIP expression and reduced activation of caspase-8 upon ER stress. Overall, our results suggest that controlling cFLIP levels in tumors may represent an adaptive strategy to prevent tumor cell demise in the unfavorable conditions of the tumor microenvironment.

Chen, X., and Cubillos-Ruiz, J.R. (2021). Endoplasmic reticulum stress signals in the tumour and its microenvironment. Nat. Rev. Cancer 21, 71–88. Smyth, P., Sessler, T., Scott, C.J., and Longley, D.B. (2020). FLIP(L): the pseudo-caspase. FEBS J. 44. Stöhr, D., Jeltsch, A., and Rehm, M. (2020). TRAIL receptor signaling: From the basics of canonical signal transduction toward its entanglement with ER stress and the unfolded protein response. Int. Rev. Cell Mol. Biol. 351.

0070-R-P

Better together: Assessing the Potential of Immunogenic Signals Triggered by TMZ, CX-4945, and Combined Treatment in GL261 Glioblastoma Cells

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Background: The cancer immune cycle has a relevant role in therapy response [1]. Accordingly, it implies that successful treatment may trigger the exposure or the release of immunogenic signals. Previous in vivo results with mice bearing GL261 glioblastoma (GB) showed that combination treatment of chemotherapy (temozolomide, (TMZ)) plus a protein kinase CK2 inhibitor (CX-4945) outperformed single treatments, provided an immune-friendly schedule was followed [2]. Our purpose was to study possible immunogenic signals released in vitro by GL261 GB cells to better understand the in vivo obtained results. 1

Methods: GL261 GB cultured cells were treated with TMZ and CX-4945 at different concentrations (25 μM – 4 mM) and time frames (12 – 72 h). Cell viability was measured with Trypan Blue and propidium iodide. Calreticulin exposure was assessed with immunofluorescence, and ATP release was measured through bioluminescence approaches.

Results: TMZ showed a cytostatic rather than cytotoxic effect, while CX-4945 presented remarkable cytotoxic effect already at low concentrations. Calreticulin exposure after 24h was detected with TMZ treatment, as well as TMZ/CX-4945 low concentration combined treatment, while high concentrations of CX-4945 produced negligible calreticulin exposure. ATP release was significantly higher with CX-4945 treatment, as well as with TMZ/CX-4945. The maximum difference between treated and untreated cells for ATP release was observed 12h after treatment.

Conclusions: The combined treatment described in this experimental setting may produce the simultaneous release of two potent immunogenic signals, which can explain the outperformance over single treatments in vivo. This highlights the relevance of a therapeutic schedule which spares the host immune system. A word of caution may be raised since in vitro conditions are not able to fully mimic pharmacokinetics observed in vivo, hence, appearance of immunogenic signals may follow a different timing.

0127-P

Testosterone administration to transgender men increase inflammation and leukocyte-endothelium interactions

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Transgender men are people who were assigned a female gender at birth but indentify as male. Gender-affirming hormone treatment is needed to reduce gender dysphoria and psychological disorders as stress or depression. However, testosterone administration can lead to metabolic alterations and obesity (1,2). Our aim was to determine if testosterone treatment affects the levels of vascular inflammation by evaluating plasmatic levels of proinflamatory cytokines and leukocyte interactions with the endothelium.

We analysed 157 transgender men (TGM) receiving 12 week testosterone undecanoate treatment (1000 mg). Anthropometrical parameters were measured, followed by blood collection for biochemical determinations and extraction of leukocytes using a Ficoll density gradient. A leukocyte aliquot was perfused over a HUVEC monolayer at physiological flux speed and the interactions were evaluated during 5 minutes. Serum was also isolated from an aliquot of peripheral blood by centrifugation, and adhesion molecules and proinflammatory cytokines were evaluated with Luminex 200 flow analyzer. Comparisons were made between 0 and 12 weeks of treatment.

We observed that the anthropometrical variables had no significant differences between 0 and 12 weeks. However, total testosterone, free androgenic index, androstendione, SHBG and AIP levels were significantly increased at 12 weeks comparing with basal levels. The adhesion assay displayed increased leukocyte-endothelium interactions in the 12-week treatment group. In addition, soluble adhesion molecules VCAM-1 and E-selectin were enhanced, but ICAM-1 showed no differences. Last, proinflammatory molecules TNFα and interleukin-6 increased after 12 weeks of testosterone treatment.

These results highlight that 12-week testosterone treatment alters the biochemical profile and has a proinflammatory effect in the organism. Caution should be taken in future testosterone treatments and a proper follow-up of the patients would be reccomended.

Acknowledgement: PI19/0838, PI19/0437, FI17/00126, GRISOLIAP/2016/015, GRISOLIAP/2019/091, ACIF/2020/371, FI17/00144, UGP-15-220, PROMETEO/2019/027, CD18/00069, CES/10/030, CPII16/00037 and the European Regional Development Fund (ERDF "A way to build Europe").

1. Irwig MS. Testosterone therapy for transgender men. Lancet Diabetes Endocrinol 2017;5:301–11. 2. Stanhewicz AE, Wenner MM, Stachenfeld NS. Sex differences in endothelial function important to vascular health and overall cardiovascular disease risk across the lifespan. Am J Physiol Heart Circ Physiol 2018;315:H1569–88.

0143-R-P

Rational combination of cyclin-dependent kinase inhibitor Dinaciclib and BH3-mimetics as a promising therapy against multiple myeloma

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Over the last decades, advances in the knowledge of multiple myeloma (MM) biology have allowed the arising of promising therapies against this hematological malignancy, among which proteasome inhibitors and anti-CD38 monoclonal antibodies clearly stand out. However, it is still an incurable disease and new pharmacological strategies need to be tested. In this regard, cyclin-dependent kinases (CDKs), whose deregulation is a hallmark of MM, have been proposed as optimal investigational targets. Apart from cell cycle regulation, CDKs play a main role in a large number of cellular processes including transcriptional regulation and mRNA maturation. Dinaciclib is a novel multi-CDKs inhibitor (CDK1, 2, 5 and 9) with anti-myeloma activity confirmed in phase II clinical trials. In this work, we have deeper explored the mechanism underlying Dinaciclib-induced apoptosis on MM cells as well as how it affected to cell cycle transition. We have observed that Dinaciclib-induced cell death really depends on intrinsic apoptotic pathway activation. Moreover, Mcl-1 down-regulation, and subsequent Bcl-2 pro-apoptotic proteins release, seemed to be a key step in Dinaciclib's mechanism of action. Our results also indicated that Mcl-1 dependency, in terms of survival, predict MM cell lines' and MM patients' response to Dinaciclib alone and Dinaciclib-based combinations with BH3-mimetics (ABT-199, S63845 and A-1155463). Those poorly dependent on Mcl-1, generally collected as Dinaciclib's resistant, responded to Dinaciclib+BH3-mimetics combinations due to simultaneous anti-apoptotic protein depletion. In addition, Dinaciclib+ABT-199 (Bcl-2 inhibitor) and Dinaciclib+A-1155463 (Bcl-X_L inhibitor) combinations led to most promising results. However, S63845 (Mcl-1 inhibitor) slightly enhanced cell death exerted by Dinaciclib as a consequence of drugs' common target.

1. S. K. Kumar et al., Nat Rev Dis Primers 3, (2017). 2. S. K. Kumar et al., Blood 125, 443-448 (2015). 3. L. Vela, I. Marzo, Curr Opin Pharmacol 23, 74-81 (2015). 4. S. Rello-Varona et al., Sci Rep 9, 3816 (2019). 5. E. A. Punnoose et al., Molecular cancer therapeutics 15, 1132-1144 (2016). 6. S. Wuilleme-Toumi et al., Leukemia 19, 1248-1252 (2005).

0146-R-P

TARGETING AURORA KINASE A AND BCL-2 FAMILY PROTEINS IN MULTIPLE MYELOMA

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Large efforts have been devoted to face up to multiple myeloma in recent years. Novel therapies include proteasome inhibitors, immunomodulatory drugs or monoclonal antibodies among others. However, despite these new agents have increased the overall survival of the patients, most of them develop resistance to the treatment. This has fostered the emergence of new approaches, including targeting different cell cycle and mitosis regulators, such as the Aurora family of proteins. In that sense, alisertib (MLN8237) is the first-in-class orally bioavailable Aurora kinase A selective inhibitor to reach clinical trials for multiple myeloma treatment. Our results demonstrate that alisertib prompts aberrant nuclear morphologies, cell cycle arrest at the G2/M phase and drives 'mitotic catastrophe' in a multiple myeloma cell line panel, which culminates in cell death or entry into senescence. Its cytotoxic activity is dose- and time-dependent, showing two diferent patterns according to the doses: a peak of toxicity at low concentrations in H929, MM.1S and U266 cells and a 'plateau effect' in RPMI 8226 and OPM-2 cells. Caspase involvement in cell death mechanism depends on the cell line as well. Additionally, we have observed that alisertib at low and high doses and certain BH3-mimetics induce autophagy in multiple myeloma cells. Finally, we have detected a heterogeneous response against both family of compounds in myeloma sublines that overexpress anti-apoptotic proteins or lack pro-apoptotic members of the Bcl-2 protein family, suggesting that a complex protein network is implicated in the cell death mechanism triggered by these drugs.

T. Harding, L. Baughn, S. Kumar, B. Van Ness, Leukemia. 33, 863–883 (2019). V. Pinto et al., Cancers (Basel). 12, 1–32 (2020). Y. Shi et al., Blood. 109, 3915–3921 (2007). G. Görgün et al., Blood. 115, 5202–5213 (2010). M. G. Manfredi et al., Clin. Cancer Res. 17, 7614–7624 (2011). Y. Tayyar, L. Jubair, S. Fallaha, N. A. J. McMillan, Crit. Rev. Oncol. Hematol. 119, 59–65 (2017).

0204-P

Obatoclax synergizes with cisplatin in Triple Negative Breast Cancer

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Background: Triple negative breast cancer (TNBC) is the most aggressive breast cancer subtype, with the worst prognosis. One of the main reasons for this is the lack of specific treatments, being chemotherapy, such as cisplatin, the standard-of-care for these tumors. For this reason, many research groups are focused on the identification of new targets or alternative therapies to improve current treatments. In this line, BCL-2 family proteins, which are involved in apoptosis processes, has been proposed as druggable target in some solid cancers. The main objective of this work has been to evaluate BCL-2 family targeting drugs alone or in combination with standard chemotherapy, which might be useful to improve the therapeutic index and diminish the adverse effects of current treatments.

Methods: MDA-MB-231 and BT549 TNBC-derived cell lines were used. MTTs, colony-forming assay, scratch area assay, and three-dimensional cultures in Matrigel were used to explore the anti-proliferative effect. Furthermore, flow cytometry and QVD pan-caspase inhibitor were used for evaluated Obatoclax apoptosis induction caspase dependency. Synergistic effects with cisplatin were evaluated by MTT and analysed with Calcusyn.

Results: Screening by MTT assay of a battery of apoptotic inhibitors uncovered Obatoclax as the most efficient BCL-2 family inhibitor in MDA-MB-231 and BT549 cells. Obatoclax also decreased invasion, migration and clonogenic abilities in these cells. In addition, a high level of caspase-dependent apoptosis was observed in both cells lines in response to this drug, as shown upon annexin V assays in the presence of the pan-caspase inhibitor QVD. Finally, combination of Obatoclax with cisplatin showed a potent synergistic effect in TNBC cell lines.

Conclusions: The use of the apoptotic inhibitor Obatoclax can be of interest in the treatment of TNBC. Moreover, a combined therapy of this inhibitor with the chemotherapy cisplatin could be a good therapeutic alternative to improve its efficacy and reduce toxic effect of this drug in TNBC patients.

0219-P

Controlling epigenetics players to overcome resistance to chemotherapy

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Introduction: Triple negative breast cancer (TNBC) is the one with the worst prognosis and the most heterogeneous among all breast cancer subtypes. TNBC lacks specific therapeutic targets, what difficult their clinical management and restrict their treatment to radio- and chemotherapy. However, patients tend to develop resistances to these therapeutic approaches. Recent findings have shown that inhibition of epigenetic readers, such as BET proteins, decreases the expression of stem cell markers in TNBC-derived cells. Moreover, these markers have been shown to be more expressed in patients who do not respond to treatment. In this work we analyzed the efficacy of these compounds in chemotherapy-resistant cell models.

Materials and methods: TNBC cells models with acquired resistance to chemotherapeutic agents were generated by pulsed-exposure. qPCR studies were performed to assess the expression of a panel of stem cells markers in these models in comparison to sensitive cell lines and to evaluate the effect of the BET inhibitor (BETi) JQ1. MTT proliferation and tumor progression assays were performed on resistant cells in response to JQ1. Apoptosis in response to this inhibitor was also evaluated through Annexin V binding analysis by flow cytometry.

Results: TNBC cells with acquired resistance to chemotherapy are enriched in stem cells markers. Despite their overexpression, JQ1 efficiently decreased the expression of these markers in the developed resistant models. Moreover, proliferation and colony formation capacities were reduced in chemo-resistant cells using BETi. In the evaluation of JQ1 exposure by flow cytometry, we found that this BETi was able to induce apoptotic events in the resistant model.

Conclusions: BETi efficiently target stem markers in chemotherapy resistant TNBC cells, what might constitute a good strategy to control resistance in TNBC.

0281-P

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 cellular components. 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 was 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 activation of the Unfolded Protein Response (UPR) pathways. Specifically, ERK5i induced expression of the ER luminal chaperone BiP (a hallmark of ER stress) and the UPR markers CHOP, ATF4 and the spliced form of *XBP1*. Pharmacological inhibition of UPR with chemical chaperone TUDC, or ATF4 silencing, impaired of 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.

Bialik et al., 2018. 131:jcs215152 Gomez et al. 2016. Front Cell Dev Biol 5:80. doi: 10.3389/fcell.2017.00080.2016 Klionsky et al. 2021. Autophagy 17:1-382 Miller et al. 2020. Cell Death Discov 6:107 Muñoz-Guardiola et al., 2020. Autophagy. doi: 10.1080/15548627.2020.1761651 Pereira & Rodrigues 2020. Trends Mol Med 26:394-407 Rashid et al. 2015. Autophagy 11:1956-1977 Salazar et al. 2009. J Clin Invest 1195:1359-72 Wang et al. 2018. ACS Chem Biol 13:2438-48 Yang et al. 2010. Cancer Cel

0328-R-P

Study of the antitumor potential of stauprimide in breast cancer

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Stauprimide, a semi-synthetic derivative of staurosporine, was characterized in 2009 as a potent differentiation-enhancing compound in embryonic stem cells [1]. Although it was first thought that this compound could maintain the properties of staurosporine as a non-selective inhibitor of protein kinases (especially potent in inhibiting tyrosine kinases), it was found that its potential as an inhibitor of these proteins was not particularly remarkable, ruling out this as its main mechanism of action for the differentiation-enhancing effect. However, a clear effect of stauprimide on embryonic stem cells was identified as an inhibitor of CMYC expression, a key factor in the maintenance of stem cell pluripotency [1]. Given the involvement of CMYC in cancer development, and the effect of stauprimide inhibiting its expression, this compound was proposed as a possible antitumor drug in the treatment of renal cancer [2].

In this work we have studied the in vitro antitumor effect of stauprimide in the context of breast cancer, exploring also the possible mechanisms of action by which stauprimide exerts its effects. The detected activity of this compound on the human adenocarcinoma model used in our studies suggests its potential usefulness in antitumor pharmacological strategies.

This work is supported by grants PID2019-105010RB-100 (Ministerio de Ciencia, Gobierno de España), UMA18-FEDERJA-220 (Junta de Andalucía and FEDER), Ayudas a Proyectos de Investigación en Salud del Plan Propio de IBIMA 2020 and BIO-267 (Junta de Andalucía).

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[1] Zhu et al. (2009). Cell Stem cell, 4:416. DOI:10.1016/j.stem.2009.04.001. [2] Bouvard et al. (2017). PNAS, 114(13), 3497- 3502. DOI:10.1073/pnas.1702663114.

0330-R-P

Intravenous immunoglobulin treatment modulate monocytes in patients with haematological malignancy

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INTRODUCTION: Secondary immunodeficiency (SID) is a common complication of haematological malignancies (HM) and their chemotherapeutic protocols. The immunological deregulation could be expressed as a specific antibody deficiency manifested by recurrent infections mainly affecting respiratory system and sepsis. Intravenous gammaglobulins (IVIg) is widely established as a prophylactic treatment in SID. Nevertheless, IVIg hold other important features as immunomodulation and homeostatic effects which are not well defined in SID patients.

AIM: This is a proof of concept study to determinate the myeloid-derived suppressor cells (MDSCs) profile in peripheral blood in SID patients receiving IVIg for the first time.

METHODS: We evaluated the myeloid profile in 6 SID patients with HM (four with non-Hodgkin lymphoma and two with chronic lymphocytic leukemia) associated to recurrent infections, before and after the first infusion of IVIg. Freshly collected blood samples were analysed by multiparametric flow-cytometry. The populations were characterized based on the surface expression of MDSCs and monocyte subsets.

RESULTS: In our cohort, we observed a significant expansion of MDSCs population (HLA-DR^{low}CD14^{+}) after IVIg infusion in comparison with baseline levels (37.42% to 59.70% p=0.0192). Regarding monocytes profile, a significant decrease in non-classical (4.18 % to 0.45% p=0.0210) and intermediate subset (11.00% to 5.73% p=0.0452), with a significant increase in classical monocytes (82.15% to 90.2% p=0.0064) was observed after the infusion.

CONCLUSIONS: Our preliminary results suggest that increased blood MDSCs could derivate from the egression of monocytes and macrophages precursors from the bone marrow due to IVIg treatment. The ability of gammaglobulin to modulate the macrophages polarization deserves further characterization in HM setting.

Domínguez-Soto, Á., et al.IVIg promote cross-tolerance against inflammatory stimuli in vitro and in vivo. The Journal of Immunology. Domínguez-Soto, A. et al.,Intravenous immunoglobulin promotes antitumor responses by modulating macrophage polarization. The Journal of Immunology Gabrilovich, D. I., & Nagaraj, S. (2009). Myeloid-derived suppressor cells as regulators of the immune system. Nature reviews immunology, 9(3), 162-174.

0332-P

Optimization of Three-dimensional Prostate Cancer Models as useful Platforms for Drug Screening

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The heterogenous nature of prostate cancer (PCa) represents a significant stumbling block to the development of effective therapeutic approaches [1]. The study of potential PCa treatments primarily employs monolayer cultures of in vitro-adapted cell lines; however, this traditional two-dimensional (2D) scenario remains far from clinical reality.

Therefore, we aimed to develop three-dimensional (3D) PCa models, which display in vivo-like characteristics that include enhanced cell-cell interactions and oxygen gradients [2, 3], as an advanced means to screen potential therapeutic strategies.

We optimized conditions for homospheroid formation using the hanging drop method with the androgen-sensitive VCaP and androgen-insensitive PC3 prostate cancer cell lines. We then compared the effect of inhibitors of poly (ADP-ribose) polymerase and androgen biosynthesis and conventional therapeutic agents via cell viability assays in 2D and 3D culture. We also optimized the formation of heterospheroids comprising VCaP-GFP and hTERT-PF179T cancer-associated fibroblasts to understand the role of the tumor microenvironment in PCa treatment responses.

Optimized formation conditions generated larger PC3 homospheroids and more compact VCaP homospheroids. Interestingly, the same concentrations of our candidate therapeutics displayed lower activity in the 3D PCa models when compared to the 2D models, suggesting increased resistance. We are currently undertaking further studies to explore the root causes of this effect. Optimized formation conditions for heterospheroid formation employed a 1:1 ratio of cells and similar conditions to homospheroid formation.

We optimized homospheroid formation using the VCaP and PC3 prostate cancer cell lines by the hanging drop method and corroborated the utility of these models in drug testing by demonstrating significantly different outcomes compared to 2D models. We also optimized heterospheroid formation by the hanging drop method, combining VCaP-GFP cells and cancer-associated fibroblasts. This model will allow us to explore the fundamental role of the tumor microenvironment in treatment response and resistance to promote the development of efficient therapeutic approaches for prostate cancer.

[1] Frame F.M. et al. (2017). J Cancer Metastasis. Treat. 2017;3:302-14. [2] Costa, E.C. et al. (2016). Biotechnol. Adv. 2016, 34, 1427–1441. [3] Mehta, G. et al. (2012) J Control Rel. 2012, 164(2):192-204.

0368-R/M-P

The impact of diet-protein content in telomerase regulation

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Higher telomere length (TL) is associated with longevity, while telomeres shortening are related with some non-communicable diseases (NCDs) such as cancer or cardiovascular disease, and with higher risk of mortality. Telomerase RNA component (TERC) and peroxiredoxin-1 (PRDX1) are involved in the regulation of Telomerase, an enzyme capable to extent TL. Diet could play a role in telomere shortening by regulation of cellular oxidative stress and by modulate the expression of certain genes involved in Telomerase Activity (TA) as presented before. This paper study how low-protein diet (LPD) and leucine deprivation (LEU(-)) in tandem with fibroblast growth factor 21 (FGF21) can affect the relative gene expression of Terc and Prdx1 in mice. Murines with and without FGF21 were fed with LPD and LEU(-), with corresponding Control Diet (CD) group for each one. Relative liver mRNA levels of Terc and Prdx1 were determined by RT-qPCR. Results suggested that diet-protein content and FGF21 could impact on Terc and Prdx1 expression by modulating oxidative stress of the cell. LPD synergized with FGF21 to increase Prdx1 mRNA levels (p=0.00096), but inconsistency and the contradictions of the findings did not allow to suggest accurate conclusions and for that reason further investigations with better designs are needed.

0378-R-P

Microvascular endothelial cell autophagy regulates neutrophil trafficking

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A key feature of an inflammatory reaction is tissue infiltration of neutrophils, a response that requires breaching of endothelial cells (ECs) that line the vascular lumen (*1*) (*2*). In recent years numerous metabolic, catabolic and redox sensitive pathways have been implicated in the regulation of leukocyte trafficking. In particular, autophagy, an evolutionary conserved process that enables the delivery of

cytoplasmic content to the lysosome for degradation, has been linked to the development of numerous inflammatory conditions (*3*). While there is ample evidence of immune cell autophagy-related genes regulating inflammation, less is known about the role of EC autophagy in this context. Here, we explored the role of microvascular EC autophagy in neutrophil trafficking within multiple acute models of inflammation.

Canonical autophagy involves the formation of dedicated double–membrane vesicles commonly known as autophagosomes. These organelles can be identified by their association with the membrane-bound lipid modified form of microtubule-associated protein light chain 3 (LC3) through development of characteristic LC3-punctae. Using high-resolution confocal microscopy, we found that inflamed postcapillary venular ECs exhibited enhanced levels of LC3-puncta that localised exclusively to EC borders, an event aligned temporally with the peak of neutrophil trafficking. Furthermore, confocal intravital microscopy revealed significantly exaggerated and faster neutrophil transendothelial migration across autophagy deficient ECs, while pharmacological induction of autophagy inhibited neutrophil migration. Mechanistically, autophagy machinery regulated the remodeling of EC junctions and expression of key EC adhesion molecules, facilitating their intracellular trafficking and degradation. Since lack of EC autophagy led to excessive neutrophil infiltration in multiple inflammatory models, our results identify EC autophagy as an essential cellular process to limit physiological inflammation.

1. S. Nourshargh, R. Alon, Leukocyte migration into inflamed tissues. Immunity 41, 694-707 (2014). 2. N. Reglero-Real, B. Colom, J. V. Bodkin, S. Nourshargh, Endothelial Cell Junctional Adhesion Molecules: Role and Regulation of Expression in Inflammation. Arterioscler Thromb Vasc Biol 36, 2048-2057 (2016). 3. V. Deretic, B. Levine, Autophagy balances inflammation in innate immunity. Autophagy 14, 243-251 (2018).

0391-P

A carbohydrate-binding trimeric fragment of lung surfactant protein SP-A neutralizes cytotoxic and pro-inflammatory effects of cathelicidin on alveolar epithelial cells

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Human cathelicidin (LL-37) is a host defense peptide with direct antimicrobial activity against several pathogens. However, LL-37 also can trigger tissue injury through binding to host membranes, causing cytotoxic or proinflammatory effects. LL-37 is secreted by epithelial and immune cells of the skin, intestine, ocular system, and lung. LL-37 levels rise in airways of chronic obstructive pulmonary disease patients, contributing to chronic inflammation. Surfactant protein SP-A is secreted by the alveolar epithelium and has essential immune functions in the lung. It is a large oligomeric protein assembled in multiples of three subunits, which contain a collagen-like domain and globular recognition domains. The objective of this study was to investigate whether either human SP-A or a trimeric recombinant fragment of the protein, which lacks most of the collagen domain (rfhSP-A), is involved in local regulation of LL-37 activity. To address this question, we studied the interaction of LL-37 with SP-A and rfhSP-A by tryptophan fluorescence and the effects of these proteins on LL-37 antimicrobial and cytotoxic activities. We found that both SP-A and rfhSP-A bound to LL-37 with high affinity in physiological conditions (K_d = 0.45 ± 0.01 nM for SP-A and K_d = 1.22 ± 0.73 nM for rfhSP-A). Such interactions result in reduction of LL-37-induced cytotoxicity and inflammation in alveolar epithelial cells. However, LL-37 antimicrobial activity against respiratory pathogens (*Klebsiella pneumoniae* K2, *Pseudomonas aeruginosa* O1, and nontypeable *Haemophilus influenzae)* was not affected by either SP-A or rfhSP-A. These results demonstrate that SP-A plays a protective role in reducing LL-37's cytotoxic and inflammatory actions, which depends on SP-A's globular/neck domains. Our studies also suggest a potential therapeutic effect of rfhSP-A on chronic inflammatory lung diseases characterized by elevated LL-37.

0393-P

Inflammasome inhibition and breast cancer

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Inflammasomes are intracellular multiprotein platforms of the innate immune system that are activated in response to pathogens or intracellular damage. The formation of ASC specks, initiated by different inflammasome receptors, promotes the recruitment and activation of procaspase-1, thereby triggering pyroptotic inflammatory cell death and pro-inflammatory cytokine release.

Here, we describe a *pan* inflammasome inhibitor, MM01 that interferes with ASC speck formation and inhibits inflammation *in vivo*. This inhibitor could be useful for the potential treatment of multifactorial diseases involving the dysregulation of multiple inflammasomes and also as a tool to explore the role of inflammation in the progression of cancer.

Inflammation is a well-established hallmark of cancer. Tumor development and progression not only depends on genetic alterations of tumor cells, but also on the inflammatory tumor microenvironment. In breast cancer, two controversial situations have been observed: the elimination of the inflammasome components and the associated reduction in pro-inflammatory cytokine release decreases tumor size and metastasis and on the other hand, the lack of inflammatory response triggers a more aggressive phenotype of the tumor. Features that tip the balance towards one type or another of response are unknown. Our aim is to identify potential biomarkers capable to classify tumors based on their response to anti-inflammatory therapies using an in vitro assay and our pan inflammasome inhibitor as predictive tool.

0396-R/M-P

BH3 mimetics sensitize bladder cancer cells to cisplatin treatment

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Bladder cancer is the ninth most common cancer in the world. About 30 percent of cases appear as muscle invasive carcinomas, which carry an increased risk of metastatic disease. These carcinomas may require radical surgery and chemotherapy with gemcitabine and cisplatin, and relapsed or refractory cases may need second-line therapies. Platinum-based drugs are currently used for the treatment of various solid cancers, however, their use is mainly limited by chemoresistance and adverse effects in normal tissues[1-3].

Bcl-2 family proteins are a group of structurally related proteins composed of pro-apoptotic members such as Bax and Bak, and pro-survival members such as Bcl-xL, Bcl-2 or Mcl-1. Overexpression of pro-survival members can lead to resistance to chemotherapeutic drugs, so the development of drugs targeting these molecules is becoming increasingly a strategy to overcome resistance to first-line cancer therapy. Two of these inhibitors, commonly known as BH3 mimetics, are Obatoclax (targeting all Bcl-2 family pro-survival proteins) and ABT-737 (selectively targeting Bcl-2, Bcl-xL and Bcl-w). The aim of this study was to analyse different combinations of these BH3 mimetics with cisplatin to identify synergies between these treatments and to develop new therapeutic strategies[4-7].

We show that the combination of ABT-737 or Obatoclax with cisplatin is able to reverse cisplatin resistance in muscle-invasive bladder cancer cells. In HT1197 cells we observe sensitisation to cisplatin after combination with ABT-737, these cells show cisplatin resistance mediated, at least in part, by autophagy. In HT1376 cells we observe sensitisation to cisplatin after combination with Obatoclax. We have also observed that the combination of Obatoclax and ABT-737 induces cell death in these cell lines. Combinations of BH3 mimetics and cisplatin may be alternative therapies in muscle-invasive bladder cancer and Bcl-2 family proteins may be predictive markers of response.

Founding: Instituto de Salud Carlos III (FIS 17/1240;FIS 20/1641)

1- Antoni, S. et al., 2017. European Urology 2- Chou, R. et al., 2016. Cancer 3- Ghosh, S., 2019. Bioorganic Chemistry 4- Banjara, S. et al., 2020. Biomolecules 5- Montero, J. 2018. Cell Death & Differentiation, 6- Flores, M. L. et al., 2012. Breast cancer research and treatment 7- Jiménez-Guerrero, R. et al., 2018. Cancers

0410-R-P

Anti-inflammatory potential of aeroplysinin-1, a bioactive compound isolated from the sponge Aplysina aerophoba

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Aeroplysinin-1 (Apl-1) is a brominated compound isolated from the marine sponge Aplysina aerophoba that has been shown to possess bioactive effects with a broad spectrum of action in *in vitro* and *in vivo* assays. Included in its pleiotropic activity are anti-tumor, anti-angiogenic, pro-apoptotic [1] and anti-oxidant effects [2], making Apl-1 a natural compound with very promising properties for its use as a potential therapeutic agent. In addition to the aforementioned effects, our group explored the role of Apl-1 in inflammation, a process related to numerous highly prevalent pathologies, such as cancer and atherosclerosis, showing the first evidence of its anti-inflammatory potential [3].

In this work, our group delves into the anti-inflammatory effect of Apl-1 in the context of vascular endothelium and provides new data regarding the molecular mechanism underlying this activity. The characterization of the mechanism of action points to the modulatory effect of Apl-1 on pathways involved in endothelial activation during the development of inflammation, experimental evidence that opens the door to the potential use of this compound as an anti-inflammatory agent.

This work is supported by funds from projects PID2019-105010RB-100 (Ministry of Science, Government of Spain), UMA18-FEDERJA-220 (Junta de Andalucía and FEDER funds), and funds from the BIO-267 group (Junta de Andalucía). M.B. is supported by a Juan de la Cierva fellowship (Government of Spain).

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is affiliated to CIBER de Enfermedades Cardiovasculares (CIBERCV, ISCIII, Spain).

[1] J.A. García-Vilas, B. Martínez-Poveda, A.R. Quesada, M.A. Medina (2015). Marine Drugs 14(1):1. [2] J.A. García-Vilas, B. Martínez-Poveda, A.R. Quesada, M.A. Medina (2018). Marine Drugs 16(9):316. [3] B. Martínez-Poveda, J.A. García-Vilas, C. Cárdenas, E. Melgarejo, A.R. Quesada, M.A. Medina (2013). PLoS One 8(1):e55203.

0424-P

Understanding and drugging the Bcl 2 interactome for tumor treatment

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The proteins of the Bcl-2 family control the permeabilization of the mitochondrial outer membrane, a triggering factor for apoptosis. The different anti- and pro-apoptotic members of this protein family interact with each other in the cytosol and in the mitochondrial membrane to decide cell fate. Understanding the mechanism of molecular interaction of the globular domains between members of this family has been crucial for the development of the first antitumor drugs directed against protein domains of the BCL2 family. Recent research has demonstrated that the carboxyl-terminal transmembrane domain (TMD) of some Bcl-2 protein family members can also modulate apoptosis. However, the transmembrane interactome of the antiapoptotic BCL2 proteins remains largely unexplored. In our laboratory, we have generated tools to comprehend thoroughly how TMDs contribute to full-length protein functions and how could be modulated. At the same time, it has been described mutations in BCL2-TMD domains of patients with clinical relevance in a tumour context, and we want to understand the role of TMD mutations in tumour progression.

0480-P

Antiproliferative and pro-apoptotic effects of Pt(II) complexes in tumour cell lines

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Universidad de Extremadura Fisiología Animal

Coordination of bioactive ligands to transition metals may lead to enhanced biological activity and reportedly constitutes a strategic approach in drug discovery Moreover, the incorporation of aromatic groups to ligands may allow an enhanced lipophilicity that can influence the cellular uptake and accumulation of the metallodrugs, thus increasing their activity. Herein, we have reported the synthesis and characterization of four Pt(II) complexes [$PtCl_2$(L)], where L= 2-(1-pyrazolyl)-2-thiazoline (PzTn), 2-(1-pyrazolyl)-1,3-thiazine (PzTz), 2-(3,5-diphenyl-1-pyrazolyl)-2-thiazoline (DPhPzTn) or 2-(3,5-diphenyl-1-pyrazolyl)-1,3-thiazine (DPhPzTz). The aim was analysing their potential anticarcinogenic ability in epithelial cervix carcinoma HeLa, human promyelocytic leukemia HL-60 and human histiocytic lymphoma U-937 tumour cell lines and checking whether the structural factors of the organic ligand may influence their biological activity. Our findings proved that PtDPhPzTn and PtDPhPzTz were far more effective in terms of cytotoxicity, especially in HeLa cells (IC_{50} =12.15 ± 0.89 and IC_{50} =14.09 ± 1.17 respectively) than their less lipophilic counterparts PtPzTn and PtPzTz (IC_{50} =140.20 ± 24.31 and IC_{50} =142.20 ± 14.26 respectively). We also check that PtDPhPzTn and PtDPhPzTz accumulate into cells much more efficiently than cisplatin (10-fold higher in both cases). All these results suggest that modulating the lipophilicity of the ligands can improve the cytotoxic effect of the complexes and their cellular uptake and accumulation.

This work was supported by Junta de Extremadura grants (ref. GR18040, GR18062 and IB18013). E. Fernández-Delgado and J. Espino hold a research pre-doctoral (jointly financed by European Social Fund, ref. PD18020) and post-doctoral (ref. TA18002). fellowship respectively, both from Junta de Extremadura.

0483-P

Pro-apoptotic and anti-migration properties of a thiazoline-containing Pt(II) complex in triple-negative breast cancer cells: role of melatonin as synergistic agent

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Universidad de Extremadura Physiology

Triple-negative breast cancer (TNBC) is an aggressive cancer that does not respond to hormonal and HER2-targeted therapies and have poor prognosis. Therefore, there is a need for the development of convenient anticancer strategies that can be effectively used for the treatment of TNBC. Herein, we reported a newly synthesized Pt(II) complex with thiazoline ring (PtDPhPzTn) and evaluated its potential anticarcinogenic by itself or in combination with melatonin, a renowned antioxidant molecule with pro-apoptotic effects in cancer cells, in the TNBC cell line MDA-MB-231. Our results showed that the complex PtDPhPzTn presented enhanced cytotoxicity compared to the classical chemotherapeutic agent cisplatin (IC_{50} = 10.4 μM vs. 56.8 μM, respectively, for MDA-MB-231 cells). Besides, induction of apoptosis by PtDPhPzTn was demonstrated in annexin-V/propidium iodide (PI) double-stained cells, which was more selective for MDA-MB-231 cells when compared to non-tumour breast epithelial cells MCF10A (~60% vs. ~30% apoptotic cells, respectively). Likewise, PtDPhPzTn produced a moderate S phase arrest and greatly impaired the ability of MDA-MB-231 cells to migrate. Melatonin alone was unable to induce either apoptosis or changes on cell cycle distribution of MDA-MB-231 cells, but significantly reduced its capacity to migrate. Most importantly, co-stimulation of TNBC cells with PtDPhPzTn and melatonin substantially enhanced the population of apoptotic cells and markedly improved the anti-migratory actions compared to the treatments with the Pt(II) complex alone. In summary, our findings provided evidence that PtDPhPzTn and melatonin could be potentially applied to TBNC treatment as powerful synergistic agents.

This work was supported by Junta de Extremadura grants (ref. GR18040, GR18062 and IB18013). J.E. and E.F.-D. hold a research post-doctoral (ref. TA18002) and pre-doctoral (jointly financed by European Social Fund, ref. PD18020) fellowships from Junta de Extremadura, respectively.

11.- Molecular Neurobiology

0004-R/M-OS

CPT1A in AgRP neurons as a modulator of endurance, muscle mass and locomotor coordination

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Exercise has multiple health benefits, including many related to body weight, appetite control and glucose homeostasis. As a result, physical activity is the best therapy to fight the current worldwide obesity epidemic. Nonetheless, the molecular mechanisms that mediate and integrate these beneficial effects are poorly understood.

In the arcuate (ARC) nucleus of the hypothalamus, the melanocortin system is at the intersection between metabolic signals (nutrients and hormones) and the neural pathways governing energy homeostasis. Specifically, orexigenic neurons expressing Agouti-related protein (AgRP) are activated upon starvation to stimulate hunger and energy conservation. The activity of these neurons changes in response to dynamic variations in the metabolic state, including those occurring during exercise. However, the potential role of AgRP neurons in mediating exercise capacity has not been evaluated yet.

Here, we use a mutant mouse model lacking carnitine palmitoyltransferase 1A (CPT1A) specifically in AgRP neurons (Cpt1a $AgRP^{-/-}$ mice). CPT1A enzyme regulates the rate-limiting step in the mitochondrial oxidation of fatty acids (FAs), and it has been previously suggested to play a key role in hypothalamic control of energy balance. To evaluate the exercise response of Cpt1a $AgRP^{-/-}$ mice, we performed differents assays: Open Field (OF) test, Elevated Plus Maze (EPM) test, Rotarod test, Treadmill exhaustion test, inverted screen test and weight test. Our results show that deletion of Cpt1a in AgRP neurons causes an increase in endurance, enhanced motor coordination and higher locomotion and exploratory behavior compared with control mice. However, no changes in anxiety-related behavior or in muscle strength were observed even though Cpt1a $AgRP^{-/-}$ mice show a reduction in the size of quadriceps and gastrocnemius muscles.

Altogether, our results suggest that CPT1A in AgRP neurons is necessary to regulate physical performance, motor coordination and muscle mass. Future studies would clarify the role of CPT1A as a potential therapy for the improvement of motor skills and muscle function.

Hawley JA, et al. Integrative Biology of Exercise. Cell. 2014 Nov 6;159(4):738-49 Zagmutt S, et al. Targeting AgRP neurons to maintain energy balance: Lessons from animal models. Biochem Pharmacol. 2018;155:224-232 Mera P, et al. Osteocalcin Signaling in Myofibers Is Necessary and Sufficient for Optimum Adaptation to Exercise. Cell Metab. 2017 Jan 10;25(1):218

0057-M-OS

Aberrant upregulation of glycolysis mediates CLN7 neuronal ceroid lipofuscinosis

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CLN7 neuronal ceroid lipofuscinosis is an inherited lysosomal storage neurodegenerative disease highly prevalent in children. CLN7/MFSD8 gene encodes a lysosomal membrane glycoprotein, but the biochemical processes affected by CLN7-loss of function are unexplored thus preventing development of potential treatments. Here, we found, in the $Cln7^{\Delta ex2}$ mouse model of CLN7 disease, that failure in the autophagy-lysosomal pathway causes accumulation of structurally and bioenergetically impaired neuronal mitochondria. In vivo genetic approach revealed elevated mitochondrial reactive oxygen species (mROS) in $Cln7^{\Delta ex2}$ neurons that mediates glycolysis activation and contributes to CLN7 pathogenesis. Mechanistically, mROS sustains a signaling cascade leading to protein stabilization of PFKFB3, a glycolytic-promoting enzyme normally unstable in healthy neurons. Pharmacological inhibition of PFKFB3 in $Cln7^{\Delta ex2}$ mouse brain in vivo and in CLN7 patients-derived cells rectified key disease hallmarks. Thus, aberrant upregulation of neuronal glycolysis contributes to CLN7 pathogenesis and targeting PFKFB3 may alleviate this and other lysosomal storage diseases.

This work was funded by Agencia Estatal de Investigación (PID2019-105699RB-I00).

0313-OS

CB1 RECEPTORS DEFICIENCY IN OLIGODENDROCYTE PRECURSORS DISRUPTS POSTNATAL OLIGODENDROGENESIS AND CAUSES HYPOMYELINATION IN MICE

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Exogenous and endogenous cannabinoid molecules have been shown to modulate oligodendrogenesis and developmental CNS myelination. However, the cell-autonomous action of these compounds on oligodendrocyte precursor cells (OPC) *in vivo* has never been explored. Here, by using OPC-specific genetic mouse models we show that selective CB_1 cannabinoid receptor depletion in OPC prevented cell differentiation and perturbed oligodendrogenesis and postnatal myelination. Moreover, early postnatal CB_1 depletion in OPC caused hypomyelination and motor alterations at adult ages in mice. Conversely, CB_1 receptor pharmacological activation promotes oligodendrocyte development and CNS myelination in *wild type* but not in OPC-CB_1-null mice. Overall, this study addresses a cell-autonomous role for CB_1 receptors in OPC modulating oligodendrogenesis that may help in understanding the complex network of signaling molecules that drives CNS myelination.

0440-R/M-OS

APC/C-Cdh1 regulates synaptogenesis and dendrite stability during postnatal development

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Cognitive brain functioning requires the synchronization of two processes involved in neuronal network formation, dendrite arborization and synaptogenesis, during postnatal neurodevelopment. Alterations in dendritic branch stability and synapse disfunction leads to neurodevelopmental pathologies, including autism spectrum disorders. Recently, we described that Cdh1, the main neuronal activator of the E3 ubiquitin ligase Anaphase Promoting Complex/Cyclosome (APC/C), is essential for maintaining neuronal network integrity in the adult brain. However, the potential role of Cdh1 on dendrite and synapse stability in the postnatal developing brain remains unknown.

Conditional Cdh1 KO (Cdh1 cKO) mice were generated by mating mice harboring a floxed allele of the Cdh1 gene with Nestin-Cre mice. Double-transgenic Cdh1 cKO mice were crossed with Thy1-YFP animals, which express yellow fluorescent protein in the pyramidal neurons in specific areas of the brain, including cerebral cortex and hippocampus. We analyzed dendrite integrity by fluorescence. Synaptic proteins were analyzed by western blotting to determine synaptic maturation. The morphology of dendritic spines, synaptic cleft and vesicles were analyzed by electron microscopy.

Cdh1 loss promoted alterations in brain structure, including microcephaly and increased lateral ventricle volume, which were more evident at 21 days after birth. Moreover, the loss of Cdh1 promoted dendrite disruption and neuronal death during postnatal brain development. Furthermore, we found decreased level expression of pre (Bassoon) and post-synaptic (PSD95) proteins in the Cdh1 cKO mice, in relation to controls. Finally, ultrastructure analysis of CA1 hippocampus layer revealed a disbalance between synaptic clefts and synaptic vesicles in the Cdh1 cKO mice, all indicating impaired synaptogenesis.

Our results suggest that Cdh1 is essential for dendritic arborization, network integrity and synaptogenesis in the developing brain after birth and describe a key role of APC/C-Cdh1 in the molecular pathogenesis of neurodevelopmental disorders.

Funded by ISCIII (PI18/00265; RD16/0019/0018) FEDER; PO FSE Castilla y Leon 2014-2020; Junta de Castilla y Leon (Escalera de Excelencia CLU-2017-03 Cofinanciada por el P.O. FEDER de Castilla y Leon). SG is funded by IBSAL.

[1] Bagni C. and Zukin RS. Neuron 101(6), 1070-1088 (2019). [2] Bobo-Jiménez V. et al. PNAS 114 (17), 4513-4518 (2017). [3] Cline H. And Haas K. J Physiol 586(6), 1509-1517 (2008). [4] Koleske AJ. Nat Rev Neurosci 14(8), 536-550 (2013). [5] Lefebvre, JL et al. Annu Rev Cell Dev Biol 31, 741-777 (2015). [6] Wefelmeyer W. et al. Trends Neurosci 39, 656-667 (2016).

0002-P

Functional differences between heteromers formed by α1a adrenoceptors and dopamine D4 receptors with relevance to ADHD

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Attention deficit hyperactivity disorder (ADHD) is a prevalent neuropsychiatric disorder in children characterized by symptoms of inattention, hyperactivity and impulsivity. It has been demonstrated that the dopaminergic system is involved in ADHD, specially the dopamine receptor D4 (D4R) variant D4.7R. The adrenergic system has also been linked to ADHD due its implication in visual attention, learning and memory. Many studies reveal that noradrenaline and dopamine have complementary effects in the reinforcement of prefrontal cortex (PFC) and striatum connections, suggesting that these coordinated effects could be mediated by interactions between their respective receptors. In particular, α1a adrenoceptors (α1aR) and different variants of D4R are expressed in PFC and striatum. We analysed whether D4.4R and D4.7R heteromerize with α1a adrenoceptor, and the possible functional differences between D4R variants. We have demonstrated D4.4R-α1aR and D4.7R-α1aR heteromerization in transfected cells by using Biolumiscence Resonance Energy Transfer (BRET) and in in rat striatum and cortex by in situ Proximity Ligation Assay (PLA). We also characterized the transmembrane domains (TM) implicated in D4.4R-α1aR heteromerization by using specific synthetic peptides corresponding to D4R and α1aR TM sequences. Functionally, we have observed a negative cross-talk and cross-antagonism in both heterodimers at the MAPK pathway level both in transfected cells and in rat tissue. Nevertheless, when intracellular calcium release and cAMP accumulation were analysed, the cross-talk and cross-antagonism were only detected in the D4.4R-α1aR heteromer. These results suggest a functional relevance of the D4R-α1a R interaction, which seems to be modified in presence of the ADHD related D4.7R variant.

0038-P

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.

0076-P

Salient brain entities labelled in P2rx7EGFP reporter mouse embryos include the septum, roof plate glial specializations and circumventricular ependymal organs

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The purinergic system is one of the oldest cell-to-cell communication mechanisms and exhibits relevant functions in the regulation of the central nervous system (CNS) development. Amongst the components of the purinergic system, the ionotropic P2X7 receptor (P2X7R) stands out as a potential regulator of brain pathology and physiology. Thus, P2X7R is known to regulate crucial aspects of neuronal cell biology, including axonal elongation, path-fnding, synapse formation and neuroprotection. Moreover, P2X7R modulates neuroinfammation and is posed as a therapeutic target in infammatory, oncogenic and degenerative disorders. However, the lack of reliable technical and pharmacological approaches to detect this receptor represents a major hurdle in its study. Here, we took advantage of the P2rx7-EGFP reporter mouse, which expresses enhanced green fuorescence protein (EGFP) immediately downstream of the P2rx7 proximal promoter, to conduct a detailed study of its distribution. We performed a comprehensive analysis of the pattern of P2X7R expression in the brain of E18.5 mouse embryos revealing interesting areas within the CNS. Particularly, strong labelling was found in the septum, as well as along the entire neural roof plate zone of the brain, except chorioidal roof areas, but including specialized circumventricular roof formations, such as the subfornical and subcommissural organs (SFO; SCO). Moreover, our results reveal what seems a novel circumventricular organ, named by us postarcuate organ (PArcO). Furthermore, this study sheds light on the ongoing debate regarding the specifc presence of P2X7R in neurons and may be of interest for the elucidation of additional roles of P2X7R in the idiosyncratic histologic development of the CNS and related systemic functions.

Ortega F, Gomez-Villafuertes R, Benito-León M, Martínez de la Torre M, Olivos-Oré LA, Arribas-Blazquez M, Gomez-Gaviro MV, Azcorra A, Desco M, Artalejo AR, Puelles L, Miras-Portugal MT. Salient brain entities labelled in P2rx7-EGFP reporter mouse embryos include the septum, roof plate glial specializations and circumventricular ependymal organs. Brain Struct Funct. 2021 Apr;226(3):715-741. doi: 10.1007/s00429-020-02204-5.

0081-P

Las convulsiones inducidas por hipertermia alteran el sistema glutamatérgico en el cerebro de rata.

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Las convulsiones febriles representan el trastorno convulsivo más frecuente en la población infantil. Los estudios epidemiológicos retrospectivos han encontrado un vínculo entre este tipo de convulsiones y un mayor riesgo de desarrollar epilepsia en la etapa adulta. El modelo animal de convulsiones inducidas por hipertermia empleado en este trabajo, ha corroborado que un porcentaje de los animales expuestos a convulsiones durante la etapa neonatal experimentaban durante la edad adulta trastornos convulsivos compatibles con la presencia de epilepsia del lóbulo temporal. El L-glutamato es el principal neurotransmisor excitatorio en el Sistema Nervioso Central donde actúa uniéndose a receptores ionotrópicos y metabotrópicos. Un exceso de L-Glutamato en el espacio extracelular provoca neurotoxicidad y ha sido asociada con desordenes neurológicos como la epilepsia. La eliminación del L-Glutamato extracelular por los transportadores de aminoácidos excitatorios (EATT) desempeña un importante papel neuroprotector. Por otro lado, un aumento en los niveles del receptor metabotrópico de glutamato $mGluR_5$ o una sobreestimulación de los mismos ha sido relacionado con la aparición de convulsiones en diferentes modelos animales y en la epilepsia del lóbulo temporal en humanos. En este trabajo, el estado de diferentes componentes del sistema glutamatérgico ha sido analizado en la corteza cerebral a corto (48 h) y largo plazo (20 días) después de las convulsiones inducidas por hipertermia. A corto plazo, detectamos un aumento en los niveles de GLT-1, una reducción en la concentración de L-Glutamato mientras que los niveles de mGluR5 no se alteraron significativamente. Tampoco se apreció una perdida neuronal significativa. A largo plazo se observó un aumento en los niveles de $mGluR_5$ que estuvo acompañado por una reducción en los niveles de GLT-1 y en los niveles de L-Glutamato. Estos cambios estuvieron asociados con la aparición de un fenotipo ansioso. Los resultados obtenidos sugieren un papel neuroprotector de los componentes glutamatérgicos $mGluR_5$ y GLT-1 a corto plazo. Sin embargo, este efecto neuroprotector parece perderse a largo plazo conduciendo a un fenotipo ansioso y sugiriendo una mayor vulnerabilidad a eventos epilépticos en la edad adulta.

0094-R/M-P

APC/C-Cdh1-ROCK2 signalling pathway: in the spotlight of new therapeutic strategies in stroke

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Stroke is a leading cause of long-term adult disability and a common cause of death in developed countries. Therefore, it is essential to establish new therapeutic strategies aimed at limiting and/or repairing ischemic neuronal damage.

The E3 ubiquitin ligase APC/C-Cdh1 is essential for neuronal survival[1,2]. Previously, we described that Cdh1 regulates the balance between proliferation, neurogenesis and apoptosis[3]. These processes play an essential role in the balance between cerebral damage and repair after ischemia. The cortical reorganization after stroke determines the recovery of the lost brain function. The RhoA-ROCK2 signalling pathway regulates the morphology, elongation and retraction of neurites, both axons and dendrites, making it an essential pathway in synaptic plasticity[4].

Recently, we demonstrated that Cdh1 regulates ROCK2 levels and activity in cortical neurons, which modulates the stability of dendrites and synapses[5]. However, whether Cdh1 plays a role in stroke remains unknown. To assess this issue, first we used an experimental in vitro model of ischemia/reoxygenation (I/R). We found that Cdh1 modulates neuronal susceptibility through the RhoA-ROCK2 signalling pathway, since ROCK2 inhibition and knock-down reduced neuronal apoptosis induced by I/R. These results were confirmed in vivo using the middle cerebral artery occlusion (MCAO) model in Cdh1-cKO and WT mice. Our results showed that Cdh1 was involved in the functional recovery after MCAO, enhancing neuronal survival and favouring both the reduction of infarct volume and the recovery of motor and neurological functions.

These results demonstrate that APC/C-Cdh1 actively participates in the neuronal response to I/R damage, enhancing neuronal survival through ROCK2 regulation. Thus, we have identified the Cdh1-ROCK2 axis as a key element in the pathogenesis of stroke and, therefore, as potential molecular targets for stroke treatment.

Funded by Instituto de Salud Carlos III (PI18/00265; RD16/0019/0018); FEDER; Junta de Castilla y León (CSI151P20; co-financed with FEDER funds) and Bodegas R. López de Heredia Viña Tondonia.

1.Almeida A, et al. J Neurosci 2005,25:8115-8121 2.Almeida A, et al. Mol Neurobiol 2012,46(3):547-554 3.Delgado-Esteban M, et al. Nat Commun 2013, 4:2879 4.Koch JC, et al. Cell Death Dis 2014,5:e1225 5.Bobo-Jimenez V, et al. Proc Natl Acad Sci USA 2017,25;114(17):4513-4518

0104-R-P

Connexin43 region 266-283, reduces neural progenitor cell proliferation promoted by EGF and FGF-2 and increases astrocytic differentiation

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Neural progenitor cells (NPCs) are self-renewing cells that give rise to the major cells in the nervous system and are considered to be possible cell of origin of glioblastoma. The gap junction protein connexin43 (Cx43) is expressed by NPCs exerting channel-dependent and -independent roles. We focused on one property of Cx43, its ability to inhibit Src, a key protein in brain development and oncogenesis. Because Src inhibition is carried out by the sequence 266-283 of the intracellular C terminus in Cx43, we used a cell-penetrating peptide containing this sequence, TAT-$Cx43_{266-283}$, to explore its effects on postnatal subventricular zone NPCs. Our results show that TAT-$Cx43_{266-283}$ inhibited Src activity and reduced NPC proliferation and survival promoted by EGF and FGF-2. In differentiation conditions, TAT-$Cx43_{266-283}$ increased astrocyte differentiation at expense of neuronal differentiation, which coincided with a reduction in Src activity and β-catenin expression. We propose that Cx43, through the region 266-283, reduces Src activity leading to disruption of EGF and FGF-2 signaling and to down-regulation of β-catenin with effects on proliferation and differentiation. Our data indicate that the inhibition of Src might contribute to the complex role of Cx43 in NPCs and open new opportunities for further research in gliomagenesis.

Santiago, M.F.; et al. The carboxyl-terminal domain of connexin43 is a negative modulator of neuronal differentiation. J Biol Chem 2010, 285, 11836-11845, doi:10.1074/jbc.M109.058750. Rinaldi, F.; et al. Cross-regulation of Connexin43 and beta-catenin influences differentiation of human neural progenitor cells. doi:10.1038/cddis.2013.546.

0112-P

Live Imaging Reveals Cerebellar Neural Stem Cell Dynamics and the Role of VNUT in Lineage Progression

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Little is known about the intrinsic specification of postnatal cerebellar neural stem cells (NSCs) and to what extent they depend on information from their local niche. Here, we have used an adapted cell preparation of isolated postnatal NSCs and live imaging to demonstrate that cerebellar progenitors maintain their neurogenic nature by displaying hallmarks of NSCs. Furthermore, by using this preparation, all the cell types produced postnatally in the cerebellum, in similar relative proportions to those observed in vivo, can be monitored. The fact that neurogenesis occurs in such organized manner in the absence of signals from the local environment, suggests that cerebellar lineage progression is to an important extent governed by cell-intrinsic or pre-programmed events. Finally,we took advantage of the absence of the niche to assay the influence of the vesicular nucleotide transporter inhibition, which dramatically reduced the number of NSCs in vitro by promoting their progression toward neurogenesis.

0149-R-P

miR-7 regulates mitochondrial metabolism and autophagy in neuronal and glial cells

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IMDEA Food Precision Nutrition and Aging

Posttranscriptional regulation of gene expression has been shown to modulate a number of signaling pathways that could lead to metabolic diseases (1). Previous studies from our lab (2) have shown that miR-7, a brain-enriched miRNA, regulates neural metabolism by inhibiting insulin signaling and is abnormally expressed in obese mice, leading us to explore its metabolic impact in neuronal cells. Initial bioinformatic analysis showed a considerable amount of mitochondrial (mt) potential targets, so we studied their mRNA levels and found they were regulated by miR-7 *in vitro*. Correlating with these results, studies of mt function revealed that miR-7 impairs glycolysis, mt respiration and ATP production, even though mt mass was increased. Electron microscopy of miR-7 overexpressing cells revealed aberrant mt morphology and organization, together with an unexpected accumulation of vesicles compared to control cells. Interestingly, *in silico* analysis predicted many targets of miR-7 related to autophagy and lysosomal function (BLOC1S4, LIMP2) (3). Study of their 3'-UTR activity indicated that miR-7 directly binds to many of these mRNAs. We then assessed autophagy function in neuronal cell lines. Our analysis showed that miR-7 inhibits mTOR and AKT expression and activates AMPK, promoting the pathways that induce initiation of autophagy, while the use of drugs that block later steps suggested that miR-7 blunts autophagy flux. Overexpression of STX17 and SNAP29 ORFs lacking their 3'-UTR rescued impairment of autophagy flux by miR-7, at least partially. Along with this result, mt-keima assay and the decreased expression of Parkin when overexpressing miR-7 suggest a defect in mitophagy, correlating with the mt dysfunction found previously. Collectively, our data suggest novel mechanisms of posttranscriptional regulation of metabolism by miR-7 through the modulation of mt function and autophagy, processes associated with insulin resistance, obesity and neurological disorders.

(1) Ramírez CM et al. Cell Cycle. (2011) (2) Fernández-de Frutos M et al. Mol Cell Biol. (2019) (3) Yu L et al. Autophagy. (2018)

0156-R-P

Role of miR-7/hnRNPK in cholesterol biosynthesis

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IMDEA Food Precision Nutrition and Aging

Cholesterol is an essential macromolecule for mammalian cells (1). In the brain, cholesterol homeostasis is tightly regulated by de novo synthesis, uptake and efflux and dysregulations of this pathway is linked with neurodegenerative pathologies such as Alzheimer disease (AD) (2). In addition to classical regulation of these processes by transcriptional factors such as Liver X receptor (LXR) or Sterol Regulatory Element Binding Proteins (SREBPs), microRNAs and RNA Binding proteins (RBP) could be key elements in cholesterol homeostasis and in AD (1). Previous studies of our group have demonstrated the role of miR-7 in regulating insulin signaling, an important metabolic pathway linked with AD (3). In this context, we aimed to explore if miR-7 could influence cholesterol biosynthesis, due to its close relationship with AD. To do so, we performed bioinformatic analysis that indicated that important biosynthetic enzymes of the pathway are potentially targeted by miR-7, including DHCR7, SC5D, DHCR24. Western blot and qPCR analysis in human (SH-SY5Y) and mouse (N2a) neuroblastoma cell lines overexpressing miR-7 showed a significant inhibition of these genes. Further analysis of the 3'-UTR activity indicated that miR-7 directly binds with these targets. To assess the functional outcome of these findings we performed cholesterol synthesis assays in miR-7 overexpressing cells. Our results showed that miR-7 inhibits cholesterol synthesis and promotes the accumulation of desmosterol in N2a, which correlates with the posttranscriptional regulation of DHCR24 enzyme. Next, we explored whether hnRNPK, an RNA binding protein that serves as a host gene for miR-7-1, could be cooperating with miR-7 in the regulation of cholesterol synthesis. Interestingly, silencing experiments in N2a cells showed that cholesterol synthesis pathway is totally blunted in absence of hnRNPK, which is accompanied by a significant decrease of SREBP2 expression. Finally, further analysis indicated that both, miR-7 and hnRNPK levels are modulated by cholesterol content, probably by transcriptional regulation of SREBP2. Altogether, these results suggest a novel and intriguing feedback loop of regulation of cholesterol homeostasis by posttranscriptional mechanisms.

(1) Goedeke et al. Neurobiology of disease (2014) (2) Martín et al. EMBO. (2014) (3) Fernández-de Frutos M et al. Mol Cell Biol. (2019)

0171-R-P

Profiling of candidate biomarkers of Alzheimer's Disease by proteomics.

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Alzheimer's disease (AD) is a progressive and chronic neurodegenerative disorder that affects wide areas of the cerebral cortex and hippocampus [1,2]. It is the most common cause of dementia worldwide with a high socioeconomic impact. Since the definitive diagnosis of AD requires *post-mortem* verification, new approaches are necessary to identify new diagnostic biomarkers and therapeutic targets of the disease [3].

Here, we aimed to identify AD-specific autoantibodies and autoantigens as blood-based biomarkers [4] using protein microarrays and mass spectrometry-based methods for their identification. ELISA, Luminescence assays, WB and/or immunohistochemistry together with serum and brain tissue samples from AD patients and controls were used for validation.

High-density (42,100) and low-density (384) protein-epitope signature tag (PrEST) planar arrays together with an immunoprecipitation protocol coupled to mass spectrometry (LC-MS/MS) analysis using either frozen brain tissue or serum samples from AD patients and healthy individuals were utilized for serum AD-related autoantibody and autoantigen identification. A total of 370 unique PrESTs corresponding to 338 target proteins from the screening phase were used for validation by antigen suspension beads arrays. Among identified PrEST target of autoantibodies, a candidate PrEST was found with a statistically significant higher seroreactivity in AD patients than in controls. Besides, two other seroreactive autoantigens specific of AD were identified by immunoprecipitation.

Both autoantigens and the candidate PrEST were further validated as full-length recombinant proteins by ELISA and Luminescence assays. The three targets of autoantibodies showed significant higher seroreactivity and altered protein levels in AD patients than in controls as observed by WB or immunohistochemistry.

Our results suggest that the combination of protein microarrays and mass spectrometry-based methods is useful for the identification of autoantigens specific of AD and protein alterations related to the disease that could be useful as biomarkers of AD.

Masters, C.L. et al.Alzheimer's disease. Nature Reviews Disease Primers.2015,1. Viña,J.; Lloret, A.Why women have more Alzheimer's disease than men: gender and mitochondrial toxicity of amyloid-beta peptide.J Alzheimers Dis.2010,20. Jack CR Jr. et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol.2010,9. DeMarshall, C. et al. Autoantibodies as diagnostic biomarkers for the detection and subtyping of multiple sclerosis. J Neuroimmunol.201

0234-R/M-P

GENETIC INACTIVATION OF PRO-GLYCOLYTIC ENZYME PFKFB3 IN ASTROCYTES TO INVESTIGATE NEURONAL FUNCTION

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Neurons use very little glucose through the glycolytic pathway for energy generation, despite their high energy expenditure during neurotransmission (1). The energy requirements of neurons are mostly supplied by oxidative phosphorylation (2). According to the astrocyte-neuron lactate shuttle (3), astrocytic-derived lactate is a major oxidizable substrate for neurons. We have demonstrated that astrocytes, in contrast to neurons, are highly glycolytic cells (4) thus explaining the continuous release of lactate for neuronal use. A key factor responsible for these metabolic shapes is the pro-glycolytic enzyme 6-phosphofructokinase/fructose-2,6-bisphosphatase, isoform 3 (PFKFB3).

PFKFB3 is the most abundant PFKFB isoform in the brain and we have shown that it is virtually absent in neurons and highly expressed in astrocytes (5). However, to date there is no experimental evidence that PFKFB3 is an important factor for the astrocytic production of lactate for neuronal use during neurotransmission. Here, we aimed to undertake this task and generated a novel genetic mouse model able to knockout PFKFB3 selectively in astrocytes *in vivo*. To do so, we used a LoxP-flanked conditional PFKFB3 mouse, which was subjected to AAV-mediated expression of a Cre recombinase governed by the astrocytic-specific glial-fibrillary acidic protein (GFAP) promoter. In this communication we show preliminary results indicating the efficacy of this novel model to reduce basal and stimulated glycolysis in astrocytes. We also show some of the consequences of decrease the glycolytic pathway in these cells, such as alterations in glucose uptake, glycogen metabolism and respiration. This tool will allow us to ascertain whether astrocytic PFKFB3, by promoting glycolysis, is responsible for the shuttle of lactate to neurons during neurotransmission.

This work was funded by Agencia Estatal de Investigación (PID2019-105699RB-I00).

1. Bolaños. J Neurochem 139, 115-125 (2016) 2. Lopez-Fabuel et al. Proc Natl Acad Sci U S A 113, 13063-13068 (2016) 3. Pellerin & Magistretti. Proc Natl Acad Sci U. S. A. 91, 10625-10629 (1994). 4. Almeida et al. Nat Cell Biol 6, 45-51 (2004). 5. Herrero-Mendez, A. et al. Nat Cell Biol 11, 747-752 (2009).

0237-P

Heneicosapentaenoic acid (HPA): a potential drug for Alzheimer's disease treatment.

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Many drugs and therapies (including immunotherapies) have been designed and developed in the past decade against neurodegenerative diseases, including Alzheimer's disease although none of them finished a phase-III clinical trial. Most therapies target the amyloid cascade (anti-amyloid therapies) however this hypothesis has recently questioned due to the failure of clinical trials of anti-amyloid therapies to date. An hydroxylated derivative of docosahexaenoic acid, 2-hydroxy-docosahexaenoic acid (DHA-H), has shown efficacy against hallmarks of AD pathology in a transgenic mouse model of AD (5xFAD). DHA-H is shown to undergo α-oxidation to generate the heneicosapentaenoic acid (HPA, C21:5, n-3) metabolite, an odd-chain omega-3 polyunsaturated fatty acid that accumulates in cell cultures, mouse blood plasma and brain tissue upon DHA-H treatment. Interestingly, DHA-H does not share metabolic routes with its natural analog DHA (C22:6, n-3) but rather, DHA-H and DHA accumulate distinctly, both having different effects on cell fatty acid composition. This is partly explained because DHA-H α-hydroxyl group provokes steric hindrance on fatty acid carbon 1, which in turn leads to diminished uptake by culture cells and accumulation as free fatty acid in cell membranes. Finally, DHA-H administration to mice elevated the brain HPA levels which in turn were directly and positively correlated with cognitive spatial scores in AD mice. This effect appeared in the apparent absence of DHA-H and without any significant change DHA levels in brain. Therefore, the evidence presented in this work suggest that the metabolic conversion of DHA-H into HPA could represent a potential event in the therapeutic effects of DHA-H against AD.

0262-R/M-P

Impact of fatty acids on astrocytic bioenergetics

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Brain fatty acids metabolism remains underexplored. Recent reports have suggested that astrocytes may utilize fatty acids (1). However, whether acetyl-CoA metabolic fate is the tricarboxylic acid (TCA) cycle to obtain energy, the biosynthesis of ketone bodies, or both metabolic pathways, have not been yet fully deciphered yet (2,3). Besides this, astrocytes contribute to neuronal energy metabolism by shuttling to neurons metabolic glucose-derived precursors, such as lactate (4). However, it is unknown whether neurons utilize astrocytic-generated ketone bodies as energy precursors to maintain neurotransmission (5,6). Here, we aimed to address this issue using a mouse genetic model unable to perform ß-oxidation, specifically in astrocytes. We used a carnitine palmitoyltransferase-1A (CPT1A) gene floxed mouse model to elaborate cortical astrocyte cultures (7). Then, we infected them with adeno-associated viruses (AAVs) that express Cre recombinase (CMV-Cre-GFP) or not (CMV-GFP) to obtain CPT1a-KO and WT astrocytes, respectively. To test whether astrocytes use fatty acids as a mitochondrial fuel, we treated CPT1a-KO and WT astrocytes with oleic acid (250 μM) for 24 hours and measured the rate of oxygen consumption (OCR) using the Seahorse technology. Our results show that oleic acid treatment increases basal respiration and ATP-linked oxygen consumption in WT, but not in CPT1a-KO astrocytes. These changes were not ascribed to possible changes in the mitochondrial population according to citrate synthase activity and TOMM20 expression levels. These results indicate that astrocytes can oxidize fatty acids to produce energy, although further studies need to be performed to decipher if astrocytes are able to synthesize ketone bodies. Ongoing work is being developed to validate these observations in vivo.

This work was funded by Agencia Estatal de Investigación (PID2019-105699RB-I00).

1.Panov A. et al. (2014). Biomed Res Int. 2014: 472459. 2.Blázquez C. et al. (1999). J Neurochem. 73(4):1674–82. 3.Nehlig A. (2004). Prostag. Leukot Essent Fatty Acids. 70:265–275. 4.Schönfeld P et al.(2013). J cereb blood flow metab;33(10), 1493-1499. 5.Mckenna M. et al (2015). Neurochem Res.40, 2527–2543. 6.Pan JW et al. (2002). J Cereb Blood Flow Metab. 22:890-898. 7.Wong BW, et al. (2017). Nature.542:49–54

0264-R/M-P

The Cdh1 Asp187Gly mutation impairs APC/C activity leading to Pfkfb3 stabilization

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The Fizzy-related protein 1 (*Fzr1*) gene encodes the Cdh1 protein, a coactivator of the E3 ubiquitin ligase Anaphase Promoting Complex/Cyclosome (APC/C), which regulates mitotic [1] and non-mitotic functions [2]. In addition to cell cycle regulation, APC/C-Cdh1 plays a key role in neuronal survival [3] and neurogenesis during brain development [4]. Recently, we described that the loss of function of APC/C-Cdh1 caused by a novel human mutation (p.Asp187Gly) in the *Fzr1* gene (c.560A > G) results in a new cause of prenatal microcephaly [5].

Here, we aim to study the molecular mechanisms involved in APC/C loss of activity due to the Cdh1 Asp187Gly mutation, focusing on Cdh1 interaction with its targets. To this end, we used the human embryonic kidney 293T (HEK293T) cell line transfected with a cDNA-containing vector of the mutated gene.

Our results showed that the Asp187Gly mutation reduces Cdh1 stability, when compared to Cdh1 wild type-expressing cells. We also observed a sequestration of mutated Cdh1 in the nucleus, which is compatible with an increased ubiquitination and proteosomal degradation of the mutant variant. Moreover, the Cdh1 Asp187Gly mutation, located in the essential protein-protein interaction WD40 domain, impairs Cdh1 ability to recognize and bind its protein targets Cyclin B1 (a key protein involved in mitosis progression) and Pfkfb3 (a crucial glycolytic regulatory protein [6]). Furthermore, both cyclin B1 and Pfkfb3 accumulated in cells expressing the mutant form of Cdh1, leading to cell cycle and glycolysis deregulation.

In conclusion, we found that the Cdh1 Asp187Gly mutation impairs Cdh1 protein stability and target recognition, thus affecting APC/C activity. These results provide new tools to understand the regulation of APC/C-Cdh1 complex activity and its involvement in important processes such as the control of cerebral cortex size.

Leticia Sancha is the recipient of a FPU contract from the Ministerio de Ciencia, Innovación y Universidades. This work was in part funded by Agencia Estatal de Investigación (PID2019-105699RB-I00).

1. Watson, E. R., et al. (2019). Trends in cell biology, 29, 117-134. 2. Almeida, A. (2012). Molecular neurobiology, 46, 547-554. 3. Maestre, C., et al. (2008). The EMBO journal, 27, 2736-2745. 4. Delgado-Esteban, M., et al. (2013). Nature communications, 4, 1-12. 5. Rodriguez, C., et al. (2019). Journal of neurochemistry, 151, 103-115. 6. Herrero-Mendez, A., et al. (2009). Nature cell biology, 11, 747-752.

0265-P

BACLOFEN-INDUCED GABAB RECEPTOR ACTIVATION IN OPCs IMPACTS ON (RE)MYELINATION IN VITRO AND IN VIVO

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Oligodendrocyte progenitor cells (OPCs) differentiation to oligodendrocytes (OLs) is a critical step that drives myelin formation in the central nervous system (CNS) during physiological processes, as development, and after demyelinating lesions, as in multiple sclerosis. Neuron-OL communication is key for the regulation of (re)myelination and different neurotransmitters, including γ-aminobutyric acid (GABA), have been identified as mediators in this process. In previous work in cultured OPCs, we have proved that GABAB receptor ($GABA_BR$) activation with its selective agonist baclofen (Bac) stimulates OPC differentiation and the expression of myelin related proteins. In this project, we delved into the Bac-induced molecular mechanisms in OPCs *in vitro* and analyzed the impact of *in vivo* systemic Bac administration in myelination during development and remyelination after demyelinating lesions, by confocal and transmission electron microscopy. First, we studied the differences in gene expression in cultured cortical rat OPCs following Bac treatment and we checked by immunoblotting alterations in the phosphorylation state of kinases/molecules relevant for OPC survival and differentiation, detecting that Bac promoted changes in their activation state. After studying $GABA_BR$ downstream mechanisms in OPCs, we examined the impact of Bac-induced $GABA_BR$ activation in myelin sheath formation *in vitro* using polycaprolactone nanofibers. In addition, for the *in vivo* studies, we first treated developing rats with Bac daily from postnatal day 6, and we determined Bac-induced effect in myelination in grey and white matter areas of the CNS

at different time points. Finally, we investigated the role of Bac in a mouse model of lysolecithin-induced spinal cord demyelination by daily injection of Bac after injury, observing acceleration in OPC differentiation and remyelination within the lesions. Overall, these results contribute to the identification of $GABA_BR$-mediated mechanisms in OPCs and support its relevance in (re)myelination.

0278-R-P

Effect of QBA in the induction of autophagy

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Autophagy is a highly conserved mechanism that plays a key role in maintaining cellular homeostasis through the turnover of cytoplasmic structures and the adaptation of cells to environmental changes. In accordance with the clearance of protein aggregates and dysfunctional organelles, autophagy has a cytoprotective role in several diseases such as neurodegenerative disorders. Therefore, a large number of natural compounds have been reported to be involved in the modulation of autophagy via multiple signaling pathways. Many of these natural products show a great potential to become therapeutic candidates for multiple human diseases. Currently, natural bee products are attracting increasing interest due to their numerous biological and pharmacological properties. Moreover, 10-hydroxy-2-decenoic acid (10-H_2DA), the major lipid component of royal jelly, possesses several beneficial effects such as anti-tumoral, anti-inflammatory and immunomodulatory activities, in mammals. Although the health benefits of 10-H_2DA are well established, the precise molecular mechanisms by which the fatty acid provides these effects are not well elucidated. Interestingly, we have observed that 10H_2DA is a modulator of SIRT1 and mTOR, both are autophagy regulators. Based on our results, we postulate that the beneficial effects of 10-H_2DA can be associated with distinct cellular processes such as autophagy activation.

0279-R/M-P

Tlr2 depletion preserves photoreceptor cells and extends visual function in two mouse models of retinitis pigmentosa.

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Retinitis pigmentosa (RP) is a rare disease responsible for most of the cases of hereditary blindness. Currently, there is not a general treatment for RP. Different RP forms despite their genetic heterogeneity, course with common traits: photoreceptor death, reactive gliosis and inflammation. We have focused in targeting retinal inflammation as a neuroprotective strategy to prolong the visual function independently of the causative mutation. The Toll like Receptors (TLRs), key components of the innate immune system, respond to tissue damage triggering an inflammatory response. It has been described that TLRs aggravate the disease progression in different brain and retinal neurodegenerative conditions.

Here, we sought to determine the role of *Tlr2* in RP progression employing two RP models. The *rd10* mouse that carries a recessive mutation in the phosphodiestaerase 6B gene and undergoes rapid retinal degeneration; and the P23H that carries a dominant mutation in the rhodopsin gene and experiences a slow disease progression. First, we analyzed the gene expression of *Tlr2* and its adaptor proteins, and found that they increased, in both *rd10* and P23H retinas, since early stages of the disease. Then, we addressed the effect of *Tlr2* depletion in *rd10* and P23H mice. The analysis of the visual function by electroretinography showed that the amplitude of the photopic- (cone response) and mixed- (rod and cone response) waves were higher in the *rd10:Tlr2*$^{-/-}$ and P23H:*Tlr2*$^{-/-}$ mice than that of their hemizygous counterparts. *Tlr2* hemizygosity did not affect visual function decline. Histological analysis showed that deletion of *Tlr2* lead to better preservation of the photoreceptor layer (ONL) thickness in both RP models. Moreover, *rd10*:*Tlr2*$^{-/-}$ displayed reduced macrophage/microglia infiltration in the ONL.

In conclusion, *Tlr2* depletion resulted in photoreceptor preservation and delay of retinal function loss, providing a new mutation-independent therapeutic target for RP treatment.

Caso JR, et al. 2007 Tlr4 is involved in brain damage and inflammation after experimental stroke. Circulation McDonald CL, et al. 2016 Inhibiting TLR2 activation attenuates amyloid accumulation and glial activation in a mouse model of Alzheimer's disease. Brain Behav. Immun. Sánchez-Cruz A, et al. 2018 Modulation of GSK-3 provides cellular and functional neuroprotection in the rd10 mouse model of RP. Mol. Neu. Verbakel SK, et al. 2018 Non-syndromic retinitis pigmentosa. Prog Retin Eye Res

0304-R/M-P

Differential regulation of autophagy in astrocytes and neurons in the cannabinoid protection against Huntington's disease

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A common pathology shared by several neurodegenerative diseases is the abnormally low autophagic flux, leading to the accumulation of autophagy substrates and toxicity. Autophagy is an evolutionary conserved proteostatic process that is essential for removal of damaged organelles and toxic or aggregated proteins for lysosome degradation, and has crucial roles in development and disease. In Huntington's disease (HD), an expansion of polyglutamine (polyQ) tract in the N-terminus of the huntingtin (HTT) protein leads to protein aggregation. Specifically, an expansion of the polyQ tract in HTT to greater than 36Q is responsible for the formation of toxic oligomers and aggregates. Therefore, autophagy has become a primary target for treatment of neurodegenerative diseases that involve aggregating proteins. Endocannabinoids act as neuromodulatory and protective cues by activation of type-1 cannabinoid receptor (CB1). These receptors are highly abundant in brain cells and play a pivotal role in the control of motor behaviour, metabolism and autophagy. Several studies have shown that downregulation of CB1 receptor is key in Huntington`s disease and demonstrate that activation of these receptor with THC attenuates disease progression in mouse models of HD [1] . In this communication we show preliminary results indicating that treatment with THC in primary cultures of astrocytes and neurons expressing wild-type (wtHTT) pEGFP-Q23 or mutant (mtHTT) pEGFP-Q74 plasmid, prevents the accumulation of cytosolic mYtHTT selectively and decreases the number of aggregates. Furthermore, we show that THC-induced autophagy impairment occurs in neurons, whereas in astrocytes it induces autophagic flux. These results may help understanding the conditions under which cannabinoid-induced autophagy can lead to cell death or cell survival, as well as the mechanisms and molecules that are involved in their action against HD.

This work was funded by Agencia Estatal de Investigación (PID2019-105699RB-I00).

Blázquez, C. et al. Loss of striatal type 1 cannabinoid receptors is a key pathogenic factor in Huntington's disease. Brain 134, 119–136 (2011).

0311-R-P

Autogaphy-mitophagy-endoplasmic reticulum stress axis regulation in human fibroblasts with parkinsonian LRRK2 R1441G mutation

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Autophagy is a mechanism responsible for the degradation of cellular components to maintain their homeostasis. However, autophagy is commonly altered and compromised in several diseases, including neurodegenerative disorders. Parkinson´s disease (PD) can be considered a multifactorial disease because environmental factors, genetic factors and aging are involved. Several genes are involved in PD pathology, among which the LRRK2 gene and its mutations, inherited in an autosomal dominant manner, are responsible for most genetic PD cases. The R1441G LRRK2 mutation is, after G2019S, the most important in PD pathogenesis. Our results demonstrate a relationship between the R1441G LRRK2 mutation and a mechanistic dysregulation of autophagy that compromises cell viability. This altered autophagy mechanism is associated with organellar stress including mitochondrial (which induces mitophagy) and endoplasmic reticulum (ER) stress, consistent with the fact that patients with this mutation are more vulnerable to toxins related to PD, such as MPP^+.

0312-P

Δ9-TETRAHYDROCANNABINOL PROMOTES FUNCTIONAL REMYELINATION IN THE MOUSE BRAIN

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Δ^9-Tetrahydrocannabinol (THC), the most prominent active constituent of the hemp plant *Cannabis sativa*, confers neuroprotection in animal models of multiple sclerosis (MS). However, the possible effect of THC on oligodendro-

cyte regeneration and myelin repair has never been studied. Here, by using oligodendroglia-specific reporter mouse lines in combination with 2 models of toxin-induced demyelination, we show that THC administration enhanced oligodendrocyte regeneration, white matter remyelination, and motor function recovery. Interestingly, THC also promoted axonal remyelination in organotypic cerebellar cultures ex vivo. THC remyelinating action relied on the induction of oligodendrocyte precursor cell cycle exit and differentiation *via* CB_1 cannabinoid receptor activation. Overall, our study identifies THC administration as a promising pharmacological strategy aimed to promote functional CNS remyelination in demyelinating disorders as MS.

0322-R/M-P

Calpain-2 nucleocytoplasmic trafficking is involved in preconditioning-induced neuronal ischemic tolerance

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The activation or inhibition of specific signalling pathways mediated by subtoxic stimulus, called preconditioning (PC), is one of the most important endogenous mechanisms responsible for increased brain tolerance against ischemia [1]. In patients, we confirmed that transient ischemic attack (TIA) prior to ischemic stroke represent a clinical equivalent of brain PC [2]. Furthermore, we described that ischemic PC (IPC) increases MDM2-p53 complex interaction, which prevents ischemia-induced p53 stabilization, leading to neuronal ischemic tolerance (IT) in both "*in vitro*" and "*in vivo*" stroke models [3].

Here, we study the potential role of calpains, an important cellular system controlling protein stability, in preconditioning-mediated neuronal IT.

We used oxygen glucose deprivation (OGD; 5 min) plus 2h of reoxygenation, as a model of IPC in primary culture of cortical neurons, followed by 30 min of OGD and 4 h of reoxygenation (IPC+OGD/R). In parallel, neurons were exposed to Normoxia or IPC. Our results showed that IPC prevented neuronal apoptosis and caspase-3 activation after ischemia. Although mRNA levels of the two higher expressed calpain isoforms in the brain, CAPN1 and CAPN2, remained unaltered, IPC prevented the increase in protein levels caused by OGD/R, suggesting posttranslational modifications. Moreover, IPC abrogated ischemia-induced nuclear translocation of CAPN2 and calpain-mediated proteolysis of fodrin, which is involved cytoskeleton integrity. Genetic depletion (siRNA) or activity inhibition (ALLN) of CAPN2 mimicked the neuroprotective function of IPC. Finally, IPC downregulated calpastatin expression levels after OGD/R, which is the only known endogenous inhibitor of calpains.

In conclusion, our results suggest that IPC controls CAPN2-mediated proteolysis of fodrin by modifying CAPN2 subcellular location, which is involved in neuronal IT.

ISCIII: PI18/00103 - PI18/00285 - RD16/0019/0018 - FEDER European regional development fund - JCyL: IES007P17 - CLU201703 P.O.FEDER CyL1420 and EDU/556/2019.

[1] Iadecola, C. and J. Anrather. Stroke research at a crossroad: asking the brain for directions. Nat Neurosci, 2011. 14(11): p. 1363-8. [2] Ramos-Araque ME, Rodriguez C, Vecino R, et al. The neuronal ischemic tolerance is conditioned by the Tp53 Arg72Pro polymorphism. Transl Stroke Res. 2019 Apr; 10(2):204-215. [3] Vecino R, Burguete MC, Jover-Mengual T, et al. The MDM2-p53 pathway is involved in preconditioning-induced neuronal tolerance to ischemia. Sci Rep. 2018 Jan 25; 8(1):1610.

0348-R-P

Development and characterization of a phosphorylation reporter of CaMKII activity: Application to study the mechanisms of synaptic plasticity

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Intracellular Ca^{2+} oscillations and subsequent activation of Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII) are common hallmarks in the signaling pathways of many cell types. In SNC neurons, CaMKII plays a central role in several forms of synaptic plasticity, including long-term potentiation (LTP), which support learning and memory functions. In the postsynaptic environment, CaMKII is a central hub that orchestrates, among others, glutamate receptor trafficking, cytoskeleton dynamics or gene expression. It is then crucial to have tools available that allow us to understand the molecular mechanisms involved in those processes.

Here, we have developed a reporter of CaMKII phosphorylation basically configured as a fusion protein that includes a fluorescent protein, a nuclear export signal, a poly-myc tag, and a short sequence of the protein Synapsin-1a, that is specifically phosphorylated by CaMKII.

An initial characterization in Hela cells expressing CaMKII confirms that the reporter is specific and sensitive to phosphorylation by this kinase. Next, we checked the reporter in cultured hippocampal neurons at DIV15-18. For that, we used a lentiviral delivery system and included the sequence of an intrabody (FingR.PSD95) to locate the reporter in the postsynaptic environment. We observed increases in the phosphorylation of the reporter after Bicuculline or NMDA stimulation. Lentiviral overexpression of CaMKII increase the phosphorylation notably, and we managed to reduce it with a CaMKII inhibitor, KN-93. Further, our data indicate that Neurogranin (Ng), an abundant Calmodulin (CaM)-binding protein in hippocampal neurons, modulates CaMKII activity. Ng is highly enriched in dendrites and spines, and its deficit in mice leads to impaired learning and memory. In cultures where endogenous Ng expression was silenced the levels of phosphorylation of the reporter increased notably, while Ng overexpression did not change the phosphorylation levels as compared with that observed in controls. These data indicate that Ng modulates CaMKII activity, acting as a brake of excessive calcium/calmodulin signaling in the postsynaptic element. Further, we hypothesize that local Ng levels are critical to postsynaptic excitability, regulating the transition between LTD and LTP response to a given stimulus.

Garrido-García, A. et al. (2019) 'Neurogranin Expression Is Regulated by Synaptic Activity and Promotes Synaptogenesis in Cultured Hippocampal Neurons', Molecular Neurobiology, 56(11), pp. 7321–7337. Gross, G. G. et al. (2013) 'Recombinant Probes for Visualizing Endogenous Synaptic Proteins in Living Neurons', Neuron, 78(6), pp. 971–985. Rossetti, T. et al. (2017) 'Memory Erasure Experiments Indicate a Critical Role of CaMKII in Memory Storage', Neuron, 96(1), pp. 207-216.e2.

0351-R-P

Calcium-Dependent Regulation of the Neuronal Glycine Transporter GlyT2 by M2 Muscarinic Acetylcholine Receptors

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The neuronal glycine transporter GlyT2 modulates inhibitory glycinergic neurotransmission and plays a key role in regulating nociceptive signal progression. The cholinergic system acting through muscarinic acetylcholine receptors (mAChRs) also mediates important regulations of nociceptive transmission being the M2 subtype the most abundantly expressed in the spinal cord. Here we studied the effect of M2 mAChRs stimulation on GlyT2 function co-expressed in a heterologous system with negligible levels of muscarinic receptor activity. We found GlyT2 is down-regulated by carbachol in a calcium-dependent manner. Different components involved in cell calcium homeostasis were analysed to establish a role in the mechanism of GlyT2 inhibition. GlyT2 down-regulation by carbachol was increased by thapsigargin and reduced by internal store depletion, although calcium release from endoplasmic reticulum or mitochondria had a minor role on GlyT2 inhibition. Our results are consistent with a GlyT2 sensitivity to intracellular calcium mobilized by M2 mAChRs in the subcortical area of the plasma membrane. A crucial role of the plasma membrane sodium calcium exchanger NCX is proposed.

0354-P

The Expression of Neurogranin, a modulator of postsynaptic excitability, is regulated by synaptic activity

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Neurogranin (Ng) is a postsynaptic protein enriched in the dendrites and spines of many telencephalic principal neurons. Its deficit is associated with poor cognitive performance in mice. Ng sequesters Calmodulin (CaM) at low calcium and releases it at higher calcium levels. In this way, Ng dynamically controls the local availability of CaM along the multiple synaptic environments. Previous work of our group using hippocampal primary cultures showed that Ng expression is specifically affected by the activity of NMDA receptors (NMDA-R), as treatments with AP5 (NMDA-R antagonist) drastically reduced Ng expression, both at protein and mRNA levels, whereas AMPA-R antagonists showed no effect. In addition, adult newborn neurons in the rodent Dentate Gyrus initiate Ng expression during their fourth-sixth week of life, a period characterized by an enhanced synaptic excitability and the integration of these neurons in the hippocampal circuits. Although Ng is expressed in most if not all the pyramidal and granular neurons of the adult hippocampus, we observed that only 10-20% of the neurons in our hippocampal cultures express adult levels of Ng.

Here, we show that hippocampal neurons cultured with media that promote synaptic activity (Neurobasal+) increase the percentage of Ng expressing neurons to 50%. One candidate to regulate Ng expression in a NMDAR dependent manner is Histone Deacetylase 4 (HDAC4), a repressor of synaptic genes such as Synapsin and vGlut. Nuclear translocation of HDAC4 antagonizes the induction of activity-dependent expression programs in neurons receiving low NMDA-R input. Using several HDAC inhibitors, we found that selective HDAC4 inhibitors induce a significant increase in Ng protein levels. We then tested to over-express HDAC4 mutants with nuclear or cytoplasmic localization and found that HDAC4-Cyto, unable to repress transcription, induced an increase in Ng expression, whereas HDAC4-Nuc, that accumulates in nucleus and acts as a constitutive repressor, decreased Ng both at the protein and mRNA levels. Further, HDAC4-Nuc abro-

gated the increase of Ng expression in cultures submitted to long-term treatment with NMDA. Together, these results strongly suggest that HDAC4 is involved in the regulation of Ng expression, that in turn, depends on proper levels and patterns of synaptic activity.

1. Garrido-García, A. et al. Neurogranin Expression Is Regulated by Synaptic Activity and Promotes Synaptogenesis in Cultured Hippocampal Neurons. Mol. Neurobiol. 56, 7321–7337 (2019). 2. Sando, R. et al. HDAC4 governs a transcriptional program essential for synaptic plasticity and memory. Cell 151, 821–834 (2012). 3. Zhu, Y. et al. Class IIa HDACs regulate learning and memory through dynamic experience-dependent repression of transcription. Nat. Commun. 10, 3469 (2019).

0356-R-P

RANOLAZINE PREVENTION OF Aβ1-42 DELETERIOUS EFFECTS ON ASTROCYTES AND NEURONS IN PRIMARY CULTURE

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Ranolazine is used as an antianginal and antiarrhythmic drug. On the nervous system have also been described as antidiabetic and anti-inflammatory effects. Based on these effects, we have studied the response to ranolazine of astrocytes and neurons in primary culture. Different concentrations of ranolazine were used (10-7, 10-6 and 10-5 M) and added to rat neurons and astrocytes in primary culture for 24 hours. We measured the inflammatory mediators IL-β and TNF-α using ELISA technique. In addition, the protein expression levels of PPAR-γ, Citocrhome c, HIF, Smac/DIABLO, Mn-SOD and Cu/Zn-SOD were determined using the Western blot technique. Under these experimental conditions, ranolazine produced a decrease in pro-inflammatory mediators (IL-1β and TNFα) in astrocytes in primary culture but not in neurons. Furthermore, ranolazine, only in astrocytes, increased the expression of the anti-inflammatory protein PPAR-γ and diminished apoptosis produced by Amyloid $\beta 1_{-42}$. On the other hand, ranolazine increased Cu/Zn-SOD and Mn-SOD proteins after addition of ranolazine (10-7, 10-6 and 10-5 M) to astrocytes in primary culture. In conclusion, ranolazine, inhibited the genesis of pro-inflammatory mediators such as IL-β and TNF-α, promoted the expression of anti-inflammatory proteins, such as PPAR-γ, diminished Citochrome c, AIF and Smac/DIABLO apoptotic proteins and increased the antioxidant proteins Mn-SOD and Cu/Zn-SOD, at concentrations corresponding to those used in clinical practice. These effects were evidenced only in astrocytes without any effect in neurons in primary culture.

0363-P

Gene expression study of potential peripheral blood biomarkers associated to psychotic processes and response to treatment in Schizophrenia

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Schizophrenia is a multifactorial psychiatric pathology with a worldwide incidence of 1%, for which standard treatment is based on antipsychotic medication [1] . However, the variability of treatment response indicates that the genetics of each individual plays a key role. Previous genetic studies have discovered schizophrenia biomarkers in post-mortem brain tissue [2] . However, having access to brain only after patient's death is one of the strongest limitations in the study of neuropsychiatric disorders, since one cannot study the progression of the disease and, also, because medication can alter the architecture and connectivity of the brain, thus conducting the researcher to misleading conclusions. As a result, recent studies have focused on new tissues that circumvent this difficulty. These studies have found differences in gene expression with evidence for an implication in brain pathophysiology in psychotic and neurodegenerative disorders [3] . Thus, the aim of the present study is to identify new markers associated with the psychotic process and response to treatment through a longitudinal study of a cohort of patients who belong to a First Psychotic Episode Unit (FSEU) at Valencian Clinical Hospital. Specifically, the expression in peripheral blood of several target genes (*AKT1*, *AKT3, SF3B1, DPYD, NDEL1* and *SEMA4D*) was analyzed in those patients by real-time quantitative PCR before the start of medication and after two years of antipsychotic treatment. Those expression levels were also compared with a cohort of age- and gender-matched healthy controls. Our results show a significantly lower expression of *SEMA4D* in patients after treatment when compared with controls, a finding that suggests the association of this gene with the progression of the pathology. In addition, *DPYD* and *NDEL1* were downregulated in patients after treatment when compared to the expression levels before starting any medication. These results provide evidence of differential gene expression that may help to elucidate psychotic mechanisms and to explain differences in response to treatment.

1. Owen, M. J., Sawa, A. & Mortensen, P. B. Schizophrenia. The Lancet (2016). doi:10.1016/S0140-6736(15)01121-6 2. Gilabert-Juan, J. et al. Semaphorin and plexin gene expression is altered in the prefrontal cortex of schizophrenia patients with and without auditory hallucinations. Psychiatry Res. (2015) 3. Chana, G. et al. Biomarker investigations related to pathophysiological pathways in schizophrenia and psychosis. Frontiers in Cellular Neuroscience (2013). doi:10.3389/fncel.2013.00095

0373-R/M-P

Mitochondrial reactive oxygen species regulate cortical organization in the developing brain

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Reactive oxygen species (ROS) participate as second messengers in different signaling pathways. Then, ROS levels regulation is essential to maintain cell physiological processes and homeostasis. In the central nervous system, ROS play key roles in cell proliferation, neuronal differentiation, and synapse maintenance (1). Moreover, mitochondrial ROS (mROS) generated by astrocytes regulate brain metabolism and behavior in the adult. Specifically, the reduction of astrocytic mROS alters neuronal structure and integrity and causes cognitive impairment (2). However, the impact of mROS generation during brain development is unknown.

We used mice genetically engineered to constitutively express a mitochondrial-tagged isoform of the antioxidant enzyme catalase (mCAT) to downmodulate ROS levels (2). We perform in vitro and in vivo experiments to follow up neurodevelopmental issues. By using western blot and immunocytochemistry, we found increased levels of mature neuron markers, TAU and MAP2, in primary mCAT neurons from 1-3 days in vitro, in comparison with wild-type neurons. Accordingly, we analyzed the expression of progenitor cell marker, NESTIN, undifferentiated neuronal marker TUJ-1, and MAP2 in different brain areas of both genotypes, including the hippocampus, cerebral cortex, and cerebellum, at early (E12, E15) and later (E18) embryonic and postnatal (P0, P7, P21) development. Although no significant differences in level expression of neuronal markers over time were observed between genotypes, we found a mROS-dependent organization of the cerebral cortex from E12. Thus, TUJ-1 positive neurons were enriched in the interzone layer of mCAT animals, compared to wild-type, which affect cortical radial distribution of MAP2-positive neurons in the neocortex from E15. However, no differences were observed in the cerebellum and hippocampus.

These results suggest a key role of mROS in cell migration that could determine the structural organization of the developing cerebral cortex. Then, endogenous mROS levels might play a critical role in layer patterning in the cerebral cortex during brain development.

ISCIII :PI18/00265-PI18/00103-RD16/0019/0018-FEDER European regional development fund- JCyL:IES007P17-CLU201703- P.O FEDER CyL1420 and EDU/556/2019.

(1) Oswald MCW, Garnham N, Sweeney ST, Landgraf M. Regulation of neuronal development and function by ROS. FEBS Lett. 2018;592(5):679-691. doi:10.1002/1873-3468.12972 (2) Vicente-Gutiérrez C, Bonora N, Bobo-Jimenez V, et al. Astrocytic mitochondrial ROS modulate brain metabolism and mouse behaviour. Nat Metab. 2019;1(2):201-211. doi:10.1038/s42255-018-0031-6,

0399-P

Potential role of mTORC2/Akt in recover axonal length and neuronal viability after ischemic damage

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mTOR (mammalian target of rapamycin) is a serine/threonine kinase that is part of two multiprotein complexes named mTORC1 and mTORC2. They regulates important cellular functions such as cell growth, survival or cytoskeleton reorganization, among others. Both complexes have different protein composition. Among them, Raptor is a scaffold protein of mTORC1, whereas and Rictor is a regulatory scaffold of mTORC2. In contrast PRAS40 acts as a mTOR inhibitor. The phosphorylation levels of some of these regulatory proteins modulate mTORC activity that can be evaluated as the phosphorylation levels of two target proteins: P70S6K and Akt (from mTORC1 and mTORC2, respectively). After brain ischemia, neurons suffer a dramatic depletion of glucose, oxygen and survival factors due to a disruption of cerebral blood flow. Therefore, brain ischemia has a high impact in mTORC activity, as we previously reported.

The aim of this work is analyze the impact of oxygen and glucose deprivation (OGD) and the effect of regeneration during 24 hours after 17 hours of OGD (OGD/R) on: i) the activity levels of mTORC1/2; ii) the phosphorylation status of some mTORC components; iii) neuronal viability; iv) neurites status (length and number per neuron).

Using primary culture neurons from mouse cerebral cortex, we observed that OGD reduces the phosphorylation of mTORC components and their activity, as indicated the western blots. Moreover, OGD induces a reduction time dependent, in neuron viability (measured by MTT assay) and an increase in apoptosis (measured as amount of cleaved caspase 3). In parallel, we observed, by immunofluorescence a shortening in length of neurites and a reduction on the number of neurites per neuron. After recovery (oxygen, glucose and growth factors) we observed a recovery in neuron viability and in their axon length, but not in the numbers and length of other neurites, and partially recover Akt phosphorylation levels. These data might suggest that mTORC2 could be involve in partially recovery of neurons after OGD.

It would be important to understand in depth the molecular

mechanisms underlying mTORC2 after OGD/R, in order to be able to propose mTORC2 as a therapeutic target for ischemic stroke

- Perez-Alvarez, M. J. et al. 2018. Role of mTORC1 controlling proteostasis after brain ischemia. Front. Neurosci.

- Saxton, R. A. and Sabatini, D. M. 2017. mTOR signaling in growth, metabolism and disease. Cell 168(6): 960-976.

0442-R-P

Elucidating the molecular mechanisms underlying glia-to-neuron direct reprogramming

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Cell reprogramming has redefined regenerative medicine by offering the possibility of theoretically obtaining any cell type of clinical interest from differentiated somatic cells. In the brain, *direct lineage reprogramming* is used to convert glial cells, such as astrocytes, into neurons, to compensate neuronal degeneration occurred in neurological diseases. During the process of glia-to-neuron conversion, cells undergo dramatic changes, including morphological, metabolic and proteomic adjustments, in order to rebuild their cellular program. The adaptation events taking place to acquire the new neuronal phenotype involve a substantial level of cellular stress. Only cells capable of managing conversion under controlled stress levels have the opportunity to become induced neurons. Cell death is shown as a major limiting factor in neuronal reprogramming.

In this context, we hypothesize that cellular pathways involved in the stress response, such as autophagy and unfolded protein response (UPR) might play fundamental roles. Autophagy is a highly regulated mechanism by which cells degrade unnecessary or dysfunctional components, allowing the recycling of molecular elements. UPR is involved in protein quality control to help cells restore normal function.

In this project, we are developing an *in vitro* and *in vivo* platform that allows us to understand the molecular mechanism by which a cell transitions to a different identity. We will investigate the role of autophagy and UPR, during the conversion of astrocytes into induced neurons by the proneural factor Neurogenin 2, using state-of-the-art reprogramming and neurobiology approaches.

Vignoles R, Lentini C, d Orange M, Heinrich C. Direct lineage reprogramming for brain repair: breakthroughs and challenges. Trends Mol Med. 2019 Jul 29;25(10):897–914. Wu Y, Li Y, Zhang H, Huang Y, Zhao P, Tang Y, et al. Autophagy and mTORC1 regulate the stochastic phase of somatic cell reprogramming. Nat Cell Biol. 2015 Jun;17(6):715–725.

0450-P

Staufen 2-dependent recognition of specific RNA secondary structures within mRNA 3'-UTR results in regulation of transport and localization to distal dendrites

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Neurons localize mRNAs near synapses where their translation can be regulated by synaptic demand and activity. This is mainly orchestrated by the regulation through RNA-binding proteins which bind RNA sequence elements within its target 3'-UTRs. However, the impact of RNA structure on gene regulation is starting to be explored.

In this regard, Staufen 2 (Stau2) is a key player in mRNA localization in polarized cells which recognizes and binds double-stranded RNA structures. Here, we used the physiological Stau2 target mRNA Rgs4 as a model to show the functional relevance of specific RNA secondary structures for gene regulation in primary neurons. By doing colocalization assays in dendrites we found that an identified structure serves as the driving force for Stau2 assembly as its deletion reduces Stau2-Rgs4 reporter colocalization. Stau2 binding through Rgs4 structure is also important for a luciferase reporter protein translation. Moreover, Rgs4 RNA localization to dendrites is dependent on Rgs4 RNA structure. Together, our data is consistent with the idea that the recognition of those RNA secondary structures by Stau2 results in mRNA transport and localization to distal dendrites, which enables subsequent activity-dependent protein synthesis at synapses.

0499-R/M-P

Neuroprotective effects of abscisic acid on the SH-SY5Y cell line under proinflammatory conditions

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Neuroinflammation and insulin resistance are key related pathophysiological features present in neurodegenerative diseases such as Alzheimer's disease [1] . Abscisic acid (ABA) is an essential phytohormone for plant physiology. However, its presence has been described in mammals, including in the brain. Previous studies have shown ABA anti-inflammatory and insulin-sensitizing properties, as well as its prevention of cognitive decline in rodent models [2]. Nevertheless, the specific intracellular mechanisms by which ABA protects neurons in a proinflammatory environment have not been elucidated.

Consequently, the first objective of the present study is to understand the effect of ABA in a well-established model of lipopolysaccharide-induced inflammation by in a human neuroblastoma cell line, SH-SY5Y. In particular, we will study whether ABA protects insulin and other trophic factors signaling under these conditions by Western blot analysis. On the other hand, it has been reported that ABA is able to induce differentiation and apoptosis in glioma cells 3 , implying an anticancer potential. Therefore, a second objective consists in evaluating the ABA ability to differentiate SH-SY5Y to neurons, by morphological analysis and Western Blot.

We expect elucidate whether ABA has a differentiating or apoptotic role in unmature SH-SY5Y cells, or a protective role in differentiated neurons by facilitating insulin signaling despite the proinflammatory environment. These objectives will clarify the mechanisms by which ABA modulates key signaling in survival and growth control in neuronal models.

1.Arnold, S. E. et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: Concepts and conundrums. Nature Reviews Neurology 14, 168–181 (2018) 2.Magnone, M. et al. Abscisic acid: A conserved hormone in plants and humans and a promising aid to combat prediabetes and the metabolic syndrome. Nutrients 12, 1–13 (2020) 3.Zhou, N. et al. Abscisic-acid-induced cellular apoptosis and differentiation in glioma via the retinoid acid signaling pathway. Int. J. Cancer 138, 1947–1958

0501-R-P

Coordinated role of imprinted genes Plagl1 and Cdkn1c in neural stem cells differentiation

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Adult neurogenesis in the mammalian brain is supported by neural stem cells (NSCs), characterized by their abilities of self-renewal and differentiation into the three lineages of the central nervous system (CNS): neurons, astrocytes and oligodendrocytes (1). Both capabilities are maintained by specific intracellular mechanisms, which are activated by extracellular signaling from the microenvironment or niche where stem cells reside *in vivo*. One of these neurogenic niches where NSCs are located is the subventricular zone (SVZ), lining the lateral ventricles. Genomic imprinting is an epigenetic process involved in gene dosage control during fetal and postnatal development (2). *Cdkn1c* is an imprinted gene expressed by the maternal allele and encodes for the P57 protein, that plays a crucial role during development and also in the maintenance of quiescence in NSCs from the adult subgranular zone (SGZ)(3, 4). On the other hand, the imprinted gene *Plagl1*, which is expressed by the paternal allele, is known as a master-regulator of an imprinted genes network (5). Among them, there is *Cdkn1c*, positively regulated by *Plagl1* during neurodevelopment (6). However, the interaction between these two genes has not been demonstrated in adult neurogenesis. This work characterizes the expression of the imprinted gene *Plagl1* and its coding protein, ZAC1, both *in vivo* and *in vitro*, showing a significant expression in the SVZ and during the differentiation of adult NSCs. In addition, the role *Cdkn1c*, is also studied by *in vivo* electroporation in *Cdkn1c* deficient SVZ. Overexpression of the *Plagl1* gene in NSCs, shows that ZAC1 positively regulates P57, proposing this strategy of overexpression of the *Plagl1* gene as a rescue mechanism for P57 deficient animals.

1. Gage, F. H. (2000) Science, 287(5457), pp.1433-1438 2. Montalbán-Loro, R. et al. (2015) World Journal of Stem Cells, 7(4), p.700 3. Tury, A. et al. (2011) Cerebral Cortex, 21(8), pp. 1840-1856 4. Furutachi, S. et al. (2013) EMBO Journal, 32(7), pp- 970-981 5. Varrault, A. et al. (2006) Developmental Cell, 11(5), pp. 711-722 6. Rraklli. V. et al. (2016) Stem Cell Research, 16(1), pp. 1-9

12.- Molecular Parasitology and Emerging Infections

0236-R-OS

LEISHMANICIDAL EFFECTS OF THE NOVEL SULPHONAMIDE COMPOUND CPS4

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In the fight against leishmaniasis, the identification of novel therapeutic compounds is a global necessity due to the limitations and resistances associated with current therapeutic options [1]. In this work, we present the characterization of the leishmanicidal activity and ADME-T properties of the novel sulphonamide CPS4.

Following previous studies, several candidates were selected due to similarity with the antileishmanial compound CPE2 [2]. Compounds were evaluated based on predicted ADME properties [3]. After purchasing them, their EC50 values in the promastigote form of *L. major* and *L. infantum* were annotated through MTT assays [4]. CPS4 emerged as a promising candidate with ADME properties compatible with oral administration and exhibiting EC_{50} values in the micromolar range.

The toxicity of CPS4 was evaluated using murine derived macrophages [4]. After proving that CPS4 was not toxic to mammal cells, its activity in the amastigote form was explored using *L. major in vitro* infections [4]. Significant reductions of the parasite burden and percentage of infected cells were observed at sub EC_{50} concentrations of CPS4.

Considering the high activity over amastigote forms, the low toxicity of CPS4 (amastigote selectivity index [S.I.] >40), and the compatibility of predicted pharmacokinetic properties to oral administration and further pharmacologic modifications, we present compound CPS4 as a promising candidate for the treatment of leishmaniasis. Future perspectives include the testing of this compound using *in vivo* infections. In summary, these results represent a valuable step to identify novel candidate drugs for the treatment of leishmaniasis.

Acknowledgements
This research was funded by Fundación “La Caixa” (LCF/PR/PR13/11080005) and Fundación Caja Navarra, Gobierno Navarra Salud (12/2017), Fundación Roviralta, Ubesol, Ministerio de Educación Cultura y Deporte (FPU17/03304), Government of Navarre, Laser Ebro, and Inversiones Garcilaso de la Vega S.L.

References 1. PMID: 32875362 2. Peña-Guerrero, et al. unpublished 3. PMID: 28256516 4. PMID: 31718000

0488-OS

eIF5A is activated by RNA virus infection and this activation is required for virus replication

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The eukaryotic initiation factor 5A (eIF5A) is an essential protein involved in translation elongation and it has been proposed to play a role in cell proliferation, differentiation, senescence, apoptosis, inflammatory processes, regulation of transcription and RNA metabolism. eiF5A is the only known protein to contain the unique amino acid hypusine, a residue that results in the mature, active form of eIF5A. Hypusin is formed by conjugation of the aminobutyl moiety of the polyamine spermidine to the lysine residue K50 of eIF5A. Polyamines have been reported to play an important role in the replication of different RNA viruses but the involvement of the polyamine-modified translation initiation factor eIF5A in virus replication is less explored. Our results show that both virus infection and treatment with synthetic double-stranded RNA (dsRNA) result in eIF5A hypusination and that activation of eIF5A is essential for the replication of several RNA viral agents including influenza, VSV, Chikungunya, Mayaro, Punta Toro or Unav virus. In addition, our data suggest that the induction of ER stress/unfolded protein response upon eiF5A inhibition may be one of the mechanisms involved in the broad sensitivity of viruses to inhibition of eIF5A. Targeting of eIF5A may be a potential strategy to control virus replication.

0492-OS

Identification of cell-type specific gene signatures associated with the development of natural acquired immunity to malaria.

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Malaria is a parasitic infection caused by *Plasmodium* that affects more than 200 million people worldwide. Endemic populations are repeatedly exposed to parasite infection, and most adults develop pre-immunition, an effective immunity to control parasitaemia and symptoms associated with the disease. However, children under 5 y/o are most at risk from severe malaria due to their scarce exposure to the parasite and, consequently, pre-immunition is inexistent or very low. Several transcriptomic studies have been conducted across different populations to study gene signatures related to immune response. Here, we aim to integrate different datasets to identify cell-type specific gene signatures associated with different immune responses to malaria.

We have re-analysed RNA-Seq and microarray data from publicly available datasets on blood samples (i.e., whole blood or PBMCs) involving individuals with different ages, immune responses and infection status to malaria to define condition-specific genes. To characterise cell types responsible for the development of immunity, we performed a deconvolution analysis defining gene signature matrix for immune cell types, including cellular sub-populations of monocytes, T and B cells.

Our results suggest a differential transcriptional regulation between individuals that are symptomatic and those who have developed pre-immunition and a degree of resistance to infection, with gene expression signatures well-kept upon infection. Moreover, we found that immune-cell populations are strongly affected by their preceding immune status and infection frequency. Finally, we identified pro-inflammatory genes and a higher proportion of innate immunity cells in symptomatic compare to asymptomatically individuals, showing the latter a higher proportion of B and Tfh cells related to their progressive acquired immunity to malaria.

0028-R-P

Erythrocyte membrane protein band 3 involved in the immune Response to P. Vivax Infection

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Introduction: Malaria is a worldwide impact disease, caused by parasites of the Plasmodium genus. During its stage in man it has contact with the erythrocyte and inside it exports proteins destined to modify the host cell and continue its survival cycle. There are still aspects that are not clear, especially regarding the proteins that are expressed in the erythrocyte membrane and the role that they play in the immune response of the infected person. The objective of this work was to identify antigenic proteins that are expressed on the red blood cell membrane in P. vivax infection, with a view to finding new molecular targets of interest.

Materials and methods: In this experimental study, the proteins of the membranes of erythrocytes infected with P. vivax were subjected to electrophoresis and Western Blot processes using primary antibodies from patients diagnosed with P. vivax malaria, which after immuno-reactive signals Proteins were characterized by mass spectrometry using MALDI TOF / TOF.

Results: The immunoreactive signal resulted in the identification of the Protein Band 3 anion transport protein (Score 323). Erythrocyte Band 3 protein could be acting as a receptor for multiple parasite proteins, forming complexes with immune response capacity.

Conclusion: During infection by different Plasmodium species, modifications generating the immune response occur in the red blood cell membrane, affecting proteins of great structural and functional importance, which merit further study to clarify the true role they play during infection and define whether these molecular targets can help find tools with a preventive function.

1. WHO (2019) World Malaria Report 2019. World Health Organization. 2. Boddey JA, Moritz RL, Simpson RJ, Cowman AF. Role of the Plasmodium export element in trafficking parasite proteins tothe infected erythrocyte. Traffic. 2009 Mar;10(3):285-99. 3. Hviid L, Jensen ATR, Rollinson D, Stothard JR. Chapter Two - PfEMP1 A Parasite Protein Family of Key Importance in Plasmodium falciparum Malaria Immunity and Pathogenesis. Advances in Parasitology. Volume 88: Academic Press; 2015. p. 51-84.

0097-R/M-P

Alternative therapeutic guanidine-based nanoparticles against Staphylococcus aureus and Pseudomonas aeruginosa

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This study aimed to determine the antibacterial effects of 3 guanidine-based nanoparticles, advancing in the development of alternative therapeutic compounds to inhibit bacterial growth and/or biofilm formation. The antibacterial activity of these new compounds was evaluated using collection strains of *Staphylococcus aureus* ATCC25923 (Gram-positive bacterium) and *Pseudomonas aeruginosa* ATCC27853 (Gram-negative bacterium). To appraise the antibacterial activity, minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), minimum biofilm inhibitory concentration (MBIC), and minimum biofilm eradication concentration (MBEC) assays were utilized. Table 1 shows

data obtained in the present study, observing different effects depending on both the type of bacteria and its growth state. Moreover, all the nanoparticles studied showed higher effects on planktonic bacteria (MIC, MBC) than on those bacteria forming biofilm (MBIC, MBEC). The results place this new family of guanidine-based nanoparticles as a starting point for the development of novel and more potent agents against Gram-positive and Gram-negative bacteria, although more studies are needed.

Table 1. Antibacterial effects of guanidine-based nanoparticles.

S. aureus				
	MIC (μg/ml)	MBC (μg/ml)	MBIC (μg/ml)	MBEC (μg/ml)
NP1	≤ 1.95	≤ 1.95	500	> 2000
NP2	15.62	31.25	250	> 2000
NP3	≤ 1.95	≤ 1.95	1000	> 2000

P. aeruginosa				
	MIC (μg/ml)	MBC (μg/ml)	MBIC (μg/ml)	MBEC (μg/ml)
NP1	15.62	15.62	125	2000
NP2	7.81	7.81	250	1000
NP3	62.5	62.5	250	1000

Chang VS, Dhaliwal DK, Raju L, Kowalski RP. Antibiotic Resistance in the Treatment of Staphylococcus aureus Keratitis: a 20-Year Review. Cornea 2015;34(6):698-703. Popescu A, Doyle RJ. The Gram stain after more than a century. Biotech Histochem 1996;71(3):145-51.

0101-R/M-P

Inhibition of heavy metal toxicity in plant seed development

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Heavy metal pollution is one the most important environmental problems in marine, terrestrial, and freshwater areas. This study evaluates the modulating effect of different commercialized green-fertilizers on the toxicity of cadmium (Cd) to plant germination. Experiments were carried out in hydroponic systems, using three day-germinated lettuce seeds. Effects of the green-fertilizers (with or without cadmium) were assessed by measuring the hypocotyl growth after four days of treatment. The Cd concentration was selected as the EC50 (32.3 μM) calculated from dose-response experiments assessing the toxicity of Cd on hypocotyl elongation. Four fertilizers were tested to know the concentrations leading to a growth improvement, although only AgroKaP-Kalibre at 0.01 and 0.1% had a positive effect on the growth (60% improvement). Furthermore, this product shown a dose-dependent protective effect against the Cd toxicity, and it was statistically significant when AgroKaP-Kalibre was used at 0.5%. The results proved the efficiency of this bioactive product in alleviating Cd toxicity in plant seed development, being potentially useful to decrease ecological problems caused by heavy metals in different contaminated environments including grapevine culture.

Chang VS, Dhaliwal DK, Raju L, Kowalski RP. Antibiotic Resistance in the Treatment of Staphylococcus aureus Keratitis: a 20-Year Review. Cornea 2015;34(6):698-703. Saeed A, Bosch A, Bettiol M, Nossa-González DL, Erben MF, Lamberti Y. Novel Guanidine Compound against Multidrug-Resistant Cystic Fibrosis-Associated Bacterial Species. Molecules 2018;23(5):1158-1169

0122-R/M-P

A novel bis(pyrazolyl)methane compound as potential agent against Gram-positive bacteria

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This study aimed to determine the antibacterial effects of a focused library of bis(triazolyl)methane (1T-7T) and bis(pyrazolyl)methane nitrogen-based compounds (1P-11P), advancing in the development of alternative therapeutic compounds to fight against bacterial pathogens and its ability to become resistant to many antibiotics. The antibacterial activity of these 18 compounds was evaluated using collection strains of *Staphylococcus aureus, Enterococcus faecalis*, *Escherichia coli,* and *Pseudomonas aeruginosa*. To appraise the antibacterial activity, minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), minimum biofilm inhibitory concentration (MBIC), and minimum biofilm eradication concentration (MBEC) assays were utilized with different concentrations (2-2,000 μg/ml) of compounds. Moreover, MTT and Resazurin viability assays at 48h were performed in both human liver carcinoma HepG2 and human colorectal adenocarcinoma Caco-2 cell lines. Among all the synthetized compounds, only 2P at non-toxic concentrations showed an inhibitory effect on Gram-positive strains growth, although a bactericidal effect was not observed at these non-toxic doses. Moreover, 2P showed a significant biofilm effect at non-toxic doses for eukaryotic cell cultures. At higher doses, 2P had a lytic effect on *S. aureus* and *E. coli* strains, although these doses resulted toxic on eukaryotic cell cultures. The results place this 2P nitrogen-based compound as a starting point for the development of novel and more potent agents against Gram-positive bacteria.

Roy R, Tiwari M, Donelli G, Tiwari V. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence 2018;9(1):522-554. Pasero C, Agostino I, De Luca F, Zamperini C, Deodato D, Truglio GI, et al. Alkyl-guanidine Compounds as Potent Broad-Spectrum Antibacterial Agents: Chemical Library Extension and Biological Characterization. J Med Chem 2018;61(20):9162-9176

0177-P

Leishmanicidal activity of novel compounds

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Currently, there is no effective human vaccine against leishmaniasis. The main treatments administered have numerous disadvantages such as their high toxicity and economic cost, difficulty in administration or the appearance of resistance. Therefore, one of the current challenges is to find therapeutic targets and new active, safe and affordable treatments[1,2]. This work has focused on the study of the leishmanicidal activity of more than thirty novel compounds not tested to date. Thirteen of them showed IC_{50} values < 35 μM in procyclic promastigotes. Five compounds were very promising and further assays were performed with them. The results of BM15, BM18, BM29, BM36 and BM39 in promastigotes and amastigotes of *Leishmania major* as well as their cytotoxicity in murine peritoneal macrophages were studied. Interestingly, BM15, BM18 and BM29 significantly reduced the percentage of infection in macrophages, as well as the number of amastigotes per macrophage when compared to untreated samples (control). Unfortunately, BM36 and BM39 did not show activity against amastigotes at tested concentrations.

The most promising compound was BM15, which reduced the percentage of infected macrophages to 36.25% and the number of amastigotes per macrophage to 2.45 at low concentration (<10 μM).

1. Fernández-Rubio, C. et al. (2019): "Leishmanicidal activity of isoselenocyanate derivatives", Antimicrob Agents Chemother, 63(2):e00904-18.
2. Vacas, A. et al (2020): "LmjF.22.0810 from Leishmania major Modulates the Th2-Type Immune Response and Is Involved in Leishmaniasis Outcome", Biomed, Nafarroa, 8(11):452.

0195-R/M-P

New essential genes regulated by heme in Leishmania major revealed by RNA-seq analysis

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Heme is an iron-coordinated porphyrin essential in most aerobic organisms.Trypanosomatid parasites such as *Leishmania* are auxotrophic for heme since they lost its complete biosynthesis pathway during evolution and must obtain this essential compound from the infected host. Exploiting this heme dependency is a rational way to find new leishmanicidal agents. The aim of this work is to identify and characterize genes/proteins differentially regulated by heme in *Leishmania major*, and evaluate their potential use as new drug target. First, we analysed the transcriptomic differences between *L. major* promastigotes cultured in the presence or the absence of heme by RNA-seq. Samples were sequenced in an Illumina Nextseq 550 using a standard protocol for paired-end 75nt libraries and sequence data generated as fastq files were analyzed using miARma-Seq. Reads were aligned against *L. major* Friedlin reference genome from TriTrypDB version 31 and summarized into gene expression levels using Feature Counts. Differentially expressed genes (DEG) were detected by edgeR with a false discovery rate-adjusted *p* value (FDR) <0.05. Our preliminary results show 1,908 DEG (802 up-regulated in the absence of heme and 1,106 down-regulated). 12 DEG were selected for qPCR validation (6 up-regulated and 6 down-regulated). In order to perform a functional interpretation of the results, gene ontology (GO) terms of each of these genes were extracted from TriTrypDB database and a functional enrichment analysis was carried out. Among the most enriched terms (having a p-value <0.05) in the GO processes we found "regulation of developmental process" and "transmembrane transport" and between the molecular functions ontology: "heme binding" and "metal ion transmembrane transporter activity". Finally, some of the most up-regulated genes in the heme depletion condition were selected for further analysis, evaluating their intracellular localization and essentiality in the parasite using the CRISPR-Cas9 technology. *In situ* tagging of genes with a fluorescent tag showed that heme-regulated proteins are located in different subcellular compartments, from plasma membrane to the mitochondrion. Our results suggest that some of these proteins are essential for the parasite survival and could be candidates to become new therapeutic targets.

0282-R/M-P

Application of SWATH-MS proteomics to the study of the initial steps of parasite invasion in fasciolosis

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Fasciolosis is a foodborne zoonotic disease caused by trematodes of the genus *Fasciola*, of which *F. hepatica* is the most geographically widespread species. It is responsible for chronic infections causing significant economic losses in animal production, as well as constituting a sanitary concern as it can also affect human hosts [1]. Once ingested, the infective forms of the parasite reach the small intestine of the host and the newly excysted juvenile worms of *F. hepatica* (FhNEJ) are liberated. They cross the host's intestinal wall and undergo a complex migratory route that drives the parasites to their definitive location inside the bile ducts. Despite its importance, host-parasite relationships during the early stages of fasciolosis have received little attention [2]. In this context, we have set up an *ex vivo* model to shed light on the intestinal wall penetration by parasites, by using FhNEJ injection into small intestine portions of mice. Then, with the aim of characterizing the changes in the proteomic repertoire of FhNEJ before and after crossing the intestinal barrier, a proteomic approach based on Sequential Window Acquisition of all Theorical Mass Spectra (SWATH-MS) was developed. This novel technique allows simultaneous identification and quantification of all peptides present in complex biological samples and, despite its advantages in terms of quantitation precision and reproducibility, it has barely been used in parasitology. Following this methodology, up to 120 FhNEJ differentially expressed proteins were identified. The obtained results revealed changes in the expression levels of proteins involved in several key aspects of the early stages of *F. hepatica* infection such as proteolysis, metabolism or disruption of the host defence mechanisms.

Funding: Projects AGL2015-67023-C2-2-R and PID2019-108782RB-C22 funded by MCIU, AEI and FEDER

1. Siles-Lucas M, Becerro-Recio D, Serrat J, González-Miguel J. Fascioliasis and fasciolopsiasis: Current knowledge and future trends. Res Vet Sci. 2021 Jan;134:27-35. doi: 10.1016/j.rvsc.2020.10.011. 2. González-Miguel J, Becerro-Recio D, Siles-Lucas M. Insights into Fasciola hepatica Juveniles: Crossing the Fasciolosis Rubicon. Trends Parasitol. 2021 Jan;37(1):35-47. doi: 10.1016/j.pt.2020.09.007.

0321-P

Plasmodium vivax malaria genotyping through CSP gene sequence analysis

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Malaria remains one of the world's most deadly diseases today. In 2019, the World Health Organization (WHO) reported more than 200 million malaria cases worldwide and more than 400,000 deaths. Although the disease has been reported since antiquity and the pathogen that causes it, a protozoan parasite of the genus *Plasmodium*, has been known since the 19th century, to date there is still no commercially available vaccine that has demonstrated sufficient efficacy in preventing it, due to the great complexity of the parasite's life cycle, its different morphological forms and its great antigenic variability. Several clinical trials are currently underway that open the door to hope, the most promising being the one developed with the circumsporozoite protein, CSP, located on the surface of the sporozoite and involved in liver cell adhesion and invasion. So far, however, this vaccine has only been shown to be partially effective in preventing the spread of malaria caused by the *Plasmodium falciparum* parasite. This is not the case for malaria caused by *P. vivax*, which is responsible for more than 3% of malaria cases worldwide, and has a wide geographical distribution (Latin America, Middle East, South and Southeast Asia, Africa and Oceania). One of the reasons for this may be the large variability in the central domain of the *P. vivax* CSP gene. This variability gives rise to two genotypes of this parasite known as VK210 and VK247. In this work, using samples from patients previously diagnosed with malaria, we have analysed the presence of these genotypes in southern Mexico by sequencing the complete gene and analysing its sequences.

Atcheson, E., & Reyes-Sandoval, A. (2020). Protective efficacy of peptides from Plasmodium vivax circumsporozoite protein. Vaccine, 38(27), 4346–4354 Coppi, A., Natarajan, R., Pradel, G., Bennett, B. L., James, E. R., Roggero, M. A., Corradin, G., Persson, C., Tewari, R., & Sinnis, P. (2011). The malaria circumsporozoite protein has two functional domains, each with distinct roles as sporozoites journey from mosquito to mammalian host. Journal of Experimental Medicine, 208(2), 341–356

0323-P

Heterologous expression of Plasmodium vivax Pv38 gene in a prokaryotic system

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Malaria continues to be one of the main threats to human beings, especially in developing countries, where it causes, according to World Health Organisation (WHO) estimates, more than 400,000 deaths a year. For this reason, in addition to enhancing appropriate disease control measures, the development of new drugs and antimalarial vaccines is essential. And not only against *Plasmodium falciparum*, which is the species responsible for more than 90% of malaria cases worldwide, but also against *P. vivax*, which has a huge geographical distribution and is responsible for most cases of severe malaria outside the African continent. Thus, finding potentially immunogenic proteins and being able to produce them in appropriate expression systems is essential for the development of antimalarial vaccines against *P. vivax*. In this work we have sequenced the *Plasmodium vivax* Pv38 gene from patients previously diagnosed with malaria. This is a gene that is expressed in the late stages of the erythrocytic phase, in mature schizonts, when the main symptoms of malaria are triggered, and has previously been shown to generate humoral immune responses in animal studies. We have carried out heterologous expression in a bacterial system, after sequence optimisation, according to the expression system. The results have been analysed on acrylamide gels and the protein encoded by Pv38 has been detected by immunochemical techniques.

Mongui, A., Angel, D. I., Guzman, C., Vanegas, M., & Patarroyo, M. A. (2008). Characterisation of the Plasmodium vivax Pv38 antigen. Biochemical and Biophysical Research Communications, 376(2), 326–330. Wang, Y., Ma, A., Chen, S. B., Yang, Y. C., Chen, J. H., & Yin, M. B. (2014). Genetic diversity and natural selection of three blood-stage 6-Cys proteins in Plasmodium vivax populations from the China-Myanmar endemic border. Infection, Genetics and Evolution, 28, 167–174.

0346-R-P

Effect of the novel synthetic compounds R13 and R27 on Leishmania major

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Leishmania disease is one of the important health problems in tropical and subtropical countries around the world [1] . Conventional treatments based on antimonials have shown associated toxicity and drug resistance development [2] . More effective treatments such as Amphotericin B, are limited by their high cost [3] . Thus, the search for alternative therapies for leishmaniasis became a priority. Inspired by natural therapeutic molecules proved to have broad range of antimicrobial activities, several researchers aim to generate better synthetic compounds that are more efficient on microorganisms and less toxic on host cells [4] . In this work, the leishmanicidal activity of two synthetic compounds (R13 and R27) was investigated on *L. major* amastigotes under *in vitro* and *in vivo* conditions. Cytotoxicity assays performed in murine macrophages showed that our compounds have no toxicity on host cells at concentrations around 4.5 μg/ml (100% of macrophages survival). We proceed then our study using a compound concentration of 1 μg/ml. Results showed that our compounds were highly active against *L. major* amastigotes *in vitro*, and significantly decreased the number of amastigotes per macrophage at 1μg/ml. Furthermore, R13 and R27 were able to reduce the expression levels of several leishmania genes, involved in cell cycle such as *CYCA*, *His-lys-N* and *CYC6*, related to drug resistance (*YIP1* and *ABCC6*) and the parasite's virulence gene *GP63*, which could explain their mechanism of action. Then, we investigated the effect of our compounds against leishmaniasis, *in vivo*. R13 and R27 significantly reduced the parasite burden in the skin lesion and the spleen of Balb/c mice infected with *L. major* and treated with 1 μg/ml of the compounds. Lastly, we showed that the combination of our compounds with the reference drugs used in the treatment of leishmaniasis, Amphotericin B (Ampho B) and Paromomycin (PM), dramatically increased their effectivity against *L. major* amastigotes, *in vitro*. Our results suggest that both R13 and R27 compounds could be considered as promising alternatives for leishmaniasis treatment, alone or combined with the drugs currently used in clinic.

[1] Marr AK et al. Identification of Synthetic and Natural Host Defense Peptides with Leishmanicidal Activity. Antimicrob Agents Chemother. 2016 [2] Fernández-Rubio C, Larrea E, Peña Guerrero J, Sesma Herrero E, Gamboa I, Berrio C, Plano D, Amin S, Sharma AK, Nguewa PA. Leishmanicidal Activity of Isoselenocyanate Derivatives. Antimicrob Agents Chemother. 2019 [3] Wortmann G et al. Lipsosomal amphotericin B for treatment of cutaneous leishmaniasis. Am J Trop Med Hyg. 2010

0408-P

NEWLY DISCOVERED ONCOGENE HOMOLOGUE INVOLVED IN LEISHMANIA VIRULENCE

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The World Health Organization (WHO) recognizes up to 20 illnesses as Neglected Tropical Diseases (NTDs), among which trypanosomatid-caused ones are included such as leishmaniasis. Current drug therapies are unsatisfactory due to their toxicity, long treatment courses and development of resistance. Therefore, researches in the field of drug development are directed towards to the study of molecular targets to achieve the control of this disease. The availability of several *Leishmania* species genome allowed the identification of *YinP*, an oncogene conserved among eukaryotes, related to ribosomal biogenesis (1), cell proliferation (2) and virulence (3, 4). Our work attempts to shed light on the involvement of this gene in parasite biology and its role as therapeutic target against pathogenic protozoan.

Firstly, by using the red fluorescent fusion protein mCherry, the Yin P protein nucleolar localization was determined. Then, the *Yin P* gene expression along *Leishmania* life cycle was studied by RT-PCR technique, showing the highest level during the infective stage. Therefore, we decided to investigate the role of this newly detected gene in the virulence using transgenic parasites overexpressing *YinP*. The percentage of infected macrophages as well as the number of intracellular amastigotes remained higher during *in vitro* infections with *YinP* overexpressing strains. Similarly, transgenic parasites caused higher and faster footpad inflammation in BALB/c mice than non- overexpressing parasites. Such an inflammation was in agreement with a great cell infiltration and higher inducible nitric oxide synthase (iNOS) protein detection in the site of the parasite inoculation.

Further studies are needed to deep into the role of this newly discovered gene in Leishmaniasis outcome. However, due to its importance for parasite virulence, *Yin P* might be considered a promising target for new drugs design against Leishmaniasis.

1. Dannheisig, D. P., et al. J. Mol. Biol. 433, 166719 (2021). 2. Fan, P., et al. Int. J. Biochem. Cell Biol. 104, 1–8 (2018). 3. Shen, J., et al. Proc. Natl. Acad. Sci. U. S. A. 105, 20918–23 (2008). 4. Kumar, R. et al. 133, 297–310 (2004).

0419-P

Modulation of the Ebola virus VP35 protein by SUMO: regulating the regulator

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Ebola virus (EBOV) is a highly pathogenic agent causing hemorrhagic fever with a high case-fatality rate in humans. The EBOV VP35 protein is a multifunctional protein that plays a role in viral replication and nucleocapsid assembly, contributes to virus scape form host innate immunity and is required for virulence. Several mechanisms mediate the inhibition of the interferon (IFN) pathway by VP35. Thus, VP35 induces SUMOylation of IRF-7 blocking IFN production. In addition, VP35 interacts with and inhibits the function of IFN regulatory factor-activating kinases IKKe and TBK-1 blocking the phosphorylation, dimerization and nuclear translocation of IFN-regulatory factor 3 (IRF-3), thereby inhibiting IFNa/b gene expression. Finally, VP35 interacts with dsRNA blocking protein kinase R (PKR) activation. We have previously demonstrated that EBOV exploits the cellular SUMOylation machinery for its own benefit, as shown by the modulation of the stability and activity of EBOV VP40 and VP24 proteins by SUMO. We then evaluated whether EBOV VP35 protein is also modulated by SUMO. Our data reveal that VP35 protein is a SUMO substrate. We demonstrate that VP35 is modified by SUMO *in vitro* and *in vivo.* Moreover, VP35 interacts with SUMO in a non-covalent manner through a SIM domain. VP35-SUMO interaction enhances the ability of VP35 to block the interferon pathway by facilitating the interaction between the viral protein and dsRNA, and the inhibition of PKR. Our results demonstrate that SUMO is a key regulator of another critical EBOV protein contributing to pathogenesis, opening the possibility to use it as a therapeutic target against EBOV infection.

0503-P

Detection of SARS-CoV-2 in aerosols by SKC Biosampler air collection followed by ddPCR: analysis of influencing factors to optimize virus quantification

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Introduction: The relevance of SARS-CoV-2 infection via aerosol transmission urges to develop a methodology to quantify the airborne virus. Several researchers have been using different protocols to collect air samples and detect the virus genome, resulting in a low percentage of positive samples or a lack of detection, which stresses the need to optimize these protocols. We selected SKC BioSampler to collect aerosols for its greater efficiency in collecting particles of 1-3 micrometers diameter [1] and droplet digital PCR (ddPCR) for its high sensitivity, able to detect SARS-CoV-2 in biological samples in which conventional PCR did not [2]. **Objective:** To analyze the influence of the change of variables in our protocol of aerosol SARS-CoV-2 quantification. **Study design and protocol:** In the study I, we collected 38 samples from 26 patient rooms of the University Hospital Son Espases from September 18th to November 11th. Between 250 and 375 L of air were collected in the presence of a PCR-positive COVID-19 patient using sterile water as collection media. In the study II, we collected 27 samples from 28 patient rooms from November 18th to May 7th. Air samples were collected only when the patient's symptoms onset was less than 8 days. 625 to 750L of air were collected in DMEM containing 10% FBS, 1% antibiotics, 0.5% BSA and 0.5% antifoam. In both studies, air sampling, RNA isolation, cDNA synthesis and ddPCR detection were performed as we described [3]. Three primer and probes sets designed to hybridize the *N* gene (*N* and *N1*) and the *ORF1ab* were used. **Results**: The mean of total isolated RNA in the studies I and II was 2760±398 and 2618±435 ng/ml of media, respectively. In the study I, 5% and 8% of the samples were positive for genes *N* and *N1,* respectively, while 11% and 19% of the samples were positive for these genes in the study II. Finally, 29% of the samples in the study I were positive for gene *ORF1ab*. **Discussion and conclusions:** The volume of collected air and the use of these media have no effect on the amount of isolated RNA, suggesting that these variables may have a low impact on SARS-CoV-2 detection following our protocol. The patient symptoms onset may contribute to explain the differences in the percentage of positive samples between the studies. The detection of *ORF1ab* sequence could be a better option for SARS-CoV-2 quantification in aerosols.

1 Kesavan J and Sagripanti JL. Evaluation criteria for bioaersol samplers. Environ Sci Process Impacts. 2015; 17(3):638-45 2 Suo T, Liu X, et al. ddPCR: a more accurate tool for SARS-CoV-2 detection in low viral load specimens. Emerg Microbes Infec. 2020; 9(1):1259-1268. 3 Truyols-Vives J, Sala-Llinàs, et al. Cuantificación del SARS-CoV-2 en muestras de aire del HUSE mediante PCR digital. 2021. 2º Congreso Nacional Multidisciplinar COVID19 de las Sociedades Científicas de España.

13.- Free Radicals and Oxidative Stress

0516-OI

Shaping bioenergetics and oxidative stress from mitochondrial cristae

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The mitochondrial ultrastructure, characterized by foldings of the inner membrane that form cristae where respiratory (super)complexes embed to account for oxidative phosphorylation, is not a mere morphological feature. Instead, cristae shape emerges as a master regulator of mitochondrial physiology and cell fate[1, 2]. Notably, the dynamic remodeling of the mitochondrial ultrastructure is central to orchestrate adaptations of the respiratory capacity to face metabolic and oxidative challenges, by still unclear mechanisms. Here, we capitalize on the key molecular cristae scaffolds F_1F_O-ATP synthase and OPA1 as determinants of mitochondrial ultrastructural changes[3], to unravel how mitochondrial cristae critically define bioenergetics and reactive oxygen species (ROS) production. Departing from a series of apoptotic, metabolic and genetic manipulations of cristae shape, we reveal how mitochondrial ultrastructure face respiratory challenges to sustain cellular viability under normal or compromised mitochondrial function.

1 F. Vogel, C. Bornhövd, W. Neupert, A.S. Reichert. Dynamic subcompartmentalization of the mitochondrial inner membrane. J. Cell Biol., 175 (2006), pp. 237-247 2 S. Cogliati, J.A. Enriquez, L. Scorrano. Mitochondrial cristae: where beauty meets functionality Trends Biochem. Sci., 41 (2016), pp. 261-273 3 R. Quintana-Cabrera, A. Mehrotra, G. Rigoni, M.E. Soriano. Who and how in the regulation of mitochondrial cristae shape and function. Biochem. Biophys. Res. Commun., 500 (2018), pp. 94-101.

0522-OI

Redox response in the liver ischemia/reperfusion injury

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Ischemia-reperfusion (IR) injury is a complex multifactorial process that causes cell damage [1]. During liver surgery, the unavoidable interruption of blood flow causes ischemia followed by a subsequent reperfusion. While ischemic oxygen deprivation depletes cellular energy, subsequent reperfusion tissue oxygenation induces many cascades. These pathways generate, among others, the production of reactive oxygen species (ROS).

The involvement of ROS in hepatic IR injury has been widely demonstrated. ROS causes cell injury through lipid peroxidation, protein oxidation, mitochondrial dysfunction, and DNA damage. Subsequently, Kupffer cells and neutrophils are recruited and cause liver inflammation. Apoptosis, autophagy, and proteasome activation have also been identified in the pathogenesis of IR injury, although such alterations and their subsequent functional significance remain controversial.

Our research has focused on different strategies to protect the liver from IR injury, such as hypothermic preconditioning [2], pretreatment with pharmacological agents, and supplementation of commercial preservatives with additives [3]. Unhealthy lifestyles associated with improper diet, along with other factors such as aging, are responsible for the higher incidence of hepatic steatosis. For this reason, we have used fatty livers. The IR damage process is aggravated in steatotic livers due to fat accumulation in the hepatic sinusoids, leading to severe obstruction of hepatic flow.

We have sought to address the progress and current knowledge on the mechanism of ROS-mediated liver IR injury. Detailed research on such mechanism could open up new insights for the development of biomarkers and new therapeutic approaches.

1. Carden, D.L.; Granger, D.N. Pathophysiology of ischaemia-reperfusion injury. J. Pathol. 2000, 190 2. Alva, N.; Bardallo, R.G.; Basanta, D.; Palomeque, J.; Carbonell, T. Preconditioning-like properties of short-term hypothermia in isolated perfused rat liver (IPRL) system. Int. J. Mol. Sci. 2018, 19 3. Bardallo, R.G.; da Silva, R.T.; Carbonell, T et al. A. Role of PEG35, Mitochondrial ALDH2, and Glutathione in Cold Fatty Liver Graft Preservation: An IGL-2 Approach. Int. J. Mol. Sci. 2021, 22

0025-M-OS

Induction of Cyclooxygenase-2 by Overexpression of the Human NADPH Oxidase 5 (NOX5) Gene in Aortic Endothelial Cells. Potential Role in Myocardial Infarction.

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Oxidative stress is one molecular mechanism underlying cardiovascular diseases (CVDs) [1]. The NADPH oxidase (NOX) family is a key source of reactive oxygen species (ROS) and its activity has been widely related with CVDs [2]. Some NOX isoforms may regulate prostaglandin (PG) synthesis (signaling lipids involved in CVDs) [3], however, little is known about the influence of NOX5 isoform in this pathway. Our aim was to evaluate NOX5 effects in PG synthesis in human aortic endothelial cells (teloHAEC) and their potential implications in myocardial infarction (MI). For that purpose, we developed an NOX5-β adenoviral overexpression *in vitro* model, and a MI model in an endothelial NOX5-β *knock-in* mouse.

The obtained results showed that our overexpression *in vitro* model generated a functional protein without affecting the cell viability. This overexpression resulted in upregulated cyclooxygenase-2 (COX-2) mRNA levels, protein expression and transcriptional activity, and in enhanced PGE_2 production in teloHAEC. As specific NOX5 inhibition with ML-090 prevented these effects, ROS levels may be responsable of them. Deeping in this pathway, NF-κB was found to be involved in NOX5/COX-2 axis, since its inhibition with PDTC blocked all previously described effects. Activation of NOX5 by protein kinase C (PKC)-mediated phosphorylation and calcium increased levels, led to enhanced expression and activity of the oxidase. This PKC-derived stimulation resulted in higher COX-2 protein levels in cells infected with NOX5-β adenovirus.

On the other hand, in the MI model, endothelial NOX5-β also altered PG homeostasis. At baseline conditions, NOX5-β induced citosolic phospholipase A_2 ($cPLA_2$) mRNA expression at cardiac level. $cPLA_2$ was boosted even more after MI in NOX5-β expressing, interestingly this enzyme is located upstream of PG biosynthesisFinally, in infarcted NOX5-β expressing mice, cardiac COX-2 and PGE_2 synthase (PGES) mRNA leves were increased compared with wildtype infarcted mice, suggesting a COX-2/PGE_2 pathway activation.

In conclusion, NOX5-β is involved in vascular endothelial COX-2/PGE_2 pathway via NF-κB. This relationship may participate in MI pathophysiology, as endothelial NOX5-β altered the expresión of different enzymes involved in PG production.

1. Costantino, S.; et al. Ageing, metabolism and cardiovascular disease. J. Physiol. 2016, 594, 2061-2073. 2. Zhang, Y.; et al. NADPH oxidases and oxidase crosstalk in cardiovascular diseases. Novel therapeutic targets. Nat. Rev. Cardiol. 2020, 17, 170-194. 3. Khanal, T.; et al. Genipin induces cyclooxygenase-2 expression via NADPH oxidase, MAPKs, AP-1, and NF-κB in RAW 264.7 cells. Food Chem. Toxicol. 2014, 64, 126-134.

0316-R/M-OS

Noise-induced vascular dysfunction, oxidative stress, and inflammation are improved by pharmacological heme oxygenase-1 induction

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Vascular oxidative stress, inflammation and subsequent endothelial dysfunction are consequences of traditional cardiovascular risk factors and all of which may trigger cardiovascular disease. Emerging environmental stressors, such as traffic noise and air pollution, may also facilitate the development of cardiovascular and metabolic diseases. In our previous studies, we investigated the influence of aircraft noise exposure on molecular mechanisms identifying oxidative stress and inflammation as central players in mediating vascular dysfunction. As well as, aircraft noise increased eNOS expression but reduced vascular NO levels due to eNOS uncoupling. Aircraft noise increased levels of nitrotyrosine, interleukin-6, NADPH oxidase subunit Nox2 and endothelin-1. The present study investigates the role of heme oxygenase-1 (HO-1) as an antioxidant response in the vascular consequences following exposure to aircraft noise. C57BL/6J mice were treated with the HO-1 inducer hemin (25 mg/kg i.p.) and the NRF2 activator dimethyl fumarate (DMF, 20mg/kg p.o.). During therapy, the animals were exposed to noise at a maximum sound pressure level of 85 dB(A) and a mean sound pressure level of 72 dB(A). Our data showed a marked protective effect of both treatments on animals exposed to noise for 4d by normalization of arterial hypertension and vascular dysfunction in the noise-exposed groups. We observed a partial normalization of noise-triggered oxidative stress by hemin and DMF therapy and found evidence that HO-1 induction has beneficial effects on systemic inflammation in noise-exposed mice. The present study identifies possible new targets for mitigation of the adverse health effects caused by environmental noise exposure. Since natural dietary constituents can achieve HO-1 and NRF2 induction, these pathways represent promising targets for preventive measures.

https://doi.org/10.3390/antiox10040625 https://doi.org/10.1093/eurheartj/ehy333

0470-OS

Mitochondrial supercomplex I+IV as a potential oxygen sensor

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Reactive oxygen species (ROS) production in the cell has emerged as an increasingly relevant phenomenon involved in pathological, but also in physiological processes, such as acute hypoxia. In a hypoxic environment, a ROS burst is paradoxically produced by cells as an adaptative response. Mitochondria generate most of the ROS produced by the cell, mainly through complex I (CI) and complex III (CIII), which are components of the electron transport chain (mETC). Our group described that during hypoxia, CI undergoes an active/deactive transition, which triggers a Na^+-dependent signaling cascade in the mitochondrial matrix and inner membrane leading to ROS production by CIII. Although the complex molecular mechanism involved has been uncovered, it remains unclear how low-oxygen levels induce CI deactivation. Indeed, the molecular identity of the oxygen sensor remains unidentified. Recently, our group has characterized a novel respiratory supercomplex, I+IV (SC I+IV), that migrates in Blue Native Electrophoresis above free CI and very close to SC $I+III_2$, but its functional role is still obscure. SC I+IV contains complex IV (CIV), which reacts with oxygen and accomplishes the last step of mETC, reducing it to water. However, CIV by itself does not explain how variations in oxygen levels are detected by the mitochondria. Here we hypothesize that SC I+IV could be an oxygen sensor, as it contains both CI, which suffers the conformational active/deactive shift triggering hypoxic ROS signaling, and CIV, whose function is affected by oxygen levels. In this work, we have verified that CIII-lacking cells are a good model to isolate SC I+IV because they lack SC $I+III_2$, which migrates very close to it and is present in a larger amount in wild type cells. We are characterizing *in vitro* the partially purified SC I+IV and the potential role of CIV in deactivating CI in response to oxygen availability.

1. Hernansanz-Agustín P, Choya-Foces C, Carregal-Romero S, Ramos E, Oliva T, Villa-Piña T, et al. Na+ controls hypoxic signalling by the mitochondrial respiratory chain. Nature. 2020;586(7828):287–91. 2. Calvo E, Cogliati S, Hernansanz-Agustín P, Loureiro-López M, Guarás A, Casuso RA, et al. Functional role of respiratory supercomplexes in mice: SCAF1 relevance and segmentation of the Qpool and SCAF1; Science Advances. 2020;6(26):eaba7509.

0509-OS

NRF2 activity is impaired in a model of ALS rendering redox dysregulation

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by motoneuron demise and muscle denervation, leading to loss of voluntary movements and eventually death. There is an urgent need for the development of disease-modifying therapies due to the limited impact of current monotherapies. Multiple pathways in ALS have been found to be dysregulated, such as oxidative and inflammatory stress, autophagy, and mitochondrial control. Return to homeostasis of these pathways could be achieved by the activation of the cytoprotective transcription factor Nuclear-related erythroid 2-related 2 (NRF2). Indeed, several reports point to an impairment of the NRF2 system in affected tissues of ALS patients. In a pilot study we found that the mRNA levels of NRF2 antioxidant targets, HMOX1 and NQO1, are diminished in blood of ALS patients compared to healthy donors. To unravel the links

between the NRF2 pathway impairment and ALS, we studied the changes in redox homeostasis in both a transient and stable cellular motoneuron model of C9ORF72-related familial ALS. We detected a concomitant increase in sensitivity to oxidative stress and a decreased activation of NRF2 response, which appear to act through non-classical mechanisms. Despite this, NRF2 enhancement through exogenous molecules such as dimethyl fumarate was able to rescue redox homeostasis. Our finding draws a connection between oxidative stress in ALS and NRF2 signalling, providing proof for the benefit of NRF2 pharmacological activation in this disease. Therefore, our results suggest NRF2 intervention could be a suitable therapeutic strategy for ALS and encourage future studies to gain insight into the interplay between the NRF2 pathway and ALS.

0034-R/M-OS

Na+ controls hypoxic redox signalling by the mitochondrial respiratory chain

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All metazoans depend on the consumption of O_2 by the mitochondrial oxidative phosphorylation system (OXPHOS) to produce energy. In addition, the OXPHOS uses O_2 to produce reactive oxygen species that can drive cell adaptations, a phenomenon that occurs in hypoxia and whose precise mechanism remains unknown. Ca^{2+} is the best known ion that acts as a second messenger, yet the role ascribed to Na^+ is to serve as a mere mediator of membrane potential. Here, we show that Na^+ acts as a second messenger that regulates OXPHOS function and the production of reactive oxygen species by modulating the fluidity of the inner mitochondrial membrane. A conformational shift in mitochondrial complex I during acute hypoxia11 drives acidification of the matrix and the release of free Ca^{2+} from calcium phosphate (CaP) precipitates. The concomitant activation of the mitochondrial Na^+/Ca^{2+} exchanger promotes the import of Na^+ into the matrix. Na^+ interacts with phospholipids, reducing inner mitochondrial membrane fluidity and the mobility of free ubiquinone between complex II and complex III, but not inside supercomplexes. As a consequence, superoxide is produced at complex III. The inhibition of Na^+ import through the Na^+/Ca^{2+} exchanger is sufficient to block this pathway, preventing adaptation to hypoxia. These results reveal that Na^+ controls OXPHOS function and redox signalling through an unexpected interaction with phospholipids, with profound consequences for cellular metabolism.

Hernansanz-Agustín P, Choya-Foces C, Carregal-Romero S,[...]Enríquez JA, Martínez-Ruiz A. Na+ controls hypoxic signalling by the mitochondrial respiratory chain. Nature. 2020 Oct;586(7828):287-291. doi: 10.1038/s41586-020-2551-y.

0062-P

NOX2 is involved in acute myeloid leukemia prognosis and survival discrimination

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NADPH oxidases have been shown to be a family of enzymes with great influence on normal and pathological haematopoietic differentiation [1]. Specifically in acute myeloid leukaemia, the involvement of the NOX2 isoform of this family of enzymes has been highlighted by some authors for its important influence on the disease[2]. Acute myeloid leukaemia is the most commonly diagnosed leukaemia among adults and is characterised by being highly heterogeneous. Genetic profile analyses of AML patients have shown mutations in up to 250 genes and more than 14 different frequent cytogenetic alterations. It is precisely this complex landscape that impedes the evolution in last 40 years of both the prognostic classification system - which is based solely on these cytogenetic alterations and mutations in three genes (*NPM1, FLT3* and *CEBPA*) - and the therapies against the disease. Currently, almost half of AML patients have no cytogenetic alterations so they are classified as intermediate prognosis group, which leads to a hotchpotch group. All of the above evinces the need to further increase molecular knowledge of this disease to ensure a more targeted treatment for its patients. Our research group has studied *CYBB* (coding for NOX2) expression in 1311 bone marrow donors collected from GSE15061, GSE14468, GSE10358 and GSE68833 datasets. Our results demonstrate that *CYBB* should be considered as an important target for AML as it is involved in AML patient prognosis and survival.

1. Prieto-Bermejo, "P22 Phox y NADPH Oxidasas En La Hematopoyesis in Vivo"; Sánchez-Sánchez et al., "NADPH Oxidases as Therapeutic Targets in Chronic Myelogenous Leukemia." 2. Adane et al., "The Hematopoietic Oxidase NOX2 Regulates Self-Renewal of Leukemic Stem Cells"; Dakik et al., "Characterization of NADPH Oxidase Expression and Activity in Acute Myeloid Leukemia Cell Lines: A Correlation with the Differentiation Status."

0069-R/M-P

The axis NOX2/NOX4 regulates mitochondrial function and content in Chronic myeloid leukaemia

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Chronic myeloid leukemia (CML) is a haematological malignancy caused by the fusion of *BCR* and *ABL* genes. The BCR-ABL protein has constitutive tyrosine kinase activity that triggers increased proliferation and blockage of differentiation, accompanied by changes in metabolism and increased ROS production via NADPH oxidases (NOX). In this sense, it is important to note that the same signalling pathways that induce overproduction of ROS via NOX cause also a metabolic change in CML cells. Therefore, we wondered whether NOX activity could be involved in the metabolic adaptation of CML cells. For this purpose, we silenced by means of shRNA NOX2 or $p22^{phox}$ subunits in K562 cell line of CML. We studied the effect of both knockdowns on the glucose use, mitochondrial bioenergetic metabolism and mitochondrial functional state.

Our results show that silencing of NOX2 causes an increase in glucose uptake, an acceleration of glucose metabolism, and a decreased accumulation of glycolytic intermediates in CML cells. Moreover, NOX2 silencing produces a raising in LDH activity without changes in lactate levels. NOX2 knockdown enhances mitochondrial respiration, an event that is surprisingly accompanied by a decrease in the mtDNA/nDNA ratio and lower protein levels of respiratory chain complexes subunits, results that might reflect a reduction in mitochondrial mass. However, most of these changes were not observed after $p22^{phox}$ silencing (essential subunit for NOX2-NOX4). We therefore considered investigating whether a possible compensatory effect could be occurring with other homologues of the NOX family. Interestingly, upon NOX2 silencing we observed an increase in NOX4 levels, the only member of the family that locates at the mitochondria. Silencing of NOX4 in the NOX2-sileced cells restores the respiratory values to those of the control cells. These data strongly suggest that NOX2 might regulate mitochondrial metabolism through the regulation of NOX4.

Understanding the molecular mechanism by which NOX2 regulates mitochondrial function in CML is a promising challenge to discover novel therapeutic strategies. Furthermore, these findings raise new questions about the retrograde regulation of two major sources of cellular ROS.

0086-R-P

Dusp1 deletion unveils its central role in the regulation of redox homeostasis and inflammation in the mouse cochlea

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Sensorineural hearing loss (SNHL) is the most prevalent sensory impairment of the elderly according to the WHO, which estimates that 1/10 people will suffer from disabling HL by 2050[1]. SNHL is mainly caused by the death of irreplaceable mechanosensory hair cells (HC) and spiral ganglion neurons (SGN). The onset and progression of SNHL rely on genetic factors not well characterized yet, often aggravated by environmental factors such as noise exposure and ototoxic agents[2].

Stress-activated protein kinases (SAPK), p38 and JNK, activation precedes cellular loss in different scenarios of cochlear insult[3]. Their inhibition has been proved to be otoprotective in animal models; thus, the therapeutic potential of small molecule inhibitors is being explored in humans[4]. *In vivo*, the activity of these kinases is tightly regulated by the Dual-Specificity Phosphatase 1 (DUSP1), an indispensable factor for long-term cochlear homeostasis whose deficit accelerates hearing loss, dysregulates redox balance and triggers the inflammatory response[5].

Here, we have further studied the link between DUSP1 loss of function leading to SAPKs sustained activation and redox imbalance in the cochlea. RNAseq was carried out in wild type and *Dusp1*$^{-/-}$ cochleae. GSEA indicated that *Dusp1*$^{-/-}$ mice present a distinct gene expression pattern of key cellular programs, including altered expression of genes of the inflammatory response and GSH metabolism. To dissociate both components, mice were treated with the antioxidant N-acetylcysteine (NAC). Antioxidant supplementation partially recovered hearing thresholds, delayed the onset of SNHL and reduced cochlear damage in *Dusp1*$^{-/-}$ mice. NAC-treated mice also showed stabilized GSH metabolism and improved redox balance. Cochleae of NAC-treated *Dusp1*$^{-/-}$ presented less TUNEL positive HC number, p-H2AX foci in SGN nuclei than untreated *Dusp1*$^{-/-}$ and caused a drastic reduction in cytokine production and macrophage recruitment.

In summary, our results contribute to unveil the role of DUSP1 in the cochlea as a node that regulates the inflammatory response following oxidative stress. We concluded that NAC-treatment of *Dusp1*$^{-/-}$ mice partially restored the antioxidant system. Interestingly, this sufficed to reduce cochlear inflammation, DNA damage and apoptosis, delaying the onset of hearing loss.

1. WHO. Deafness and Hearing Loss. Fact sheet. April 2021. 2. Gates GA and Mills JH. Presbycusis. Lancet 2005, 366, 1111–20. 3. Wong AC and Ryan AF. Mechanisms of Sensorineural Cell Damage, Death and Survival in the Cochlea. Front Aging Neurosci 2015, 7, 58. 4. Wang J and Puel JL. Toward Cochlear Therapies. Physiol Rev 2018, 98, 2477–2522. 5. Celaya AM, et al. Deficit of Mitogen-Activated Protein Kinase Phosphatase 1 (DUSP1) Accelerates Progressive Hearing Loss. Elife 2019, 8.

0091-P

Effect of long-term exposure to microplastics and depuration period in Sparus aurata Linnaeus, 1758: liver and blood biomarkers

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The increasing in plastic pollution has attracted great attention in recent years due to its potential negative effects on marine organisms. While the consequences of ingestion of large plastic litter are mostly understood, the impacts resulting from a long-term exposure and a recovery period of microplastics (MPs) are still limited. The aims were to monitor oxidative stress, detoxification and inflammatory biomarkers in liver, plasma and erythrocytes of *Sparus aurata* exposed during 90 days to low-density polyethylene (LDPE)-MPs enriched diet (10% by weight) followed by 30 days of depuration. Exposure to LDPE-MPs progressively activates the antioxidant and detoxification system and induces an inflammatory response in liver and plasma, whereas no significant changes were observed in erythrocytes. The plasma activities of catalase (CAT), myeloperoxidase (MPO), lysozyme and the levels malondialdehyde (MDA) as maker of lipid peroxidation significantly increased after exposure to LDPE-MPs for 90 days compared to the control group. The activities of all antioxidant enzymes – CAT, superoxide dismutase, glutathione peroxidase and glutathione reductase -, the detoxification enzyme glutathione *s*-transferase, MPO, the production of reactive oxygen species and the levels of MDA were also significantly increased in liver after MPs exposure. Additionally, all these biomarkers tended to recover during the depuration period, most of them reaching similar levels to those of the control group. In conclusion, the ingestion of a diet containing LDPE-MPs for 90 days induced a progressive increase in oxidative stress and inflammation biomarkers in liver and plasma of *S. aurata* but not in erythrocytes, which tended to regain control values when not exposed to MPs for 30 days. The present study contributes to a better understanding of the toxic effects of MPs in *S. aurata* and highlights the usefulness of plasma that can be obtained in a minimally invasive way to monitor these effects.

Capo, X., et al., 2021. Long-term exposure to virgin and seawater exposed microplastic enriched-diet causes liver oxidative stress and inflammation in gilthead seabream Sparus aurata, Linnaeus 1758. Sci. Total Environ. 767, 144976. Solomando, A., et al., 2020. Long-term exposure to microplastics induces oxidative stress and a pro-inflammatory response in the gut of Sparus aurata Linnaeus, 1758. Environ. Pollut. 266, 115295.

0098-R/M-P

Importance of NADPH oxidase activity on haematopoiesis in vivo

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Despite their harmful effects, reactive oxygen species (ROS) are increasingly recognized as important molecules in the regulation of cell signalling. Because of their capacity to reversibly modify the activity of a multitude of proteins in response to different stimuli they can now be considered as second messengers[1] . The discovery of the NADPH oxidase (abbreviated as Nox) family of enzymes, the only known specific ROS-producing system, has been essential for understanding redox signalling. There are 7 members in mammals (Nox1 to Nox5, Duox1 and 2), which may differ in their subcellular localization and activation stimuli, but which can be expressed simultaneously in the same cell types [2] , suggesting that they also have some specificity of function.

Nox are involved in the control of important biological processes such immune defence, inflammation, maintenance of vascular pressure, or cell differentiation[2]. Haematopoietic differentiation is a paradigmatic example of cell differentiation in the adult, in which all the blood cell lineages come from a single cell type, the haematopoietic stem cells (HSCs). ROS levels have great influence on the homeostasis of HSCs, decreasing their quiescence and self-renewal capacity, and promoting their differentiation, thus reducing their ability to maintain haematopoiesis[3]. However, we still do not know what influence Nox have on this system, so our aim has been focused on studying the role of NADPH oxidase activity on haematopoiesis *in vivo*.

Our analysis[4] showed that mouse Lin^- progenitor cells express three members, Nox1, Nox2 and Nox4, all of them dependent on $p22^{phox}$ for activity, so we generated mice deficient in $p22^{phox}$ coding gene (*Cyba*). $Cyba^{-/-}$ mice manifest an increase in the number of immature HSCs and in their proliferation capacity. We then performed competitive bone marrow transplants to test the haematopoietic regenerative capacity of these cells. $Cyba^{-/-}$ cells show a repopulation advantage over control cells, which is maintained in the long term after secondary transplantation. Finally, we studied the regenerative capacity of cells deficient in each of the Nox separately, and the results reveal that both Nox1 and Nox2 are involved in this effect on haematopoietic regeneration. In contrast, Nox4 may have an influence on the long-term maintenance of HSPCs.

1 Rhee et al., 2000. “Hydrogen Peroxide: A Key Messenger That Modulates Protein Phosphorylation through Cysteine Oxidation” 2 Bedard and Krause, 2007. “The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology” 3 Ito et al., 2004. “Regulation of Oxidative Stress by ATM Is Required for Self-Renewal of Haematopoietic Stem Cells” 4 Prieto-Bermejo et al., 2021. “Cyba-Deficient Mice Display an Increase in Hematopoietic Stem Cells and an Overproduction of Immunog

0119-R/M-P

Effect of microbiota-derivated short-chain fatty acids on intestinal oxidative status

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Intestine is a complex organ that performs numerous functions to provide us nutrients while acting as a protective barrier. Due to its high metabolic rate, intestine is a crucial source of reactive oxygen species, generating oxidative stress when it faces inflammatory responses as the result of its continuous contact with external agents, toxins and potential pathogens. Microbiota that colonizes the intestine is responsible for several functions critical to the maintenance of homeostasis. Among them is the fermentation of indigestible foods and transformation into absorbable metabolites such as short-chain fatty acids.

The objective of this work was to study the effect of short-chain fatty acids (acetate, butyrate and propionate) from the microbial fermentation of fiber on the intestinal oxidative status. To do this, we used the Caco-2 cell line as an in vitro model of intestinal epithelium. The oxidative status was quantified by measuring carbonyls as the protein oxidation index and MDA + 4-HDA as a reflection of lipid oxidation. Antioxidant defense was assessed by analyzing the activity of the main antioxidant enzymes: catalase, superoxide dismutase and glutathione peroxidase.

Our results showed that treatment with short-chain fatty acids for 24 hours did not modify the intestinal oxidative status, with the exception of acetate, which increased lipid and protein oxidation, possibly due to a reduction in the antioxidant activity of glutathione peroxidase and superoxide dismutase. To simulate an inflammatory situation, the cells were treated with TNFα. TNFα induced a significant increase in both lipid and protein oxidation with a reduction in the antioxidant activity of superoxide dismutase. These effects were reversed by the combined treatment of TNFα with propionate or butyrate.

Our results confirm that the microbiota could have a protective role against intestinal oxidative stress, where short-chain fatty acids are able to restore the redox balance. These results may be useful to clarify the processes involved in intestinal inflammation and contribute to the design of specific therapies in the treatment of inflammatory bowel diseases acting on the redox balance.

0130-P

PBMCS Oxidative stress and plasma inflammatory biomarkers in adults with normal weight, overweight and obesity

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Obesity is an important pathology in public health worldwide. Obese patients are characterized by higher cardiovascular risk and a pro-inflammatory profile. The aim was to evaluate the oxidative stress in peripheral blood mononuclear cells (PBMCs) and inflammatory biomarkers in plasma in adults with normal weight, overweight and obesity. 150 adults (55-80-years-old; 60% women) living in the Balearic Islands, Spain, were recruited and classified according to body mass index (BMI). Anthropometric measurements were carried out, fasting blood samples were collected and plasma and PBMCs were obtained. Biochemical parameters, hemogram, antioxidant enzyme activities and protein levels, malondialdehyde (MDA), and cytokine (tumour necrosis factor, TNFa, and interleukin 6, IL-6) levels were assessed. Glycaemia, triglyceridemia, abdominal obesity, and waist to height ratio (WHtR) were higher, and HDL-cholesterol was lower in obese patients. MDA and TNFa plasma levels were higher in the obese compared to normal-weight group, while the levels of IL-6 were higher in both obese and overweight respect to normal-weight. The activities of all antioxidant enzymes in PBMCs progressively increased with BMI. The protein levels of catalase in PBMCs were higher in obese and glutathione reductase in obese and overweight subjects compared to normal-weight peers. No other differences were observed. In conclusion, the current results evidenced that overweight and obesity are associated with an increase in oxidative stress and proinflammatory status in plasma and PBMCs. The studied biomarkers may be useful for diagnostic purposes in clinical practice.

Ataey, A.; Jafarvand, E. et al. The Relationship Between Obesity, Overweight, and the Human Development Index in World Health Organization Eastern Mediterranean Region Countries. J. Prev. Med. Public Heal. 2020, 53, 98–105. García-Sánchez, A.; Gámez-Nava, J.I.; et al. The effect of visceral abdominal fat volume on oxidative stress and proinflammatory cytokines in subjects with normal weight, Overweight and Obesity. Diabetes, Metab. Syndr. Obes. Targets Ther. 2020, Volume 13, 1077–1087.

0160-P

THE DEGREE OF HEPATIC STEATOSIS IS RELATED TO A DECREASE IN ANTIOXIDANT ENZYMES IN PBMCS AND A PROINFLAMMATORY STATE

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Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the western world, and is characterized by excessive fat accumulation, especially triglycerides in hepatocytes. If the pathology is not properly treated, it can progress to non-alcoholic steatohepatitis (NASH) and continue to fibrosis, cirrhosis or carcinogenesis. The aim of the current research was to identify peripheral blood mononuclear cells (PBMCs) and plasma markers related to liver damage and inflammation that facilitate the early diagnosis and monitoring the progression of the disease. Antioxidant and inflammatory biomarkers were measured in PBMCs and plasma of patients diagnosed of NAFLD (n=100 adults; 40-60 years old) living in the Balearic Islands, Spain. Patients were classified attending to the stage of liver steatosis measured by Magnetic Resonance Imaging (MRI). Circulating glucose, glycosylated haemoglobin, triglycerides, low-density lipoprotein-cholesterol, aspartate aminotransferase and alanine aminotransferase were higher in patients with a higher degree of steatosis compared to those with less fat accumulation. Although leukocytes did not increase significantly, there is a significant increase in lymphocytes, basophils and eosinophils as the degree of steatosis increases. The activities of antioxidants enzyme activities in PBMCs tended to decreases as the degree of steatosis, although this decrease was only significant in superoxide dismutase. No differences were observed in malondialdehyde (MDA) levels in PBMCs. Plasma levels of the pro-inflammatory interleukin-6 (IL-6) and MDA, and the biomarker of liver damage cytokeratin 18 progressively increased with the degree of steatosis, whereas the pro-resolving mediator resolvin D1 levels were reduced. The present data show that the severity of NAFLD is associated with an increase in oxidative stress and proinflammatory status.

Monserrat-Mesquida, M. et al. Oxidative Stress and Pro-Inflammatory Status in Patients with Non-Alcoholic Fatty Liver Disease. Antioxidants (Basel). 2020 Aug 16;9(8):759. doi: 10.3390/antiox9080759. PMID: 32824349. Busquets-Cortés, C. et al. Peripheral Blood Mononuclear Cells Antioxidant Adaptations to Regular Physical Activity in Elderly People. Nutrients. 2018 Oct 20;10(10):1555. doi: 10.3390/nu10101555. PMID: 30347790.

0166-P

Acute NCLX inhibition prevents HIF-1α stabilization in hypoxia

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Reactive oxygen species (ROS) generated by mitochondria are a well-known example of mitochondria-derived signals that can drive cell adaptations or trigger cell damage. This is the case of the increase in ROS during the first minutes of hypoxia. Recently, we have discerned the molecular pathway leading to mitochondrial ROS production upon acute hypoxia, triggered by the mitochondrial sodium/calcium exchanger (NCLX) activation, which is used in specialized cells to trigger acute responses to hypoxia.

However, the long-term adaptation to hypoxia relies on hypoxia-inducible factors (HIFs). During normoxia, the α subunit of HIFs is hydroxylated by the prolyl-hydroxylases (PHDs) which target it to ubiquitination and degradation by the proteasome. In hypoxia, however, PHDs are inactivated and the α subunit stabilizes, what allows initiation of the hypoxic transcriptional programme. Although PHDs depend on O2 concentration to perform the hydroxylation reaction, HIF-α stabilization may also depend on ROS production by mitochondria.

We show that NCLX inhibition, which blocks hypoxic ROS production by mitochondria without affecting overall mitochondrial respiration, is able to inhibit hypoxic HIF1α stabilization and the expression of HIF targets. Interestingly, the prevention of HIF-1α stabilization during hypoxia occurred only after acute inhibition of NCLX, either pharmacologically or genetically. In contrast, under chronic NCLX knock-down, HIF-1α stabilization in response to hypoxia was maintained. Our results point out to NCLX as a key regulator of mitochondrial redox signalling, linking acute and long-term responses to hypoxia.

0189-R-P

Oxidative damage in reperfusion after stroke: ferroptosis and the role of the mitochondrial sodium/calcium exchanger NCLX

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Ferroptosis is a form of regulated cell death driven by iron-dependent accumulation of lipid hydroperoxides that cause membrane damage. It is now known to play a critical role in important pathological mechanisms such as ischemic cerebral stroke, where brain iron concentration and lipid peroxides levels are increased.

Lipid peroxidation can be initiated by reactive oxygen species, which are known to be produced by the mitochondria during hypoxia - reoxygenation. The mitochondrial sodium/calcium exchanger NCLX is involved in this process, since its activation during acute hypoxia drives superoxide production at complex III. The inhibition of Na+ import through NCLX is enough to block this pathway and inhibit reactive oxygen species production during hypoxia.

Taking this into account, our hypothesis is that NCLX could be involved in the production of lipid peroxides during hypoxia-reoxygenation, which would lead to membrane integrity loss and subsequent cell death by ferroptosis.

In order to test this idea, we developed a method for detecting lipid peroxidation in cell culture models of hypoxia-reoxygenation and ischemia-reperfusion by labelling the cells with Bodipy^{W} C11, a lipophilic fluorescent ratio-probe used for indexing levels of lipid peroxides.

By using this model and other ferroptosis readouts, we are assessing the role of NCLX in triggering ferroptosis in hypoxia-reoxygenation and ischemia-reperfusion.

0203-R/M-P

Effects of different tissue plasminogen activators on the oxidative stress response and neuronal damage, after neuronal ischemia-reperfusion injury

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Stroke is one of the leading causes of death and long-term disability, which makes it a clinically and epidemiologically highly relevant and impactful disease. Intravenous rt-PA (Actilyse) remains the sole standard treatment for distal vessel occlusions. rt-PA however, offers significant drawbacks, such as the risk of intracranial haemorrhages following treatment, a limited therapeutic window and an increase brain damage upon reperfusion. The main mechanism of damage after reperfusion is the increase in reactive oxygen species (ROS). Therefore, it is crucial to find new and improved methods of treatment providing greater efficacy as well as decreasing the severity and prevalence of side effects. Tenecteplase (TNK, Metalyse) is an alternative fibrinolytic drug generated by mutations in three aminoacids of the alteplase sequence. There is controversy regarding the beneficial effect of TNK *vs* rt-PA in clinical trials, with some of them showing a significantly better reperfusion and clinical outcome whilst others demonstrated worse functional outcome.

We wanted to evaluate the effect of TNK vs rt-PA over the toxicity after reperfusion in an *in vitro* model of stroke. We used primary neuronal cultures that underwent an oxygen/glucose deprivation (OGD) followed by up to 24 hours of reperfusion. We administered rt-PA and TNK at similar concentration to those found in brains from stroke patients during fibrinolytic therapy. We assessed the apoptotic neuronal death by flow cytometry and found that rt-PA induced a higher neurotoxicity compared to TNK. This effect was corroborated by an increase in caspase-3 activity and p53 stabilization in the rt-PA treated group compared to TNK. Furthermore, the mitochondrial superoxide anion production and hydrogen peroxide as well as HIF-1-alpha were also increased in the rt-PA condition at earlier time points, hinting at ROS overproduction by rt-PA as the culprit of the increased neuronal apoptosis under these experimental conditions.

These results show that rt-PA produces an increase in apoptosis during reperfusion compared to TNK. These higher apoptotic levels were, at least in part, induced by ROS overproduction.

Funded by Instituto de Salud Carlos III (PI19/01398) and Junta de Castilla y León PhD grant co-financed by Fondo Social Europeo.

0210-R/M-P

NRF2 function is required for osteocyte differentiation and bone homeostasis.

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Osteocytes are the most abundant type of cells in mature bone. No longer considered passive placeholders in bone, osteocytes have recently emerged as major orchestrators of bone remodeling, physical mechanosensors, hematopoietic niche cells, and endocrine regulators of whole-body metabolism.

Osteocytes are derived from osteoblasts. At the end of the bone formation phase, osteoblasts can either become quiescent as bone lining cells, die by apoptosis, or undergo an active process of differentiation called osteocytogenesis. During osteocytogenesis, dramatic morphological and metabolic changes occur, concurrently with the acquisition of a unique gene expression profile. Cells downregulate key molecular markers specific for osteoblasts, such as Collagen type one (*Col1a1*), alkaline phosphatase (*Alpl*), and osteocalcin (*Ocn*). Meanwhile, they start to express some osteocyte-specific markers, including dentin matrix protein 1 (*Dmp1*), matrix extracellular phosphoglycoprotein

(*Mepe*), and sclerostin (*Sost*). Bioinformatic studies show that those osteocyte-specific genes are organized in a few "topologically associated domains" (TADs).

Osteocyte differentiation occurs in parallel to mitochondrial biogenesis, increases reactive oxygen species (ROS) levels and consequently enhances NRF2 activity. Whole-genome NRF2-ChipSeq data (GSE113497G) shows that TADs with osteocyte-specific genes are enriched in binding sites for NRF2. ChiP assays confirm the binding of NRF2 to the regulatory region of *Dmp1* and *Sost*. Moreover, binding of NRF2 promotes osteocyte-specific expression of *Dmp1*, *Mepe*, and *Sost* in IDG-SW3 cells and primary osteocytes. On the other hand, ablation of *Nfe2l2* in osteocytes generates osteopenia and decreases the expression of most osteocyte-specific genes. Finally, treatment with dimethyl fumarate, an activator of NRF2, prevents the deleterious effects of ovariectomy in trabecular bone masses of mice. Altogether, we uncovered the role of NRF2 activity in osteocytes during the regulation of osteocyte gene expression and maintenance of bone homeostasis.

0218-P

Effects of moderate beer intake on cognitive impairment associated with aging: comparison of alcoholic beer and non-alcoholic beer

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Cognitive decline associated with aging is one of the main limiting factors in older people. Dietary interventions such as the use of phenolic compounds are one of the strategies that have been shown to slow down the brain aging process, largely due to their antioxidant and anti-inflammatory effects (1). Beer is a widely consumed beverage rich in bioactive compounds such as polyphenols and antioxidants (2). The aim of the present study was to analyze in an old rat model the chronic exposure to beer in attenuating brain deterioration and the cognitive decline characteristic of aging, and analyzing whether alcohol content can reduce its possible beneficial impact. Female Wistar rats were assigned to control, alcoholic beer (AB) or beer 0'0 (Non-Alcoholic, NAB) groups (n=6/group). The animals received the equivalent dose of two beers for human once per day during four months. Once a month, rats performed the Barnes and radial mazes (cognition) and the rotaRod test (motor coordination). At the end of the treatment, the rats were sacrificed and the left frontal brain and liver were used to evaluate antioxidant enzymes activities and lipid peroxidation levels. Improved performance tendency for the Barnes test was observed among months for all groups; and statistical differences were observed for NAB respect to month 0 and compared to AB, suggesting less cognitive impairment in short-term memory. Motor coordination was better for AB, although without significant differences. In the radial maze test less errors were done by NAB. The biomarkers of oxidative stress -catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST) activities-, as well as activity of myeloperoxidase (MPO) and malondialdehyde (MDA) levels did not show significant differences between groups in the brain or liver. In conclusion, a better cognitive status was observed in non-alcoholic beer group without an imbalance of the antioxidant system and a hermetic effect may occur in AB.

(1) Sarubbo F, Moranta D, Asensio VJ, Miralles A, Esteban S. Effects of Resveratrol and Other Polyphenols on the Most Common Brain Age-Related Diseases. Curr Med Chem. 2017;24(38). doi:10.2174/0929867324666170724102743. (2) Martínez-Gómez A, Caballero I, Blanco CA. Phenols and melanoidins as natural antioxidants in beer. Structure, reactivity and antioxidant activity. Biomolecules. 2020;10(3). doi:10.3390/biom10030400.

0287-P

Oral nitrate activates antioxidant and mitochondrial dynamics genes after moderate intensity acute exercise in metabolic syndrome patients

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Moderate intensity exercise can induce a pro-inflammatory response in aged metabolic syndrome subjects. Dietary nitrate supplementation has shown anti-inflammatory and antioxidant effects on plasma markers in response to acute exercise. We aimed to evaluate the influence of dietary nitrate on the response of the antioxidant and mitochondrial dynamics genes to acute exercise in peripheral blood mononuclear cells (PBMCs) from metabolic syndrome patients.

Metabolic syndrome patients participated in a crossover study in which they consumed a beverage containing 16mM sodium nitrate or a placebo beverage (with the same composition except for the absence of nitrate) before performing a submaximal test for 30 minutes. Venous blood samples were extracted from the antecubital vein of participants before and after performing the two submaximal tests at 60-70% of their maximal heart rate. The first test was performed after the intake of 500 mL of the placebo beverage or the nitrate rich beverage, and the second test was performed after the intake of 500 mL of the opposite beverage. We measured plasma nitrate plus nitrite levels using a NO analyser, detecting the chemiluminescence produced by the reaction between ozone and nitric oxide. PBMCs were isolated using a density gradient with Ficoll®Paque PLUS and resuspended in Tripure for RNA extraction. The mRNA expression was determined by real time PCR with human 18S ribosomal as the reference gene.

The intake of nitrate increased about 8-fold the nitrate plus nitrite plasma levels and induced the up-regulation of catalase, superoxide dismutase, glutathione peroxidase, mitofusin 2 and PGC1α in PBMCs after exercise. These responses to acute exercise were not observed after the intake of the placebo beverage.

In conclusion, the intake of nitrate by metabolic syndrome patients previous to an acute bout of moderate intensity exercise allows an antioxidant and mitochondrial dynamics response to exercise, which is not observed in the absence of nitrate intake.

0358-P

Atomic Quantum Clusters, potential radiosensitizers for cancer treatment

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Radiotherapy (RT) remains a cornerstone of cancer treatment: 50% of patients will receive RT during the course of their illness, with the majority of them being treated with conventional RT using photons (1). Technological improvements in conventional RT together with the introduction of new RT modalities (protons or heavy ions), have been translated into better clinical results. However, resistance and tumor recurrence are still observed.

Atomic Quantum Clusters (AQCs) are characterized by their small size (<1nm), which is responsible for the loos of its typical metallic character and the acquisition of a molecular-like behavior, leading to the emergence of new physico-chemical properties exclusive of these AQCs. The potential of AQCs for cancer treatment has been demonstrated for Silver AQCs of three atoms (Ag3-AQCs) which intercalates into the DNA augmenting chromatin accessibility to DNA-binding drugs, thus increasing the therapeutic index of chemotherapy (2); and for silver AQCs of five atoms (Ag5-AQCs) which catalyzes the oxidation of thiol groups of specific proteins in cells with high ROS levels resulting in a rapid cell death.

The aim of this study was to investigate the radiosensitizing potential of Ag-AQCs in combination with photon and proton radiation. As Ag5-AQCs preferentially kill cells with high ROS levels we hypothesize that Ag5-AQCs will enhance RT efficacy. All Ag5-AQCs concentrations used resulted in significant enhancement of the sensitivity to RT without increase DNA damage. In case of Ag3-AQCs, we propose that the increase in chromatin accessibility would render DNA more accessible to both, direct and indirect DNA damage caused by X-rays. Reduction in survival in cancer cells treated with Ag3-AQCs and X-rays is higher than those treated with X-rays alone.

In conclusion our results show that Ag3-AQCs and Ag5-AQCs have a radiosensitizing effect when combined with photon and proton beams.

1., Garibaldi, C. et al. 2017. Recent advances in radiation oncology. ecancermedicalscience. Cancer Intelligence. 2-. Porto, V. et al. 2018. 2. Silver Atomic Quantum Clusters of Three Atoms for Cancer Therapy: Targeting Chromatin Compaction to Increase the Therapeutic Index of Chemotherapy. Advanced Materials.

0369-P

Ag5, a new therapeutic tool in hard-to-treat solid cancers

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Deregulated redox homeostasis is a hallmark of cancer cells and is implicated in malignant progression and resistance to treatment. Cancer cells exhibit persistently high reactive oxygen species (ROS) levels compared with normal cells. To counteract ROS-mediated oxidation cancer cells are equipped with potent antioxidant machinery. Glutathione (GSH)- and Thioredoxin (Trx)-dependent pathways play an active role in protecting cancer cells from cell death. Disabling antioxidant defense systems thus represents a therapeutic option for cancer treatment (1, 2).

Atomic Quantum Clusters (AQCs) constitute a new class of molecules characterized by their small size (< 1nm) and their unique physico-chemical properties. The potential of AQCs for cancer treatment has been demonstrated for silver AQCs of three atoms (Ag3) which intercalates into the DNA augmenting chromatin accessibility to DNA-binding drugs, thus increasing the therapeutic index of chemotherapy (3). Here we present silver AQCs of 5 atoms (Ag5), a new molecule that can catalyze thiol oxidation in the presence of different oxidant species.

Our results demonstrate that Ag5 catalyze the selective oxidation of the thiol groups of thioredoxins, peroxiredoxins and targets of the glutathione pathway in cells generating high ROS. Since redox homeostasis is fundamental to maintain normal cellular functions and ensuring cell survival, Ag5 cause rapid cell death by induction of apoptosis specifically in cancer cells. Using an orthotopic mouse models we demonstrate the efficacy of Ag5 *in vivo*. These results highlight the potential of Ag5 as antitumor agents constituting an innovative tool to solve one of the major problems in cancer treatment.

1.Panieri, E, Santoro, M. "ROS homeostasis and metabolism: a danger-

ous liason in cancer cells". doi.org/10.1038/cddis.2016.105 2.Moloney JN, Cotter TG. "ROS signalling in the biology of cancer". doi: 10.1016/j.semcdb.2017.05.023 3.Porto, V., Borrajo, E., Buceta, D., Carneiro, C., Huseyinova, S., Domínguez, B., et al. "Silver Atomic Quantum Clusters of Three Atoms for Cancer Therapy: Targeting Chromatin Compaction to Increase the Therapeutic Index of Chemotherapy". doi.org/10.1002/

0381-P

Effect of bioaccesible melanoidins fraction on Nrf-2 and NF-kB in Caco-2 and HUVEC cells

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Nrf2 and NF-kB are the transcription factors that regulate cellular response to oxidative stress and inflammation, respectively. There are a functional cross-talk between these two pathways and can to be modulated by bioactive compounds such as melanoidins. The aim of the study was to evaluate the effect of bioaccesible melanoidins (BM) extract (0,4 mg) obtained from crust bread, subjected to *in vitro* gastrointestinal digestion, on intestinal cell (Caco-2) and endothelial cell (HUVEC) lines. The BM fraction activated the gene expression of NF-kB and Nrf2 in HUVEC cells but decreased it in Caco-2 cells. Further, a decrease in SOD gene expression in Caco-2, and an increase in HUVEC was observed. The evaluation of the GSH/GSSG levels only showed a decrease in Caco-2 treated with the BM fraction. Both transcription factors are activated by tert-butylhydroperoxide, and the effect of BM in these oxidant conditions was dependent of the cell type. In Caco-2, NF-kB gene expression decreased while Nrf2 increased, and the opposite takes place in HUVEC cells. For the BM treated cells, the SOD gene expression increased in both cell lines, and an increase in the GSH/GSSG was observed in HUVEC cells with not change in Caco-2 cells. These results indicate a different cell effect of melanoidins that could be attributed to the different oxidative stress level in basal condition.

0385-P

Detection of intracellular reactive oxygen and nitrogen species in skeletal muscle fibres using fluorescence genetically-encoded biosensors

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Reactive Oxygen and Nitrogen Species (RONS) play an essential role in pathophysiological processes. Hydrogen peroxide (H_2O_2) might act as a signalling molecule and modulate different crucial cellular signalling pathways, such as glucose uptake in skeletal muscle. Nitric oxide (NO) may play a prominent role on glucose uptake in skeletal muscle associated with the effect of insulin and in the development of insulin resistance. Glutathione (GSH) is part of the non-enzymatic antioxidant defense system of the cell, and the redox potential GSH/GSSG indicates cellular oxidative stress.

HyPer3, mito-HyPer, nuc-HyPer and GFP2-Orp1 are hydrogen peroxide biosensors. Mito-Grx1-roGFP2 and cyto-Grx1-roGFP2 biosensors are used for monitoring redox potential GSH/GSSG and G-geNOp is a nitric oxide biosensor. The coding sequences of these biosensors were microinjected and electroporated in muscle fibres isolated from the *flexor digitorum brevis* (FDB), or Viromer RED transfected in myoblasts. Isolated fibres or myoblast differentiated to myotubes in culture that expressed one of the biosensors were settled in the incubation chamber coupled to the fluorescence microscope. The chamber maintains temperature (37°C), environmental CO_2 (5%) and humidity.

Cells were exposed to different agents (H_2O_2, DTT, SNAP and CO_2) and intracellular H_2O_2, NO or GSH/GSSG were registered in real time using fluorescence emitted by biosensors and microscopy imaging analysis. We observed that when there were environmental CO_2 (5%) fluctuations, due to initial medium stabilization or occasional interruption of CO_2 supply, the biosensors showed changes in the fluorescence emission, which were registered. The main consequence of CO_2 fluctuations is the change in the pH of medium. The main part of the biosensor structure is a fluorescent protein, GFP in GFP2-Orp1, cytoGrx1-roGFP2, mitoGrx1-roGFP2 and GgeNOp, and YFP in de case of mito-HyPer, nuc-HyPer and HyPer3. It has been reported that these fluorescent proteins are sensitive to pH and this might be a disadvantage for the biosensors. However, we propose that this pH sensitivity should be considered as an additional property of these biosensors, since they provide information in real time about the rapid changes of pH due to environmental fluctuation of CO_2 and likely other gases such as O_2 or N_2.

0387-R/M-P

Interference with mitochondrial activity drives the on-set of cardiovascular disease following long-term treatment with SGAs

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The chronic intake of second generation antipsychotics (SGAs) has been related to increased risk of cardiovascular diseases. Taking into account the importance of redox balance in vascular function, we analysed the effect of Ari and Ola in the bioenergetics of primary bovine aortic endothelial vascular cells (BAEC). The results indicate that both Ola and Ari can interfere with mitochondrial function after 3h treatment, increasing the mitochondrial production of O2-. After 24h, we observed a higher recovery capacity in Ola than of Ari treated cells, suggesting a higher mitochondrial toxicity or a blunted capacity to induce compensatory systems in Ari treated cells. The effects of these drugs on mitochondrial respiration were also measured in in peripheral blood mononuclear cells of healthy volunteers treated with Ari or Ola. We found a stronger effect on respiration and increased proton leak, normally associated with increased ROS production and deficient ATP-linked respiration, with changes being more significant for Ari than for Ola. To analyse whether this SGAs can accumulate in mitochondria, we isolated liver mitochondria from mice ip injected with Ari or Ola and found that both Ari and Ola accumulated in the mitochondrial membranes, with Ola showing a trend for higher levels but also faster turnover. To evaluate the physiological effects of this inhibition we treated a mouse model of mitochondrial dysfunction (PGC-1-/-) with Ari and Ola for 6 months and we observed a reduction in O2 consumption, cardiac fibrosis, left ventricular remodeling and exacerbated cardiac I/R Injury, with all parameters being more evident in Ari than in Ola treated mice. Remarkably, Ola treated mice showed increased mitochondrial content, suggesting that Ola allows a partial compensation by increasing mitochondrial mass. These results suggested that both Ari and Ola interfered with mitochondrial function, and thus lead to increased risk of cardiovascular disease.

0417-P

CXCR8: A key factor between inflammation and mitochondria in breast cancer.

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Interleukin-8 (IL-8), also CXCL8, is a proinflammatory CXC chemokine, that mediated effects through the binding of IL-8 to its receptor (CXCR8). Increased IL-8 synthesis and secretion in tumor cells has been described and increase proliferation and survival of breast cancer cells. Mitochondria, main source of reactive oxygen species (ROS), play an important role in proliferation and apoptosis. IL-8 modulates growth and invasiveness of breast cancer cells, but its effects over mitochondria and oxidative stress in breast cancer it has not been studied yet.

The aim of this study was to determine the mRNA levels of mitochondrial biogenesis, inflammation, and oxidative stress by relating them to CXCR mRNA levels. Total RNA was isolated and purified (Tri-Reagent®) from 31 women (ages range: 45-90) with an invasive ductal carcinoma. The expression level of CXCR8 was determined by qPCR, using the median value to create two groups: low and high CXCR8 groups. Mitochondrial biogenesis, inflammation and antioxidant enzymes expression were analyzed by qPCR. ERα, ERβ and GPER mRNA levels were analyzed too.

High levels of CXCR8 are correlated, first, with high levels of other pro-inflammatory cytokines (IL-8, IL-6, IL6R, TNFα and COX2), strengthen the idea of autocrine signaling that increase malignancy of tumor cells. This malignancy is usually accompanied by energy demand, corroborated by high levels of mitochondrial biogenesis genes (SIRT1, PGC1α, NRF1, mtSSB and ATPase), observed in high CXCR8 group. To avoid excess ROS production, UCP5 mRNA levels are greatly increased in tumors with high CXCR8 levels. Likewise, some antioxidant enzymes such as GPx, CuZnSOD and the NRF2 factor, present higher expression levels in tumors with higher levels of CXCR8, suggesting that oxidative stress could be controlled. Finally, ERα mRNA levels did not show statistically significant changes between groups, but ERβ and GPER shown greater levels in high CXCR8 group.

Could there be a relationship between the IL-8 signaling pathway and estrogen receptors? If so, CXCR8 could be used as a biomarker for breast cancer prognosis, along with estrogen receptors.

*Supported by IdISBa-Proyectos intramurales Primus 2018 (PRI18/04). Proyecto Investigación en Cáncer de Mama (InCaM), FUEIB. Feim Camí per Viure, Santa Maria del Camí.

Qian Liu et al. (2016). The CXCL8-CXCR1/2 pathways in cancer. Cytokine & Growth Factor Reviews 31 (2016) 61–71. Helen Ha et al. (2017). Role of the CXCL8-CXCR1/2 Axis in Cancer and Inflammatory Diseases. Theranostics 7, 6, (2017) 1543-1588.

0425-P

Leukocytes from type 1 diabetic patients present oxidative stress, mitochondrial alterations, and increased autophagy markers

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Cardiovascular diseases (CVD) are the main cause of death in patients with Type 1 Diabetes (1). Hyperglycaemia is an important risk factor for the development CVD, it promotes the overproduction of reactive oxygen species (ROS), leading to increased oxidative stress, inflammation, and cellular death [1]producing oxidative stress, the formation of advanced glycation end products (AGEs . These stress conditions activate autophagy, a catabolic process that protects cells from apoptosis [2] . However, depending on the cellular or tissue environment, autophagy can exacerbate the disease [3] . The objective of this study was to evaluate oxidative stress rate, mitochondrial function and autophagic flux in leukocytes and serum levels of inflammatory parameters in T1D patients.

We recruited 63 healthy volunteers and 51 T1D patients. Anthropometric measurements were taken, and biochemical determinations and molecular analysis were performed in blood samples. Levels of proinflammatory cytokines (TNF-α and IL-6) and myeloperoxidase (MPO) were measured in the patients' serum. Mitochondrial function and several oxidative stress parameters were determined with fluorescent probes: DCFH-DA for total ROS production, MitoSOX for mitochondrial ROS production and TMRM for mitochondrial membrane potential. The relative expression of autophagy markers SQSTM1/p62 and LC3 were evaluated by Western Blot.

As expected, T1D patients presented higher glucose levels and HbA1c-DCCT than controls (both $p<0.001$). Serum levels of TNF-α ($p<0.01$) and MPO ($p<0.05$), but not of IL-6, were higher in T1D patients. Furthermore, leukocytes from T1D patients presented oxidative stress and mitochondrial alterations, expressed through increases in levels of total and mitochondrial ROS production (both $p<0.05$) and in mitochondrial membrane potential ($p<0.05$). Reduced protein levels of SQSTM1/p62 and an increased LC3-II/I ratio (both $p<0.05$) confirmed enhanced autophagic flux in the patients' leukocytes.

In conclusion, oxidative stress and mitochondrial alterations seem to induce inflammation and autophagy activation in leukocytes of T1D patients.

Acknowledgements: PI19/0838, PI19/0437, FI17/00126, GRISOLIAP/2016/015, GRISOLIAP/2019/091, FI17/00144, UGP-15-220, PROMETEO/2019/027, CD18/00069, CES/10/030, CPII16/00037 and the European Regional Development Fund (ERDF "A way to build Europe").

[1] Volpe, C.M.O., et al. Villar-Delfino, P.H., dos Anjos, P.M.F., Nogueira-Machado, J.A., 2018. Cellular death, reactive oxygen species (ROS) and diabetic complications. Cell Death & Disease 9(2): 1–9. [2] Galluzzi, L., et al., 2017. Molecular definitions of autophagy and related processes. The EMBO Journal 36(13): 1811–36. [3] Yun, H.R., Jo, Y.H., Kim, J., Shin, Y., Kim, S.S., Choi, T.G., 2020. Roles of Autophagy in Oxidative Stress. International Journal of Molecular Sciences 21(9).

0431-P

Estrogen receptor beta maintain mitochondrial functionality after obesity-associated inflammation treatment.

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Breast cancer development and progression could be increased by estrogen, leptin and proinflammatory cytokines that adipose tissue produces. In this process, estrogen receptor ratio (ERα/ERβ) takes on a relevant role, being an important cellular response factor in breast cancer. The objective of this study has been to analyze the role of ERα/ERβ ratio on mitochondria in an obesity-associated inflammation situation in breast cancer cell lines. Estrogen (10 nM), leptin (100 ng/ml), IL-6 (50 ng/ml) and TNF-α (10 ng/ml) cocktail treatment were studied on two breast cancer cell lines: MCF7 (higher ERα/ERβ ratio) and T47D (lower ERα/ERβ ratio). To do this, the expression of genes related to mitochondrial biogenesis and dynamics (qPCR), ROS production (Fluorimetry), OXPHOS complexes protein levels and oxidative damage (Western blot) were analyzed. Mitochondrial biogenesis mRNA levels in MCF7 cell line following cocktail treatment were decreased. However, in T47D cell line there were no change or showed an increase in the expression of ERRα and Twinkle mRNA levels. Furthermore, protein levels of the OXPHOS system decreased in MCF7 and not in T47D cell line after cocktail treatment. Mitochondrial dynamics markers expression showed a decrease in MCF7 cell line after treatment, but the opposite occurs on T47D cell line. ROS production increased especially in MCF7 cell line, showing, in addition, greater oxidative damage after treatment, while there were no changes in T47D cell line. These results were modulated in part by ERβ, as seen in silence/overexpression situation, highlighting these important genes related with estrogen signaling and mitochondria in breast cancer. In conclusion, this study suggests that ERβ could play an important role in maintaining mitochondrial biogenesis and dynamics, thus avoiding oxidative stress in an obesity-associated inflammation situation in breast cancer, and highlights some genes regulated by ERβ that could be new targets to better understand breast cancer biology.

*Supported by Fundació Institut d'Investigacio Sanitària Illes Balears (IdISBa)-Proyectos intramurales Programa "Primus" 2018 (PRI18/04).

Proyecto Investigación en Cáncer de Mama (InCaM), Fundació Universitat Empresa de les Illes Balears (FUEIB). Feim Camí per Viure – Santa Maria del Camí.

0435-R-P

Protective effects of melatonin on age-induced oxidative stress and inhibition of surfactant synthesis in rat type II pneumocytes

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Aging is associated with an increase in inflammation and oxidative stress. The aging lung is particularly affected since it is continuously exposed to environmental oxidants while antioxidant machinery weakens with age. Melatonin, a highly potent free radical scavenger, is known to counteract oxidative stress in healthy cells from several tissues. This study aimed to elucidate the role of melatonin in age-related inhibition of surfactant synthesis and oxidative stress in rat type II pneumocytes. Male Wistar rats were assigned to four experimental groups: young control (2 months old), old control (24 months old), old treated with 2.5 mg/kg/day melatonin and old treated with 5 mg/kg/day melatonin. After 10 weeks of treatment, animals were sacrificed and their lungs were collected in order to isolate and culture type II pneumocytes. Levels of CO (haemoglobin:carboxyhaemoglobin ratio after haemoglobin addition), NO (Griess reaction), LPO (ELISA), cGMP (ELISA), and *de novo* phosphatidylcholine production (incorporation of 10 mmol/L D-[U-14C] glucose into phosphatidylcholine) were measured. Type II pneumocytes showed an increase with age in the levels of NO, cGMP, CO and LPO ($p < 0.05$) as well as a decrease in pulmonary surfactant synthesis (phosphatidylcholine) ($p < 0.05$). Age-associated changes were reversed by melatonin treatment ($p < 0.05$). In conclusion, aging decreased PC synthesis and altered the redox status in type II pneumocytes, which were partially restored by melatonin supplementation.

Chung HY et al. 2006. The Molecular Inflammatory Process in Aging. Antioxidants & Redox Signaling. 8(3–4):572–581. Hardeland R. 2018. Melatonin and inflammation-Story of a double-edged blade. J Pineal Res. 65(4):e12525. Harman. 1956. Aging: a theory based on free radical and radiation chemistry. J Gerontol. 11(3):298–300. Veldhuizen RAW et al. 2019. The effects of aging and exercise on lung mechanics, surfactant and alveolar macrophages. Exp Lung Res. 45(5–6):113–122.

0494-R/M-P

Non-invasive analysis of metabolism biomarkers aiming to discriminate thyroid hyperplasias vs carcinomas prior to thyroidectomy

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The metabolic syndrome contributes to tumor development. However, the use of metabolic biomarkers has not yet been implemented. Taking into account that the metabolic syndrome is associated with mitochondrial dysfunction and the accumulation of mutations in mtDNA we aimed to evaluate its value as biomarkers in thyroid cancer.

Methods. The study was conducted in two phases, with cohorts of 26 and 45 patients respectively, undergoing a thyroidectomy due to suspicion of thyroid cancer. Tumor development and metabolic alterations were analyzed in tumor and surrounding healthy tissue as well as in peripheral blood mononuclear cells (PBMCs). **Results.** We noted a significant correlation between metabolic disorders at the systemic level, measured in PBMCs and tumor tissue. Two mitochondrial DNA fragments were identified in plasma samples. The origin of these fragments and their correlation with metabolic markers in both the tumor tissue and PBMCs was evaluated. One of them, a fragment of the mitochondrial ND1 gene was found to be associated with markers of good general or systemic metabolic status, with balanced mitochondrial activity and antioxidant levels. The second fragment, derived of the mitochondrial ND4 gene, was associated with bad metabolic status markers and originated from the tumor. **Conclusion.** Therefore, these results suggest that the assessment of metabolic parameters in PBMCs can inform both metabolic state systemic and its impact on the tumor, and that circulating mitochondrial DNA fragments can be used as biomarkers for the stratification of patients according to their level of metabolic risk of thyroid cancer

14.- Regulation of Gene Expression and Genome Dynamics

0511-OI

New insights into DNA damage signaling during replication in eukaryotes

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CABIMER Genetics

DNA can be damaged by numerous endogenous and exogenous factors. To safeguard the genome against these insults, cells have evolved DNA damage checkpoints that sense the presence of damaged DNA, block cell cycle progression and ensure that DNA is fully repaired before resuming the cell cycle. However, in many cases, unrepaired lesions remain in the DNA when cells enter S-phase. In this scenario, cells employ DNA damage tolerance mechanisms to complete genome replication and prevent fork breakage. Importantly, these pathways are not restricted to the site of stalling but can also function behind the fork at single-stranded(ss)DNA gaps originated by re-priming of DNA synthesis downstream of lesions.

While it is well known that ssDNA is the signal that triggers the checkpoint response, it is less clear how and where ssDNA actually arises. Generally, it is assumed to accumulate at stalled replication forks by an uncoupling between replicative helicase and polymerase movement. However, in a recent study in yeast, we found that ssDNA gaps left behind replication forks, and extended by processing factors such as the exonuclease Exo1, constitute the predominant signal that leads to checkpoint activation in response to damaged DNA templates during S-phase (García-Rodríguez et al, *EMBOJ,* 2018). Interestingly, not only ssDNA gap processing seems important for checkpoint signaling but also for the template switching pathway of DNA damage tolerance. Our findings regarding the role of Exo1 in yeast led us to investigate whether its function in checkpoint activation and damage tolerance during replication is conserved in human cells.

Garcia-Rodriguez, N., Morawska,M., Wong,R.P ., Daigaku,Y . and Ulrich,H.D. (2018) Spatial separation between replisome- and template-induced replication stress signaling. EMBO J., 37, e98369.

0512-OI

Chromatin dynamics in stem cells

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A central question in biology is how pluripotent cell retains the plasticity to acquire different transcriptional states upon differentiation. Notably, the chromatin structure and composition oscillate dynamically during the cell cycle of pluripotent cells, hence offering windows to engage either self-renewal or exit of pluripotency and differentiation. How the oscillation of chromatin factors across the cell cycle impacts on pluripotency exit remains largely unknown. Here, by combining quantitative proteomics, functional genomics, and single-cell transcriptomics, we have identified a chromatin dynamic regulatory axis that regulates the cell cycle-dependent transcriptional priming of pluripotent cells. We believe that our new data provide novel insights into the molecular mechanisms governing cell fate specification during early embryo development, which might be at the basis of multilineage specification of adult proliferating progenitors.

0117-OS

REPLICATIVE AGE IMPACTS PROLIFERATION CAPACITY SINCE EARLY STAGES

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Clonal cell populations exhibit proliferative heterogeneity in all kind of organisms, from unicellular microorganism to human tissues. This is especially relevant in, for example, tumoral cells, where this proliferative heterogeneity leads to drug resistance (Levy et al., 2012) .

Yeast cell can be trapped in regular alginate microparticles by microfluidic microencapsulation, allowing to study proliferative heterogeneity at a single-cell level. When a clonal yeast culture is encapsulated, each single cell forms a microcolony inside the capsule and the very first divisions can be studied in detail. We have already shown that these microcolonies differ in size as a consequence of the heterogeneity in the proliferation capacities of their founder cells (García-martínez et al, 2016). We wonder what is causing that two clonal cells in the same environmental conditions proliferate at different rates.

Our previous transcriptomic results showed that the subset of microcolonies with the lowest proliferation rate were enriched in the expression of two gene categories: respiratory metabolism and cell cycle regulation. The highest enriched gene that we found was *WHI5*, which encodes a cell cycle repressor of the G1-S transition and the yeast functional homologous of human Retinoblastoma.

Here we present results showing that the smallest microcolonies are not usually founded by newborns but by cells that have already undergone more than one division. This indicates that proliferative age, since early stages, is a potential explanation for heterogeneity of cell populations.

Since very small microcolonies are enriched in *WHI5* expression and are frequently founded by non-newborn cells, we wondered whether expression of *WHI5* increases with cell division rounds in mother cells. Our results confirmed this hypothesis, suggesting a role for Whi5 in cell aging, not just in late stages, but in the first cycles of the lifespan.

García-Martínez, J., Delgado-Ramos, L., Ayala, G., Pelechano, V., Medina, D.A., Carrasco, F., et al. (2016). The cellular growth rate controls overall mRNA turnover, and modulates either transcription or degradation rates of particular gene regulons. Nucleic Acids Res 44(8), 3643-3658. doi: 10.1093/nar/gkv1512. Levy, S.F., Ziv, N., and Siegal, M.L. (2012). Bet hedging in yeast by heterogeneous, age-correlated expression of a stress protectant. PLoS Biol 10(5), e1001325.

0140-R-OS

CTCF binding affinity associates with distinct regulatory functions across the genome

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CTCF is a DNA-binding protein involved in establishing long-range interactions that define chromatin architecture and regulate transcriptional programs. In B cells, CTCF regulates both VDJ recombination and class switch recombination, which are key events for the immune response. However, the relationship between CTCF-mediated contacts and their transcriptional implications in mature B cells is not well understood. To address this question, we used a conditional mouse model where CTCF is deleted specifically in mature B cells and performed ChIPseq analysis. We found a subset of CTCF-binding sites that are particularly resistant to CTCF depletion (*retained* CTCF sites), enriched in canonical CTCF-binding motifs, which are often in close proximity to each other forming tandem CTCF sites. *Retained* CTCF sites are highly conserved across different cell types and are enriched in activation epigenetic marks, characteristic of transcriptional enhancers. In contrast, those sites that are lost after CTCF depletion (*lost* CTCF sites) tend to be cell-type specific and are enriched in promoter-specific marks. Additionally, *retained* CTCF sites are more often localized at topologically associating domains (TADs) boundaries, suggesting that CTCF has more affinity for binding sites involved in constitutive chromatin architecture.

To analyze the link between differential CTCF binding and regulation of gene expression we performed RNAseq analysis and developed an algorithm that identifies regions that can be transcriptionally regulated by CTCF-dependent loops. This algorithm has allowed the identification of clusters of genes predominately flanked by *retained* CTCF sites, whose expression significantly changes after CTCF depletion, suggesting that they can be coordinately regulated by CTCF-mediated loops. Therefore, our approach can predict and classify CTCF interactions based on distinct gene expression patterns. Our study represents a step forward in the understanding of CTCF regulation and its impact on the organization and expression of genes.

0497-OS

Histone H1 regulates non-coding RNA turnover on chromatin in a m6A-dependent manner

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Centro de Biología Molecular Severo Ochoa (CBMSO) Dinámica y Función del Genoma

Linker histones are highly abundant chromatin-associated proteins with well-established structural roles in chromatin and as general transcriptional repressors. In addition, it has been long proposed that histone H1 exerts context-specific effects on gene expression. Here, we have identified a new function of histone H1 in chromatin structure and transcription using a range of genomic approaches. We show that histone H1 is required to prevent the accumulation of nascent non-coding RNAs, suggesting that it regulates non-coding transcript turnover on chromatin. Interestingly, we found that unscheduled non-coding transcripts have reduced levels of m6A modification, causing replication-transcription conflicts. Accordingly, impairing m6A demethylase activity rescues the replicative stress phenotype of H1 loss. This work unveils unexpected regulatory roles of histone H1 on non-coding RNA turnover and m6A deposition, highlighting the intimate relationship between chromatin conformation, RNA metabolism and DNA replication to maintain genome performance.

0071-R/M-P

NOVEL MOLECULAR TOOLS FOR GENOMIC ANALYSIS AND DROUGHT TRAIT PREDICTION IN Vicia sativa

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INIA CBGP

Loss in crop yield due to climate change and associated drought is one of the main problems faced by current crops, including the legume common vetch (*Vicia sativa* L.). This legume has high protein content and nitrogen fixation capacity, which makes this crop very relevant from an economic and ecological point of view.

Recent efforts in transcriptome analysis using next generation sequencing technology provide a relatively cost-effective and useful source for molecular markers in unsequenced species, as vetch. An RNA-Seq approach has been used to analyze the genetic basis of drought response in this plant. *De novo* transcriptome assembly, annotation and analysis of differential expressed genes (DEGs) have allowed the identification, not only drought regulated genes, but also and most relevant, genes potentially involved in drought resilience. The presence of polymorphic variants, such single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs) on DEG genes between drought-sensitive and tolerant genotypes has enabled the design of molecular markers that would be used in trait prediction association analysis and the identification of potential drought-tolerant genotypes (De la Rosa *et al.*, 2020).

In our lab, the combined use of multiplex-PCR technics with fluorochrome marked primers has allowed the simultaneous analysis of hundreds of genotypes with different SSR markers in a high throughput and cost-effective way. Similarly, large-scale qPCR-based SNP genotyping has been developed and implemented with rhAmp technology. The screening *V. sativa* collection of CRF genebank comprising Spanish, but also West Mediterranean and Middle East accessions with these drought-related molecular markers would be useful to select candidates for drought resilience, adaptability or yield by the screening of germplasm crop collections.

Our final goal will be the evaluation of functional parameters of drought tolerance, using physiological, agronomical, yield and eco-geographical parameters, to validate the use of these markers in the selected candidates. The integration of this functional and genetic information would be used in future strategies to accelerate breeding programs and in the development of predictive genotypic and phenotypic strategies for further use in genebanks.

De la Rosa, L., Zambrana, E., Ramirez-Parra, E. (2020) Molecular bases for drought tolerance in common vetch: designing new molecular breeding tools. BMC Plant Biology 20, 71. DOI: 10.1186/s12870-020-2267-z

Acknowledgements & Funding: This work was supported by Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA [Grant number AT2016-009] and by the Spanish Government MICINN (RTI2018-094037-R-I00 and PTA2017- 13541-I).).

0102-R/M-P

The CDK Pho85 blocks Whi7 to promote Start activation

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Start (the G1/S transition) is the main decision point in the eukaryotic cell cycle at which cells irreversibly commit to a new round of cell division by activating the Start transcriptional programme. In budding yeast, triggering Start involves the inactivation of the Start transcriptional repressors (Whi5 and Whi7[1]) which are inactivated by the CDK Cdc28 dependent phosphorylation. Pho85 is a CDK that regulates the cellular response to phosphate levels and diverse stresses[2]. Pho85 is also linked to G1 control and key Start regulators have been identified as Pho85 targets[3,4,5]. Here we unveil a new mechanism by which Pho85 promotes Start. Pho85 specifically downregulates Whi7 but not Whi5 protein levels. We demonstrate that CDK Pho85-cyclins regulates Whi7 levels in two parallel ways: Pho85-Pho80 represses Whi7 expression and Pho85-Pcl1,Pcl2,Pcl9,Pho80 promotes its instability. *WHI7* transcriptional activation in a *pho85* mutant is dependent of the Pho4 transcription factor but, unexpectedly, Pho4 promotes *WHI7* expression independently of the phosphate signaling pathway. Alike Whi5, Whi7 overexpression is toxic in *pho85* mutant cells. Importantly, we show that the G1 delay observed in a *pho85* mutant is dependent on the Whi7 repressor. We seek to identify the cellular stress conditions which inactivate Pho85 to unlock Whi7 repressor. Fluorescence microscopy revealed Pho4-GFP nuclear signal under cell wall stress and DNA damage. On these lines, the lethality of a *pho85* mutant upon cell wall stress is rescued by inactivating Whi7. We conclude that Pho85 inhibits Whi7 to promote Start activation. We propose that this new mechanism of Start control may be crucial to ensure G1 temporary arrest under adverse conditions and promote cell adaptation to stress.

[1] Gomar-Alba, M., et al. (2017). Nature communications, 8(1), 1-13. [2] Huang, D., et al. (2007). Molecular microbiology, 66(2), 303-314. [3] Wysocki, R., et al. (2006). Nature structural & molecular biology, 13(10), 908-914. [4] Huang, D., et al. (2009). PLoS Biol, 7(9), e1000188. [5] Valk, E., & Loog, M. (2013). Molecular and cellular biology, 33(7), 1270-1272.

0111-P

Human prefoldin modulates co-transcriptional pre-mRNA

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Prefoldin is a co-chaperone, present in archaea and all eukaryotic organisms. Although known for its cytoplasmic role in the folding of actin and tubulin monomers during cytoskeleton assembly, prefoldin can also be found in the nucleus. We have previously described that prefoldin plays functions related to transcription elongation and chromatin dynamics in *Saccharomyces cerevisiae* (1).

In human cells, we have found that prefoldin perturbation generates changes in gene expression over the genome. These transcriptional alterations are stronger in long genes with a high number of introns, which is consistent with the co-transcriptional splicing defect detected in prefoldin knockdown cells.

Prefoldin is localized to transcribed genes over the genome, accumulates around the transcription start site (TSS), and follows a similar distribution to RNA pol II. Furthermore, its accumulation correlates with the negative impact of prefoldin-depleted cells on gene transcription, which supports a local contribution of prefoldin to gene expression.

Lack of prefoldin also generates a decrease in the levels of Ser2-phosphorylation of the RNA polymerase II CTD domain, and in the presence of the Ser2P kinase CDK9 in transcribed genes. Moreover, splicing factors PRP19 and U2AF65, which are known to be co-transcriptionally recruited, were also less present in transcribed chromatin of prefoldin KO cells.

Taken altogether, these results demonstrate that prefoldin contributes to human gene expression by preserving RNA pol II Ser2-phosphorylation and thereby modulating co-transcriptional splicing.

1. Millán-Zambrano, G., Rodríguez-Gil, A., Peñate, X., de Miguel-Jiménez, L., Morillo-Huesca, M., Krogan, N., & Chávez, S. (2013). The prefoldin complex regulates chromatin dynamics during transcription elongation. PLoS genetics, 9(9), e1003776.

0123-P

Bisphenol A, Bisphenol F and Bisphenol S affect differently 5α-reductase expression and dopamine-serotonin systems in the prefrontal cortex of juvenile female ra

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Early-life exposure to the endocrine disruptor bisphenol A (BPA) affects brain function and behavior, which might be attributed to its interference with hormonal steroid signaling and/or neurotransmitter systems. Alternatively, the use of structural analogues of BPA, mainly bisphenol F (BPF) and bisphenol S (BPS), has increased recently. However, limited *in vivo* toxicity data exist. We investigated the effects of BPA, BPF and BPS on 5α-reductase (5α-R), a key enzyme involved in neurosteroidogenesis, as well as on dopamine (DA)- and serotonin (5-HT)-related genes, in the prefrontal cortex (PFC) of juvenile female rats. Gestating Wistar rats were treated with either vehicle or 10 µg/kg/day of BPA, BPF or BPS from gestational day 12 to parturition. Then, female pups were exposed from postnatal day 1 through day 21 (PND21), when they were euthanized and RT-PCR, western blot and quantitative PCR-array experiments were performed. BPA decreased 5α-R2 and 5α-R3 mRNA and protein levels, while both BPF and BPS decreased 5α-R3 mRNA levels in PFC at PND21. Further, BPA, BPF and BPS significantly altered, respectively, the transcription of 25, 56 and 24 genes out of the 84 DA and 5-HT-related genes assayed. Of particular interest was the strong induction by all these bisphenols of *Cyp2d4*, implicated in corticosteroids synthesis. Our results demonstrate for the first time that BPA, BPF and BPS differentially affect 5α-R and genes related to DA/5-HT systems in the female PFC. *In vivo* evidence of the potential adverse effects of BPF and BPS in the brain of mammals is provided in this work, raising questions about the safety of these chemicals as substitutes for BPA.

0132-R/M-P

CD90 as a predictive marker for dexamethasone response in GBM patients

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Gliomas are the most common malignant tumor of the central nervous system (CNS). Glioblastoma (GBM)-a grade IV glioma-is the most aggressive and malignant primary brain tumor in adults, with an overall survival (OS) of 8 months in patients without treatment. Nowadays, the therapy consists on surgery followed by radiotherapy plus concomitant and adjuvant chemotherapy (temozolomide). Prior to tumor resection the most common drug prescribed is dexamethasone, which cease cephaleas, and the cerebral associated edema that damage the cerebral parenchyma. Despite its high mortality, poor advances have been developed in GBM treatment, which only increases the OS until 20,9 months due to its high inter- and intra-tumoral heterogeneity. In order to evaluate if there are some predictive markers to dexamethasone treatment, we analyzed CD90, CD133, CD44 and OCT3/4 stem cells markers expression in a cohort of 25 patients. Then we determined clinical response according to RANO criteria, the EVA's scale and the Glasgow's scale. We identified that patients with over-expression of CD90 had a better response to dexamethasone. Hence, the over-expression of CD90 could be used as a predictive biomarker for the response to dexamethasone in GBM patients.

1) Keskin DB, Anandappa AJ, Sun J, et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. Nature. 2019;565(7738):234-239. doi:10.1038/s41586-018-0792-9 2) Wirsching HG, Galanis E, Weller M. Glioblastoma. Handb Clin Neurol. 2016;134:381-97. doi: 10.1016/B978-0-12-802997-8.00023-2. PMID: 26948367.

0142-R/M-P

RNA polymerase II assembly and mRNA decay regulation are mediated and interconnected via CTD Ser5P phosphatase Rtr1 in Saccharomyces cerevisiae.

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Rtr1 is an RNA pol II CTD-phosphatase that influences gene expression by acting during the transition from transcription initiation to elongation, and during transcription termination. Rtr1 has been proposed to mediate the nuclear RNA pol II import during the biogenesis of the enzyme, and to participate in mRNA decay by autoregulating the turnover of its own mRNA. In addition, the interaction of Rtr1 with RNA pol II depends on the phosphorylation state of CTD, which also influences Rpb4/7 dissociation during transcription. In this work, we demonstrate that Rtr1 acts in RNA pol II assembly, likely in a final cytoplasmic RNA pol II biogenesis step, and mediates the Rpb4 association with the rest of the enzyme. However, we do not discard a role in the Rpb4 association with RNA pol II in the nucleus. This role of Rtr1 interplays RNA pol II biogenesis and mRNA decay regulation. In fact, *RTR1* deletion alters RNA pol II assembly and leads to the chromatin association of RNA pol II lacking Rpb4, in addition to whole RNA pol II, likely decreasing mRNA-Rpb4 imprinting and, consequently, increasing mRNA stability. Our data also indicate that Rtr1 mediates mRNA decay regulation more broadly than previously proposed. Interestingly, these data include new layers in the crosstalk between mRNA synthesis and decay.

0157-R/M-P

Specificity for the orphan base opposite an abasic (AP) site suggests complementary DNA repair roles for AP endonucleases and AP lyases

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Abasic (apurinic/apyrimidinic, AP) sites are ubiquitous DNA lesions that arise from spontaneous base loss, but also as intermediates during base excision repair (BER). AP sites may be processed either by AP endonucleases or AP lyases, but the relative roles of these two types of enzymes are not well understood. We hypothesized that the sequence flanking the AP site and/or the orphan base on the opposite DNA strand may influence the probability that the lesion is processed either by an AP endonuclease or an AP lyase. In this work we have analyzed the activity of human and plant AP endonucleases and AP lyases on DNA substrates containing an abasic site opposite either G or C in different sequence contexts. AP sites opposite G are common intermediates during repair of deaminated cytosines, whereas AP sites opposite C arise during repair of oxidized guanines. We found that in all tested contexts the major *Arabidopsis* AP endonuclease (ARP) displayed a significantly higher activity on AP sites opposite G. In contrast, the major plant AP lyase (FPG) consistently showed a higher preference for AP sites opposite C. The major human AP endonuclease (APE1) preferred G as the orphan base, but only in some sequence contexts. Our results suggest that plant AP endonucleases and AP lyases perform complementary functions in the maintenance of C:G pairs, counteracting the potential mutagenic consequences of C deamination and G oxidation, respectively.

0158-P

Fused in Sarcoma (FUS) is involved in the response to topoisomerase I-induced transcriptional stress

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Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease, characterized by progressive degeneration of motor neurons. Some of the genes associated with this disease encode proteins involved in RNA processing, including FUS (Fused in Sarcoma). Autosomal dominant mutations in FUS have been associated with both familial and sporadic ALS. It has been proposed that ALS mutations cause pathological changes in FUS-regulated gene expression and RNA processing, due either to loss of normal FUS function, toxic gain of function, or both. FUS is a nuclear RNA/DNA binding protein that plays a key role in multiple steps of RNA metabolism and the DNA Damage Response. However, the nature of the endogenous sources of DNA damage that might trigger a requirement for FUS and/or other RNA-processing factors is unknown. Of particular threat to neural maintenance and function is DNA damage induced by topoisomerases, a class of enzymes that remove torsional stress from DNA by creation of transient DNA strand break. Here, using a variety of different cell types, including human spinal motor neurons, we showed that FUS is a component of the cellular response to topoisomerase I (TOP1)-induced DNA breakage. FUS relocalised from nucleoplasm to sites of nucleolar rRNA synthesis in response to RNA polymerase II transcriptional stress induced by abortive TOP1 DNA breakage. This relocalisation was rapid and dynamic, reversing following the removal of TOP1-induced breaks and coinciding with the recovery of global transcription. The molecular role of this response is unclear, but we propose that FUS moves from sites of stalled RNA polymerase II to sites of RNA polymerase I activity either to regulate pre-mRNA synthesis and/or processing during transcriptional stress, or to modulate some yet unidentified aspect of rRNA biogenesis. Finally, we found that HeLa cells and ALS patient fibroblasts expressing mutant FUS are hypersensitive to TOP1-induced DNA breakage, highlighting the possible relevance of our findings to ALS disease pathology.

0183-P

Cdc14 phosphatase facilitates recombinational DNA repair by avoiding DNA2 over-resection

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After a DNA lesion, one of the first events taking place during the execution of the repair pathway is the nucleolitic degradation of one strand of the DNA molecule at both sides of the break. This process, known as resection, generates long 3' single-stranded DNA (ssDNA) tails that are used for invading a homologous sequence to copy the lost information [1,2] .

We show that Cdc14 is transiently accumulated in the nucleus and activated in response to a DNA lesion. Once activated, Cdc14 recognize several Cdk targets of the repair machinery[3] cells have developed a coordinated signalling network called DNA damage response (DDR . Elimination of Cdc14 results in the accumulation of long ssDNA tracks at both sides of the DNA lesion, suggesting a role of the phosphatase in the control of resection length. Importantly, the elimination of Dna2 restores the hyper-resection phenotype observed in the absence of the phosphatase, indicating that this exonuclease is the target of Cdc14 during resection inhibition. To understand the importance of these phosphorylation changes in Dna2 activity, we generated different phospho-mutant versions of Dna2. The hyper-resection phenotype described above was eliminated when using a phospho-deficient Dna2, indicating that these residues control Dna2 activity in response to a DNA break. On the other hand, the expression of a phosphomimetic Dna2 developed a hyper-resection phenotype similar to that observed in Cdc14 deficient cells. Importantly, while Cdk-dependent phosphorylation of Dna2 ensures its nuclear accumulation, activation of Cdc14 during the damage response excludes the exonuclease from the nucleus, suggesting that the negative control that this phosphatase exerts over Dna2 is executed by stimulating its transport to the cytoplasm.

Overall, we propose a model whereby the subsequent activation and deactivation of Dna2 by the Cdk/Cdc14 module acts as a molecular switch that ensures the accurate length of resection. Importantly, the accumulation and activation of Cdc14 at the nucleus is also subjected to a spatiotemporal regulation during the damage response. We propose that Cdc14 nuclear enrichment in response to DNA damage ensures the timely inhibition of resection only when the ssDNA tracks are long enough to promote recombinational events, and therefore, avoids the accumulation of excessive long resection intermediates that might affects the repair of the DNA lesion.

1. Liu T, Huang J. DNA End Resection: Facts and Mechanisms. Genomics Proteomics Bioinformatics. 2016;14(3):126-130. doi:10.1016/j.gpb.2016.05.002 2. Symington LS. Mechanism and regulation of DNA end resection in eukaryotes. 2016;51(3):195-212. doi:10.3109/10409238.2016.1172552 3. Villoria MT, Ramos F, Dueñas E, Faull P, Cutillas PR, Clemente-Blanco A. Stabilization of the metaphase spindle by Cdc14 is required for recombinational DNA repair. EMBO J. 2017;36(1):79-101. doi:10.15252/embj.2015

0202-P

Papel de receptores nucleares y citoquinas inflamatorias en la regulación de las enzimas peroxisomales implicadas en la biosíntesis de ácidos biliares

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Antecedentes: La expresión de CYP7A1, la enzima limitante de la biosíntesis de los ácidos biliares (ABs)[1], está regulada por receptores nucleares como FXR[2] e interleucinas inflamatorias como IL-1β[3]. Sin embargo, se desconoce el papel de estos factores en la regulación de otras enzimas involucradas en el metabolismo de los ABs.

Objetivo: Caracterizar los mediadores fisiológicos y fisiopatológicos implicados en la regulación transcripcional de las enzimas peroxisomales que participan en la biosíntesis de los ABs.

Métodos: Se determinó la expresión de ABCD3, AMACR, HSD17B4, ACOX2, SCP2 y BAAT mediante RT-qPCR en células de hepatoblastoma humano (HepG2) y hepatocitos humanos inmortalizados (IHH) cultivados en presencia de agonistas de FXR, LXR y PPARs, de FGF19 recombinante humano o de las citoquinas recombinantes IL-6, oncostatina M (OSM), IL-1β y TNFα. La biotransformación de ABs en células HepG2 y células de hepatoma humano (HepaRG) se analizó mediante HPLC-MS/MS[4].

Resultados: La activación de los receptores nucleares estudiados no modificó significativamente la expresión de los genes que codifican para las enzimas implicadas en la ruta peroxisomal de biosíntesis de los ABs, salvo en el caso del tratamiento con bezafibrato, un agonista inespecífico de PPARs, que indujo un incremento significativo en la expresión de ABCD3 y HSD17B4. El péptido hormonal FGF19 causó una reducción, moderada aunque significativa, de la expresión de ACOX2 a través de una vía dependiente de la activación de ERK1/2. Sin embargo, las citoquinas ensayadas mostraron un efecto más potente, principalmente la OSM, que redujo en un ≈80% la expresión de CYP7A1, AMACR, ACOX2 y BAAT. Además, el tratamiento con OSM provocó una disminución de la capacidad de las células HepG2 para sintetizar ácido cólico a partir de ácido trihidroxicolestanoico, así como de las células HepaRG para conjugar ácido cólico.

Conclusión: Las citoquinas inflamatorias juegan un papel relevante en la regulación transcripcional de enzimas implicadas en la vía peroxisomal de biosíntesis de los ABs, lo que podría contribuir a la alteración de la homeostasis de estos esteroides, que acompaña a ciertas hepatopatías que cursan con inflamación.

1) Zhang L et al. Mol Endocrinol 2009; 23(2): 137–145. 2) Goodwin B et al. Mol Cell 2000; 6(3): 517-526. 3) Li T.et al. Hepatology 2006; 43(6): 1202-1210. 4) Nytofte NS et al. J Med Genet 2011;48:219–225.

0238-P

The RING domain of human Nse1 participates in Smc5/6 complex and genomic integrity in an E2-independent manner

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To achieve a faithful transmission of the genetic information, eukaryotic cells need to accurately replicate and segregate their chromosomes during cell division. This arduous task is orchestrated by the conserved Structural Maintenance of Chromosome (SMC) complexes. The Smc5/6 complex plays a crucial role in the maintenance of genomic integrity by ensuring proper chromosome segregation and facilitating DNA repair1.

To further pinpoint the essential function of SMC5/6 complex, we focused on one of the subunits of the complex – Nse1 – which contains a RING domain, with a potential ubiquitin ligase activity2. To analyze the role of the RING domain in a more comprehensive manner, we deleted the C-terminal RING domain by using CRISPR/Cas9. Mutant cells show no detectable levels of Nse1 and other subunits of the complex, except Nse2. These mutant cells have a genomic instability phenotype, characterized by slow growth, prolonged mitosis, spontaneous endogenous DNA damage, slowdown of replication fork progression and sensitivity to MMS, a genotoxic drug.

To better understand the role of the RING domain in Nse1, we created a series of mutations at conserved residues in the RING domain, predicted to be important for zinc ion coordination or to disrupt the E2-E3 interaction3.Our results indicate that the RING domain of NSE1 is necessary for the stability of the SMC5/6 complex and genome integrity, in a non-ubiquitin-ligase dependent manner.

1.Aragón L. The Smc5/6 Complex: New and Old Functions of the Enigmatic Long-Distance Relative. Annu Rev Genet. 2018;52:87-107.doi:10.1146/annurev-genet-120417- 031353 2.Solé-Soler R, Torres-Rosell J. Smc5/6, an atypical SMC complex with two RING-type subunits. Biochem Soc Trans. 2020;48(5):1-13.doi:10.1042/BST20200389 3.Garcia-barcena C, Osinalde N, Ramirez J, Mayor U. How to Inactivate Human Ubiquitin E3 Ligases by Mutation. Front Cell Dev Biol. 2020;8(39):1-14. doi:10.3389/fcell.2020.0

0244-P

HMGB1 silencing promotes dysregulation of genes related to RNA processing and ribosome biogenesis in prostate and ovarian cancer cell lines

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Prostate cancer is the second most commonly occurring cancer in men, and ovarian cancer is the second cause of death from gynecological cancer in women. In cancers from different origins, including prostate and ovarian cancer, the overexpression of HMGB1, member of the High Mobility Group (HMG) protein family, has been detected and related to main cancer hallmarks as epithelial-mesenchymal transition, angiogenesis, invasion and migration, as well as bad prognosis (Barreiro-Alonso *et al.*, 2016; Raboport,B.L. *et al.*, 2020). The multiple roles of HMGB1 depend on its oxidation state, its subcellular location (nuclear, cytoplasmic, or extracellular) and their interaction with other proteins. The mass spectrometry technology has been used to identify novel HMGB1 binding partners in prostate and ovarian cancer cell lines. Interestingly, several proteins related to RNA processing and ribosome biogenesis have been detected. In order to study the possible functional relationships between HMGB1 and these interacting partners, the mRNA expression levels of the partners have been analysed by qRT-PCR, in HMGB1-silenced prostate and ovarian cancer lines. Dysregulation of these genes supports a new ribosome-related function of HMGB1, that could play an important role in the development of cancer.

Barreiro-Alonso, A., Lamas-Maceiras, M., Rodríguez-Belmonte, E., Vizoso-Vázquez, Á., Quindós, M., & Cerdán, M. E. (2016). High Mobility Group B Proteins, Their Partners, and Other Redox Sensors in Ovarian and Prostate Cancer. Oxidative medicine and cellular longevity, 2016, 5845061. Rapoport, B. L., Steel, H. C., Theron, A. J., Heyman, L., Smit, T., Ramdas, Y., & Anderson, R. (2020). High Mobility Group Box 1 in Human Cancer. Cells, 9(7), 1664.

0251-R-P

Non-coding mutations affecting glioblastoma-specific enhancer elements

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IdISBa Biología celular del cáncer

Gliomas are divided into low-grade gliomas (LGG) and glioblastomas (GBM) (1). GBM is the most aggressive type of primary brain tumor with a higher recurrence rate and a worse prognosis than LGG (2). Besides their clinical features, LGG and GBM exhibit different molecular signatures. Epigenetic mechanisms play a key role in cancer progression and resistance traits. These changes are due, in part, to changes in cis-acting gene regulatory elements, such as enhancer elements (EEs), which modify the expression pattern of proximal or distal gene networks. Non-coding mutations affecting EEs promote cancer initiation and progression (3-4). Here, we identified GBM-specific EEs bearing non-coding mutations and characterized the affected gene networks and transcription factors (TFs).

We integrated ATAC-seq data of GBM (N=9) and LGG (N=11) patients generated by The Cancer Genome Atlas (TCGA) and explored the differential accessibility of dynamic EEs determined by the FANTOM5 project. GBM-specific EEs with non-coding mutations (ICGC Data Portal) and binding of multiple TFs (ENCODE ChIP-seq datasets) were identified using the GALAXY Server. R was used to identify differentially expressed genes between GBM and LGG using TCGA RNA-seq data. Finally, the Factorbook was employed to estimate the differential TF binding affinity between wild-type and mutant EEs.

We identified 22 GBM-specific EEs with at least five non-coding mutations, which were bound by multiple TFs. 108 genes encoded up to 500kb away of these EEs were differentially expressed in GBM. Based on GBM oncogenic gene expression patterns, we selected two EEs that modulate the expression of *SOCS3*, a gene involved in unfavorable GBM prognosis (5). We identified that the mutant versions of these EEs enhance the binding affinity of the CBX5 and XRCC3 TFs, respectively. Further expansion of our analysis and in-vitro validation of our findings will provide clues about the mechanisms underlining pathological non-coding genetic alterations in GBM.

1.Bray, F. et al. CA. Cancer J. Clin. 68, 394–424 (2018). 2.Brown, R., Curry, E., Magnani, L., Wilhelm-Benartzi, C. S. & Borley, J. Nat. Rev. Cancer 14, 747–753 (2014). 3.Khurana E, et al. Science. 2013; 342:1235587. [PubMed: 24092746] 4.Khurana, E., Fu, Y., Chakravarty, D., Demichelis,F., Rubin, M.A., and Gerstein, M. (2016). Nat.Rev. Genet.17, 93–108 5.Zheng S, Li Z. Aging (Albany NY). 2020;12(17):17380-17392. doi:10.18632/aging.103737

0291-R-P

Paired guide RNA CRISPR-Cas9 screening for protein-coding genes and lncRNAs involved in transdifferentiation of human B-cells to macrophages

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CRISPR-Cas9 screening libraries have arisen as a powerful tool to identify both protein coding (pc) and non-coding genes playing a role along different processes. In particular, the usage of a nuclease active Cas9 coupled to a single gRNA has proven to efficiently impair the expression of pc-genes by generating deleterious frameshifts. Here, we first demonstrate that the usage of a second gRNA targeting the same gene synergistically enhances the capacity of the CRISPR-Cas9 system to knock out pc-genes. We next take advantage of our paired-guide (pgRNA) system DECKO (1), and the software CRISPETa (2), to design a library to simultaneously target 874 pc-genes and 166 lncRNAs, which are known to change expression during the transdifferentiation from pre-B cells to macrophages (3, 4). We show that this system is able to identify known players in this process, and also predicts 26 potential novel ones, of which we select four for deeper characterization (5). Two of these, FURIN and NFE2, code for proteins related to cell differentiation and macrophage function; the other two, LINC02432 and MIR3945HG, are lncRNAs associated with cancerous and infectious diseases, respectively. The CRISPR-Cas9 coupled to pgRNAs system is, therefore, a suitable tool to target simultaneously pc-genes and lncRNAs, and also to promote deletions of other regulatory regions, such as enhancers.

(1) Aparicio-Prat et al., 2015, BMC Genomics. doi: 10.1186/s12864-015-2086-z (2) Pulido-Quetglas et al., 2017, PLoS Comput Biol. doi: 10.1371/journal.pcbi.1005341 (3) Borsari B. et al., 2020, BioRxiv. doi: https://doi.org/10.1101/2020.11.20.391524 (4) Rapino F. et al., 2013, Cell Rep. doi: 10.1016/j.celrep.2013.03.003 (5) Ullrich S. et al., 2021, BioRxiv. doi: https://doi.org/10.1101/2021.04.26.441397

0314-R/M-P

PRODUCTION AND EVALUATION OF HMGB1 KNOCKOUTS IN SEVERAL OVARIAN CANCER CELL LINES

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HMGB1 is known to contribute to virtually all hallmarks of cancer, being involved in events such as replenishing telomeric DNA and maintaining cell immortality, autophagic increase, evasion of apoptosis, cell proliferation and invasion, dedifferentiation during epithelial to mesenchymal transition (EMT) via RAGE/NF-κB signaling pathways, or in angiogenesis (1). In the present work, our group produced HMGB1 knockouts in different ovarian cancer cell lines via CRISPR/Cas9 technology to analyze the effect on the transcriptional regulation of several oncogenic biomarkers related to the onset and progression of the disease.

(1) Barreiro-Alonso A et al. (2019) Characterization of HMGB1/2 Interactome in Prostate Cancer by Yeast Two Hybrid Approach: Potential Pathobiological Implications. Cancers. doi:10.3390/cancers11111729

0331-R/M-P

New insights into the FurC regulon from Anabaena sp. PCC7120: Genome-wide screening for novel FurC-DNA binding sites and its experimental validation.

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FurC from the cyanobacterium *Anabaena* sp. PCC7120 is a key transcriptional regulator that was previously described as the Peroxide Stress Regulator (PerR) [1] . However, recent studies revealed that FurC regulation goes beyond the response to oxidative stress, modulating the expression of genes involved in photosynthesis [2] , iron homeostasis and nitrogen metabolism and heterocyst differentiation (unpublished results). In this work, *in silico* approaches and its experimental validation were performed to extend our knowledge of FurC regulon. For the prediction of novel FurC-DNA binding sites, we performed MEME analyses (https://meme-suite.org/meme/tools/meme) to build a position-weight-matrix with the sequences of a selection of previously defined FurC-binding sites. The obtained FurC matrix was scanned in the retrieved putative promoter regions from *Anabaena* sp. PCC7120 genome using FIMO (https://meme-suite.org/meme/tools/fimo). A selection of the obtained putative FurC-binding sites was further validated by EMSA assays revealing 24 new direct targets of FurC. These genes belonged to the expected categories such as iron metabolism, photosynthesis, oxidative stress, and heterocyst differentiation but also to novel categories, highlighting many genes involved in carbon metabolism and regulatory functions. Finally, the regulation of the new direct targets was further validated by Real Time RT-PCR comparing its differential expression between a *furC*-overexpressing strain "EB2770FurC" and the wild-type strain *Anabaena* sp. PCC7120.

1. Yingping, F. et al. Unravelling the cross-talk between iron starvation and oxidative stress responses highlights the key role of PerR (alr0957) in peroxide signalling in the cyanobacterium NostocPCC 7120. Environ. Microbiol. Rep. 6, 468–475 (2014). 2. Sevilla, E. et al. Regulation by FurC in Anabaena Links the Oxidative Stress Response to Photosynthetic Metabolism. Plant Cell Physiol. 60, 1778–1789 (2019).

0339-R/M-P

Unraveling regulatory networks performed by FUR proteins in Anabaena sp. PCC7120

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FUR (ferric uptake regulator) proteins are metalloregulators present in most prokaryotes. The cyanobacterium *Anabaena* sp. PCC 7120 contains three FUR paralogues: FurA (Fur), FurB (Zur) and FurC (PerR), which are key regulators that control a wide set of cellular processes, ranging from photosynthesis to nitrogen metabolism. Comparative transcriptomics studies of misregulation strains of *furA*, *furB* and *furC* unveil significant alterations in the transcription of more than 200 transcriptional regulators and two component systems. This suggests that FUR proteins could modulate a large number of regulators not yet characterized, whose targets and activities remain unknown. Moreover, some of these regulators do not show orthologs in heterotrophic bacteria, indicating that at least some of them could be specific of cyanobacteria.

In this work we have identified several genes with regulatory functions containing FurA, FurB and FurC binding boxes and we have performed electrophoretic mobility shift assays (EMSA) to study the binding of FUR proteins to their promoter regions. This has allowed us to identify nearly 30 genes with regulatory functions directly regulated by FUR proteins, including adenylate cyclases, transcriptional regulators, sigma factors, sensor kinases and response regulators. Besides, we have carried out transcriptional analyses of some of these genes under stress conditions such as nitrogen deficiency and oxidative stress, which suggest that their expression could be dependent on abiotic stresses. Taken together these results open the door to a better understanding of regulatory networks in cyanobacteria, in particular those controlled by FUR proteins, and its possible participation in the response to abiotic stresses.

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0349-R/M-P

Genetic interaction of Dot1 and DNA damage checkpoint

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Intra S-phase checkpoint is sensed by the kinase Mec1 and transduced until the effector kinase, Rad53. The transduction of the signal depends on Rad9 –DNA damage checkpoint-, or on Mrc1-tof1-Csm3- DNA replication checkpoint, and the activation of one of these pathways depends on the type of genotoxic stress induced [1] . Generally, HU deplete the pool of dNTPs and activates DNA replication checkpoint, being Mrc1 essential for this function while Tof1 and Csm3 have partial roles. One of the main targets characterized for this pathway is the regulation of the activation of replication origins [2] . When genotoxic stress is induced with MMS, phleomycine or other derivatives that creates lesions on the DNA, the transducer preferred is Rad9. We found a synthetic grow defect in the *tof1 rad9*, in presence of MMS and HU, compared with the respective single mutants. Indeed, a serious impaired DNA replication progression and recovery after genotoxic exposure was observed by pulse field gel electrophoresis analyses. These phenotypes are mediated in part by Dot1, a histone methyltransferase involved in translesion polymerases recruitment. Rad52 foci analyses points to a role of Dot1-TLS polymerases pathway in damage induced by MMS, while a different mechanism should be involved in answer to the nucleotides depletion induced by HU. To identify partners involved in HU stress, TAP-tag analyses performed with Tof1 and Dot1 are ongoing.

1. Bacal, J. et al. Mrc1 and Rad9 cooperate to regulate initiation and elongation of DNA replication in response to DNA damage. EMBO J. 37, (2018). doi: 10.15252/embj.201899319. 2. Forey, R. et al. Mec1 Is Activated at the Onset of Normal S Phase by Low-dNTP Pools Impeding DNA Replication. Mol. Cell (2020) doi:10.1016/j.molcel.2020.02.021.

0357-R-P

A putative transcription factor involved in the regulation of neurosporaxanthin biosynthesis in the fungus Fusarium fujikuroi.

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Fungi are used as source of secondary metabolites (SM) for biotechnological industry with diverse beneficial properties. Our group is focused on the regulation by light and different stresses of carotenoid biosynthesis in the filamentous fungus *Fusarium fujikuroi*. The main carotenoid produced by this fungus is a xanthophyll called neurosporaxanthin with a high antioxidant potential [1] . In the last years, we have described the genes involved in the neurosporaxanthin biosynthesis pathway, called *car* genes [2]. Transcription of these genes in this fungus is upregulated by light and nitrogen starvation and it is downregulated by the RING finger protein CarS [2] . The molecular mechanism by which CarS modulates transcription of the *car* genes remains to be clarified, but it does not bind to the *car* genes promoters and it presumably interacts with other regulatory proteins currently under investigation.

SM biosynthetic clusters frequently include regulatory genes. Adjacent to the *car* cluster there is a gene encoding a predicted transcription factor with a typical fungal TF_MHR and Zn2-Cys6 binuclear cluster domains, however, with no known function. We postulate that this gene, that we have called *carZ*, could be involved in the regulation of neurosporaxanthin biosynthesis. To check this hypothesis, we have obtained *carZ* deletion mutants in a wild strain and in a *carS* overexpression strain [3]. Preliminary analyses of the mutants in the wild type background suggest an alteration in the induction of the synthesis of carotenoids to light, a response mostly dependent on the White Collar photoreception system [2]. A detailed phenotypic characterization of the expression of the *car* genes in these mutants in the dark or after illumination, as well as the synthesis of carotenoid under different illumination conditions, will be presented.

1. Parra-Rivero, O. et al. Neurosporaxanthin overproduction by Fusarium fujikuroi and evaluation of its antioxidant properties. Antioxidants 2020, 9,528. 2. Avalos, J. et al. Carotenoid biosynthesis in Fusarium. J. Fungi 2017,3. 3. Marente, J. et al. Controlled transcription of regulator gene carS by Tet-on or by a strong promoter confirms its role as a repressor of carotenoid biosynthesis in Fusarium fujikuroi. Microorganisms 2021, 9, 1–17.

0423-R/M-P

AFM visualization of different single-stranded RNA molecules

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In the past years, scientists have made great efforts into unveiling the non-coding RNA paradigm: why some RNA molecules are being actively transcribed by cells but do not code for any protein. Surprisingly back then, it was discovered that many of these molecules possess enzymatic, structural or regulatory activities in crucial processes such as gene regulation, viral defense or genomic regulation as in the inactivation of the X chromosome [1]. In these molecules, structure is determinant for its intrinsic activity, however, RNA structural characterization nowadays is mostly based on computational predictions that may differ from their real physiological structure. In this work, we prove atomic force microscopy (AFM) to be of great use in the visualization and characterization of different RNA molecules [2]. We synthesized three different single-stranded RNA molecules ranging from ~ 600 to ~ 2000 bases and found the best conditions to visualize them. Also, we studied how we could use a ~ 300 nucleotides long unstructured RNA poly-A tail to tag the 3´-terminal end of these molecules. All in all, we have demonstrated that AFM is a fast technique to check the structure of long RNA molecules, what could shed light into the functioning of non-coding RNAs or viral RNA packaging.

[1] Alberts B, Johnson A, Lewis J, et al (2014) Molecular Biology of the Cell 6e [2] Gilmore JL, Yoshida A, Hejna JA, Takeyasu K (2017) Visualization of conformational variability in the domains of long single-stranded RNA molecules. Nucleic Acids Res 45:8493–8507. https://doi.org/10.1093/nar/gkx502

0433-P

The meiosis-specific AAA+ ATPase Pch2 supports the meiotic recombination checkpoint from the cytoplasm

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The meiotic recombination checkpoint ensures the accurate chromosome distribution to the gametes during meiosis by blocking cell cycle progression in response to synapsis and/or recombination defects, thus preventing the formation of aneuploid gametes. The meiotic recombination checkpoint in *Saccharomyces cerevisiae* is activated in the absence of Zip1, the main protein of the central region of the synaptonemal complex. Thus, the *zip1Δ* mutant is a useful tool to provoke synapsis and/or recombination defects that trigger this meiotic checkpoint response.

The evolutionarily conserved Pch2^{TRIP13} AAA+ ATPase from *S. cerevisiae* participates in the checkpoint meiotic arrest by promoting Hop1 phosphorylation at threonine 318. Analysis of Pch2 distribution in *zip1Δ* revealed that Pch2 localizes to the nucleolus and the cytoplasm. In an *orc1* mutant, where Pch2 is prevented from targeting to the rDNA region, the meiotic recombination checkpoint remains fully active. This demonstrates that Pch2 nucleolar localization is dispensable for the meiotic arrest triggered by *zip1Δ* and raises the possibility of a chromosome-independent fraction of Pch2 that might be relevant for the functionality of the checkpoint.

To resolve this issue, we have further explored the relationship between Pch2 subcellular localization and its function. We have artificially forced Pch2 localization to different subcellular compartments by adding a nuclear export signal (NES) or a nuclear localization signal (NLS) to a N-terminally GFP-tagged Pch2 protein.

The meiotic arrest of *zip1Δ* when Pch2 is outside the nucleus by addition of a NES demonstrates that Pch2 cytoplasmatic population is sufficient to support checkpoint function. On the other hand, forced Pch2 nuclear accumulation by adding an ectopic NLS leads to checkpoint defects. Our unexpected discoveries indicate that the cytoplasmatic fraction of Pch2 promotes the phosphorylation of Hop1, a protein typically localized at chromosome axis.

To further analyze the impact of Pch2 localization on the meiotic checkpoint, we have also developed a genetic system where leptomycin B is used to study the effect of blocking Pch2 nuclear-cytoplasmic transport. This system will be employed in the future to further characterize the functional impact of Pch2 subcellular distribution.

Herruzo, E., Lago-Maciel, A., Baztán, S., Santos, B., Carballo, J. A., & San-Segundo, P. A. (2021). Pch2 orchestrates the meiotic recombination checkpoint from the cytoplasm. BioRxiv, 2021.04.13.439596. https://doi.org/10.1101/2021.04.13.439596

0478-R/M-P

Ischemia-induced oxidative stress triggers DNA double strand breaks leading to neuronal apoptosis

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The maintenance of DNA integrity is essential for neuronal homeostasis. Increased oxidative stress and DNA double strand break (DSB) generation occur in neurological diseases, including neurodegenerative diseases and stroke [1]. However, the interplay between both phenomena and their implication in neuronal death is largely unknown. Here, we aim to investigate the impact of ischemia-induced DNA DSB on neuronal death and the role played by oxidative stress on DNA repair. For this purpose, mouse cortical neuron in primary culture were exposed to an experimental model of ischemia in vitro (oxygen-glucose deprivation, OGD) for 30 minutes followed by 4 hours of reoxygenation [1]. Next, we evaluated DNA DSB generation, protein expression and neuronal apoptosis by using western blot, flow cytometry, and immunostaining. We found that OGD time-dependently increased the expression of the DNA DSB marker, phospho-H2AX [2], which paralleled with a higher expression levels of apoptotic markers, active caspase-3 and p53. Moreover, OGD promoted oxidative stress and neuronal apoptosis, as revealed by the increase in both annexin/7AAD staining and TUNEL assay. Finally, to study the implication of oxidative stress in DNA damage, we used neuronal cultures from embryo mice that constitutively express the catalase in the mitochondria (mCAT), which downmodulates the endogenous generation of reactive oxygen species [3]. We found that mCAT neurons were more resistant to OGD-induced DNA damage and neuronal apoptosis than wild type neurons.

Our results demonstrate that oxidative stress might play an important role in DNA DSB generation, which is involved in neuronal apoptosis caused by ischemia.

Funded by:
ISCIII: PI18/00103;PI18/00285;RD16/0019/0018; FEDER European regional development fund; JCyL: CSI151P20; CLU201703 P.O.FEDER CyL1420 and EDU/556/2019.

[1] Sanchez-Moran, I., Rodriguez, C. et al (2020). Nuclear WRAP53 promotes neuronal survival and functional recovery after stroke. Science Advances, 6(41). [2] KUO, L. J., & YANG, L.-X. (2008). γ-H2AX - A Novel

Biomarker for DNA Double-strand Breaks. In Vivo, 22(3). [3] Vicente-Gutierrez, C., Bonora, N. et al (2019). Astrocytic mitochondrial ROS modulate brain metabolism and mouse behaviour. Nature Metabolism (Vol. 1, Issue 2, pp. 201–211).

0500-P

Deconstructing JunB transcriptional networks in cell proliferation and cancer progression

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The transcription factor AP-1 is composed of dimeric combinations of Jun, Fos and ATF family members. It is an essential mediator of gene expression in response to a wide variety of extracellular stimuli. It can also exert oncogenic functions playing a crucial role in cell transformation and invasion. JunB, a Jun family member, behaves either as a tumour suppressor or as an oncogene depending on the cellular context. It is overexpressed in some human cancers contributing to neoplastic development, specifically in anaplastic large cell lymphoma, certain Hodgkin's lymphomas and also, in breast and stomach cancers. It has also been involved in the pathogenesis and resistance to chemotherapy of malignant pleural mesothelioma (MPM), an extremely aggressive human cancer. However, the mechanisms whereby JunB promotes neoplastic growth are still unknown.

We have studied the phenotypic consequences of JunB silencing in tumorigenic cell lines. Depletion of JunB reduces the number of cancer cells in S phase and promotes G1 arrest. In order to understand the molecular mechanisms underlying these phenotypes we have performed ChIP-seq analysis in combination with transcriptomic analysis in U2OS cells to identify JunB transcriptional targets. We have identified more than 3500 target genes containing JunB binding sites and around 1800 differentially expressed genes in JunB-silenced cells. Among the differentially expressed genes, we have found an overrepresentation of genes related to cell differentiation, pluripotency, epithelial to mesenchymal transition (EMT) and cell cycle. We have selected a set of genes that are important for these processes for further validation and we have confirmed that the expression of EMT pivotal drivers, and key cell cycle regulators is affected by JunB levels. Unraveling the transcriptional networks controlled by JunB in these cellular processes will help to understand the mechanisms by which this transcription factor contributes to tumorigenesis.

1- Eferl, R. and E.F. Wagner, AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer, 2003. 2- Farràs, R., et al., JunB breakdown in mid-/late G2 is required for down-regulation of cyclin A2 levels and proper mitosis. Mol Cell Biol, 2008 3- Yang, M.Y., et al., JunB gene expression is inactivated by methylation in chronic myeloid leukemia. Blood, 2003

15.- Metabolic Regulation and Nutrition

0521-OI

Impact on gene expression and metabolic homeostasis of bioactive compounds-enriched diets.

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The prevention/treatment of metabolic syndrome (MetS) and obesity is primary based on the follow-up of a healthy lifestyle, which includes, among other recommendations, a healthy diet. In this context, the Mediterranean Diet (DietMEd) has shown beneficial effects on these pathologies by reducing chronic low-grade inflammation, improving endothelial function and reducing cardiovascular risk [1–3] .

DietMed is characterized by a high consumption of foods rich in bioactive compounds such as polyphenols to whose have been attributed a large part of the health effects of this diet [4,5]no studies have evaluated the relation between all polyphenol subclasses and the incidence of diabetes. Objective: We aimed to prospectively examine the associations between the intake of total polyphenols and different groups of polyphenols (flavonoids, phenolic acids, stilbenes, lignans, and others . Polyphenols are the most abundant phytochemicals in nature and are widely distributed in fruits, vegetables, and highly present in foods like legumes, cocoa, some cereals as well as in some beverages, such as tea, coffee and wine [6] . Even polyphenols are not essential nutrients for humans research in nutrition has shown that long-term and acute intakes of these bioactive compounds exert beneficial effects on body weight management and pathologies such as cardiovascular diseases, obesity, type 2 diabetes, the onset and development of some cancers and cognitive function [7] .

From the last years, our research has been focused on describing the molecular mechanisms underlying the anti-obesity effects of polyphenols by themselves or within a food matrix in diet-induced obese rodents [8–10] . We analyze the metabolic impact in liver and adipose tissue, including white adipose tissue and brown adipose tissue of different nutritional interventions that include different sources and kinds of polyphenols.

One of our main results is that while the administration of polyphenols in the context of a food matrix is beneficial to combat obesity and its comorbidities, the administration of isolated polyphenols can have a negative impact on the individual, worsening insulin sensitivity and inducing kidney fibrosis.

1. Serra-Majem, L. Nutr. Rev. 2006, 64, S27-47. 2. Bendall, C.L. Crit. Rev. Food Sci. Nutr. 2018, 58, 3070–3084. 3. Sofi, F. BioFactors 2013, 39, 335–342. 4. Tresserra-Rimbau, A. J. Nutr. 2016, 146, 767–777. 5. Medina-Remón, A. Nutr. Metab. Cardiovasc. Dis. 2015, 25, 60–67. 6. Manach, C. Am. J. Clin. Nutr. 2004, 79, 727–747. 7. Sandoval, V. Nutrients 2020, 12, 2393. 8. Sandoval, V. Mol. Nutr. Food Res. 2018, 62. 9. Sandoval, V. Antioxidants 2019, 8, E360. 10. Sandoval, V. Ant

0525-R/M-OI

Impact of astrocyte clock in energy balance

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The endogenous timekeeping system evolved to anticipate the time of the day through the 24 hours cycle of the Earth's rotation. In mammals, the circadian clock governs rhythmic physiological and behavioral processes, including the daily oscillation in glucose metabolism, food intake, energy expenditure, and whole-body insulin sensitivity.

Disruption of the circadian cycle is strongly associated with metabolic imbalance and reduced longevity in humans. Also, rodent models of circadian arrhythmia, such as the constitutive knockout of the clock gene *Bmal1*, leads to metabolic disturbances and early death.

Although astrocyte clock regulates molecular, physiological and behavioral circadian rhythms, its involvement in the metabolic dysfunctions associated with alterations of the timekeeping system are unknown. Here, we summarize our findings on the astrocyte clock regulation of energy balance and glucose homeostasis. Our results suggest astrocytes as pharmacological targets to prevent the metabolic dysfunctions and shortened lifespan associated with alterations of circadian rhythms.

Barca-Mayo, O, et al. Deletion of astrocytic BMAL1 results in metabolic imbalance and shorter lifespan in mice. GLIA, 2019. 68:1131-1147 Barca-Mayo, O, et al. Astrocytes and circadian rhythms: an emerging astrocyte-neuron synergy in timekeeping processes. Methods in Mol Biol, 2019 (Clifton, NJ) 1938:131-154. Book chapter. Barca-Mayo, O et al. Astrocyte deletion of Bmal1 alters daily locomotor activity and cognitive functions via GABA signaling. Nat. Comm. 2017, 8:14336.

0186-R/M-OS

Differential effects of saturated and unsaturated fatty acids on hypothalamic regulation of brown fat thermogenesis

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Fatty acid (FA) metabolism in neurons signals the energy status of the organism. Depending on the level of saturation and chain length, FAs differently provide physiological responses, but these effects in hypothalamic regulation of obesity are unclear. Our aim is to elucidate the hypothalamic effects of saturated and unsaturated FAs on body weight, food intake and brown adipose tissue (BAT) thermogenesis.

Male C57BL/6J mice were fed standard diet (SD) or high fat diet (HFD), enriched with monounsaturated (MUFA) or saturated (SFA) fatty acids for 7-28 days. Unsaturated (oleic, linoleic) and saturated FAs (palmitic, stearic) were also intracerebroventricularly (ICV) administrated. BAT thermogenesis was analyzed by infrared thermography and mRNA thermogenic markers. Neuronal activity was tested in different hypothalamic nuclei by c-fos immunohistochemistry.

Administration of both HFD led weight gain and food intake compared to control group. Interestingly, although MUFA diet triggered greater food intake, weight gain was lower than SFA and similar to SD groups. This result was associated to higher BAT thermogenesis activation in MUFA versus SFA group. ICV administration of FAs correlated with these results, since unsaturated FAs reduced body weight and activated BAT thermogenesis, but these effects were not observed with saturated FAs. ICV of unsaturated FAs and MUFA diet feeding induced neuronal activation in paraventricular nucleus of the hypothalamus whereas this activation was abrogated in response to saturated FAs or SFA diet. Central unsaturated FAs, but not saturated FAs, induced hypothalamic expression of fatty acid synthase (FAS), indicating raised levels of malonyl-CoA, an indicator of the energy status of neurons. Altogether, saturated and unsaturated FAs have different effects on central regulation of BAT thermogenesis, which could be driven by modulation of malonyl-CoA levels via FAS. Further studies are needed to elucidate the exact molecular pathway and hypothalamic circuits involved.

0246-R/M-OS

Effect of endothelial Nox5 expression in mice fed with high fat diet and in 3T3-L1 adipocytes treated with glucose and palmitic acid

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Obesity is a global health issue associated with insulin resistance and altered lipid homeostasis as well as other pathologies. One of the main mechanisms involved in the development of these pathologies is the increased production of reactive oxygen species (ROS). These increased levels of ROS could act as drivers of redox signalling or even induce oxidative stress when exceeding the balance between their production and degradation. One of the main producers of ROS in the organism is the family of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. Within this family, NOX5 represents the underexplored most recently discovered member. The aim of the present work is to describe the role of endothelial NOX5 expression over adipose tissue in obesity conditions, using two experimental systems. An *in-vivo* model based on NOX5 conditional knock-in mice fed with a high-fat diet and an *in-vitro* system developed with 3T3-L1 adipocytes cultured for 24 h with conditioned media of NOX5-expressing endothelial cells. bEnd.3 endothelial cells were previously treated with glucose and palmitic acid for 24 h to simulate obesity conditions. Animals expressing NOX5 presented lower body weight gain and reduced mesenteric and epididymal adipose mass compared to control mice fed with the same diet. NOX5-expressing mice also showed significantly lower glycaemia and improved insulin-induced glucose uptake measured by an intraperitoneal glucose tolerance test. In addition, mRNA and protein expression of *Glut4* and Caveolin 1 (*Cav1*) were significantly increased in the adipose tissue of these animals. Moreover, 3T3-L1 adipocytes treated with conditioned media from NOX5-expressing endothelial cells, previously incubated with high glucose and palmitic acid, presented a reduction in lipid accumulation and an increase in insulin-stimulated glucose uptake. Likewise, a significant increase in the mRNA and protein expression of *Glut4* and *Cav1* was also observed in these cells. As a result, in response to obesogenic conditions, NOX5 endothelial activity may regulate glucose sensitivity and lipid homeostasis in the adipose tissue.

Heyn, G. S.; Correa, L. H.; Magalhaes, K. G. The impact of adipose tissue-derived miRNAs in metabolic syndrome, obesity, and cancer. Front Endocrinol (Lausanne) 2020, 11, 563816. Furukawa, S.; Fujita, T.; Shimabukuro, M.; Iwaki, M.; Yamada, Y.; Nakajima, Y.; Nakayama, O.; Makishima, M.; Matsuda, M.; Shimomura, I. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004, 114 (12), 1752-61.

0427-M-OS

Vitamin C activates pyruvate dehydrogenase (PDH) targeting the mitochondrial tricarboxylic acid (TCA) cycle in hypoxic KRAS mutant colon cancer

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In hypoxic tumors, positive feedback between oncogenic KRAS and HIF-1α involves impressive metabolic changes correlating with drug resistance and poor prognosis in colorectal cancer.

Up until now, KRAS-targeting molecules are not showing clear benefits in terms of patient overall survival (POS), so pharmacological modulation of aberrant tricarboxylic acid (TCA) cycle in hypoxic cancer may constitute a metabolic vulnerability of KRAS-driven tumors.

Pyruvate dehydrogenase (PDH) is one the main metabolic sentinels controlling the flux of metabolites between glycolysis and the TCA cycle and it is inhibited by Pyruvate Dehydrogenase Kinase 1 (PDK-1) in metastatic CRC.

Here we show that pharmacological doses of vitamin C downregulates PDK-1 in KRAS mutant CRC cells and murine xenografts through hydroxylation (Pro402) of the Hypoxia inducible factor-1(HIF-1)α, correlating with decreased expression of the glucose transporter 1 (GLUT-1) in both models.

Moreover, vitamin C induced remarkable ATP depletion, rapid mitochondrial Δψ dissipation and diminished Pyruvate Dehydrogenase E1-α phosphorylation at Serine 293, then boosting PDH activity.

Summarizing, we report a striking and previously non reported role of vitamin C in the modulation of mitochondrial metabolism in KRAS mutant colon cancer.

Potential impact of vitamin C in the clinical management of anti-EGFR chemoresistant colorectal cancer should be further considered.

Cenigaonandia-Campillo A, Serna-Blasco R, Gómez-Ocabo L, Solanes-Casado S, Baños-Herraiz N, Puerto-Nevado LD, Cañas JA, Aceñero MJ, García-Foncillas J, Aguilera Ó. Vitamin C activates pyruvate dehydrogenase (PDH) targeting the mitochondrial tricarboxylic acid (TCA) cycle in hypoxic KRAS mutant colon cancer. Theranostics. 2021 Jan 25;11(8):3595-3606. doi: 10.7150/thno.51265. PMID: 33664850; PMCID: PMC7914362.

0005-R-P

In vivo activation of lipid metabolism in brown adipose tissue with CPT1AM

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Current lifestyle and continued excess of macronutrients intake alarmingly increases the incidence of obesity worldwide. Obesity is characterized by an imbalance between food intake and energy expenditure. Brown adipose tissue (BAT) is recognized as an important regulator of energy expenditure in obesity by burning fuels such as glucose and lipids to control thermogenesis. Carnitine Palmitoyltransferase 1A (CPT1A), the key enzyme in fatty acid oxidation (FAO), has been involved in the control of energy homeostasis. However, the specific role of CPT1A in modulating BAT metabolism to control obesity is still unknown. Previous results from our group showed that expression of a constitutively active form of CPT1A (CPT1AM) in cultured brown adipocytes increased thermogenesis and mitochondrial activity. Therefore, here we aim to analyze whether the *in vivo* expression of CPT1AM in BAT can increase FAO and protect against obesity and diabetes in mice.

We used adeno-associated viral (AAVs) vectors and the BAT-specific UCP1 promoter to express CPT1AM specifically in BAT of normal or high-fat diet (HFD)-treated mice. CPT1AM-expressing mice successfully showed enhanced CPT1AM mRNA and protein levels. Importantly, CPT1AM-expressing mice under HFD showed reduced body weight and blood glucose levels when compared to normal chow mice. Our results highlight AAV-mediated CPT1AM gene therapy in BAT as a therapeutic approach for the treatment of obesity and diabetes.

1. World Health Organization. Global Health Observatory (GHO) data. 2016. 2. Calderon-Dominguez M, et al. Carnitine palmitoyltransferase 1 increases lipolysis, UCP1 protein expression and mitochondrial activity in brown adipocytes. PLoS One. 2016;11(7):1–17. 3. Jimenez Veronica, et al. In Vivo Adeno-Associated Viral Vector–Mediated Genetic Engineering of White and Brown Adipose Tissue in Adult Mice. Diabetes. 2013; 62: 4012-4022.

0026-R/M-P

Impact of maternal diet in the prevalence of intrauterine growth restriction and the associated cardiovascular remodeling.

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Background – Intrauterine growth restriction (IUGR) affects 5-10% of newborns. It has been associated with adverse perinatal outcomes such as cardiovascular remodeling (CVR) and dysfunction, in accordance to higher levels of BNP. IUGR increases the risks of intrauterine demise, neonatal morbidity, and death. It has been recently suggested that some metabolic sensors highly influenced by diet, such as GDF15, might have a role as cardiomyokines, involved in oxidative stress and metabolic regulation. However, until date, no studies have evaluated their effect in IUGR.

Aim – To investigate the value of GDF15 in the early diagnosis and the modulation of diet to prevent IUGR. Methods – A single-site, cross-sectional, observational study was conducted for two years. It included 20 IUGR pregnancies and 29 uncomplicated pregnancies with appropriate for gestational age newborns (CTL). Maternal serum (at first trimester and delivery), and cord blood were collected to measure BNP and GDF15 levels. Clinical and dietary data were also collected to evaluate IUGR and CVR association with maternal diet intake. Obtained data was analysed through non-parametric tests.

Results – Compared with the CTL group, IUGR newborns had higher BNP levels (23.1±3.4 vs 14.24±1.6 pg/ml), confirming CVR and validating the source of the studied sample; GDF15 was also increased in IUGR newborns compared to CTL (6595±1116 vs 3831±347.2 pg/ml; p-value 0.027). Additionally, IUGR mothers presented a significant increase of GDF15 levels at delivery compared to first trimester of gestation, not found in CTL. From the dietetic point of view, IUGR consumed significantly less monounsaturated fatty acids (39.38±9.4 vs 56.80±4.8 g/day; p-value 0.045) and tend to consume less lipids, polyunsaturated fatty acids, vitamin E and olive oil (1.75±0.8 vs 4.84±0.7 g/

day; p-value 0.029).

Conclusions – According to dietary differences of IUGR pregnant women (fatty acids, lipids, vitamin E and olive oil consumption), healthy pattern diet, including olive oil consumption, might be potential preventive tools for this obstetric complication. Cardiomyokine GDF15 emerge as promising biomarker for IUGR, both at maternal and neonatal level. The present outcomes set the path for the further development of strategies for early detection and prevention of IUGR.

0031-R/M-P

Preserved energy balance and glucose homeostasis in mice lacking Retinoid-related Orphan Receptor gamma (RORγ1) specifically in adipocytes

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White adipose tissue (WAT) acts as a crucial integrating element in the regulation of energy balance and glucose homeostasis. Hence, WAT dysfunction is associated with the development of metabolic diseases. Although key transcription factors (i.e. C/EBPs, PPARγ) that control adipocyte differentiation are well known, the precise role that others transcription factors expressed in adipose cells play on fine-tuning adipocyte differentiation/function remains to be elucidated. The mRNA expression of *RORγ1,* a member of the ROR subfamily of hormone nuclear receptors, dramatically increases during differentiation of 3T3-L1 adipocytes. *In vivo*, the expression of *Rorγ1* in WAT is mostly restricted to the adipocyte fraction, whereas others members of the ROR family are similarly distributed amongst the stromal vascular (SVF) and the adipocyte fractions. These data suggest that RORγ1 could play an essential role in the biology of WAT by controlling the expression of gene networks essential for proper adipocyte differentiation or function. To assess the role of RORγ1 in WAT, we have generated a mouse model devoid of RORγ1 specifically in adipocytes (RORγ-FAT-KO mice). RORγ-FAT-KO mice fed with a chow diet or a high fat diet (HFD) did not exhibit significant differences in body weight, adipose mass or adipocyte morphology when compared to wild type (Wt) littermates. Glucose homeostasis was also similar between Wt and RORγ-FAT-KO mice, regardless of the feeding conditions. To unravel the function of RORγ1 in WAT, we compared the gene expression profile of WAT from Wt and RORγ-FAT-KO mice fed with a HFD using DNA microarrays. Pathways related to the inflammatory response appeared altered. Analysis of WAT resident immune cell populations revealed a reduction in NK cells and an increase in γδT cells, but no changes in other immune cells, in RORγ-FAT-KO mice. Our findings demonstrate that lack of *Rorγ1* in adipocytes can alter the resident immune landscape in WAT although is not sufficient to bring out a metabolic phenotype.

0044-R/M-P

Leptin, acting at central level, increases FGF21 expression in white adipose tissue via PPARβ/δ

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The altered function of adipose tissue can result in obesity, insulin resistance and its metabolic complications. Leptin, acting on the central nervous system, modifies the composition and function of adipose tissue. To date, the molecular changes that occur in epididymal white adipose tissue (eWAT) during chronic leptin treatment are not fully understood. Herein we aimed to address whether PPARβ/δ could mediate the metabolic actions induced by leptin in eWAT. To this end, male 3-months-old Wistar rats, infused intracerebroventricularly (icv) with leptin (0.2 µg/day) for 7 days, were daily co-treated intraperitoneally (ip) without or with the specific PPARβ/δ receptor antagonist GSK0660 (1 mg/kg/day). In parallel, we also administered GSK0660 to control and/or pair-fed rats without leptin infusion. Leptin, acting at central level, prevented the starvation-induced increase in circulating levels of FGF21, while induced markedly the endogenous expression of FGF21 and browning markers of eWAT. Interestingly, GSK0660 abolished the anorectic effects induced by icv leptin leading to increased visceral fat mass and reduced browning capacity. In addition, the pharmacological inhibition of PPARβ/δ alters the immunomodulatory actions of central leptin on eWAT. In summary, our results demonstrate that PPARβ/δ is involved in the up-regulation of FGF21 expression induced by leptin in visceral adipose tissue.

Mora, C., C. Pintado, B. et al. Central leptin regulates heart lipid content by selectively increasing PPAR β/δ expression. J. Endocrinol. 2018, 236, 43–56. Rubio, B., C. Mora, et al. The nutrient sensing pathways FoxO1/3 and mTOR in the heart are coordinately regulated by central leptin through PPARβ/δ. Implications in cardiac remodeling. Metabolism 2021, 115, 154453.

0056-R/M-P

Anti-inflammatory effects of (-)-epicatechin and the colonic metabolite 2,3-dihydroxybenzoic acid in renal proximal tubular cells

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A chronic low-grade inflammation is present in diabetes, and contributes to the pathogenesis of the diabetic kidney disease. Epicatechin (EC), which is the main flavanol in cocoa, and a main colonic phenolic acid derived from flavonoid intake, 2,3-dihydroxybenzoic acid (DHBA) have been suggested to exert anti-diabetic effects. The aim of this work was to study the potential anti-inflammatory properties of EC and DHBA in renal proximal tubular NRK-52E cells incubated with high glucose (HG) and lipopolysaccharide (LPS). Pre-treatment of cells with EC and DHBA (5 μM) reduced the increased levels of pro-inflammatory cytokines namely interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α) and monocyte chemoattractant protein 1 (MCP-1) stimulated by HG+LPS. In addition, EC and DHBA pre-incubation diminished the enhanced values of intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), as well as mitogen-activated protein kinases (MAPKs)-extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK) and -p38 protein kinase (p38) provoked by the HG+LPS challenge. Pre-treatment of cells with EC and DHBA also protected against HG+LPS-induced oxidative stress by preventing the increase in ROS generation and NADPH-oxidase-4 (NOX-4) levels. Specific inhibitors of p38 and NOX-4 demonstrated that both proteins were involved in the EC- and DHBA-mediated protection against inflammation and associated oxidative stress. All together suggests that EC and DHBA exert beneficial effects in renal proximal tubular NRK-52E cells by preventing the inflammatory-induced milieu and the accompanying redox unbalance, wherein NOX-4/p38 signalling plays a crucial role.

Acknowledgments: This work was supported by AGL2015-67087-R and RTI2018-095059-B-I00 grants (MINECO/FEDER, UE) grants. D. Álvarez-Cilleros is a FPI fellow from the predoctoral program of MINECO (BES-2016-076721).

0067-M-P

Long-term consumption of conventional (but not organic) red grape polyphenols modulates brown adipose tissue gene expression in a photoperiod-specific manner in healthy rats

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Grape consumption provides several health benefits including regulation of energy metabolism. However, we have recently demonstrated that grape's polyphenol profile and its subsequent bioavailability depend on both the type of cultivation and the photoperiod in which consumers are exposed. Since brown adipose tissue (BAT) is considered a strong modulator of energy expenditure in mammals, we aimed to investigate in healthy rats, exposed to different photoperiods, if the thermogenic activation of BAT induced by consumption of red grape polyphenols differed between the consumption of non-organic (conventional) and organic grapes.

Fifty-four Fischer 344 rats were acclimated for 4 weeks to short-day (L6), long-day (L18) or standard-day (L12) photoperiods. After adaptation, three groups from each photoperiod (n=6) were daily supplemented either with 100 mg/kg of body weight of conventional red grapes (CG), organic red grapes (OG) or vehicle (VH) for 10 weeks. Gene expression of key thermogenic and adipogenic regulators in BAT were analyzed by qPCR.

During L6, which corresponds to grape harvest photoperiod, we did not detect any significant changes in thermogenic activity of BAT in CG and OG groups compared to VH group. We only observed that consumption of both grapes decreased the expression of some transcription factors involved in adipogenesis such as *Ppara* and *Pparg* in comparison to VH animals. In contrast, during L12, our results showed that CG group presented a clear downregulation of mRNA levels in several critical genes involved in thermogenesis (*Ucp1, Dio2, Cpt1b* and *Pgc1a*), adipogenesis (*Ppara* and *Pparg)* and lipid transport (*Lpl* and *Fatp1*), suggesting a significant reduction of BAT functionality in this photoperiod. Notably, this loss of thermogenic capacity was not observed in the OG group, indicating that the specific polyphenol composition of each type of grape might be involved in the latter effects of its out-of-season consumption. Finally, CG animals exposed to L18 showed a significant up-regulation of gene expression of some lipid transporters including *Cd36* and *Fatp1* compared to VH group, which was not detected in OG animals.

Overall, our results showed a different effect from both

fruits, also influenced by photoperiod, which might be explained by the different polyphenol composition of CG and OG.

Ibars M et al., Seasonal consumption of polyphenol-rich fruits affects the hypothalamic leptin signaling system in a photoperiod-dependent mode. Sci Rep. 2018 Sep 11;8(1):13572. Iglesias-Carres L et al., Exposure of Fischer 344 rats to distinct photoperiods influences the bioavailability of red grape polyphenols. J Photochem Photobiol B. 2019 Oct;199:111623.

0080-P

Usefulness of PBMC to study overweight/obesity impact on key genes of lipid metabolism and to analyse metabolic recovery after weight loss intervention

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Peripheral blood mononuclear cells or PBMC are widely used as a source of transcriptomic biomarkers in nutrition and obesity studies because of their capability to reflect gene expression profile of internal tissues in response to changes in feeding conditions or to changes in body weight. In our pilot proof-of-concept study we analysed in humans if, as we previously suggested in rodents, PBMC could be a surrogate tissue to study overweight/obesity impact on the expression of key genes of lipid metabolism. Moreover, we aimed to analyse if PBMC are able to reflect lipid metabolism gene expression pattern recovery as a result of weight loss.

To fulfil our objectives, pre-selected key lipid metabolism genes based in our previous preclinical studies were analysed in PBMC of normoglycemic normal-weight (NW), and overweight-obese (OW-OB) subjects (19-44 years old), and of the OW-OB subjects after a 6-month weight-loss plan. Gene expression in PBMC was correlated with relevant anthropometric and clinical parameters to establish the potential usefulness of the analysed genes as biomarkers of metabolic impairment/recovery.

PBMC mRNA levels of *CPT1A*, *FASN* and *SREBP-1c* increased in the OW-OB group, according with what has been described in liver and adipose tissue of humans with obesity. This altered expression pattern it could be reflecting an attempt to increase fatty acid oxidation to prevent adiposity, as well as increased fatty acid synthesis characteristic of obesity. Moreover, it could be related to early signs of metabolic impairment (fatty liver and insulin resistance indexes). A minor body weight/adiposity decrease produced metabolic but not gene expression recovery in PBMC of OW-OB group, suggesting more severe alterations requiring additional body fat loss.

Thus, human PBMC reflect lipid metabolism expression profile of energy homeostatic tissues, as well as early obesity-related alterations in subjects that could be at higher metabolic risk. Further studies are needed to understand the usefulness of PBMC for analysis of metabolic recovery in weigh management programs.

• Oliver P, Reynés B, Caimari A, Palou A. Peripheral blood mononuclear cells: a potential source of homeostatic imbalance markers associated with obesity development. Pflugers Arch. 2013 Apr;465(4):459-68. • Reynés B, Díaz-Rúa R, Cifre M, Oliver P, Palou A. Peripheral blood mononuclear cells as a potential source of biomarkers to test the efficacy of weight-loss strategies. Obesity (Silver Spring). 2015 Jan;23(1):28-31.

0083-R/M-P

Magnesium accumulation upon cyclin M4 silencing ameliorates NASH.

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Perturbations of intracellular magnesium (Mg^{2+}) homeostasis have implications for cell physiology. In particular, deficiencies of the cation have been identified in cirrhosis and liver cancer [1] , whereas the supplementation of the cation has proved to reduce mortality derived from liver diseases [2] . Moreover, perturbations in systemic Mg^{2+} have been described in many comorbidities from non-alcoholic steatohepatitis (NASH) such as insulin resistance, obesity and cardiovascular diseases [3] . Among all magnesiotropic proteins, the cyclin M family, CNNM, perform key functions in the transport of Mg^{2+} across cell membranes. Although they have been previously characterized to interact with phosphatases of regenerating liver (PRLs) involved in tumour development [4]also known as protein tyrosine phosphatase 4A (PTP4A , the role of CNNMs in the liver remains poorly understood.

In the present work it has been performed a clinical characterization of serum Mg^{2+} levels and hepatic CNNM4 expression. Studies have been realized in primary hepatocytes cultured under methionine and choline deprivation, in order to mimic NASH phenotype and in two *in vivo* rodent NASH models: 0.1% methionine and choline-deficient and choline-deficient high-fat diets. *Cnnm4* was silenced using siRNA, in vitro with DharmaFECT and in vivo with Invivofectamine or siRNA conjugated to N-acetylgalactosamine that allows a specific delivery to the liver.

Patients with NASH showed hepatic CNNM4 overexpression and dysregulated Mg^{2+} levels in the serum. *Cnnm4* silencing ameliorated hepatic lipid accumulation, inflammation and fibrosis in the rodent NASH models. Mechanistically, CNNM4 knockdown in hepatocytes induced cellular Mg^{2+} accumulation, reduced endoplasmic reticulum stress and increased microsomal triglyceride transfer activity, which promoted hepatic lipid clearance by increasing the secretion of very-low-density lipoprotein. The conjugation of siRNA with GalNAc allows a stable and effective delivery to, the liver.

In conclusion, hepatic CNNM4 is a valuable therapeutic target for treating NASH.

[1] Liu M, et al. Magnesium and liver disease. Ann Transl Med 2019;7:578. [2] Wu L, et al. Magnesium intake and mortality due to liver diseases: Results from the Third National Health and Nutrition Examination Survey Cohort. Sci Rep 2017;7:17913. [3] Simón J, et al. Magnesium, Little Known But Possibly Relevant: A Link between NASH and Related Comorbidities. Biomedicines 2021;9. [4] Wei M, et al. Targeting phosphatases of regenerating liver (PRLs) in cancer. Pharmacol Ther 2018;190:128–3

0096-R-P

TP53 Induced Glycolysis and Apoptosis Regulator (TIGAR) mediates human T lymphocytes activation and proliferation

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TP53-Inducible Glycolysis and Apoptosis Regulator (TIGAR) is a protein which has been described to be overexpressed in several types of tumours and to have a role in the metabolic switch in cancer cells. In this paper, we analysed the role of TIGAR in the activation of primary human T lymphocytes. Concanavalin A (ConA) was used in order to activate T lymphocytes. Under this conditions, there is a rise of TIGAR expression in treated lymphocytes compared to control ones, and that TIGAR is located in the cytoplasm. This induction was prevented in the presence of PI3K/AKT pathway inhibitors. In addition, when TIGAR is silenced, a decrease in proliferation and an increase in ROS was observed. Moreover, TIGAR was silenced in T lymphocytes before induction and the results obtained suggested that TIGAR is involved in leading carbon flux to the pentose phosphate pathway (PPP) at the expense of glycolysis. At the same time, TIGAR protects the cell from oxidative stress and prevents autophagy.

In conclusion, we demonstrated that TIGAR plays a role in human physiological lymphocyte activation and proliferation through the PI3K/AKT signalling pathway affecting mainly to oxidative stress by pentose phosphate pathway.

Bensaad, K. et al. (2006). TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell Ko, Y. et al. (2016). TP53-inducible Glycolysis and Apoptosis Regulator (TIGAR) Metabolically Reprograms Carcinoma and Stromal Cells in Breast Cancer. JBC Maciolek, J. et al. (2014) Metabolism of activated T lymphocytes. Curr Opin Immunol Simon-Molas, H. et al. (2018). PI3K-Akt signaling controls PFKFB3 expression during human T-lymphocyte activation. Mol Cell Bio.

0144-P

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.

0152-P

Palmitic and oleic acids differently modify the miRNA content of exosomes released by hypothalamic astrocytes and with POMC neurons responding differently to these exosomes

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Hypothalamic astrocytes participate in the maintenance of metabolic homeostasis[1]. Metabolic hormones can act directly on these glial cells to modify their metabolism, morphology and function, which in turn can influence neighboring neurons. It has recently becomes clear that astrocytes can communicate with neurons through the release of exosomes[2]. Exosomes are macrovesicles that contain different molecules, including microRNAs that can influence the function of target cells. Exosome content can be modulated by the metabolic environment and thus transmit essential information about energy homeostasis. In this study our objectives were: 1) To determine if miRNA content changes in hypothalamic astrocyte-derived exosomes in response to oleic acid (OA) and palmitic acid (PA) and 2) Analyze the response of POMC neurons to exosomes secreted from astrocytes treated with PA or OA. Hypothalamic astrocyte cultures were exposed to PA (0.5 mM), OA (0.5 mM) or vehicle (V) for 24 hours. Exosomes were purified from the media and miRNAs analyzed. Moreover, these exosomes were used to treat a POMC neuronal cell line. The levels of specific miRNAs were modified by both OA and PA compared to V exosomes, but in a different manner. The response of POMC neurons exposed to exosomes from female astrocytes for 24 hours depended on the fatty acid environment of the astrocytes. The expression of POMC mRNA was reduced in response to non-treated astrocytes and increased in response to exosomes from both OA and PA treated astrocytes. These results suggest that hypothalamic astrocytes can modulate neurons involved in metabolic control through exosomes and that the nutrient environment controls the messages contained within these exosomes.

1. Frago LM, Chowen JA. Involvement of Astrocytes in Mediating the Central Effects of Ghrelin. Int J Mol Sci. 2017;18(3):536. Published 2017 Mar 2. doi:10.3390/ijms18030536 2. Pascual M, Ibáñez F, Guerri C. Exosomes as mediators of neuron-glia communication in neuroinflammation. Neural Regen Res. 2020;15(5):796-801. doi:10.4103/1673-5374.268893

0178-P

Insulin regulates Colon Adenocarcinoma progression inducing PFKFB3 gene expression through PI3K/Akt and ERK1/2 signaling pathways

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Dietary and lifestyle factors associated with insulin resistance and hyperinsulinemia have also been related to a significant increase in colorectal cancer risk. It has been shown that PFKFB3 is required for the survival and growth of multiple cancer types.

However, few studies have been done on the mechanism of PFKFB3 modulation in insulin-related colon cancer and the signaling cascades responsible for these insulin effects.

PFKFB3 is a homodimeric bifunctional enzyme, belonging to the family of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases, that controls the conversion of fructose-6-phosphate to fructose-2,6-bisphosphate (Fru-2,6-P2). This metabolite is important for the dynamic regulation of glycolytic flux by allosterically activating the rate-limiting enzyme of glycolysis phosphofructokinase-1 (PFK-1). In the present study, we investigate the mechanism connecting insulin, glucose metabolism, and PFKFB3 in human colon adenocarcinoma.

We demonstrate that PI3K/Akt and ERK kinases signaling pathways mediates glycolysis and PFKFB3 modulation by insulin. Results suggest a multimodal mechanism of insulin affecting PFKFB3 transcriptional regulation and kinase activation by protein phosphorylation. qRT-PCR experiments showed progressive induction of PFKFB3 mRNA after insulin treatment, being significant already in 1h and reaching a maximum at 3h that is still maintained significantly at 6 and 10 h. Other glycolytic genes such as Glut1 and HK-II are also induced while other PFKFB isoforms remained unchanged. PFKFB3 protein levels showed a parallel induction pattern. The effect of insulin on PFKFB3 correlates with Fru-2,6-P2, lactate changes, and stimulation of glycolytic flux. Insulin effects on PFKFB3 also affect the proliferation of colon cancer cells. Hyperinsulinemia may have an important role in the progression of colon cancer being PFKFB3 a key point of metabolic reprogramming.

Cirillo F et al. Obesity, Insulin Resistance, and Colorectal Cancer: Could miRNA Dysregulation Play A Role?. Int J Mol Sci. 2019. Lu CC. et al.. Insulin induction instigates cell proliferation and metastasis in human colorectal cancer cells. Int J Oncol. 2017;50(2). Rodríguez-García A. et al. TGF-β1 targets Smad, p38 MAPK, and PI3K/Akt signaling pathways to induce PFKFB3 gene expression and glycolysis in glioblastoma cells. FEBS J. 2017;284(20).

0179-R-P

Androgen-receptor transcriptional response promotes survival against glucose deprivation in androgen-sensitive PCa cells.

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Most tumors are characterized by showing a glycolytic phenotype; however, that is not the case in prostate cancer (PCa). PCa is a commonly diagnosed with a high mortality rate. While the healthy prostate is mainly glycolytic and shows a partial inhibition of the tricarboxylic acid cycle, tumor glands shift towards oxidative phosphorylation, at least during the first stages of carcinogenesis, when the androgen receptor is still functional (1) . After the first-line treatment base on several anti-androgen therapies, many tumors develop into a resistant phenotype known as castration-resistant prostate cancer (CRPCa), whose underlining metabolic mechanisms have not been well described yet (2) .

In addition, our own research has demonstrated higher dependence on glucose uptake by androgen-independent PC-3 cells than by androgen-responsive LNCaP cells. AR activation, which is triggered by glucose removal, leads to a transcriptional response that includes an increase in the glucose transporter 1 (GLUT1) levels, which promotes survival in androgen-responsible cells (3) . Since survival seems to be correlated with the hormone resistance acquisition, we aim to characterize the molecular mechanisms that are regulated by the AR transcriptional response under glucose withdrawal.

For this purpose, human androgen-sensitive LNCaP and androgen-isensitive PC-3 cells were cultured under glucose deprivation or with H2O2 or endoplasmic reticulum (ER) stress inductors to induce cell death. The antioxidant NAC and the ER stress inhibitor Salubrinal were employed. Our findings show that PC-3 cells are more sensitive to glucose-deprivation-induce cell death, which mainly cause due to redox disbalance and consequently activates ER stress, leading to autophagy. LNCaP cells are able to better survive under these stress conditions, thanks to AR transcriptional response and GLUT1 upregulation. In conclusion, cell death induced by glucose deprivation in prostate cancer is mediated by ER stress response and it is dependent on androgen sensitivity.

1. Verze P et al. The role of the prostate in male fertility, health and disease. Vol. 13, Nature Reviews Urology. Nature Publishing Group; 2016 2. Bader DA et al. Tumour metabolism and its unique properties in prostate adenocarcinoma. Vol. 17, Nature Reviews Urology. Nature Research; 2020 3. Gonzalez-Menendez P et al. GLUT1 protects prostate cancer cells from glucose deprivation-induced oxidative stress. Vol.17, Redox Biol; 2018

0197-P

Sex-specific differences in white adipose tissue expansion in the transition from healthy to unhealthy obesity.

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During the development of obesity, the way how adipose tissue expands plays a key role in the onset of comorbidities. Hypertrophic adipocytes together with extracellular matrix fibrosis limit the accumulation of fat within the tissue, changing the biology of adipocytes and leading to hypoxia, inflammation, oxidative stress and ectopic accumulation of fat, among other processes. Thus, an increased ability to expand adipose tissue could explain the presence of morbid obese patients who show a healthier metabolic profile than expected according to their BMI (known as metabolically healthy obese, MHO, in opposition to metabolically unhealthy obese patients or MUO). However, the mechanisms that trigger a healthy or unhealthy expansion are unknown. In addition, there is an increased percentage of MHO women compared to men, which suggests that, even when both men and women share the same pathological processes, obesity progression is different in both populations. To analyze these differences, we performed a structural analysis and an RNA microarray in the visceral adipose tissue biopsies from male and female morbid obese patients who exhibited differential metabolic profile. First, we analyzed the transition from MHO to MUO and we only found 3 genes that were differentially expressed between MHO and MUO in our population of study. However, a sex-based stratification revealed 28 genes upregulated in MUO vs MHO males, mostly related to extracellular matrix remodeling and inflammatory processes. In women, MUO vs MHO analysis revealed differences in the expression of another set of 24 genes, including mostly immunoglobins. These sex-based differences were confirmed when we analyzed MUO and MHO male vs female (712 genes differentially expressed between men and women in MHO and 333 in MUO). These results confirmed that obesity follows a different progression in men and women, so sex-based differences should be considered in the prevention and treatment of the disease.

Slagter SN, Corpeleijn E, van der Klauw MM, Sijtsma A, Swart-Busscher LG, Perenboom CWM, et al. Dietary patterns and physical activity in the metabolically (un)healthy obese: the Dutch Lifelines cohort study. Nutr J. 2018 Feb 12;17(1):18. Vasanthakumar, A., Chisanga, D., Blume, J.,

Gloury, R., Britt, K., Henstridge, D. C., et al. (2021). Author Correction: Sex-specific adipose tissue imprinting of regulatory T cells. Nature, 591(7851), E26.

0205-P

AE1 (Slc4a1) prevents inorganic phosphate uptake in Xenopus laevis oocytes

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Inorganic phosphate (Pi) homeostasis control mainly relies on the regulation of intestinal Pi absorption, bone storage and renal excretion mechanisms, acting on several known sodium dependent Pi transporters. Nevertheless, several transport systems involving both, sodium-dependent and independent mechanisms, remain to be molecularly identified. In a search of new Pi transporters, our group is screening the members of the Slc4 and Slc26 families based on the inhibition pattern of Pi transport. Here, we show our findings on Slc4a1 (AE1, Band3), the best-known Cl/HCO_3 exchanger.

Slc4a1 was heterologously expressed in *Xenopus laevis* oocytes by cRNA injection, which provoked an almost 50% decrease of ^{32}Pi uptake respect to the water-injected oocytes. Although Slc4a1 is a Cl/HCO_3 exchanger, this inhibition was chloride independent.

Coexpression of Slc4a1 with either the rat Na/Pi cotransporters *NaPi2a (Slc34a1*) or PiT1 (Slc20a1) completely abolished the Pi accumulation observed in oocytes expressing NaPi2a or PiT1 alone. Western-Blot (WB) analysis of biotinylated proteins from oocyte plasma membrane of NaPi2a/Slc4a1-coexpressing oocytes revealed that both transporters were correctly expressed.

In order to characterize the possible Pi efflux mechanism, several Slc4a1 inhibitors known to act through different mechanisms were tested: DIDS, SITS, phloretin, dipyridamole, DNFB and niflumic acid. None of them prevented the effect of Slc4a1 on Pi uptake.

To know if the effect in oocytes could be reproduced in cell lines, we used the Opossum Kidney cell line, which shows a high expression of NaPi2a. Transfection of rat Slc4a1 cDNA, however, did not affect Pi transport.

Expression of Slc4a1 in rat jejunum and kidney cortex was determined by qPCR and Western blot. To study the involvement of Slc4a1 in the control of Pi homeostasis, the protein abundance of this transporter was determined in jejunum mucosa and renal medulla from rats fed diets with different Pi content. WB revealed no significant changes of AE1 expression as a response to dietary Pi changes in these tissues.

In conclusion, our preliminary results show that heterologous expression of Slc4a1 in oocytes abolishes Pi accumulation by either preventing endogenous Pi influx or acting as a Pi efflux transporter. The relevance *in vivo* also remains to be elucidated.

0209-R/M-P

Neddylation inhibition reduces liver steatosis in MAFLD mice models by promoting hepatic fatty acid oxidation via DEPTOR-mTOR axis

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Metabolic-associated fatty liver disease (MAFLD) is a complex liver disease and comprehends a group of conditions being the massive accumulation of fat in the liver the main feature. Mechanistic target of rapamycin (mTOR) pathway plays an essential role in lipid metabolism and development of MAFLD [1] . In recent years, the regulation of DEP domain-containing mTOR-interacting protein (DEPTOR), a negative regulator of mTOR pathway, has been involved in the alteration of lipid homeostasis. It is known that DEPTOR is degraded by SCF (Skp1-Cullin-F box proteins) E3 ubiquitin ligase, which needs to be neddylated to be active [2] . Neddylation is a reversible ubiquitin-like post-translational modification upregulated in many diseases [3] , including MAFLD. Therefore, we decided to evaluate the potential use of Pevonedistat (MLN4924), a neddylation inhibitor, in MAFLD therapy through regulation of mTOR signaling. Neddylation inhibition was evaluated in mouse isolated hepatocytes and in male adult C57BL/6 mice (3-month old) fed either with 0.1% methionine and choline deficient diet (0.1%MCD diet) for 4 weeks or with a choline-deficient high fat diet (CD-HFD) for 6 weeks. Pevonedistat (60mg/Kg) was administered last 2 or 3 weeks of diet by oral gavage each 4 days. Neddylation inhibition using Pevonedistat, as well as silencing Nedd8, reduced lipid accumulation in oleic acid-stimulated mouse primary hepatocytes. Likewise, pharmacological neddylation inhibition and Nedd8 hepatic knockdown ameliorates liver steatosis preventing lipid peroxidation, oxidative stress and inflammation in mouse models of MAFLD. Increased Deptor levels and concomitant repression of mTOR signalling when neddylation is inhibited, is associated with augmented fatty acid oxidation and reduced lipid content. Deptor silencing in isolated mouse hepatocytes abolishes the anti-steatotic effects mediated by neddylation inhibition. Finally, serum NEDD8 levels correlate with hepatic neddylation both during disease progression in the clinical setting and during disease regression by therapeutic approaches in pre-clinical models. Overall, upregulation of DEPTOR, driven by neddylation inhibition, is proposed as a novel effective target and therapeutic approach to tackle MAFLD.

1.Eslam, M. et al. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. Gastroenterology 158, 1999-2014.e1 (2020). 2.Zhao, Y., Xiong, X., Jia, L. & Sun, Y. Targeting Cullin-RING ligases by MLN4924 induces autophagy via modulating the HIF1-REDD1-TSC1-mTORC1-DEPTOR axis. Cell Death & Disease 3, e386–e386 (2012). 3.Zubiete-Franco, I. et al. Deregulated neddylation in liver fibrosis. Hepatology 65, 694–709 (2017).

0230-R-P

Effect of angiotensin II type 2 receptor (AT2R) activation in brown adipose tissue expansion and thermogenic activity in a mouse model of obesity

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The thermogenic activity of brown adipose tissue (BAT) has emerged as a promising therapeutic tool against metabolic diseases. Recently, the angiotensin II type 2 receptor (AT2R) has been proposed as a new target to enhance thermogenesis in lean mice by activating BAT and promoting white adipose tissue browning. AT2R belongs to the protective arm of the renin-angiotensin system and its local activation exerts anti-inflammatory and antioxidant effects, although its expression is relatively low and only increases under pathological situations. However, it is still unknown if AT2R activation would have an impact on obese brown adipose tissue. To analyze the potential role of AT2R activation in obesity, we administered C21, a specific AT2R agonist, to HFD-fed obese mice for 6 weeks. Both body weight and BAT mass increased with HFD. C21 did not modified weight gain in HFD mice, but increased BAT mass through lipid accumulation. qPCR analysis revealed that obesity increased the expression of the deleterious AT1R, together with markers of lipid uptake (CD36, LDLR), fatty acid oxidation and thermogenesis (PGC1a, PPARa, CPT1b, UCP1). HFD-C21 mice exhibited a reduced expression of AT1R together with an increased AT2R. However, it did not have an impact on the aforementioned markers, whose levels were similar to those found in the HFD mice. These data were confirmed by Western Blot, showing an upregulation of UCP1 and electron transport chain complexes by HFD, with no visible effects of C21 supplementation. Taken together, these data suggest that chronic oral C21 treatment is not enough to promote BAT thermogenesis, although it is still unrevealed if it may play a role in brown adipocyte differentiation.

Than A, Xu S, Li R, Leow MK, Sun L, Chen P. Angiotensin type 2 receptor activation promotes browning of white adipose tissue and brown adipogenesis. Signal Transduct Target Ther. 2017 Jun 23;2:17022. Quiroga DT, Muñoz MC, Gil C, Pffeifer M, Tobli JE, Steckelings UM, Giani JF, Dominici FP. Chronic administration of the angiotensin type 2 receptor agonist C21 improves insulin sensitivity in C57BL/6 mice. Physiol Rep. 2018 Aug;6(16):e13824.

0231-P

Neuron-specific CPT1C regulates bis(monoacylglycero)phosphate (BMP) metabolism by modulating ABHD6 activity

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Our group has identified carnitine palmitoyltransferase 1C (CPT1C) as an important nutritional indicator that regulates energy metabolism and cognition through malonyl-CoA sensing. CPT1C has no catalytic activity but is able to modulate other proteins activity (Casals et al., 2016)and they show differences in their kinetics and tissue expression. Although CPT1C exhibits high sequence similarity to CPT1A and CPT1B, it is specifically expressed in neurons (a cell-type that does not use fatty acids as fuel to any major extent . Our recent findings have confirmed that CPT1C negatively regulates the hydrolase activity of α/β-hydrolase domain containing 6 (ABHD6), a new member of the endocannabinoid system involved in the pathogenesis of obesity (Miralpeix et al., 2021) . Regarding lipid metabolism, ABHD6 has a role in monoacylglycerol degradation and in endolysosomal lipid sorting by regulating bis(monoacylglycero)phosphate (BMP) catabolism in vitro and in vivo (Grabner et al., 2019, 2020; Pribasnig et al., 2015) . Here, we aimed to demonstrate whether CPT1C regulates BMP metabolism in brain tissues through its interaction with ABHD6.

We have measured BMP levels and endogenous ABHD6 activity in brain tissues (hypothalamus and hippocampus) and liver of WT and CPT1C-KO mice after short-term feeding standard or high fat diet (HFD) (7 days).

CPT1C-KO mice show decreased BMP levels and increased ABHD6 hydrolase activity in brain tissues, but significantly higher liver BMP levels compared to WT mice under standard diet. When fed a HFD, total BMP levels decreased in hypothalamus and hippocampus of WT but remained unchanged in CPT1C-KO mice. Levels of specific species of BMP were also differently modified by CPT1C neuronal deletion in both brain tissues (i.e. 40:8, 44:12) and liver (i.e. 40:7, 44:12). Total BMP levels correlated with

ABHD6 activity in hypothalamus since its hydrolase activity increased after 7 days of HFD administration in WT but not in CPT1C-KO mice.

These results reveal the physiological role of CPT1C and ABHD6 interaction in BMP metabolism in brain tissues and highlight these proteins as potential targets in neuronal regulation of lipid metabolism, particularly in endolysosomal lipid sorting.

Casals N, et al. Prog Lipid Res. 2016;61:134–48. Grabner GF, et al. J Lipid Res. 2019;60(5):1020–31. Grabner GF, et al. J Lipid Res. 2020;61(7):995-1003. Miralpeix C, et al. Br J Pharmacol. 2021;178(7):1507–23. Pribasnig MA, et al. J Biol Chem. 2015;290(50):29869–81.

0239-M-P

Role of Purine metabolism in cellular senescence and ageing

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Introduction: Cellular senescence is a hallmark of ageing and characterized by cell cycle arrest and production of cytokines, interleukins, lipid mediators and extracellular vesicles. Exosome-like particles are a group involved in the transmission of paracrine senescence and rejuvenation and they are an attractive target to therapeutic application. In the last years, the metabolic changes had revealed crucial in the senescence signature in ageing and premature ageing and our group has focused to determined what metabolism pathways were altered in Hutchinson-Gilford progeria syndrome (HGPS). This disease is a very rare fatal disease characterized for accelerated aging.

Objective: Because of that, we focused our study on how the alteration of purine-metabolism could affect the SASP mediated by exosome-like particles.

Methods: In this study, we work with several models of cellular senescence: oncogenic-induced senescence and DNA-damage induced senescence (DDis) in mesenchymal stem cells, chondrocytes and fibroblasts from human origin. During the senescence induction the medium was supplemented with S-adenosyl-methionine (SAMe), a metabolite that is an alternative source of purine and besides the exosome-like particles from that senescent cell models were used to treat proliferative cells.

Results: It was observed that the production of exosome-like particles characterized by NTA from senescence signature models were decreased in a significantly way. It was observed that the production of exosome-like particles characterized by NTA from senescence signature models were decreased in a significantly way. Besides, the transmission of paracrine senescence, which occurs through exosome-like particles, it is no happens when cells are treated with SAMe as indicated in our results on proliferation using crystal violet and b-galactosidase activity.

Conclusion: Altogether, our data suggests that purine metabolism is altered in premature aging through SASP mediated by exosome-like particles. This opens a new window for the therapeutic treatment of the age-related disease and HGPS.

0263-R-P

Neonatal overfeeding in mice permanently programs obesity through early white adipose tissue hypertrophy

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Most children with obesity will remain obese until adulthood, which in turn, increases the risk of developing co-morbidities later in life, including diabetes and cardiovascular disease[1,2]. The mechanisms behind the persistence of obesity are poorly characterized. We aimed to explore the mechanisms by which increased adiposity is extended from childhood to adulthood, and its relationship with metabolic disturbances.

We developed a mouse model of neonatal obesity (*i.e.* childhood obesity) by litter size reduction. At birth, litters were adjusted to 8 pups per control-dam (C) and 4 pups per small litter-dam (SL). At weaning, all mice were maintained on a standard chow diet. We characterized inguinal (iWAT) and epididymal (eWAT) white adipose tissue biology in young (2-week old) and adult mice (6-month old).

SL mice remained heavier and maintained higher fat mass than controls until adulthood, despite showing similar food intake/energy expenditure after weaning. SL iWAT and eWAT adipocytes were hypertrophic and remained larger than C. Hypertrophy could be largely attributed to increased lipogenesis, as assessed by increased mRNA expression of *Fasn*, *Scd1*, *Plin1, Glut4*, and *Chrebp*. In the adults (6-month old), the expression of lipogenic and adipogenic genes was largely normalized. *In vitro* pre-adipocyte differentiation (iWAT) from young and adult mice showed no differences, suggesting that pre-adipocyte commitment and differentiation is not impaired in SL mice.

These data led us to propose that early hypertrophic adipose tissue accrual is primarily mediated by enhanced lipogenesis. Long-term adiposity (in the context of childhood obesity) cannot be attributed to changes in energy expenditure and/or defects in adipocyte proliferation/differentiation. Therefore, here we propose that stable adipose hypertrophy could be mediated by slow adipocyte turnover. We are currently testing this hypothesis *in vivo*. Together, we speculate that low turn-over might explain why treating obesity in young children is so challenging.

1. Llewellyn, A., Simmonds, M., Owen, C. G., & Woolacott, N. (2016). Childhood obesity as a predictor of morbidity in adulthood: A systematic review and meta-analysis. Obesity Reviews, 17(1), 56–67. doi: 10.1111/obr.12316 2. W.H.O. (2013). Global Strategy on Diet, Physical Activity and Health. Childhood overweight and obesity. In W.H.O. updates. Retrieved from: http://www.who.int/dietphysicalactivity/childhood/en

0268-R/M-P

Concomitant activation of nutrient and growth factor signaling in the liver synergistically compromises hepatic homeostasis and metabolism

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The mechanistic target of rapamycin complex I (mTORC1) is a master regulator of cell growth and metabolism by the integration of two major regulatory inputs: nutrients, which activate mTORC1 pathway through the family of Rag GTPases, and growth factors, which inhibit the Tuberous Sclerosis Complex 1 (TSC1) to allow mTORC1 activation. To understand the physiological fundamentals on how the mTOR pathway integrates signals by two related but independent cues, and how mTOR orchestrates metabolism accordingly, we have engineered a mouse model in which systemic and cellular nutrient signaling are deregulated in hepatocytes (Li-TSC1^{KO} RagA$^{GTP/\Delta}$ mice). Our results show that while single activation of mTORC1 through either nutrients or growth factors dominantly activates the mTORC1 pathway and abrogates fasting metabolism, simultaneous genetic activation of nutrient and hormonal cues elicited diverse features of severe hepatic damage without a mirror increase in mTORC1 pathway activity. In particular, we have observed increased concentration of liver injury markers in serum, histological alterations and loss of hepatic zonation together with perturbations in glucose homeostasis. Moreover, these mice have a reduced lifespan due to the development of heterogeneous liver tumors. Pharmacological inhibition of mTORC1 completely rescues the strong phenotype of Li-TSC1^{KO} RagA$^{GTP/\Delta}$ mice: rapamycin administration corrects hepatic damage, recovers defects in glucose metabolism and leads to an extension in survival. We are currently aiming at the molecular determinants of such synergic effects of this aberrant liver phenotype that does not mirror the mTORC1 signaling in the Li-TSC1^{KO} RagA$^{GTP/\Delta}$ mice.

0272-P

Modulation of brain lipid metabolism in specific brain cells by C75-CoA loaded polymeric nanoparticles

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Carnitine palmitoyl transferases are a family of proteins involved in the metabolism of fatty acids. CPT1A in the mitochondria catalyses the conversion of long-chain fatty acids into acylcarnitines. It is expressed in peripheral tissues but also in the hypothalamus, where it is involved in energy homeostasis. Inhibition of hypothalamic CPT1A reduces feeding, whereas overexpression increases food intake and adiposity. Furthermore, CPT1A and CPT1C are overexpressed in glioblastoma, contributing to cell proliferation and metabolism. Pharmacological manipulation of CPT1 proteins activity in specific brain cells could therefore be useful in treating diseases such as obesity or glioblastoma.

C75 is a racemic lactone originally described to inhibit FASN. [RRR1] Later it was described that (+)-C75 inhibits CPT1A, while the physiologically formed adduct (-)-C75-CoA inhibits FASN. To target CPT1A in specific brain cells, we have developed a strategy involving polymeric nanoparticles targeting hypothalamic neurons or glioblastoma cells. We have prepared PEG-Pasp(DET) nanoparticles which can encapsulate racemic C75 and its enantiopure forms, and can be modified with ligands designed to overcome the blood-brain-barrier and target specific cells of interest.

In vitro characterization of C75-CoA-loaded PEG-Pasp(-DET) nanoparticles in cell lines of hypothalamic neurons and glioblastoma cells, showed long stability and low polydispersity, as well as stability during *in vitro* and *in vivo* administration in mice. The uptake profile of the nanoparticles was higher in hypothalamic than in glioblastoma cells. Furthermore, these nanoparticles decreased viability and modulates the lipid metabolism of *in vitro* hypothalamic and glioblastoma cell lines compared to free drug. Currently, *in vivo* biodistribution studies are being performed by intracerebroventricular and intranasal administration of model fluorescent nanoparticles. Altogether, polymeric nanoparticles constitute an interesting strategy to modify lipid metabolism of specific brain cells.

[1] Sierra, AY et al., Journal of Biological Chemistry, (2008),11, 6878-6885. [2] Casals, N et al., Progress in Lipid Research, (2016), 61, 134-148. [3] Mera, P et al., Biochemical Pharmacology, (2009), 6, 1084-1095. [4] Makowski, K et al., Chirality, (2013) 25, 281–287. [5] Quader, S et al. Journal of Controlled Release, (2017), 258, 56-66.

0274-R/M-P

The influence of nutrigenetic factors on the impact of the first wave of COVID-19 in Europe

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The COVID-19 pandemic has highlighted the need to know in greater depth factors that can promote the proper functioning of the immune system, such as the nutritional status. Hence, the European Food Safety Authority (EFSA) considers that the vitamins D, A, C, B_6, B_9 and B_{12} and, the minerals Iron, Zinc, Copper and Selenium are essential for the normal functioning of immune system. Therefore, their optimal intake would be essential to avoid or effectively deal with infectious diseases. Thus, the aim of this study was to analyze the association of the intake levels of these micronutrients with the COVID-19 incidence and mortality in the European framework. For that, published data on the intake levels of the 10 nutrients and COVID-19 epidemiological indicators (from *worldometers*), from Spain, Italy, France, UK, Denmark, Germany, Finland, Belgium, Netherlands and Portugal, have been analyzed to assess potential relationships between them. Besides, the potential influence of genetics on the status of these 10 nutrients has also been considered by analyzing the population frequency of risk alleles that could promote suboptimal status (data from *1000genomes)*. The results show that vitamin D, C, B_{12}, Iron and Zinc intake levels inversely correlated with incidence and/or mortality from COVID-19, pointing out that those countries with lowest intake are those that suffered the worst consequences of the pandemic first wave. In addition, genetic analyses pointed out that countries with higher frequency of risk alleles for specific nutrient suboptimal status would be associated with increased risk of COVID-19 beyond nutritional indicators. In conclusion, this ecological study highlights the links among optimal nutrition, genetic factors and the proper immune system function, now also associated with potential benefits against COVID-19. This nutrigenetic knowledge can be a fundamental tool to help in the implementation of nutrition precision advices in order to strengthen the immune system and better prepare the population to fight COVID-19 and against other upcoming infectious diseases.

0276-R/M-P

E2F1 and E2F2 promote the lipid-rich environment necessary for the development of MAFLD-related HCC

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Lipid metabolism rearrangements in metabolic associated fatty liver disease (MAFLD) contribute to disease progression. Moreover, MAFLD has emerged as a major risk for hepatocellular carcinoma (HCC), where metabolic reprogramming is a hallmark. In this context, identification of metabolic drivers might reveal therapeutic targets to improve the treatment of MAFLD-related HCC. Here, we investigated the contribution of transcription factors E2F1 and E2F2 to MAFLD-related HCC development and their involvemnent in metabolic reprogramming during disease progression. To achieve that, *E2f1*$^{-/-}$, *E2f2*$^{-/-}$, *E2f1/E2f2* (DKO) and WT mice were used. Liver disease in mice were induced by diethylnitrosamine (DEN) administration and feeding a high-fat diet (HFD). Adeno-associated viruses serotype 8 (AAV8) were used to overpress or silence *E2f2* specifically in the liver. E2F2 were also overexpressed *in vitro* in hepatocytes through adenovirus infection. ChIP analysis were carried out in tissue and cells. Expression and protein levels of E2F1 and E2F2 were also analyzed in human cohorts. Results showed that in human MAFLD, E2F1 and E2F2 protein levels were increased and positively correlated. The same was observed when analyzing *E2F1* and *E2F2* expression in human HCC. Consistently, *E2f1* and *E2f2* were upregulated in DEN-HFD WT mice while *E2f1*$^{-/-}$ and *E2f2*$^{-/-}$ mice were resistant to DEN-HFD-induced hepatocarcinogenesis as well as to the associated lipid accumulation. Administration of DEN-HFD in *E2f1*$^{-/-}$ and *E2f2*$^{-/-}$ mice enhanced fatty-acid oxidation (FAO) and increased expression of *Cpt2*, an enzyme essential for FAO whose downregulation is linked to MAFLD-related hepatocarcinogenesis. These results were also observed following *E2f2* knockdown in liver, and overexpression of *E2f2* elicited opposing effects. ChIP analyses revaled that E2F2 binding to the *Cpt2* promoter was enhanced in DEN-HFD-administered mouse livers compared to controls, implying a direct role for E2F2 in transcriptional repression. In human HCC, *E2F1* and *E2F2* expression inversely correlated with *CPT2* expression. Collectively, these results indicate that activation of the E2F1-E2F2-Cpt2 axis provides a lipid-rich environment required for hepatocarcinogenesis. Thus, E2F1 and E2F2 arise as new potential therapeutic targets for MAFLD-related HCC.

0325-P

Anti-Helycobacter pylori activity of melanoidins by prevention of Caco-2 cell adhesion

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Helicobacter pylori infection is the single most important risk factor worldwide for the development of gastritis and gastric ulcers. *H. pylori* adheres to epithelial cells by injecting toxins to activate proinflammatory signaling cascades and cause cell death. Data from *in vitro and in vivo* studies suggested that melanoidins possess prebiotic properties. Melanoidins are Maillard reaction compounds that may inhibit the growth of pathogenic bacteria such as *H. pylori* evaluated *in vitro*. The aim of this study was to evaluate the capacity of melanoidins obtained from crust of biscuit, loaf and soft bread by membrane ultrafiltration. A concentrated melanoidins was recovered through the dead-end ultrafiltration of the extract using 10 kDa membrane with 76 mm of diameter in polyethersulfone material. The samples were characterized by browning index and UV-visible spectra and their cytotoxicity were assayed in Caco-2 monolayers. The ability of melanoidins to interfere with the adhesion of *H. pylori* was determined by *in situ* immunofluorescence after preincubation with melanoidins extracts. No Caco-2 cell cytotoxicity was observed to different concentrations (25 mg/mL, 50 mg/mL, 100 mg/mL, 200 mg/mL) of the three types of melanoidins, the values of cell viability was greater than 80 %. All melanoidins decreased significantly the levels of *H. pylori* adhesion to intestinal epithelial cell Caco-2 being the greatest inhibitory effect for loaf bread melanoidins.This study is financed by Autonomous Government of Castilla y León and FEDER (JCyL/FEDER) BU243P18.

Diaz-Morales, N., Cavia-Saiz, M.; Salazar, G.; Rivero-Pérez, MD.; Muñiz, P. (2021) Cytotoxicity study of bakery product melanoidins on intestinal and endothelial cell lines. Food Chemistry. 343- (128405). Fogliano, V., Morales, F. J. (2011). Estimation of dietary intake of melanoidins from coffee and bread. Food and Function, 2(2), 117-123. Ecrolini, D., Fogliano, V. (2018) Food design to feed the human gut microbiota. J. Agri. Food Chem. 3754-3758

0343-P

Resveratrol supplementation to suckling mice impacts muscle and liver lipid and energy metabolism in the long-term

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Introduction: Increased awareness of the importance of early life nutrition, added to the high prevalence of obesity and associated comorbidities, is boosting interest in preventive strategies based on dietary agents and mechanisms of metabolic programming. Benefits of resveratrol (RSV) supplementation to adult animals and humans are well known, yet metabolic programming activity of RSV is less known, especially when directly administered to the lactating animals. We previously showed that RSV supplementation to suckling mice enhances in the male progeny a more oxidative and thermogenic phenotype of white adipose tissue in adulthood(1). We here extended the study to eventual programming effects in non-adipose tissues.

Objectives: To study long-term effects of mild RSV supplementation during lactation on muscular and hepatic lipid and energy metabolism in adulthood.

Methods: Newborn male mice received RSV (2mg/kg/day) or vehicle (controls) from day 2 to 20 of age, were weaned onto a chow diet on day 21, and were assigned to either a high-fat diet (HFD) or a normal-fat diet on day 90 of age for 10 weeks. Histological and gene expression analyses on skeletal muscle (gastrocnemius) and liver samples were conducted.

Results: RSV-treated mice showed in adulthood protection against HFD-induced triacylglycerol accumulation in skeletal muscle, enhanced muscular capacities for fat oxidation and mitochondria activity, and signs of activation of sirtuin 1 and AMP-dependent protein kinase pathways in muscle. Liver triacylglycerol content was unaffected, yet RSV-treated mice showed increased fat oxidation capacities and a decreased capacity for lipogenesis in liver compared with controls.

Conclusions: RSV supplementation in early postnatal life may help preventing later diet-related disorders linked to ectopic lipid accumulation in muscle and liver tissues, and represent a multi-target-directed strategy to prevent obesity and related metabolic disorders.

1. Serrano A et al. Mol Nutr Food Res. 2018;62:e1800463

0365-R/M-P

Oleocanthal and oleacein, two polyphenols from olive oil, inhibit angiogenesis

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Extra Virgin olive oil polyphenols contribute to the beneficial effects on health attributed to the Mediterranean diet. However, while there is much evidence about the biological effects of polyphenols such as hydroxytyrosol, tyrosol or oleuropein, secoiridoids like oleocanthal and oleacein remain less explored. In this study, we examined the anti-angiogenic potential of oleocanthal and oleacein using a series of assays. Oleocanthal and oleacein inhibited proliferation and migration of endothelial cells, as well as the secretion of extracellular matrix-degrading proteases involved in their migration. These compounds also prevented the formation of blood vessel-like structures by endothelial cells and induced a stop in S/G2/M phase of the cell cycle, and their apoptosis. Taken altogether, these results point towards the anti-angiogenic effect of the polyphenols oleocanthal and oleacein, making them good pre-clinical candidates for the treatment of diseases such as cancer, psoriasis, macular degeneration, and a list of angiogenesis-related orphan diseases. Additional experiments to uncover the *in vivo* antiangiogenic potential of these compounds and their possible mechanisms of action are underway. [This work was supported by grants PID2019-105010RB-I00 (Spanish Ministry of Science, Innovation and Universities), and UMA18-FEDERJA-220 (Andalusian Government and FEDER) and funds from groups BIO-267 and FQM-182 (Andalusian Government). The “CIBER de Enfermedades Raras” is an initiative from the ISCIII (Spain)].

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[1] J. Lozano-Castellón, A. López-Yerena, J. F. Rinaldi de Alvarenga, J. Romero del Castillo-Alba, A. Vallverdú-Queralt, E. Escribano-Ferrer, R. M. Lamuela-Raventós, Crit. Rev. Food Sci. Nutr. 2020, 60, 2532–2548. [2] M. R. Emma, G. Augello, V. Di Stefano, A. Azzolina, L. Giannitrapani, G. Montalto, M. Cervello, A. Cusimano, Int. J. Mol. Sci. 2021, 22, 1–22.

0372-R/M-P

Ghrelin and des-acyl ghrelin actions in glucose metabolism and autophagy in a caloric restriction model.

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Ghrelin is a hormone synthesized mainly in the stomach in fasting situations and induces an increase in food intake, body weight and adiposity (1). Thereby, it was initially proposed as an anti-obesity target. However, it has been seen that it has a role in the regulation of glucose homeostasis, moreover it has the ability to maintain euglycemia in cases of negative energy balance, allowing survival. In addition, ghrelin stimulates the secretion of growth hormone (2) and its effect over glucose could be regulated in a GH dependent or independent manner. On the other hand, des-acyl ghrelin (UAG), the other ghrelin isoform, was thought to be inactive, but there is evidence pointing out that it may also have physiological effects on energy homeostasis, potentially via a receptor that still needs to be identified (3).

Aim: To know the implication in the control of glycemia and autophagy in a caloric restriction condition of central UAG and compare it with ghrelin, analysing the impact on peripheral organs, such as liver.

Methods: We used male C57BL/6 mice, fed a standard diet. We administered intracerebroventricular vehicle, ghrelin or UAG with mini-osmotic pumps for 7 days. During this time, mice are subjected to a 60% caloric restriction. We monitored glucose, body weight and food intake daily. The effects on glucose metabolism were evaluated by protein expression using western blot, histo-morphological analysis and serum insulin and IGF-1 levels by ELISA.

Results: In caloric restriction ghrelin generates a lower decrease in body weight and an increase in fat depots compared to mice treated with vehicle and UAG. The analysis of glucose metabolism shows that ghrelin and UAG are able to increase gluconeogenesis, but only ghrelin produces increase of insulin levels and IGF-1. The analysis of autophagy and glucose pathways in liver proteins showed how ghrelin and UAG in caloric restriction are able to increase gluconeogenesis and autophagy, although UAG is not able to activate the GH pathway.

Conclusion: The data obtained supports the regulation of glycemia by ghrelin in caloric restriction, and provides new data on the influence of UAG on glucose control and autophagy, with different mechanism of ghrelin, such a GH independent manner.

1.Cummings, D.E.; Purnell, J.Q.; Frayo, R.S.; Schmidova, K.; Wisse, B.E.; Weigle, D.S. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001; 50: 1714–1719 2.Müller, T. D. et al. Ghrelin. Mol. Metab. 2015; 4: 437–60. 3.Poher AL, Tschöp MH, Müller TD. Ghrelin regulation of glucose metabolism. Peptides. 2017; 12.015

0376-P

Efectos del alperujo en la dieta del pez cebra. Análisis morfométrico y actividades de enzimas digestivas y antioxidantes

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La acuicultura es uno de los sectores productivos de mayor desarrollo en el último cuarto de siglo, pero su crecimiento está seriamente comprometido por la disponibilidad de piensos sostenibles. Por otra parte, el proceso de extracción de aceite de oliva genera cada año una ingente cantidad de alperujo, un subproducto de escaso valor económico pero alto impacto ambiental. Este residuo además de poseer una alta concentración de azúcares, ácidos grasos insaturados y polialcoholes de elevado valor nutritivo, contiene compuestos fenólicos con propiedades antioxidantes, antiinflamatorias y anticancerígenas [1]. Por todo ello se está investigando su utilización como complemento en la dieta de animales de granja y también podría constituir una alternativa sostenible, con elevado potencial nutracéutico, en la dieta de peces de piscifactoría [2-3]. El pez cebra (*Danio rerio*) es uno de los animales modelo más utilizados en diferentes ámbitos de investigación, incluidos estudios nutricionales en peces [4]. En este trabajo hemos estudiado, en el pez cebra, el efecto de dietas con diferente proporción de alperujo (entre 5 y 50%) a lo largo de un periodo de 15 semanas a partir de los 40 dpf. Hemos valorado su efecto sobre el crecimiento y sobre la actividad de diferentes enzimas relacionadas con el estado nutricional y la respuesta antioxidante. El análisis de parámetros morfométricos indica una tolerancia de hasta un 15% de alperujo en la dieta sin un efecto significativo sobre el crecimiento. A concentraciones superiores se observa una disminución significativa en el peso. No obstante, el análisis de la tasa instantánea de incremento de peso mostró que este retraso podría deberse a la necesidad de un periodo de adaptabilidad a la nueva dieta. Estudios preliminares indican ausencia de efecto del alperujo en la actividad de las enzimas digestivas esterasa y fosfatasa alcalina, en cambio, la actividad proteasa total se incrementó notablemente con las dosis más altas. Por otro lado, se ha observado un ligero efecto protector del alperujo hasta la dosis de 25% sobre la oxidación de proteínas, en consonancia con una menor actividad glutatión reductasa. Las actividades G6PDH y SOD también se vieron significativamente afectadas por las concentraciones más altas. (Financiación: Universidad de Córdoba XXIII PP Mod. 4.1)

[1] Difonzo, G. el al. (2021) J Sci Food Agric 101:15-26 [2] Ben-Salem, H. et al. (2008) Anim Feed Sci Tech 147:2006-2222 [3] Nasopolou, C. et al. (2011) Food Chem 129:1108-1113 [4] Ulloa, P.E. et al. (2011) Rev Fish Biol Fisheries 21:649-666

0382-R/M-P

The role of p107 in the regulation of glucose metabolism in the liver

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Homeostasis is a physiological process seeking to keep a stable metabolism and avoiding significant changes. Through food intake, physiological mechanisms, nutrient balance and cellular oxidative processes, the body is able to guarantee energy homeostasis. An important organ contributing to this function is the liver. It responds to hormones in order to regulate glucose and fatty acid metabolism. On the other hand, current studies demonstrate that tumor suppressors play an important role in liver metabolism in mice. p107, member of Rb family, is a representative of the “Pockets Protein“ whose main activity is to restrict the G1-S transition through the regulation of genes responsive to E2F. Studies carried out by our group have shown that total-p107-KO-mice and liver-specific-p107-KO-mice do not develop liver steatosis when treated with a high-fat diet. In addition, p107 deficient mice had better glucose tolerance. However, it is not yet evident how p107 acts on glucose metabolism in liver. Our aim is to explore the action of p107 specifically in glucose metabolism in mice liver. Therefore, we evaluate the effects of p107 on glucose metabolism pathways in the liver of p107 KO mice on a HFD. Also we perform an insulin tolerance test in order to analyze proteins involved in insulin-sensitivity. Our current results reveal the involvement of p107 with key enzymes in glucose homeostasis. However, further experiments are needed to elucidate the specific mechanisms that involve p107 in glucose homeostasis.

Blanchet E, Annicotte JS, Fajas L. Cell cycle regulators in the control of metabolism. Cell Cycle. 2009. Lopez-Mejia IC, Castillo-Armengol J, Lagarrigue S, Fajas L. Role of cell cycle regulators in adipose tissue and whole body energy homeostasis. Cell Mol Life Sci. 2018 Cunarro J, Buque X, Casado S, Lugilde J, Vidal A, Mora A et al. p107 Deficiency Increases Energy Expenditure by Inducing Brown-Fat Thermogenesis and Browning of White Adipose Tissue. Molecular nutrition & food research 2019.

0402-R/M-P

GLUT8 silencing impacts lipid metabolism in human hepatocytes and hepatic stellate cells differentially

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Introduction: Excessive consumption of high-fructose diets is associated with insulin resistance, obesity, and non-alcoholic fatty liver disease (NAFLD). Although the eighth member of facilitated glucose transporters (GLUT8) has been linked to fructose-induced macrosteatosis, the specific contribution to lipid metabolism in the different liver cells remains to be elucidated.

Objectives: The major aim of this study was to evaluate the impact of GLUT8 knockdown on lipid metabolism under fructose-enriched medium by using well-established hepatic cell lines.

Methods: LX-2 human hepatic stellate (HSCs) and THLE2 human hepatic cell (HCs) lines were cultured under different nutritional conditions. Following, cells were transfected with specific small-interfering RNA (si-RNA) to knock down the expression of GLUT8. Nontargeting siRNA was used as a negative control. After silencing GLUT8, the medium was replaced with a fresh high-fructose medium (25 mM fructose) for another 24 h until cells were collected for protein and RNA extraction and immunofluorescence and Oil Red analyses.

Results: The efficiency of GLUT8 silencing was around 50% in LX2 and 75% in THLE2 cells. GLUT8 knockdown in LX2 cells significantly decreased IL6 gene expression, indicating a reduction of inflammatory signal. Interestingly, whilst the human cytochrome P450 monooxygenase CYP4F2, which predominantly catalyzes the omega-oxidation of long Fas and VLCFAs, was significantly reduced in GLUT8 silenced LX2 cells; CYPA11 and CYP4F3, which are involved in the metabolism of various endogenous substrates, including fatty acids (FAs), were upregulated. In THLE2 cells, GLUT8 knockdown cells significantly decreased IL1b and IL18 gene expression. Importantly, the gene expression of the three cytochrome P450 monooxygenases analyzed was downregulated. GLUT8 silencing mainly reduced fructose-induced DNL in THLE2 cells, since the fatty acid synthase (FASN) gene expression and FAS and ACC protein levels were downregulated. Enhanced beta-oxidation mediated by CPT1A and lipolysis by ATGL was only observed in LX2 cells.

Conclusions: GLUT8 silencing differently reduced the FA content in both LX2 and THLE2, as confirmed by Oil Red staining, probably due to reduced lipogenesis in HCs and enhanced lipid oxidation and lipolysis HSCs.

0403-R-P

Gastronomía Bioactiva: compuestos bioactivos en alimentos y quimioprevención en enfermedades de alta prevalencia

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La quimioprevención de enfermedades basada en la dieta ha surgido en los últimos años como un enfoque interesante para la prevención, e incluso la ralentización de fases iniciales, en patologías de alta prevalencia como son las enfermedades cardiovasculares, neurodegenerativas y el cáncer. En el caso concreto del cáncer, una de las dianas farmacológicas utilizadas en clínica es la inhibición de la angiogénesis, dado el papel fundamental que este proceso tiene en el desarrollo de tumores y su diseminación. Derivada del anterior concepto, la *angioprevención* propone el uso de inhibidores de la angiogénesis en la prevención del cáncer. Precisamente, el potencial antiangiogénico que exhiben muchos compuestos naturales contenidos en diferentes alimentos de la dieta mediterránea hace que este patrón dietético sea especialmente interesante como fuente de agentes quimiopreventivos, definidos dentro de la estrategia de angioprevención [1].

En línea con esto, la gastronomía tradicional española, basada en la dieta mediterránea, emerge como una potencial herramienta al servicio de la quimioprevención, por lo que acuñamos el nuevo concepto de *gastronomía bioactiva,* relativa a los beneficios de nuestra gastronomía bajo el prisma de la quimioprevención mediante compuestos bioactivos de los alimentos.

Este trabajo está financiado por fondos de los proyectos PID2019-105010RB-100 (Ministerio de Ciencia, Gobierno de España), UMA18-FEDERJA-220 (Junta de Andalucía y fondos FEDER), y fondos del grupo BIO-267 (Junta de Andalucía). El trabajo cuenta con el aval del sello Gastrocampus de Innovación, Facultad de Turismo, Universidad de Málaga.

Afiliaciones adicionales: A.D.M., A.R.Q, M.A.M. y B.M.-P. Instituto de Investigación Biomédica de Málaga (IBIMA); A.R.Q. y M.A.M. CIBER de Enfermedades Raras (ISCIII); B.M.-P. CIBER de Enfermedades Cardiovasculares (ISCI-II).

[1] Beatriz Martínez-Poveda et al. (2019) Mediterranean diet, a rich source of angiopreventive compounds in cancer. Nutrients, 11, 2036; doi:10.3390/nu11092036

0416-P

Sexual dimorphism in response to caloric restriction in skeletal muscle of young rats

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Caloric restriction (CR), defined as a decrease of 30% to 60% ad libitum intake without malnutrition, can extend lifespan, prevent or delay the onset of age-associated diseases, and decelerate the functional decline in a wide range of species. CR effects on aging appear to be mediated by inhibition of mTOR, reduction of inflammation and oxidative stress, induction of autophagy, and prevention of cellular senescence. However, little is known about the effects of CR when started in early life. The aim of this study was to analyze the effects of CR in the skeletal muscle of young Wistar rats. For this, 3-month-old male and female rats were subjected to 40% CR or fed ad libitum for 3 months. Gastrocnemius muscles were collected and frozen with liquid nitrogen, placed in a chilled mortar, and pulverized with a pestle to extract RNA and protein. Western blot and RT-qPCR were performed to determine the levels of classical markers of autophagy (LC3, p62), inflammation (IL-6, IL1R, IL1B NFκB, IκB, TNFα), antioxidant (SOD, NRF2), nutrient-sensing (HIF1, FOXO3, mTOR, IRS1, LDH, GSK, IDH2, AKT, AMPK, LKB1, ERRα, SIRT3, SIRT6) mitochondrial dynamics (DRP1, FIS1), and senescence pathways (p53, CASP3, p16, p21). Results showed that CR decreased total weight and skeletal muscle weight in both males and females. Interestingly, we did not find differences in most of the inflammation, antioxidant, and nutrient-sensing pathways. Interaction effects between gender and diet were observed for DRP1, CASP3, p53, SIRT6, and HIF1 levels. In female rats, SIRT3, SIRT6, LC3, p53, p21, and NFκB levels increased with CR, while IL6 levels decreased. On the other hand, CR male rats showed decreased levels of p53, HIF-1α, and SIRT6. These results suggest a sexual dimorphism in markers of inflammation, autophagy, and senescence in response to CR in young rats. Our data show that young caloric restricted female rats exhibit similar expression patterns to the ones observed in previous studies in old animals under CR, promoting significant pro-longevity effects, while in male rats these effects are diminished. More studies are needed to understand how late or early in life CR might exert positive effects on healthspan and lifespan.

This work was supported by a grant from Programa postdoctoral Margalida Comas - Comunidad Autónoma de las Islas Baleares (PD/050/2020).

Colom, B. et al (2007). Cellular Physiology and Biochemistry, 19(1-4), 205-212. Serna, JDC. et al (2020). Journal of Bioenergetics and Biomembranes, 52, 269-277. Chen, CN. et al (2019) GeroScience 41, 871–880. Faitg J. Et al (2019). Frontiers in Physiology, 10:420. Mitchell, Sarah J et al (2016). Cell metabolism, 23(6): 1093-1112.

0434-P

Vitamin A Status is Correlated with Epithelial-Mesenchymal Transition in the Lung

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Vitamin A and its derivatives, mainly retinoic acid (RA), play a key role in cell proliferation, cell differentiation and organ development and vitamin A deficiency (VAD) has been shown to contribute to the development of chronic lung disorders. In previous studies, we have shown that alterations in lung function and architecture in VAD are associated with modifications in extracellular matrix and basement membranes, which play an important role in cell adhesion and tissue morphogenesis. In addition, we have also observed that VAD induced hyper-activation of TGF-β signaling, increased oxidative stress, ectopic collagen I deposition by epithelial cells and alteration of several cell-junction proteins which suggests that disruption of endogenous RA signaling leads to an epithelial-mesenchymal transition (EMT) process in lung. In order to elucidate the role of lung vitamin A status in EMT and cell migration and invasion abilities we have analyzed several protein markers of EMT transition by western blotting, immunocytochemistry and quantitative RT-PCR (qPCR) in the lung of VAD rats and in VAD rats treated with RA. In addition, we have analyzed by qPCR the mRNA of the proprotein convertase furin, which activates a wide variety of precursor proteins, such as TGF-β and matrix metalloproteinases and regulates the levels of adhesion molecules. Our western blotting, immunocytochemistry and qPCR analysis results showed a switch from E-cadherin to N-cadherin in VAD lungs. These results support our previous studies in which we reported that EMT is occurring in the VAD lung. Treatment of VAD rats with RA only partially reversed the alterations. When we analyzed the expression of furin, we observed that it increased in VAD rats. Retinoic acid treatment did not restore furin expression to the control values, as it continues to increase much more markedly in VAD rats treated with retinoic acid. A. Since it has been shown that the EMT and increased expression of N-cadherin and furin correlate with cell migration and tissue invasion, our results emphasize the importance of maintaining vitamin A tissue levels in a physiological range.

Funding: Generalitat Valenciana (grant PROMETEO/2018/167)

1-Esteban-Pretel G., Marín MP., Renau-Piqueras J., Barber T.,

Timoneda J. (2010) J. Nutr. Biochem. 21:227-236. doi: 10.1016/j.jnutbio.2008.12.007. 2-Barber T., Esteban-Pretel G., Marín MP., Timoneda J. (2014) Nutrients 6:4984-5017. doi:10.3390/nu6114984. 3-Timoneda J., Rodríguez-Fernández L., Zaragozá R., Marín MP., Cabezuelo MT., Torres L., Viña JR., Barber T. (2018) Nutrients 10:1132. doi: 10.3390/nu10091132. 4-Braun E., Sauter D. (2019) Clin. Transl. Immunology 8: e1073.doi:10.100

0436-P

Carbamoyl Phosphate synthetase of Escherichia coli: study of its regulation

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Carbamoyl phosphate (CP) is a crucial metabolite in *Escherichia coli* (*E. coli*) as it allows to synthetize pyrimidine nucleotides and L-arginine, which are considered to be important precursors for desoxyribonucleic acid (DNA) and proteins synthesis, respectively. That is why production of CP in *E. coli* needs to be highly regulated, existing a balanced between both metabolic pathways. The enzyme which is capable of synthetizing these metabolites is carbamoyl phosphate synthetase (CarAB), a heterodimer composed by two subunits. The smaller one, CarA, hydrolizes L-glutamine in ammonia, whereas the biggest one (CarB), uses this ammonia for CP production by using moreover bicarbonate and ATP-Mg2+ [1]. CP formation is highly regulated by several modulators. IMP and L-ornitine activates CarAB, but its activity is reduced by UMP [1] . These effectors are able to bind to the C-terminal subunit of the biggest subunit, specifically to Lys 993, which is a vital residue to UMP inhibition [1].

The activity of CarAB may be affected by lysine acetylation. Ne-acetylation can occur in prokaryotes and its role is protein function regulation. This can be carried out by using Acetyl-CoA or Acetyl phosphate (AcP) in a chemical way; or by an enzymatic manner employing an acetyltransferase [2] .

The aim of this work is to know if lysine acetylation could have any effect in CarAB activity and its regulation by UMP. For this purpose, site-directed incorporation of acetyl-lysine unnatural amino acid to specific residue has been carried out using the genetic code expansion concept with pRSF-Duet-1 modified plasmid. The results deepen the knowledge of CarAB regulation.

[1] V. Fresquet, P. Mora, L. Rochera, S. Ramón-Maiques, V. Rubio, and J. Cervera, “Site-directed mutagenesis of the regulatory domain of Escherichia coli carbamoyl phosphate synthetase identifies crucial residues for allosteric regulation and for transduction of the regulatory signals,” J. Mol. Biol., vol. 299, no. 4, pp. 979–991, 2000. [2] S. Zhao et al., “Regulation of cellular metabolism by protein lysine acetylation,” Science (80-.)., vol. 327, no. 5968, pp. 1000–1004, 2010.

0437-P

The inhibition of carnitine palmitoyltransferase 1 (CPT-1) impairs boar sperm function in the absence of exogenous energy substrates

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The study of sperm metabolism has usually focused on ATP production through glycolysis and oxidative phosphorylation powered mainly by exogenous glucose and lactate. This study evaluates if blocking fatty acid transport into the mitochondrial matrix modifies boar sperm function *in vitro*.

Boar spermatozoa samples (n = 6) were exposed to etomoxir (300 µM), an inhibitor of carnitine palmitoyltransferase 1 (CPT-1), in a non-capacitating medium depleted of exogenous energy substrates. Rotenone (10 µM), an inhibitor of mitochondrial Complex I, was used as a positive control and to analyse if etomoxir non-specifically inhibits Complex I. After 1 h of incubation, sperm motility was measured by Computer-Assisted Sperm Analysis (CASA) and flow cytometry was used to study sperm viability, mitochondrial membrane potential (ΔΨm) and the production of reactive oxygen species (ROS).

Exposure to etomoxir impaired sperm motility, significantly reducing their curvilinear velocity (VCL, 52 %), straight-line velocity (VSL, 66 %) and average path velocity (VAP, 63 %). Other sperm motility parameters, such as linearity (LIN), beat-cross frequency (BCF) and straightness of trajectory (STR) also decreased significantly after etomoxir treatment. Sperm viability, the population of spermatozoa with high ΔΨm and the sperm production of ROS were not altered by etomoxir exposure. Meanwhile, the incubation with rotenone significantly decreased all motility parameters; in most cases, its effect was significantly lower than the effect caused by etomoxir. Sperm viability was not modified after treatment with rotenone, but the percentage of spermatozoa with high ΔΨm showed a non-significant increase compared to the control group (21 %), while the production of ROS was significantly increased (65 %).

This work suggests that etomoxir is not likely to affect sperm mitochondrial Complex I nor produce its effect by increasing oxidative stress in boar spermatozoa. Therefore, the inhibition of fatty acid metabolism must play a significant role in boar sperm function, regulating at least their motility

0443-P

Identification of ARMCX3 as negative regulator of brown/beige adipose tissue activity

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ARMCX3 is a protein encoded by a member of the Armcx gene family. It was found to be involved in mitochondrial function in the nervous system (1) and we recently reported that it mediates the susceptibility to hepatocarcinoma promoted by high-fat diet-mediated lipotoxic insults in mice (2). We found that Armcx3-null mice are protected against the effects of a high-fat diet eliciting obesity and glucose intolerance, despite no reduction in food intake. Surprisingly, Armcx3-null mice showed a massive spontaneous browning of subcutaneous and epididymal white adipose tissues, as well as minor signs of activation of brown fat, consistent with increased energy expenditure. In wild-type mice, ARMCX3 mRNA and protein levels were markedly downregulated in brown and white adipose tissues in response to physiological (cold) and pharmacological (the beta3-adrenergic activator CL316,243) thermogenic stimuli. In adipocytes in culture, ARMCX3 expression was repressed in association with brown/beige differentiation. Adenoviral-driven vector over-expression of ARMCX3 in mouse adipocyte cell cultures repressed the expression of marker genes of thermogenic function, such as *Ucp1*, *Cidea* or *Bmp8b*. *ARMCX3* gene expression correlates negatively with the expression of marker genes of oxidative/thermogenic function such as *PPARGC1B*, *CIDEA*, *CPT1B* and mitochondrial DNA-encoded components of the respiratory chain in subcutaneous fat in a cohort of human patients spanning a wide range of body mass index. In conclusion, ARMCX3 is identified as a novel, previously unsuspected, molecular actor in the control of adipose tissue plasticity, being a negative regulator of white adipocyte browning processes. Ongoing investigation will establish the intracellular molecular mechanisms mediating the ARMCX3 effects on repressing the thermogenic adipocyte phenotype, as well as the involvement of adipose browning repression on the susceptibility of mice to lipotoxic-promoted hepatocarcinogenesis.

(1) López-Doménech G et al. The Eutherian Armcx genes regulate mitochondrial trafficking in neurons and interact with Miro and Trak2. Nat Commun. 2012 May 8;3:814. doi: 10.1038/ncomms1829. (2) Mirra S, Gavaldà-Navarro A, et al. ARMCX3 Mediates Susceptibility to Hepatic Tumorigenesis Promoted by Dietary Lipotoxicity. Cancers (Basel). 2021 Mar 5;13(5):1110. doi: 10.3390/cancers13051110.

0449-P

Grape Seed Proanthocyanin Extract modulates circadian rhythms of clock genes and inflammation mediated by oxidative stress in dietary obese rats

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Obesity is associated with health complications such as metabolic syndrome, altering the metabolism due to a low-grade inflammatory condition, and modifying the central and peripheral circadian rhythms. Furthermore, phenolic compounds seem to be able to modulate circadian rhythms. For this reason, we wonder if the improvement in oxidative stress parameters observed with the supplementation of Grape Seed Proanthocyanin Extract (GSPE) in obese rats could be associated with a circadian-dependent modulation of inflammation and oxidative stress. Hence, the aim of this study was to study the effects of GSPE on liver peripheral clock genes and oxidative stress parameters related to inflammation in an obese context.

Ninety-six 12-week-old male Fischer rats were housed by pairs under standard laboratory conditions (temperature 22 ºC, 12 h light/dark cycle) with *ad libitum* access to food and drinking water and divided into 6 groups: 2 groups were fed a standard diet (STD) and 4 groups were fed a cafeteria diet (CAF) for 5 weeks. Then, a daily oral administration of GSPE (25mg/kg body weight) or vehicle (VH) were administered at *zeitgeber* time (ZT) 0 or 12 to CAF groups for 4 weeks. The STD groups were given VH at ZT0 or 12. Animals were sacrificed at ZT 1, 7, 13 and 19 (n=4). Liver samples were collected and snap frozen until further analysis.

The qPCR results showed an alteration in the circadian rhythm of clock genes *Bmal1* and *Nampt* in CAF group. Interestingly, CAF-GSPE induced a restoration of circadian clock genes, as well as a reduction of oxidative stress genes such as *Catalase* or *Glutathione Peroxidase 1* (*GPx1*) at ZT12. Moreover, Western Blot analysis showed a significant decrease in inducible Nitric Oxide Synthase (iNOS) in CAF-GSPE *vs.* CAF-VH group at ZT1 when GSPE is administered at ZT12, showing similar values to STD-VH. Additionally, GSPE administration at ZT12 influenced biochemical parameters showing a reduction of total lipid content in the liver. Hence, these results suggest that GSPE can function as *zeitgeber* for the molecular clock machinery regulating these genes expression in the liver of obese rats and decreasing the oxidative stress-mediated inflammation.

Acknowledgement: Martí i Franquès predoctoral Fellowship. Universitat Rovira i Virgili, Tarragona

Arola-Arnal, A et al. Nutrients 2019, 11, 2602. Scarpellini E. & Tack J. Dig Dis 2012, 30, 148-153.

0479-R/M-P

miR-222 transfection in preadipocytes alters pathways related to lipid homeostasis and insulin sensitivity – a transcriptomic approach

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A role for miR-222 in obesity, insulin resistance and diabetes has been proposed, as its levels rise on these pathological situations in both animals and humans, especially in the adipose tissue [1] . Moreover, its concentration in breastmilk increases in rat dams fed with an obesogenic diet during lactation. The offspring that received greater levels miR-222 through lactation displayed insulin resistance and greater adiposity in the adulthood [2] . However, the molecular effects of this microRNA remain unclear.

The aim of this study was to determine the global impact of miR-222 on gene expression in preadipocytes.

To achieve this objective, 3T3-L1 preadipocytes were transfected with either a mimic molecule of miR-222 (mim222 group) or a negative control of the mimic molecule (C-mim group). A control (C) group with no transfection performed was also included. After 48h of exposure, cells were collected. Total RNA was extracted and transcriptomic analysis was performed through a microarray technique. Differences in gene expression were calculated comparing C-mim versus mim222 groups. In addition, analysis of signalling routes with Hipathia was conducted.

A total of 2045 genes were significantly affected by miR-222 mimic transfection. Results show that miR-222 exerts a uniform effect on the transcriptome, as mim222 samples clustered together and their gene expression was significantly different from those from C-mim. Regarding pathway analysis, routes related to Type II Diabetes Mellitus, PPAR signalling pathway, Insulin signalling pathway and Adipocytokine signalling pathway show a significant change in mim222 group compared to C-mim. These alterations could be linked to insulin insensitivity and altered lipid homeostasis, offering a mechanistic explanation to the outcomes seen in *in vivo* studies.

This *in vitro* approach shows how solely an acute dose of miR-222 exerts effects on global gene expression on preadipocytes that could be linked to obesity and insulin resistance. Further research should assess these effects *in vivo* and their relevance on metabolic programming.

1. Deiuliis, J. A. Int. J. Obes. 40, 88–101 (2016). 2. Pomar, C. A. et al. Mol. Nutr. Food Res. 63, 1800928 (2019). 3. Hidalgo, M. R. et al. Oncotarget 8, 5160–5178(2017).

0502-P

The blockade of carnitine palmitoyltransferase 1 (CPT-1) inhibits boar sperm motility without affecting sperm viability

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One of the unknown metabolic pathways contributing to sperm function is lipid catabolism. This study investigates the effects of blocking fatty acids transport into mitochondria in sperm function, including motility. Boar spermatozoa isolated from commercial boars ejaculates were incubated 1 h in TBM medium with 300 µM Etomoxir (ETO), a carnitine palmitoyltransferase I (CPTI) inhibitor) or 10 µM Rotenone (ROT), a mitochondrial Complex I inhibitor.

Sperm motility was measured by computer-assisted sperm analysis (CASA) and viability, mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) by flow cytometry. Etomoxir treatment reduces main sperm motility parameters: % of motile spermatozoa by 30 %, significantly inhibits the % of rapid and progressive spermatozoa by 82% and significantly reduces any sperm velocity, curvilinear (VCL), straight-line (VSL) and average path velocity (VAP). Sperm viability, ROS (mitochondrial or cytosol) levels and MMP are not affected by ETO incubation. Moreover, ROT exposure causes a greater inhibition of sperm motility parameters than ETO treatment: significantly reduces (55 %) the % of motile spermatozoa and rapid progressive spermatozoa (94 %) as well as sperm velocities VCL, VSL and VAP. ROT treatment does not affect sperm viability or the mitochondrial ROS production but significantly increases MMP (37 %) and ROS levels in cytosol (30 %).

In summary, inhibition of the fatty acids transport into mitochondria does not affect boar sperm viability while it does in motility, suggesting that β-oxidation likely plays a role in this important sperm function. Moreover, etomoxir effect is not mediated by oxidative stress and is not likely affecting the sperm mitochondrial Complex I.

16.- Cellular Signalling

0515-OI

HER2-CB2R heteromers as new therapeutic targets and patient screening tools in breast cancer

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There is a specific subtype of breast cancer that is characterized by the over-expression of the receptor tyrosine kinase HER2. Although most patients with this diagnosis benefit from HER2-targeted treatments, some do not respond to these therapies and others develop resistance with time. New tools are therefore warranted for the treatment of this patient population, and for early identification of those individuals at a higher risk of developing innate or acquired resistance to current standards of care.

Our group has recently demonstrated that HER2 forms heteromer complexes with cannabinoid receptor CB_2R, protecting the former from degradation and therefore promoting its pro-oncogenic signaling via c-SRC. In line with the idea of the HER2-CB_2R complex being an oncogenic driver, we have also demonstrated that the expression of these structures correlates with poor patient prognosis, and that their disruption induces antitumor responses by the inactivation of HER2 and its subsequent degradation by the proteasome.

Collectively, our results support HER2-CB_2R heteromers as new therapeutic targets and patient screening tools in HER2+ breast cancer.

Blasco-Benito et al., Therapeutic targeting of HER2-CB2R heteromers in HER2-positive breast cancer. PNAS USA 116 (9) 3863-3872. 2019
Pérez-Gómez et al., Role of cannabinoid receptor CB2 in HER2 pro-oncogenic signaling in breast cancer. Journal of the National Cancer Institute 107:djv077. 2015

0524-OI

Understanding the role of RAS signalling in the tumour microenvironment of KRAS-driven lung cancer

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Lung cancer is the leading cause of cancer-related death with a survival rate of less than 5%, mostly due to patients presenting with metastatic disease and developing resistance to therapy. Recently, molecularly-targeted agents (e.g. against EGFR or ALK) and immunotherapies (anti-PD1 antibodies) have been approved for treatment of NSCLC. However, it remains a challenging disease, particularly in KRAS-mutated cases, which are associated with an even worse prognosis and do not benefit from targeted agents. KRAS inhibitors currently in clinical development hold promise, but only for patients with a specific, relatively uncommon, mutation (G12C).

We have found that RAS signalling through PI3K has a significant impact on tumour progression by acting over the tumour microenvironment. Proteomic analysis of KRAS-driven lung tumours lacking RAS-PI3K interaction suggested a dependency for this signalling pathway in functions typically related to CAFs. Further analysis confirmed that TGF-b activated fibroblasts deficient for RAS-PI3K interaction displayed changes in CAFs markers such as YAP1, a-SMA, vimentin or fibronectin. This was accompanied by a reduction in the ability to contract collagen gels, impairment in cytoskeleton rearrangement and formation of thinner and more disorganised matrices. Furthermore, our data also revealed that proliferation of KRAS mutant lung tumour cells is highly impaired in those matrices generated by CAFs lacking RAS-PI3K. *In vivo* analysis of tumour stiffness showed that lung tumours lacking RAS-PI3K interaction are softer.

In summary, our data suggest an overarching effect of RAS signalling through PI3K in the formation of a pro-tumorigenic extracellular matrix by controlling CAF activation and function to modulate tumour cell behaviour. The high prevalence of RAS mutations in human cancer and the presence of CAFs in all tumours means that these results have far-reaching implications and point to new ways to tackle RAS-driven tumours.

0412-R/M-OS

Regulation of selective autophagy of ER through persulfidation of ATG18a

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Hydrogen sulfide (H_2S) is an endogenously generated gaseous signalling molecule, which recently has been implicated in autophagy regulation in both plants and mammals through persulfidation of specific targets. Persulfidation has been suggested as the molecular mechanism through which sulfide regulates autophagy in plant cells. Autophagy is an extremely important conserved mechanism involved in multiple biological processes. Latest studies have reported its regulation through different posttranslational modifications. H_2S acts as an inhibitor of autophagy and recently several ATG proteins have been reported to be modified by persulfidation. ATG18a is a core autophagy protein that binds to phosphoinositides, involved in autophagosome biogenesis during phagophore expansion, and ER stress-induced autophagy is dependent on the function of ATG18a. Our data revealed the role of sulfide in ER stress as a negative regulator of autophagy in plants during ER stress through the persulfidation of ATG18a by regulating its PtdIns(3)P-binding affinity.

Angeles Aroca is a postdoc researcher at IBVF (CSIC-US). Her current research topic is the study of the molecular mechanisms underlying the physiological effects of sulfide signaling in Arabidopsis, mainly focused on the proteomic analysis of these effects on the molecular targets that are regulated by Persulfidation. She has been granted with the European Marie Curie Global fellowship, to address the contribution of sulfide to regulation of selective autophagy in plants through Persulfidation.

0482-R/M-OS

The dsRNA analogue Poly(I:C) induces inflammation and calcification via JAK/STAT pathways in aortic valve interstitial cells

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Calcific aortic valve disease (CAVD) is the most prevalent heart valve disease, yet surgical replacement is the only available therapy. Its underlying mechanisms involve inflammatory and metabolic processes, such as infiltration of immune cells, lipid deposition, matrix remodeling, angiogenesis, as well as calcification. In human aortic valve interstitial cells (VIC), the dsRNA analogue Poly(I:C) promoted type I interferon (IFN) secretion, inflammation, and osteogenesis via Toll-Like Receptor 3. Moreover, Janus kinase (JAK)/Signal transducers and activators of transcription(STAT) pathways have been recently shown to promote inflammation and calcification in VIC.

In the present study, we aimed to investigate whether IFN signaling mediates dsRNA responses in human VIC. Our results showed that Poly(I:C) promoted a type I IFN response, including the secretion of IFN-β. Poly(I:C) also triggered a pro-inflammatory phenotype, characterized by the expression of adhesion molecules and the secretion of cytokines. In addition, Poly(I:C) promoted an IFN-like pro-osteogenic phenotype and triggered in vitro calcification under high phosphate conditions in an apoptosis-dependent manner. Pharmacologic experiments revealed that these effects were abrogated by ruxolitinib, a JAK1/2 inhibitor. Furthermore, calcification was also blunted by a type I IFN receptor neutralizing antibody. Finally, Poly(I:C) cooperated with IFN-γ to further promote osteogenesis via JAK-STAT/ERK and HIF-1α routes.

In conclusion, our study demonstrates that dsRNA-triggered inflammation, apoptosis and calcification are mediated by JAK-STAT pathways, which may contribute to a positive autocrine loop in the presence of IFN-γ. Therefore, clinically used JAK inhibitors may be a promising therapeutic avenue for CAVD.

Garcia-Rodriguez et al.(2018).Toll-Like Receptors,Inflammation and Calcific Aortic Valve Disease,Front Physiol Mathieu et al.(2015).Innate and Adaptive Immunity in Calcific Aortic Valve Disease,J Immunol Res Rajamannan et al.(2011)Calcific aortic valve disease:not simply a degenerative process,Circulation Zhan et al.(2017)Double-stranded RNA upregulates the expression of inflammatory mediators in human aortic valve cells through the TLR3-TRIF-noncanonical NF-kB pathway,Am J Physiol Cell Physi

0485-R-OS

SUR8 AND S100A9 DEPLETION ENHANCE EPIDERMAL PROINFLAMMATORY STATE

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The outmost layer of the skin is the epidermis. It has a physical, chemical, and immunological barrier function thanks to different cells junctions and allowing paracrine signaling among keratinocytes and immunological cells. This communication is about chemoattraction mediated by chemokines and cytokines, which accelerate the immune response in case of wounds. An important cytokine implicated in inflammation is S100A9. It forms a heterodimer with S100A8 termed calprotectin that is mainly segregated by neutrophils, but also, monocytes, macrophages and keratinocytes. S100A9 is commonly overexpressed in psoriasis, hidradenitis suppurative and works as a systemic inflammatory biomarker. The RAF-MEK-ERK pathway and its scaffold proteins have a key role in the development, differentiation, and homeostasis of the epidermis. One of the scaffold proteins is Sur8, a positive modulator that accelerates RAS-RAF interaction and the resulting signal carried out by ERK pathway contributes to proper skin functions and structure. Besides, genetic disorders of the RAS/MAPK pathway, termed RASopathies, produce numerous abnormalities, including keratodermas. Our main aim is to study the relation between keratinocytes and the immune system when Sur8 and S100A9 are absent and assess their impact in skin diseases and inflammation. In order to study the effect of Sur8 in the skin, our group has stablished a conditional animal model *msur8$^{\Delta ep}$ K5-Cre* in skin (KO animals), and 3D culture from skin derivatives have been carried out. We have observed a steep increase of several alarming cytokines in KO animal by RNAseq and ELISA test. In addition, we have developed a conditional animal model in C57BL/6J based on the Cre-Lox P system, by generating male mice lacking Sur8 in epidermal cells (*msur8$^{\Delta ep}$K5-Cre* or KO) and crossing them with constitutive S100A9 KO female (*S100A9$^{-/-}$*) resulting *msur8$^{\Delta ep}$-S100A9$^{-/-}$K5-Cre* (DKO) animals. Our group have studied DKO animals' survivals, skin injuries, and barriers defects by toluidine assays and RNA and protein detection. Histological analysis has shown epidermal thickening alteration, impaired proliferation and immune infiltration. We propose that Sur8 protein is an important skin mediator of inflammation modulating the inflammatory response.

(1) Schonthaler, et al. (2013). S100A8-S100A9 protein complex mediates psoriasis by regulating the expression of complement factor C3. Immunity, 39(6), 1171-1181. (2) Hyein J. et al. (2020) The Leucine-Rich Repeats Signaling Scaffolds Shoc2 and Erbin: Cellular Mechanism and Role in Disease. The FEBS journal 288 721-739. (3) Proksch, E., et al. (2008). The skin: an indispensable barrier. Experimental dermatology, 17(12), 1063-1072.

0024-R/M-P

Phosphoproteomic and functional analyses reveal sperm-specific molecular mechanisms underlying G-protein coupled receptors in human spermatozoa

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Human spermatozoa express G protein coupled receptors (GPCRs) which actively participate in the regulation of their fertilizing capacity. Due to the incapacity of human spermatozoa to express new proteins, they may possess different signaling pathways underlying GPCRs compared to those present in somatic cells. Therefore, the aim of this study is to reveal which are those specific molecular mechanisms activated downstream GPCR in human spermatozoa. In order to study the signaling pathways underlying GPCRs, and specifically the kappa-opioid receptor (KOR), we performed phosphoproteomic and functional approaches. Human spermatozoa were stimulated at different time points with U50488H, the specific agonist. After sample processing, we performed phosphopeptide enrichment with TiO_2 beads followed by the chemical labeling with TMT to further analyze them by a Q Exactive Mass spectrometer (Odense, DK). MS raw files were processed with MaxQuant software v1.3.0.7 and Perseus bioinformatic program. Functional assays were conducted by flow cytometry using the anti-CD46 antibody. According to our results, U50488H regulates the phosphorylation of novel phosphosites belonging to human sperm specific proteins. These phosphosites could be involved in the regulation of a novel calcium signaling pathway which differs from the one found in somatic cells. These results support the idea that GPCRs present unique features in their molecular mechanisms in human spermatozoa. This finding could describe the first steps of the signaling pathways activated via KOR and which lasts in an inhibition of the acrosome reaction, as the functional studies confirm. As a conclusion, the human sperm GPCRs regulate different signaling pathways comparing to the ones described in somatic cells via the phosphorylation of sperm specific proteins. As the spermatozoa are transcriptionally and translationally silent cells, the post-translational modifications such as phosphorylations play a key role in the fulfillment of the different physiological functions.

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0032-R/M-P

Muscle p38 MAPKs: novel targets for the regulation of inter-organ communication in obesity and type 2 diabetes

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Obesity is a well-known risk factor for several chronic diseases, including type 2 diabetes, cardiovascular diseases, fatty liver disease and cancer [1]. Physical inactivity and changes in dietary habits have increased the incidence and prevalence of obesity in current societies, which has become a major health problem [2]. Although several pharmacological treatments to lose weight through a reduction in food intake have been proposed, understanding the molecular mechanisms that lead to increased energy expenditure may help to find new therapies. In fact, exercise has the capacity to improve the metabolic status in obesity and type 2 diabetes, being skeletal muscle an important regulator of glucose homeostasis and an essential contributor to exercise-induced changes in metabolism [3], in part through the release of secreted factors termed myokines [4].

Previous studies have demonstrated the relevance of the mitogen-activated protein kinases (MAPKs) pathway in the control of obesity and whole-body metabolism [5, 6]. However, most of them have focused on the JNK and ERK MAPKs, being the role of the p38 MAPKs pathway less understood. In this work, using a conditional mouse model lacking p38α in striated muscle, we have attempted to increase knowledge about the role of muscle p38s signaling both at the local and the systemic level. Our results show that the deficiency of p38α in striated muscle decreases body weight and protects mice against high-fat diet-induced obesity by increasing energy expenditure. The molecular alterations caused by this deficiency lead to skeletal muscle metabolic remodeling, increasing mitochondrial oxidative metabolism. Importantly, lack of p38α results in the hyperactivation of another p38 family member in skeletal muscle, p38γ, which is essential for their improved glucose and energy homeostasis. Altogether, these molecular and metabolic changes in skeletal muscle are physiologically manifested as increased locomotor activity together with improved glucose homeostasis, decreasing the risk of developing type 2 diabetes and liver steatosis, therefore linking local and systemic manifestations of p38α deficiency. In conclusion, this study provides insight into a novel role of stress signaling in skeletal muscle in inter-organ crosstalk in the context of metabolic regulation.

[1] J. R. Speakman. J. Nutr. 134, 2090S-2105S (2004). [2] World Health Organization (WHO). Obesity and overweight. [Online]. Available in: http://www.who.int/mediacentre/factsheets/fs311/en/ (2020). [3] Egan, B., Zierath, J. R. Cell Metab. 17, 162-184 (2013). [4] Priest, C., Tontonoz, P. Nat. Metab. 1, 1177-1188 (2019). [5] Kyriakis, J. M., Avruch, J. Physiol. Rev. 92, 689-737 (2012). [6] Nikolic, I., Leiva, M., Sabio, G. Nat. Rev. Endocrinol. 16, 697-716 (2020).

0043-R-P

AhR absence induces stemness-related genes expression pattern in liver regeneration

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Under homeostatic conditions, the adult liver is in a differentiated, non-proliferative and polyploid state with low cell renewal. Injuries and pathological processes can damage this organ. However, the liver has an efficient regenerative capacity to recover its size, architecture and function (1, 2). The 2/3 partial hepatectomy model in rodents allow the understanding of the complex and organized network of signaling pathways that are critical to achieving liver regeneration (3). In this process, cell proliferation is not produced essentially by the activation and proliferation of stem cells, although these also participate and expand during regeneration (4).

The Aryl hydrocarbon receptor (AhR) participates in primordial signaling pathways in liver function and development. Thus, Knockout mice models for AhR have a severe impact in this organ presenting an altered development of the liver, a reduced size and a intrahepatic portosystemic shunt (5). The liver functions of AhR related to detoxification against toxic and carcinogenic compounds are the best known and studied. However, several studies have revealed that this receptor is essential in cell differentiation, pluripotency and reprogramming, regulating the balance between pluripotency-differentiation under physiological and patological conditions (6, 7, 8).

To investigate the role of AhR in liver regeneration, a surgical resecting of two-thirds of the liver was performed in mice and then the changes in the differentiation state of the liver were analysed. To this aim, a knockout mouse model for AhR (*AhR-/-*) was employed. This work revealed that AhR depletion improved regeneration, motivating the expansion of cells that express markers of stemness. These results are interesting since the endogenous activation of the receptor shows its importance in physiological processes independent of its detoxifying function. Thus, its pharmacological inhibition could stimulate the recovery of the functionality and structure of the liver after damage by toxins or pathological processes such as hepatocarcinoma, and even accelerate liver regeneration in recipients following liver transplantation.

(1) Fausto, 2004 doi.org/10.1002/hep.20214 (2) Taub, 2004 doi.org/10.1038/nrm1489 (3) Higgins et al., 1931. (4) Duncan et al., 2009 doi.org/10.1053/j.gastro.2009.05.044 (5) Fernandez-Salguero et al., 1995 doi.org/10.1126/science.7732381 (6) Ko et al., 2016 doi.org/10.1002/stem.2456 (7) Mulero-Navarro & Fernandez-Salguero, 2016 doi.org/10.3389/fcell.2016.00045 (8) Roman, et al., 2017 doi.org/10.1016/j.pharmthera.2017.12.003

0048-R-P

Full caveolar interactome identifies a stress-driven Cavin-1 nuclear entry pathway governed by Importin-7

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Caveolae are nano-invaginations of the plasma membrane essential for cell mechanoprotection and mechanoadaptation. The caveolar core components -caveolins, cavins, EHD2 and Pacsin2- were identified several years ago; however, whether additional proteins are part of the complex or contribute to its regulation is unknown. Here, we have performed a proximity ligation-based screening to identify the complete caveolar interactome using all 7 core caveolar components as independent baits. We detected previously described interactions among the caveolar core members, validating the approach. Among the new potential interactors for Cavin-1 we have identified several nuclear proteins. We demonstrate that Cavin-1 shuttles to the nucleus in cells and *in vivo*. Cavin-1 nuclear translocation is induced by several types of stress, including mechanical stretching, hypo-osmotic shock and oxidative stress. In addition, Cavin-1 has a cytoplasmic retention signal that maps to its HR1 domain. Furthermore, we have identified Importin-7 (Imp7) as a new binding partner of Cavin-1. We show that Imp7 and Cavin-1 directly bind and Imp7 is sufficient to drive Cavin-1 nuclear translocation *in vitro* and in cells. A constitutive nuclear localized Cavin-1 mutant binds several ribosomal proteins, suggesting a link of this membrane protein with ribosome biology. Indeed, Cavin-1 KO cells exhibit lower levels of the 47S pre-ribosomal RNA compared to reconstituted cells. Our study confirms previously described interactions between the caveolar core components and identifies a mechanism by which cell stress sensed at the plasma membrane is rapidly transduced into the nucleus.

0066-P

COMPREHENSIVE REGULATORY NETWORKS IN HUMAN ENDOTHELIAL CELLS EXPOSED TO ESTRADIOL

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Objective. Estrogens play an important role in the regulation of endothelial function. MicroRNAs (miRNAs) are small non-coding RNA that modulate post-transcriptional expression of their target messenger RNAs (mRNAs). miRNAs, along with transcription factors are the major drivers of gene expression and signaling networks. Thus, the objective of this study was to perform a comprehensive analysis to identify regulatory networks (miRNA-transcription factor-genes) that controls the transcriptomic changes observed in endothelial cells exposed to estradiol.

Design and Method. Differentially expressed ($p<0.05$) miRNAs and mRNA were assembled using our previous microarray data of cultured human umbilical vein endothelial cells (HUVEC) treated with 1 nmol/l E2 for 24 hours (1, 2). miRNA-target pairings and canonical pathways were determined using Ingenuity Pathway Analysis software (Qiagen, 2019 Fall release). Transcription factors were identified using Panther Gene List Analysis (www.pantherdb.org). Enrichr software (maayanlab.cloud/Enrichr/) were used to identify the transcription factors target genes.

Results. Bioinformatic analysis determined specific miRNA-target interactions. As miRNAs negatively regulates mRNAs expression, opposite expression pairing between miRNAs and mRNA levels was implemented. 102 miRNAs were paired with 758 mRNA targets using experimentally observed and highly predicted pairings. Among them, 39 predicted targets were classified as transcription factors, including JUN and STAT3. Data analysis revealed significant canonical pathways important for endothelial function such as Hypoxia, Integrin, Ephrin receptor, and CXCR4 signaling pathways.

Conclusions. This study identifies regulatory networks obtained by integrative microarray analysis and provides additional insights by which estradiol could regulate endothelial function.

Acknowledgments. Supported by Spanish Ministerio de Ciencia, e Innovación (MICINN), Instituto de Salud Carlos III (ISCIII) - FEDER-ERDF (PI16/00229 and PI19/01714), Generalitat Valenciana (AICO 2020/030), and the COST Action (CA17129: CardioRNA). D.P-C. is a “Juan de la Cierva - Incorporación” fellow (grant number IJC2019-040237-I) from MICINN. A.B.P. is a predoctoral researcher (FI18/00323) from ISCIII.

(1) SOBRINO, A.; MATA, M.; LAGUNA-FERNÁNDEZ, A.; NOVELLA, S.; OVIEDO, P.J.; GARCÍA-PÉREZ, M.A.; TARÍN, J.J.; CANO, A. & HERMENEGILDO, C. Estradiol stimulates proangiogenic and metabolic pathways in cultured human endothelial cells. PLoS One. 2009; 4: e8242. (2) VIDAL-GÓMEZ, X.; PÉREZ-CREMADES, D.; MOMPEÓN, A.; DANTAS, A.P.; NOVELLA, S. & HERMENEGILDO, C. miRNA as crucial regulators of gene expression in estradiol-treated human endothelial cells. Cell Physiol. Biochem. 2018; 45: 1878-18

0100-R-P

UPREGULATION OF LYSOSOMAL DEGRADATION PATHWAY IN MYOTONIC DYSTOPHY TYPE 1

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease caused by a nucleotide repeat expansion CTG in the 3'UTR of the DM protein kinase (*DMPK*) gene. It is a multisystemic disorder that affects many organs and tissues; however DM1 is characterized by a progressive weakness and muscle wasting. Autophagy impairment is a risk factor for the progression of muscle wasting. Indeed, it is a degradative process that ensures the clearance of toxic proteins and damaged organelles into lysosomes. Although there were no significant differences in endosomal-lysosomal proteins between DM1 patients and healthy subjects, there was more degradation of epidermal growth factor receptor (EGFR) in DM1. This increase in degradation could be attributed to the activity of lysosomal enzymes such as Cathepsin D (CTSD). Moreover, the enhancement of autophagosome formation, upon rapamycin or bafilomycin treatment, displays that basal autophagy is upregulated in DM1 cells. Therefore, the increase of autophagy-lysosomal pathway may contribute to DM1 progression.

Acknowledgements:

E.A-C is supported by a Grant (IB18048) from Junta de Extremadura, Spain. E.U-C is supported by a FPU predoctoral fellowship FPU16/00684 from Ministerio de Educación, Cultura y Deporte. M.P-B was a recipient of a Fellowship from the Universidad de Extremadura, Spain. M.N-S was funded by "Ramon y Cajal" Program (RYC-2016- 20883) Spain. G.M-C was supported by ONCE Foundation. J.M.F. received research support from the Instituto de Salud Carlos III, CIBERNED (CB06/05/004) and Isabel Gemio Foundation. This work was also partially supported by "Fondo Europeo de Desarrollo Regional" (FEDER) from the European Union.

0121-P

Modulation of adenosinergic system after resveratrol exposure in HeLa and SH-SY5Y cells

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Adenosinergic pathway is a major immunosuppressive mechanism, whose blockade has emerged as a promising approach for cancer therapy. Adenosine is overproduced by the tumor to promote proliferation, invasion, angiogenesis and metastasis. This purine exerts its immunosuppressive effect through the activation of adenosine receptors (A1, A2A, A2B and A3). Resveratrol (3,4',5 trihydroxystilbene) or RSV, a natural phytoalexin present in grapes, peanuts and red wine, is well recognized for its antioxidant, anti-inflamatory and antitumoral properties. Furthermore, this polyphenol has the capacity to target various tumor microenvironment components. Recently, it has been demonstrated that RSV acts as a non-selective adenosine receptor agonist in rat C6 glioma cells, but its molecular mechanisms are still unknown. The aim of the present work was to study the antitumoral effect of RSV and the possible mechanism involving adenosine receptors in two different human cell lines: HeLa epithelioma cervix cells and SH-SY5Y neuroblastoma cells. To this end, cell viability by XTT method, adenosine receptors quantification by Western-blotting, and gene expression by real time PCR were assayed. The possible modulation of purine metabolism has also been analyzed by 5'-Nucleotidase (5'-NT) and adenosine deaminase (ADA) activities assays and high performance liquid chromatography (HPLC). Results herein showed a significant decrease in HeLa and SH-SY5Y cell viability after RSV treatment in a concentration-dependent manner. Accordingly, there was a reduction in the number of treated cells. In addition, RSV caused an increase in A1 and A2A gene expression and a decrease in A2B protein level in HeLa cells. However, these parameters remained unaltered in SH-SY5Y cells. Furthermore, 5'-NT activity in plasma membrane was significantly reduced in both cell lines. Nevertheless, ADA activity was not affected in both cell lines. Finally, intracellular guanosine levels were decreased in HeLa and SH-SY5Y cells, whereas inosine levels were decreased and adenosine levels were increased in SH-SY5Y cells. As RSV is a non-selective adenosine receptors agonist, results suggest a possible involvement of adenosinergic system in RSV antitumoral effects in HeLa and SH-SY5Y cells.

0125-P

Pharmacological concentrations of melatonin diminish the proliferation of pancreatic stellate cells subjected to hypoxia through activation of apoptosis, induction of ER stress and MAPKs modulation.

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Pancreatic stellate cells (PSCs) are major players in the fibrosis that develops in pancreatic cancer (PC). Due to the rapid growth of the tumoral tissue and the aberrant blood vessels formation in the tumor, the consumption of oxygen by cells in the tumor mass leads to a hypoxia condition under which cells included in the tumor mass survive, upon adaptation. Additionally, melatonin has gained attention as an agent with therapeutic potential against PC. The anti-inflammatory and anticancer activity of melatonin have been well documented, but the antifibrotic action of this indolamine is less known. In this study, we have investigated the effect of hypoxia on PSCs proliferation and whether melatonin exerts any modulatory action under these conditions.

Our result show that PSCs subjected to hypoxia increased their proliferation, as evidenced by the rises in cell viability, BrdU incorporation to DNA and Cyclin D detection. ER-stress response was down-regulated by hypoxia. The phosphorylation state of c-Jun NH2-terminal kinase (JNK) was increased by hypoxia, whereas that of p44/42 and p38 was decreased. By contrast, melatonin reduced the viability of PSCs subjected to hypoxia, as well as BrdU content and the expression of Cyclin A and D. Moreover, under these conditions, melatonin induced the activation of caspase 3. Several ER-stress response markers were increased by melatonin treatment. Finally, melatonin evoked increases in the phosphorylation of p44/42 and of p38, whereas that of JNK was decreased.

Under hypoxia, PSCs exhibit an increased proliferative status compared with that noted in normoxic conditions. Treatment with melatonin reduces this proliferative rate involving modulation of cell cycle, MAPKs signalling and activation of apoptosis. Therefore, melatonin could be taken into consideration as potential therapeutic agent for pancreatic fibrosis.

This study was partly funded by Ministerio de Economía y Competitividad (BFU2016-79259-R), Ministerio de Ciencia, Innovación y Universidades (EQC2018-004646-P), Fundación Valhondo Calaff and Junta de Extremaura-FEDER (GR18070).

0126-P

SCF(FBXW7) is a negative regulator of the MRN complex stability

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A major problem in cancer therapies is the appearance of acquired and intrinsic resistance mechanisms. One of the most important mechanisms of resistance to chemotherapy is related to an increased ability to repair DNA damage. This is the basis for the use of repair inhibitors to enhance the therapeutic effects of DNA damaging drugs. In eukaryotic cells, double strand breaks (DSBs) in DNA are repaired by two major mechanisms, the homologous recombination and the classical non-homologous end joining, as well as several secondary pathways. Depending upon the cellular context, the DSBs can either be recognized by the MRE11/RAD50/NBS1 (MRN) or the Ku70/Ku80 complexes. Alteration of stability or function of some of the proteins of the MRN complex has been reported to increase the sensitivity of cells to chemotherapy or radiotherapy.

By analyzing the Flag FBXW7 immunoprecipitation using mass spectrometry, we identified some peptides corresponding to MRE11 not found in control samples from non-immune IgG immunoprecipitation. This finding led us to study the potential role of SCF (FBXW7) in the stability of MRE11 and its effect on tumor cells sensitization to chemotherapy treatments. FBXW7 is a subunit of the SCF (SKP1-CUL1-F-box) ubiquitin ligase responsible for recruiting substrates. FBXW7 is often considered a tumor suppressor protein because it targets several known oncoproteins for degradation by the proteasome or lysosome. Furthermore, *FBXW7* is frequently mutated in human cancers. In this work, we studied the Flag FBXW7 and the MRE11 immunocomplexes, respectively, by Western blot assays and found that FBXW7 associates not only to endogenous MRE11, but also to the whole MRN complex. To know whether these proteins are substrates of SCF (FBXW7), we carried out *in vivo* ubiquitination experiments. We found that all of them are polyubiquitinated by SCF (FBXW7). Next, we used lentivirus to overexpress *FBXW7* in U2OS cell line and discovered that FBXW7 induced degradation of the MRN complex. The physiological implications of this finding will be discussed.

0136-P

New role of β2-chimaerin in the regulation of glucose homeostasis

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β2-chimaerin is a negative regulator (GAP) of the small GTPase Rac1. This GTPase has been recently identified as an important regulator of processes of crucial importance for maintaining glucose homeostasis, such us pancreatic release of insulin and the resulting insulin-stimulated glucose uptake into skeletal muscle and adipose tissue. Rac1 exerts these functions through the control of actin cytoskeletal reorganization. In pancreatic β-cells, Rac1-mediated actin polymerization is necessary for the translocation of the insulin-containing vesicles to the plasma membrane (1). In skeletal muscle and adipocytes, insulin activates Rac1 which induces the redistribution of the glucose transporter GLUT4 from intracellular GLUT4 storage vesicles to the plasma membrane, thus favoring glucose uptake. (2-4).

The regulatory mechanisms that mediate Rac1 activation and function in these tissues are incompletely characterized. The Rac1 activators (GEFs) Tiam1 and Vav2 are involved in glucose-induced Rac1 activation in β-cells and FLJ00068 mediates Rac1 activation by insulin in muscle and adipocytes (5). Among the Rac1 inhibitors, genetic studies support that β2-chimaerin is a key element of proximal insulin signaling *in vivo*, since polymorphisms of the β2-chimaerin gene are associated with the development of diabetes and its complications (6). To corroborate this function, we are studying the role of β2-chimaerin in glucose homeostasis *in vivo* using β2-chimarin-KO mice that we generated in our laboratory (7). We are investigating the effect of knocking down β2-chimaerin by performing intraperitoneal glucose tolerance test (Ip-GTT) and intraperitoneal insulin tolerance test (IP-ITT), in β2-chimaerin KO mice fed with standard diet. β2-chimaerin-KO showed improved glucose clearance after glucose load. These mice also have reduced glucose levels after insulin injection than WT animals. The levels of blood glucose and the body weight in fasting and postprandial conditions did not show statistically significant differences between β2-chimaerin-KO and WT mice. We did not find any significant difference between males and females in all parameters studied. These results clearly show that genetic ablation of β2-chimaerin leads to improved glucose tolerance and increased insulin sensitivity.

1. Asahara, S., Shibutani. y, et al. Diabetologia (2013) 56, 1088-1097 2. JeBailey, L., Rudich, A. et al. (2004) Mol Endocrinol 18, 359-372 3. Sylow, L., Jensen, te. et al. (2013) Diabetes 62, 1865-1875 4. Takenaka, N., Nakao, M., et al. (2019) Int J Mol Sci 20, 5443 5. Takenaka, N., Nakao, et al. (2020) FEBS Letters doi: 10.1002/1873-3468.13939. 6. Suliman, SG., Stanik, J. et al. (2009) Diabetes 58, 2954-2961 7. Casado-Medrano, V., Barrio-Real, L. et al. (2016) Oncotarget 7, 28301-2831

0173-R/M-P

DISCOVERY OF CBGA AND CBGV AS NOVEL MODULATORS FOR CANNABINOID CB1 AND CB2 RECEPTORS

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Cannabis Sativa L. plantis one of the worldwide most consumed psychoactive drugs. It contains multiple phytocannabinoids which interact with our endocannabinoid system (ECS) providing several beneficial effects. Despite its therapeutic potential, the use of cannabis is illegal in the vast majority of countries because it also generates psychoactive effects which represent a barrier to the development of new drugs or treatments for several diseases. Cannabinoids can activate two different cannabinoid receptors: $CB_1$and CB_2. Both receptors are G protein-coupled receptors (GPCR) that couple Gi protein, inhibiting adenylate cyclase (AC) and decreasing cAMP intracellular levels. They can also induce MAP kinases phosphorylation and activate ion channels like voltage-gated calcium channel. It is considered that CB_1R is responsible of the psychoactive effects while CB_2R provides neuroprotection and analgesic effects.

More than 140 phytocannabinoids have been extracted from *Cannabis Sativa L.* However, most of them remain unknown. The research of new phytocannabinoids could open new avenues to discover potential compounds with important therapeutic effects and without the psychoactive side-effect. One of these new compounds is cannabigerol (CBG) which show pharmacological interest but it has low affinity for both cannabinoid receptors. In the present study it has been investigated two CBG derivates: cannabigerolic acid (CBGA) and cannabigerivarine (CBGV). By the analysis of cAMP intracellular levels, MAPK phosphorylation, Dynamic Mass Redistribution (DMR) and b-arrestin recruitment, it has been demonstrated that CBGV is a potent agonist on both CB_1 and CB_2 cannabinoid receptors while CBGA is an interesting antagonist on cannabinoid signaling.

0174-R/M-P

GHSR-1A ANTAGONIST YIL781, POTENTIATES CB2R SIGNALLING IN HIGH FAT DIET MICE

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One of the worldwide most consumed psychoactive drugs is D^9-tetrahydrocannabinol or D^9-THC, the main component of cannabis sativa plant. D^9-THC was isolated in 1964 and its structure and synthesis were described. This molecule binds to endocannabinoid receptors CB_1 and CB_2. Both of these proteins are G protein coupled receptors (GPCR) constituted by seven transmembrane domains. CB_1R is the most abundant receptor in the Central Nervous System (CNS) and responsible of psychoactive effects induced by cannabinoid compounds. While CB_2R is predominantly distributed in cells and tissues of the immune system, including the thymus, tonsils, B lymphocytes and T lymphocytes, even though, it can also be located on the CNS.

D^9-THC consume is characterized by showing euphoria, anxiety and at high doses hallucinations. Nonetheless, it also increases the feeling of appetite. Thus, the main purpose of this project consists in describing a possible interaction between cannabinoid CB_2 and ghrelin GHS-R1a receptors. Ghrelin is a hormone, that acts as a neuropeptide, whose levels in the Central Nervous System respond to food intake and are abnormally regulated in obesity. Cumulative evidence suggests that obesity and anorexia/bulimia are partly due to imbalance in the reward mechanisms, which are also altered by D^9-THC consumption. Interestingly, our reports show that ghrelin GHS-R1a receptor, which is expressed in the basal ganglia and, also, belongs to the GPCR family, colocalize at the plasma membrane level and form heteromeric complexes with cannabinoid CB_2 receptors. In this study, it has been shown a negative crosstalk between CB_2R and GHS-R1a receptors in cAMP accumulation signaling and a partial crossantagonism in calcium release assays in HEK-293T co-transfected cells and in high fat diet mice.

0175-R/M-P

THCA and THCV characterization over cannabinoid receptor 1

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More than 400 compounds can be isolated from a single plant of which the most studied is Δ^9-tetrahydrocannabinol (Δ^9-THC). *Cannabis sativa L.* plant contains other compounds that have structural similarities with Δ^9 -THC. Currently, it is accepted that most of the pharmacological properties of Δ^9-THC engage the activation of the cannabinoid receptor CB1R, which belong to the G-protein-coupled receptor (GPCR) superfamily. To contribute to this field, we have studied the mode of action of tetrahydrocannabinolic acid (THCA) and tetrahydrocannabivarin (THCV) in this receptor of the endocannabinoid system. THCA is a precursor of Δ^9-THC, while THCV is an homologue. Pure THCA and THCV from *Cannabis sativa L.* are differentially acting on CB_1R. To determine the affinity of phytocannabinoids for cannabinoid receptors we perform a homogenous time resolved fluorescence resonance energy transfer (HTRF) assay. In order to functionally evaluated the effects promoted by these compounds when interacting with this cannabinoid receptor, we carried out four different functional outputs: determination of cAMP levels, of extracellular-signal-related-kinase phosphorylation, of label-free dynamic mass redistribution (DMR) and of ß-arrestin recruitment. The results obtained show that the affinity of THCV is like the affinity of Δ^9-THC, while THCA have less affinity. Δ^9-THC is the only compound that can activate the CB_1R canonical pathway. Although the three compounds can activate the MAPK pathway. Interestingly, THCV acts as an antagonism in the presence of the selective agonist ACEA.

0181-R-P

Role of mammalian PKCδ isoform in the DNA damage response

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The protein kinase C (PKC) family plays important regulatory roles in numerous cellular processes. *Saccharomyces cerevisiae* contains a single PKC, Pkc1, whose main function is cell wall integrity maintenance. In mammals, the PKC family contains 9 isoforms, classified in classical, novel and atypical. We have described that Pkc1 and the novel isoform PKCδ, and at a much less extent its closes relative isoform PKCθ, control DNA integrity checkpoint activation, indicating that this mechanism is conserved from yeast to humans. In this work, we combined studies from two different model organisms in order to characterize the role of PKCδ in the DNA damage response.

First, we used yeast to study, in absence of other mammalian PKC isoforms, PKCδ specific requirements compared to PKCθ for its function in the DNA integrity checkpoint. We obtained truncated versions and point mutants in key activating residues that reveal differences between PKCδ and PKCθ in their activation mechanisms. Also, we show that the catalytic fragment of PKCδ, but not that of PKCθ, is sufficient to activate the checkpoint effector kinase Rad53.

In parallel, we studied the functional relevance of PKCδ in mammalian cells. Our previous results show that down-regulation of PKCδ activity in HeLa cells caused a defective activation of the DNA damage checkpoint. To further explore the function of PKCδ in a non-tumor cell line, we obtained PKCδ knocked-out mouse stem cells using CRISPR-Cas9 technology. Results from these studies suggest that activation of the effector kinase CHK1 is also reduced in the absence of PKCδ. Our results support the important role of PKCδ as a player in the DNA integrity checkpoint pathway and, in addition, highlight the benefits of combining distinct research models.

Soriano-Carot M, Quilis I, Bañó MC and Igual JC. Protein kinase C controls activation of the DNA integrity checkpoint. 2014. Nucleic Acids Research. 42(11):7084-95. doi:10.1093/nar/gku373.

0190-P

The MAPKAP kinase Srk1 regulates Lsk1 kinase, which control phosphorylation of RNA pol II-CTD domain in response to perturbation of the actomyosin ring in Schizosaccaromyces pombe.

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In *Schizosaccharomyces pombe*, cytokinesis is mediated through the assembly and constriction of a contractile, actomyosin ring[1]. Minor perturbation of the actomyosin ring triggers a checkpoint mechanism that has the capacity to both delay G2/M progression and stabilize the actomyosin ring[1].

Critical components of the monitoring system include the septation initiation network (SIN), the Cdc14 family phosphatases Clp1 and the Lsk1 kinase (which phosphorylates the carboxy-terminal domain (CTD) of the RNA pol II) [2,3]. The cytokinesis checkpoint is activated in mutants affecting several components of the actomyosin ring as well as by treatment with drugs such as latrunculin B (LatB), which disrupt the rate of actin polymerization.

The checkpoint G2 delay is associated to Clp1-dependent activation of Wee1 and destabilization of Cdc25[2]. Moreover, the molecular mechanism leading to actomyosin ring maintenance is associated to the activity of Lsk1 kinase[3].

Our studies demonstrate that the MAPKAP kinase Srk1[4,5] is hypersensitive to LatB at concentrations, which do not affect the viability of wild-type cells. Srk1 is activated by the MAPK Sty1 in LatB conditions. Moreover, Srk1 is necessary to maintain actomyosin ring stability and the achievement of cytokinesis, as shown by the deletion of *srk1* in response to LatB.

Furthermore, in a screening to identify Srk1-binding proteins we have found the Lsk1 kinase. We have confirm the binding between Srk1 and Lsk1, and the phosphorylation of the N-terminal domain of Lsk1 by Srk1. Ongoing studies are designed to uncover whether Srk1 is a cytokinesis checkpoint component acting through Lsk1 kinase.

1. Balasubramanian MK, Bi E, Glotzer M (2004) Curr Biol 14:R806–818 2. Karagiannis J, Bimbo A, Rajagopalan S, Liu J, Balasubramanian MK (2005) Mol Biol Cell 16: 358–371. 3. Krapp A, Simanis V (2008) Biochem Soc Trans 36: 411–415 4. López-Avilés S, Grande M, González M, Helgesen AL, Alemany V, Sanchez-Piris M, Bachs O, Millar JB, Aligue R. (2005)Mol Cell.17:49-59 5. López-Avilés S, Lambea E, Moldón A, Grande M, Fajardo A, Rodríguez-Gabriel MA, Hidalgo E, Aligue R. (2008) Mol Biol

0191-R/M-P

Metabolic rewiring by increased mitochondrial respiration drives immune evasion in liver cancer

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Recent evidence supporting the need of a mitochondria-based metabolism for tumor growth (1) prompted us to study the role of MCJ, an endogenous negative regulator of mitochondrial complex I (2), in the context of hepatocellular carcinoma (HCC). This work aims to study the metabolic reprogramming of cancer cells at different stages, to prove increased malignancy in mitochondria-based tumors, and to analyze the differential immune response driven by metabolic changes.

Wt and *Mcj*$^{-/-}$ mice were injected intraperitoneally with 25 mg/kg body weight of diethylnitrosamine (DENA) at 14 days of age and they were monthly monitored, tumor growth was followed by ultrasound analysis and blood samples were extracted. DENA-treated mice, and the corresponding controls, were sacrificed at 5, 8, and 12 months post injection. Mortality, tumor number and size, liver metabolism, mitochondrial activity and tumor infiltrating immune cell analysis were then assessed.

The initial *in silico* approach using UALCAN revealed reduced *Mcj* expression in stage IV HCC patients. *In vivo*, lack of MCJ increased both the presence of liver tumors and mortality rate after DENA treatment. A highly oxidative phenotype was confirmed in *Mcj*$^{-/-}$tumors, as mitochondrial respiration was significantly higher, along with elevated intracellular ATP, NAD$^+$ and NADPH levels. We then studied tumor infiltrating immune cells, observing a reduction in effector CD4$^+$ T lymphocytes (CD44+ CD62L-) and neutrophils (GR1$^+$CD11b$^+$) in *Mcj*$^{-/-}$ mice 5 months after DENA injection; similar results were also visible at 12 months, including significantly reduced PD-1. Analysis of PDL-1 revealed significantly increased hepatic protein levels in *Mcj*$^{-/-}$ mice, hinting a possible immune evasion. Serum analysis of cytokines highlighted reduced levels of inflammatory IFN-g and TNF in *Mcj*$^{-/-}$ mice. Besides, highly ROS producing neutrophils were found in DENA-treated Wt mice.

Reduced MCJ levels, also visible in HCC patients, increase oxidative respiration and drive a metabolic reprogramming that enables immune evasion, through PD-1/PDL-1 axis. As elevated NAD$^+$ and PDL-1 are known to increase sensitivity to anti-PDL-1 treatment (3), tumor MCJ expression may serve as a predictive biomarker for HCC immunotherapy efficacy.

1. Cassim, S. & Pouyssegur, J. Tumor microenvironment: A metabolic player that shapes the immune response. Int. J. Mol. Sci. 21, (2020). 2. Hatle, K. M. et al. MCJ/DnaJC15, an Endogenous Mitochondrial Repressor of the Respiratory Chain That Controls Metabolic Alterations. 33, 2302–2314 (2013). 3. Lv, H. et al. NAD+ Metabolism Maintains Inducible PD-L1 Expression to Drive Tumor Immune Evasion. Cell Metab. 33, 110-127.e5 (2021).

0211-R/M-P

Role of the spermidine-hypusine-eIF5A axis in cancer progression

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Polyamines are essential metabolites required for eukaryotic cell growth, and their metabolism is frequently deregulated in cancer. One key target of the polyamine spermidine is the translation factor eIF5A, an essential protein highly conserved. eIF5A is the only known protein that undergoes a posttranslational modification, required for its activity in the ribosome, named 'hypusination'. This modification generates the hypusine residue, which is derived from the transfer of the aminobutyl moiety of the spermidine to a conserved lysine residue by the action of the enzyme deoxyhypusine synthase (DHS) and subsequently by deoxyhypusine hydroxylase (DOHH). Human cells contain two genes encoding eIF5A, namely eIF5A1 and eIF5A2. eIF5A2 is located on the genomic locus 3q26, a region frequently amplified in many tumors, including non-small cell lung cancer (NSCLC). eIF5A2 overexpression is associated with bad prognosis in several cancers. The highly selective modification by hypusination of eIF5A is susceptible of pharmacological inhibition, which makes this pathway a very attractive therapeutic target.

Our research project aims at the characterization of the pathogenic role of eIF5A2 and the spermidine-hypusine pathway in NSCLC. Here we show that eIF5A2 plays a role in the actin cytoskeleton, cell proliferation, cell migration and invasion in lung cancer cells. We also show that the expression of eIF5A2 and its hypusinated form is induced by TGFβ1 signalling, and the overexpression of eIF5A2 induces the expression of proteins involved in epithelial-mesenchymal transition (EMT) such as Fibronectin and SNAI1, in a TGFβ1-dependent manner. Moreover, the hypusination inhibitor GC7 reduces eIF5A2 activity in cells treated with TGFβ1. This result suggests that eIF5A2 is a mediator of TGFβ1 signaling and therefore could promote EMT, invasion and metastasis in NSCLC. Modulation of the enzymatic activities by which EIF5A2 stability and function are controlled in the context of EMT will reveal candidate therapeutic targets for the prevention of metastasis of early-stage NSCLC cancers. Downstream effectors of the EIF5A2 protein may also prove to be potential therapeutic targets, and will provide insight into the cellular mechanisms underlying EMT that may be translatable to a broad range of solid tumors.

0249-P

New role for nuclear CAPN2 in breast cancer cells through LIMK-1 cleavage

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Calpain-2 (CAPN2) is a calcium-dependent protease ubiquitously expressed in mammalian tissues. The enzyme produces specific cleaves on its substrates, modifying them and altering their original distribution, interactions with other proteins or its regulation. In this sense, CAPN2 plays a role in several signaling pathways, and its functions will be determined by its subcellular localization. However, CAPN2 functions in the cell nucleus are not completely understood. Identifying the components of CAPN2-mediated interactions in the cell nuclei will provide a better understanding of CAPN2 functions in this compartment. Triple-negative breast cancer cells had high levels of nuclear CAPN2, specifically showing a nucleolar localization at interphase. A proteomic approach was used to elucidate the CAPN2-dependent components of nucleolar proteome in MDA-MB-231 cells. Among the different proteins identified we focused on the actin-severing protein cofilin-1 (CFL1). CAPN2 promotes the phosphorylation and subcellular distribution of CFL1 by direct cleavage and activation of LIM Kinase-1 (LIMK1). Upon CAPN2 knockdown, CFL1/LIMK1 binding was inhibited; LIMK1 accumulated at the cell periphery and perinucleolar region and, the CFL1 phosphorylation and localization was altered during cell division, leading to aberrant mitosis and cell multinucleation. These findings show a mechanism for the role of CAPN2 during mitosis, and, identifying LIMK1 as a new CAPN2-target, provides a novel mechanism for LIMK1 activation. CFL-1 is crucial for cytoskeleton remodeling, but also for the maintenance of nuclear structure, chromosomes alignment during mitosis and the modulation of transcription often altered in cancer cells. Therefore, the role of CAPN2 in the nuclear compartment might be extended to other actin-associated biological and pathological processes.

This work was supported by the Consellería de Educación [grant number GVPROMETEO 2018-167 (to JRV)]

Sorimachi, H. et al. J. Biol. Chem. 393, 853-871 (2012). Ono, Y. & Sorimachi, H. Biochem. Biophys. Acta.1824, 224-236 (2012). Telechea-Fernández, M. et al. Oncotarget. 9, 9100-9113(2018). Raynaud, F. et al. Int. J. Dev. Biol. 52, 383-388 (2008). Amano, T. et al.J. Biol. Chem. 277, 22093-220102 (2002). Mizuno, K. Cell Signal. 25, 457-469 (2013).

0258-R-P

Demonstration of a physical interaction between adenylate cyclase and adenosine A2A-dopamine D2 heteromer

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protein-coupled receptors (GPCRs), G proteins and adenylyl cyclase (AC) comprise one of the most studied transmembrane cell signaling pathways. However, it is unknown whether the ligand-dependent interactions between these signaling molecules are based on random collisions or the rearrangement of pre-coupled elements in a macromolecular complex. Furthermore, it remains controversial whether a GPCR homodimer coupled to a single heterotrimeric G protein constitutes a common functional unit. Using a peptide-based approach, we here report evidence for the existence of functional pre-coupled complexes of heteromers of adenosine A2A receptor and dopamine D2 receptor homodimers coupled to their cognate Gs and Gi proteins and to subtype 5 AC. We also demonstrate that this macromolecular complex provides the necessary frame for the canonical Gs-Gi interactions at the AC level, sustaining the ability of a Gi-coupled GPCR to counteract AC activation mediated by a Gs-coupled GPCR.

0280-R/M-P

Oleanolic acid as a potential molecule to treat wounds: molecular effects and its complexation with modified cyclodextrins

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Oleanolic acid (OA), a plant produced triterpenoid molecule, shows numerous benefits over wound healing, a tightly regulated process which is crucial for skin integrity. Over this, epithelial cell migration is fundamental for wound healing and oleanolic acid have shown a promoting effect on it. Using epithelial cell models to study OA molecular mechanisms, we have recently showed that OA enhances migration linked to epidermal growth factor receptor (EGFR), MAP kinases and c-Jun stimulation. These proteins constitute a pathway which is activated during OA-enhanced treatment, leading to a migratory gene expression profile. Going deeper into this, using a specific EGFR inhibitor (PD153035), we have shown that OA stimulation of c-Jun is independent on this receptor. Moreover, using a detailed time course, we observed a time-uncoupling between c-Jun and EGFR activation. On the other hand, due to its lipophilic nature, OA delivery to epithelial cells should be improved. To overcome this, we complexed OA with modified cyclodextrins as a way to improve its solubility and bioavailability. The drying of the OA/cyclodextrin complexes during their collecting was done by lyophilization or novel technology Spray-drying, and both processes gave products with a similar biological activity on wound healing scratch assays. Spray-drying was preferred for its faster time-processing. Additionally, angiogenesis is a process relevant for wound healing to allow tissue restoration. Thus, we tested OA on endothelial cell line HUVEC. We found that OA is able to improve HUVECs motility and tube network formation. All together, these results encourage us to pursue on OA/cyclodextrins as an alternative potential natural agent to apply on wounds to improve healing.

1. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen Off Publ Wound Heal Soc Eur Tissue Repair Soc. octubre de 2008;16(5):585-601. 2. Bernabé-García Á, Armero-Barranco D, Liarte S, Ruzafa-Martínez M, Ramos-Morcillo AJ, Nicolás FJ. Oleanolic acid induces migration in Mv1Lu and MDA-MB-231 epithelial cells involving EGF receptor and MAP kinases activation. PLOS ONE. 23 de febrero de 2017;12(2): e0172574.

0289-R/M-P

SOS1/2 ablation triggers alterations of adipose tissue in mice

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Prior studies showed that deletion of both SOS1 and SOS2 mammalian RAS-GEFs in mouse embryonic fibroblasts (MEFs) leads to defect in cellular proliferation, migration and oxidative stress. In vivo studies showed that loss of both SOS1 and SOS2 in mice leads to dramatic weight loss, reduction of fat tissues and sudden death. To analyse the role of SOS RAS-GEFs in adipose tissue metabolism, tamoxifen-inducible SOS1 KO, constitutive SOS2 KO and SOS1/2 DKO mouse strains were used. A phenotypic switch in inguinal white adipose tissues (iWAT) from energy-storing white adipocytes to thermogenic beige adipocytes was observed in single and double mutants of SOS GEFs. Compared with WT, hematoxylin and eosin (HE) staining of iWAT showed more multilocular adipocytes in SOS1 KO and SOS2 KO mice and a complete loss of fat depots (lipid droplets) in SOS1/2 DKO mice. Coupled with those results, immunofluorescence staining showed upregulation of Uncoupling Protein 1 (UCP1) and Adipose Triglyceride Lipase (ATGL), as well as more mitochondria-rich adipocytes in the iWAT of SOSless mice. Moreover, deletion of both SOS1 and SOS2 induced a significant loss of several types of white adipose tissues (iWAT, gWAT, vsWAT) and a dramatic decrease in body temperature. Furthermore, apparent changes in the histological structure of the brown adipose tissue (BAT) were observed only in SOS1/2 DKO mice. mRNA expression of thermogenesis genes including UCP1, Elvovl3 and CIDEA was significantly upregulated in the BAT of SOS1 KO mice. Our data indicate that SOS GEFs may have a relevant role in thermogenesis and adipose tissue homeostasis.

Liceras-Boillos P, et al.Sos1 disruption impairs cellular proliferation and viability through an increase in mitochondrial oxidative stress in primary MEFs. Oncogene 2016;35(50):6389-6402. Baltanás FC, et al. Functional redundancy of Sos1 and Sos2 for lymphopoiesis and organismal homeostasis and survival. Mol Cell Biol. 2013;33(22):4562-78.

0305-R/M-P

BMP9 promotes an epithelial phenotype and a hepatocyte-like gene expression profile in adult hepatic progenitor cells

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Adult hepatic progenitor cells, known as oval cells (OC) in rodents, have a key role in chronic liver diseases (CLDs). Their expansion and bi-potential capacity to differentiate into cholangiocytes or hepatocytes in the damaged liver contributes to fight against cell loss and liver functional impairment and to regenerate the liver. Bone Morphogenetic Protein 9 (BMP9), a member of the TGF-b superfamily, has emerged as a new player in CLDs. In fact, its increased levels in fibrotic liver act promoting fibrogenesis and it has also proved to be an important regulator of oval cell biology in vivo and in vitro. Here we analyze the effect of the chronic exposure of oval cells in culture to BMP9. Our results indicate that B9T-OC (BMP9-treated OC) acquire proliferative and survival advantages. In addition, we show that B9T-OC display a more epithelial phenotype and move further in their differentiation process toward a mature hepatocyte as evidenced by gene expression profile of hepatocytic cell markers and functional approaches. Since our previous studies had brought to light a functional crosstalk between BMP9 and the HGF/c-Met signaling pathways operating in OC to regulate cell survival, we analyzed the possible role of HGF/c-Met in BMP9-induced long term effects in OC. Using OC lines harboring an inactive Met tyrosine kinase (Met-/- OC) we show that an active c-Met signaling is necessary to obtain maximum effects of long-term BMP9 treatment in terms of hepatocytic differentiation potential, further evidencing a functional cooperation between these two pathways. In conclusion, our work suggests a role for BMP9 as a differentiation factor and promoter of epithelial phenotype in OC, providing additional mechanisms contributing to increase OC regenerative potential that could be therapeutically modulated in CLD.

0306-P

Estudio de la interacción de PSTPIP1 con LYP, WASP y pirina mediante BIFC.

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Mutaciones en la proteína PSTPIP1 causan enfermedades autoinflamatorias como el síndrome PAPA. El estudio de su interacción con otras proteínas involucradas en trastornos hereditarios del sistema inmunitario, tales como LYP (artritis), WASP (síndrome de Wiskott-Aldrich) o pirina (fiebre mediterránea familiar), así como su localización *in vivo*, puede ayudar a conocer como las mutaciones de PSTPIP1 causan dichas enfermedades autoinflamatorias.

Para analizar la interacción de PSTPIP1 con estas proteínas hemos utilizado la técnica BiFC (*bimolecular fluorescence complementation)*. Esta técnica permite, frente a otras técnicas utilizadas hasta el momento, detectar interacciones directas, así como observar la localización de dichas interacciones *in vivo*. De esta manera, encontramos que PSTPIP1 interacciona consigo misma y con las proteínas LYP, WASP y pirina de forma directa y que dichas interacciones tienen lugar en la membrana plasmática.

0317-P

ALTERATIONS IN THE COMPONENTS OF THE WNT/ β-CATENIN PATHWAY IN HUMAN CIRCULATING LYMPHOCYTES PRODUCED BY HIGH CONCENTRATIONS OF CHOLESTEROL

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The existence of molecular cues that facilitate the development of colorectal cancer (CRC) in the population with type 2 diabetes (T2D) is supported by substantial epidemiological evidence. A high percentage of these tumors exhibit increased Wnt/β-Catenin signalling through LRP/Fzd receptors. Our group has shown that high glucose levels in diabetic patients enhances β-Catenin nuclear accumulation in cancer cells, this being an indicator of poor prognosis. Moreover, recent studies suggest that Wnt signaling performs an essential function in immune cell modulation and counteracts various disorders. Nonetheless, the emerging role and mechanism of action of this signaling cascade in immune cell regulation, as well as its involvement in various cancers, remain debatable. Our objective is to evaluate if high circulating levels of glucose and/or cholesterol alter Wnt/β-Catenin signalling in peripheric lymphocytes.

Lymphocytes of healthy individuals, diabetic individuals and CRC patients were analyzed for this study. Levels of plasma HbA1c1 and cholesterol were measured. We analyzed the main receptor for Wnt proteins, LRP6 by measuring changes in gene expression (qPCR), protein levels (WB), and exhibition (flow cytometry). The influence of antidiabetics and statins are also analyzed.

Diabetic patients show alterations in LRP6 receptor exhibition in lymphocytes that cannot be related to HbA1c1 or cholesterol levels. However, patients with CRC showed changes in lrp6 gene expression, protein levels and exhibition that correlate with cholesterol levels. Thus, high cholesterol in CRC patients correlated with high levels and exhibition of LRP6 in circulating lymphocytes. Future research will elucidate if this alteration mediates or alters Wnt / β-Cat signalling in the lymphocytes of these individuals and how this interfers with CRC progression.

García-Jiménez, C et al.: A new link between diabetes and cancer enhanced Wnt/β catenin signaling by high glucose. J. Mol Endocrinol. 52(1): R51-66, 2013 Chocarro-Calvo A. et al.: Glucose-induced β-catenin acetylation enhances Wnt signaling in cancer. Mol Cell. 49(3): 474-86, 2013. Borrell-Pages M et al.: LRP5 and plasma cholesterol levels modulate the canonical Wnt pathway in peripheral blood leukocytes. Immunol Cell Biol. 93(7):653-61 (2015 Aug;93(7):653-61)

0333-P

A new axis: ROS/AMPK/EP300/β-catenin drives glucose-mediated colorectal cancer cell proliferation

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Diabetic patients have an increased risk of suffering certain types of cancer, including colorectal cancer (CRC). AMP activated protein kinase (AMPK) is a critical metabolic adaptator, and we have shown a 'non-canonical' activation of AMPK in gastrointestinal (GI) cancer cells dependent of reactive oxygen species (ROS) derived from glucose metabolism. Our lab has shown that hyperglycemia (HG) amplifies Wnt signalling in GI cancer cells through EP300-mediated acetylation of β-catenin, a key driver of tumour evolution in CRC. AMPK controls EP300 stability and substrate choice through phosphorylation and we hypothesize the existence of an axis activated by HG that leads to increased proliferation and tumorigenesis in GI cancer cells.

Methodology: Human GI cancer cells exposed to HG and treated with different inhibitors (CoenzymeQ10 to inhibit ROS; Compound C to inhibit AMPK or C646 to inhibit EP300) to analyse cell proliferation and cell cycle. 95 stage II human colorectal cancer and healthy mucosa sections were examined by immunohistochemistry.

Results: As expected, HG accelerated cell cycle progression, to increase the proliferation rate of GI cancer cells. Blockade at each level of the axis ROS/AMPK/EP300/β-catenin abolished the effects of HG on GI cancer cells proliferation, highlighting its importance mediating the proliferative effects of HG. The pro-proliferative effects of the axis were validated in vivo (mice) and in an array of human colorectal cancer and healthy mucosa sections by immunohistochemistry. A positive and significant correlation was found at each level of the axis: between ROS and pAMPK (T172) levels, between pAMPK (T172) and pEP300 (S89) and between pEP300 (S89) and β-catenin in human tumour sections.

Conclusions: The new ROS/AMPK/EP300/β-catenin axis ensures enhanced GI cancer cell proliferation following increased nutrient availability (HG). Since AMPK is a critical therapeutic target in diabetes and cancer, our results may have significant implications for the selection of AMPK activators or inhibitors for different cancer stages or cancers according to their metabolism.

0350-R-P

Negative effects of the herbicide Round up® Ultra Plus in motility and PKA and GSK3 pathways in mammalian spermatozoa.

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The worldwide use of glyphosate-based herbicides as Roundup® (RUP) has become a matter of concern due to its potential toxic effects for human and animal health caused by serious environment contamination. This work aims to investigate the impact of in vitro exposure of boar spermatozoa to the herbicide RUP and its active ingredient glyphosate (GLY) at concentrations 100 times lower than those recommended for agricultural use. We have used pig spermatozoa as a validated model to investigate cell toxicity and also as a well-demonstrated model in human sperm studies. Semen samples from boars were pooled to minimize individual variations and incubated in a non-capacitating medium (1h, 38.5 °C, 5% CO2) with several RUP dilutions (0.0025%, 0.005%, 0.01%) or equivalent concentrations of GLY (41, 82 and 164 µM). None of the RUP dilutions studied have a significant effect on sperm viability but cause a clear concentration-dependent reduction in the percentages of motile spermatozoa that is statistically significant at 0.01% RUP dilution, where only 35% of spermatozoa remain motile. RUP (0.01%) also causes a significant increase in plasma membrane lipid disorganization. Nevertheless, none of GLY concentrations has a significant effect on sperm motility, viability or plasma membrane disorganization. In order to know effects on signaling pathways that control sperm motility, PKA substrates and GSK3α/β phosphorylation were studied. Sperm treatment with the highest RUP concentration reduces GSK3α/β phosphorylation and two specific PKA substrates. However, sperm incubation with equivalent concentrations of GLY has not any effect in phosphorylation. To conclude, concentrations of herbicide present as environment contaminants have adverse effects on sperm motility without affecting sperm viability. This adverse RUP effect could be likely due to a detrimental effect in the plasma membrane integrity and to inhibition of phosphorylation of both, GSK3α/β and specific PKA substrates. The negative herbicide effects cannot be attributed to its active ingredient GLY.

Anifandis G, Amiridis G, Dafopoulos K, Daponte A, Dovolou E, Gavriil E, et al. The In Vitro Impact of the Herbicide Roundup on Human Sperm Motility and Sperm Mitochondria. Toxics. 2017;6. Nerozzi C, Recuero S, Galeati G, Bucci D, Spinaci M, Yeste M. Effects of Roundup and its main component, glyphosate, upon mammalian sperm function and survival. Sci Rep. 2020;10:11026.

0355-R/M-P

Development of new three dimensional models to study tumour environment role in melanoma progression

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Cellular heterogeneity is currently the biggest obstacle to cancer healing. Despite the evolution of available antitumour therapies frequently appear resistance and recurrence, a reflection of their ineffectiveness in combating all tumour cell phenotypes [1]. Melanoma cells interact bidirectionally with the tumour environment, which is actively involved in tumour progression and represents an attractive therapeutic target [2], [3]. Our data indicate that human adipose tissue, present in the stroma of most tumours, induces melanoma primary cell invasion in 2D systems. It is essential to develop new in vitro three-dimensional (3D) models that recreate more accurately tumoral in vivo structure, cell-cell and cell extracellular matrix (ECM) interactions, to study the role of the tumor environment in tumor progression [4], [5]. This work focus on the development of new in vitro melanoma 3D.

Methods: Primary and metastatic melanoma cells and human preadipocytes were used to generate 3D multicellular spheroid. Standard cell biology techniques were used to characterize spheroids.

Results: We have developed a new scaffold free method to generate multicellular human melanoma/adipocytes spheroids. This method allows the fully differentiation and induction of adipocyte differentiation in vitro, resulting in larger lipid droplets. In addition, we have fine-tuned the optimal culture conditions to avoid adipocyte dedifferentiation, one of the main problems of adipocyte tissue in vitro culture.

Conclusion: Our multicellular spheroid model offers an improved culture system to study cellular and molecular mechanisms implicated in the adipose tissue contribution to melanoma metastatic progression.

[1] R. Somasundaram. 2012. Advances in Pharmacology (65): 335–359. [2] J. M. Brandner. 2013 Pathology (45): 443–452. [3] P. Friedl. 2012 Cell (147): 992–1009. [4] K. Beaumont. 2013 Healthcare (2): 27–46. [5] A. Marconi. 2018 Exp. Dermatol (27): 578–586.

0374-R/M-P

Tumour microenvironment induced phenotype-switch in melanoma cells via β-catenin signaling

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Tumour progression obeys to the interactions of the cancer cell genome with external factors from the tumor micro- and macro-enviroment (TME) that provides driving forces

for dynamic adaptations leading to net cancer cell proliferation and invasion (1, 2), even under therapeutic pressure providing drug resistance (3, 4). The TME provides for example WNT proteins, growth factors that promote proliferation and invasion of tumour cells and represents an attractive therapeutic objective. Despite the fact that obesity correlates with larger melanoma tumours and increased metastases in mice models (5-8), few works have studied the role of adipose tissue, abundant in the stroma of most tumours, in melanoma progression.

This work aims to study the role that adipose tissue plays in the induction of phenotypic changes in melanoma cells, focusing on the molecular mechanism.

METHODS: Primary and metastatic human melanoma cells and human adipose tissue explants were co-cultured. Standard cell biology techniques were used to identify the molecular mechanism and the biological consequences of lipid uptake in melanoma cells.

RESULTS: Adipose tissue induces invasion of primary but not metastatic melanoma cells. Lipids are transferred from human adipose tissue explants to primary melanoma cells in the co-culture. Lipid uptake regulates post-translational modifications that alter Wnt signalling and mediate melanoma invasion induced by lipids. We have identified a subset of fatty acids that recapitulate these effects and induce melanoma invasion.

CONCLUSION: Our results indicate that adipocytes are an exogenous source of lipids with the capacity to drive phenotype-switch in melanoma cells via Wnt signaling. Future work will elucidate the molecular markers and the therapeutic consequences of lipid transfer from adipocyte to melanoma cells.

1. Bowman RL, et al. Cell Rep. 2016; 17(9):2445–59. 2. Tammela T, et al. Nature. 2017; 545(7654):355–59. 3. Brandner JM, Haass NK. Pathology. 2013; 45(5):443–52. 4. Bissell MJ, Radisky D. Nat Rev Cancer. 2001; 1(1):46–54. 5. Zhang M, et al. Cancer Discov. 2018; 8(8):1006–25. 6. Jung JI, et al. Int J Cancer. 2015; 136(2):258. 7. Yang H, et al. Oncotarget. 2016; 7(13):16227–47. 8. Mori A, et al. Int J Cancer. 2006; 119(12):2760–7.

0375-P

Control of directional polymerization of microtubules to fine-tune metabolism of human CD4 T cells by CCT cytosolic chaperonin

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T lymphocyte activation requires the formation of immune synapses (IS) with antigen-presenting cells (APC). This cell-cell communication structure depends on the dynamics of membrane receptors, signaling scaffolds, microfilaments, and microtubules. We have observed that the centrioles re-orientate inside the centrosome through cryocorrelative microscopy by using soft-X-Rays. Resonant scanning-based confocal analysis of CD4 cells forming IS showed that this arrangement of centrioles allows polarized polymerization of microtubules towards the IS. The conformation of the IS fine-tunes the potency of T cell activation and subsequent immune response. The cytosolic chaperonin CCT (chaperonin-containing TCP1) is in charge of folding protein partners coming from new synthesis. CCT regulates the changes in the reciprocal orientation of the centrioles and polarization of the tubulin dynamics induced by T cell receptor in T lymphocytes forming an IS. CCT also controls the mitochondrial ultrastructure and the metabolic status of T cells, regulating the 'de novo' synthesis of tubulin. These changes ultimately determine the function and organization of the mitochondria, as shown by analysis of mitochondria respiration and three-dimensional reconstruction of resting and stimulated primary T cells using cryo-soft x-ray tomography and STED microscopy. Through this mechanism, CCT governs T cell activation and polarity.

Martin-Cofreces et al.2020 The chaperonin CCT controls T cell receptor-driven 3D configuration of centrioles. Sci Adv DOI: 10.1126/sciadv.abb7242. Cuellar et al.2019 Structural and functional analysis of the role of the chaperonin CCT in mTOR complex assembly. Nat Commun DOI: 10.1038/s41467-019-10781-1. Wolf et al.2020 Dynamics in protein translation sustaining T cell preparedness. Nat Immunol 21, 927-937.

0386-R-P

Deciphering the role of Fusarium fujikuroi cwhA, the ortholog of Saccharomyces cerevisiae CWH43.

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Fusarium fujikuroi is a filamentous fungus that synthesizes a wide variety of secondary metabolites, which include carotenoids. The synthesis of these pigments is downregulated by the protein CarS that has two RING finger domains, characteristic of proteins with E3 ubiquitin ligase activity. CarS also has a LON domain that could allow the interaction with its possible target proteins. A previous yeast-two hybrid assay showed that CarS could interact with different proteins. One of them is CwhA, orthologous to Cwh43p of *Saccharomyces cerevisiae*. Cwh43p is a conserved transmembrane protein that is involved in lipid homeostasis and in remodeling of GPI lipids to ceramide in glycosylphosphatidylinositol (GPI)-anchored proteins (1).

Previous transcriptomic analysis showed higher levels of *cwhA* in a *carS* mutant, either in the dark or after 1 h light pulse (2), suggesting that CarS could control transcription of *cwhA*. The purpose of this work is to understand the role of CwhA in *F. fujikuroi* and the possible regulatory connection between CarS and CwhA. To investigate CwhA function, we generated *cwhA* deletion strains in the wild type and in a *carS* mutant. Growth studies in minimal and nitrogen starvation media showed a retarded colony-forming ability of Δ*carS cwhA* mutant due to lower levels of conidia germination of this strain. Moreover, the conidia of the Δ*cwhA* mutant also exhibited a lower germination rate in nitrogen starvation, but a lower colony forming ability was not detected in this case, suggesting a more severe Δ*cwhA* mutant phenotype in the absence of CarS. Once the colonies were formed, no differences were appreciated on solid or in liquid cultures between wild and mutant strains, indicating that phenotypic effects have been only found at the germination level. In a similar way to that observed with the *S. cerevisiae* mutant Δ*CWH43*, the *cwhA* deletion did not affect the sensitivity to Calcofluor white. To check a possible role of CarS protein on CwhA function, ubiquitination assays are in progress to determine if CarS is an E3 ubiquin ligase and if CwhA is one of its target proteins. Moreover, lipids will be analyzed to investigate a possible role of CwhA on the lipid metabolism of *F. fujikuroi*.

(1) Ghugtyal, V., Vionnet, C., Roubaty, C. and Conzelmann, A. (2007). CWH43 is required for the introduction of ceramides into GPI anchors in Saccharomyces cerevisiae. Mol. Microbiol. 65, 1493-1502. (2) Ruger-Herreros, M., Parra-Rivero, O., Pardo-Medina, J. et al. (2019) Comparative transcriptomic analysis unveils interactions between the regulatory CarS protein and light response in Fusarium. BMC Genomics 20, 67

0397-P

The phosphatase DUSP1 impairs cell migration and invasion in prostate cancer cells through the downregulation of Snail and the inactivation of JNK and ERK signaling pathways

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Prostate cancer (PC) is the second leading cause of cancer-related deaths among males. Most of these deaths are associated with metastasis, in which the epithelial–mesenchymal transition is crucial. One of the hallmarks of this process is the overexpression of the transcription factor Snail, which regulates cell polarity, the expression of epithelial and mesenchymal markers, as well as migration and invasion. We have previously shown that Dual Specificity Phosphatase 1 (DUSP1) acts as a tumor suppressor in PC by negatively regulating the activity of MAPKs. Thus, DUSP1 promotes apoptosis in PC cells through p38MAPK inhibition (1) and participates in the pro-apoptotic effects of the chemopreventive molecule resveratrol (2). Here we have studied the role of DUSP1 in PC cell motility, analyzing its effects on the regulation of Snail expression and the underlying mechanisms mediated by MAPK inhibition. To this purpose, migration and invasion assays, western blot, immunofluorescence analyses and reporter assays were conducted in PC cells overexpressing or lacking DUSP1 or incubated with specific MAPK inhibitors. Our data demonstrate that DUSP1 decreases Snail expression, as well as cell migration and invasion. These results are similar to those obtained following specific inhibition of DUSP1 molecular targets, JNK and ERK. Moreover, our findings show that the mechanism by which DUSP1 overexpression and specific inhibition of these MAPK pathways induce Snail downregulation involves the export of this transcription factor from the nucleus and its subsequent degradation by the proteasome in the cytosol. Finally, we also demonstrate that dual inhibition of ERK and JNK pathways cooperate to regulate Snail expression, cell migration, and invasion, supporting the idea that DUSP1 affects both processes through the specific inactivation of these two signaling pathways. In summary, we consider that our findings suggest new opportunities to improve current therapeutic strategies for the diagnosis and treatment of PC.

(1) Gil-Araujo B, Toledo Lobo M-V, Gutiérrez-Salmerón M, Gutiérrez-Pitalúa J, Ropero S, Angulo JC, Chiloeches A, Lasa M (2014) Mol Oncol 8(1):27-38. (2) Martínez-Martínez D, Soto A, Gil-Araujo B, Gallego B, Chiloeches A, Lasa M (2019) Food Chem Toxicol 124:273-279.

0400-P

Deciphering an interplay between ISG15 and SUMO

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Interferon (IFN)-stimulated gene 15 (ISG15) is a 15 kDa protein that belongs to the family of ubiquitin-like proteins induced by type I IFN. Type I IFN also induces the upregulation of ISG15 E2 and E3 enzymes, promoting ISGylation. In addition to ISGylation, IFN treatment also enhances global levels of SUMOylation, a post-translational modification that involves the addition of small ubiquitin-like modifier (SUMO) to a target protein, suggesting a putative interplay between these two ubiquitin-like proteins. Recently it has been reported that SUMO2/3 overexpression leads to the stabilization of different components of the ISG15 conjugation machinery in IFN-treated cells. Furthermore, an enhance in the IFN-induced global cellular ISGylation by SUMO2/3 has been reported. However, so far, a direct interplay between SUMO and ISG15 has not yet been demonstrated. We have evaluated a putative direct interplay between SUMOylation and ISGylation. Our results demonstrate that ISG15 protein is regulated by covalent and non-covalent interaction with SUMO2/3 proteins. In

addition, our data reveal that this interaction modulates the subcellular localization of ISG15, its secretion, and its conjugation to target proteins. Finally, we show that this interaction is regulated in response to different stimuli, including IFN treatment, suggesting that this interaction may contribute to the activity of ISG15.

0401-R-P

NOTCH4 exhibits anti-inflammatory activity in activated macrophages by interfering with interferon-γ and Toll-4 signaling

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NOTCH4 is a member of the NOTCH family of receptors whose expression in macrophages is intensively induced after their activation by Toll receptors and/or interferon-γ. Most of the NOTCH receptors act as positive signals of inflammation. However, our results show that NOTCH4 acts as a negative regulator of macrophage proinflammatory activation by diminishing the expression of proinflammatory cytokines as IL-6 and IL-12, since it inhibits the activation of key transcription factors involved in this response such as NF-κB and STAT1. Regarding interferon-γ signaling, our results show that NOTCH4 reprograms the macrophage response to IFN-γ, favoring STAT3 over STAT1 phosphorylation without affecting their expression, resulting in a lower expression of STAT1 dependent genes like IRF1. On the other hand, NOTCH4 inhibits canonical NOTCH signaling induced by LPS; however, it is able to reverse the inhibition that IFN-γ exerts on NOTCH signaling, favoring the expression of NOTCH target genes such as HES1, which is a transcriptional repressor that modulates Il6 and Il12 expression. Moreover, Hes1 seems to mediate, at least in part, the potentiation of STAT3 activation by NOTCH4.

Our study provides new data on the mechanisms that control macrophage proinflammatory activation and present NOTCH4 as a new regulatory element that could be used as a target for the control of pathologies in which there is an excess of inflammation.

(Grant SBPLY/17/180501/000301 by Junta de Castilla-La Mancha, Spain; and PID2019-109421RB-100 by Ministerio de Ciencia e Innovación, Spain)

0452-P

G protein-coupled receptors in skin cells: effect of plant extracts on bitter taste receptors signaling

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The presence of a class of G protein-coupled receptors (GPCRs), the bitter taste receptors, in extra-oral tissues has been demonstrated, including non-gustatory organs, like the skin. We have found specific taste receptors expressed in skin cells, particularly TAS2R4, TAS2R20 and TAS2R31 in human dermal fibroblasts, and TAS2R20, TAS2R31 and TAS2R43 in human keratinocytes. We have focused our analysis on TAS2R4 expression and function in human dermal fibroblasts. TAS2R4 was found to be expressed in fibroblasts, both at the transcription and transduction levels, and its functionality was assessed by a positive calcium signal upon exposure to an agonist. We analyzed the effect of 15 different substances (pure molecules and plant extracts) that were screened against TAS2R4. Compounds were assayed for cytotoxic effects on primary fibroblasts and the compounds ability to activate the studied GPCRs was analyzed using a calcium influx assay. We tested TAS2R4 specific inhibitors in order to determine which compounds were specifically activating TAS2R4. Of the different extracts analyzed, Humulus lupulus (hop) signal was avoided by two of the three inhibitors used. Hop was studied in more depth because of its putative skin protective potential effect on dermal fibroblasts through its ability to activate TAS2R4. Regarding the hop effect on cell gene expression, 8 of the 22 studied genes showed downregulated expression, including MMP1, MMP3, TIMP1 SOD2 and CAT. Reduction of MMPs and TIMP-1 allows the cell to have higher ratios of the structural proteins that these enzymes degrade, like fibronectin or elastin (whose expression is also downregulated) without modifying gene expression and as consequence, resulting in less cell metabolic stress. P21 was detected to be overexpressed, which is associated with cell senescence and promotes cell arrest when activated through the P53 pathway. COL1A1, COL3A1 and COL2A1 genes showed a similar peculiar behavioral pattern that would deserve further investigation.

Brockhoff, A. et al., 2010. Structural requirements of bitter taste receptor activation. Proc. Natl. Acad. of Sci. U.S.A., 107(24), 11110–11115. Meyerhof, W. et al., 2009. The molecular receptive ranges of human TAS2R bitter taste receptors. Chem. Senses, 35(2), 157–170. Reszka, E., 2015. Expression of Bitter Taste Receptors in the Human Skin In Vitro. J. Clin. Res. Bioeth., 6(2). 1000218

0463-R/M-P

Activation of stress signal in macrophages controls liver metabolism

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Obesity is a global health problem that contributes to the development of metabolic syndrome. This pathology is characterized by a chronic low inflammatory state in multiple tissues where macrophages play an important role. Particularly, ectopic lipid accumulation in the liver promotes hepatic steatosis and liver dysfunction that has been associated with increased macrophage infiltration. Stress-activated kinases, which include JNK and p38s, are reported also as crucial factors in the inflammatory response associated to obesity. However, the precise mechanisms involved in liver-macrophage crosstalk during obesity and whether myeloid p38s has an important contribution to these processes remain unknown.

We have found that mice lacking the main upstream activators of p38s, MKK3 and MKK6, in myeloid cells (MKK3/6$^{Lyzs\text{-}KO}$) were more susceptible to obesity and diabetes when feeding a High Fat Diet due to a reduction of hepatic and circulating FGF21 levels that affect whole body metabolism. Interestingly, these changes in FGF21 levels correlated with drastic alterations in the liver macrophage pool of MKK3/6$^{Lyzs\text{-}KO}$ mice. Thus, we investigated whether liver macrophage remodeling during obesity affects FGF21 production. To test this idea, we first characterized *in vitro* the inflammatory response of bone-marrow derived macrophages (BMDM) lacking MKK3 and MKK6 and found that the complete inhibition of p38 pathway leads to a more pro-inflammatory response. Then, we exposed hepatocytes to conditioned medium (CM) from WT BMDM previously stimulated with palmitate and LPS and found that BMDM after immune activation directly inhibits the expression of *Fgf21* in hepatocytes. According to this and the more pro-inflammatory phenotype of BMDM lacking MKK3/6, we found a higher downregulation of *Fgf21* when hepatocytes are exposed to CM from BMDM lacking MKK3/6 comparing to the effect exerted by CM from control BMDM.

As a summary, in this work we found that macrophages modulate hepatocyte Fgf21 expression being p38 activation in macrophages crucial for this macrophage-hepatocyte crosstalk and for the regulation of whole-body metabolism through hepatic FGF21 during obesity.

Funding: MINECO-FEDER, PID2019-104399RB-I00, Fundación AECC PROYE19047SABI, Fundación AECC INVES20026LEIV.

Manieri, E., & Sabio, G. (2015). Stress kinases in the modulation of metabolism and energy balance. Journal of molecular endocrinology, 55(2), R11–R22. Saklayen M. G. (2018). The Global Epidemic of the Metabolic Syndrome. Current hypertension reports, 20(2), 12. Wen, Y., Lambrecht, J., Ju, C., & Tacke, F. (2021). Hepatic macrophages in liver homeostasis and diseases-diversity, plasticity and therapeutic opportunities. Cellular & molecular immunology, 18(1), 45–56.

0464-R/M-P

Novel pathway activated by two platinum iodido prototypes in cancer cell death

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Platinum-based cancer chemotherapy continues being one of the most used treatments in clinic, whether in monotherapy or in combination with other drugs. Despite the high activity and good response in clinical use of cisplatin on certain tumors, its serious side effects and the cellular development of resistance in some of the tumors (intrinsic or acquired) have conducted research into new complex designs.

Trying to solve these problems, our group have been working in the synthesis and study of platinum(II) complexes where the chloride ligand is replaced by iodide (bulkier than cisplatin's one).

It was long believed that iodido analogues of cisplatin were poor pharmacological agents, their inactivity being ascribed to the greater stability and lower reactivity of Pt-I bonds. However, we were first to report a broad family of Pt-I complexes which showed excellent cytotoxic activity against various tumor cell lines, especially in those with greater resistance to cisplatin.(1) Further studies were performed at molecular level that allowed to determine a different reactivity versus small models of DNA and proteins. Unlike cisplatin, these iodide analogues have other biological targets beyond the DNA, showing only the covalent interaction at high concentration. The interaction study versus model proteins showed, in some cases, a non-conventional reactivity towards S-donors bioligands, binding through the metal but releasing a different part of the platinum drug.(2)

For this work, we selected two iodido prototypes: *cis* and *trans*-[PtI2(ipa)2], and we have tried to explore its mechanism of action and potential clinical application. In order to analyze the signaling pathways towards a broader spectrum of interactions (before and after DNA damage), we began studying the in vitro cytotoxicity against MKN45 and HCT116 to later identify the role of p53 in the cell death mechanism. Then we analyzed the apoptotic pathway induced by the drug and finally, we propose that iodido drugs increased cell death independent of p53, which is very promising for tumors with p53 mutated (>50% of tumors). (3)

These results offer a big spectrum of possibilities, and even though more and specific studies are required, turn these initially "underestimate" Pt-I complexes into a promising antitumoral agents.

(1) L. Messori, A.G. Quiroga et al. ACS Med. Chem. Lett. 2010, 1, 381–385. (2) L. Messori, A.G. Quiroga et al. Inorg. Chem. 2012, 51, 1717–1726. (3) A.G. Quiroga, I. Sánchez-Pérez et al. ACS Omega. 2019, 4, 21855–21861.

0467-R-P

Sur8, a determinant protein in colorectal cancer tumor progression

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Colorectal cancer (CRC) has the highest incidence rate in the Spanish population. The most important challenge consists on the discovery of efficient disease treatments, due to high mortality rates in highly developed stages. Sur8 is a scaffold protein that positively modulates ERK signaling pathway, which has a major role in the progression and metastasis in colorectal cancer. The main goals of our research are to determine the role that Sur8 plays in the development and progression of CRC and to analyze its possible therapeutic potential. For this purpose, our group has developed an inducible conditional mouse model msur8$^{f/f}$VillinCre^{ERT2}. In order to determine Sur8 action in the colonic tissue, we have developed organoids from the colon epithelium of healthy mice and have analyzed gene expression pattern by an RNAseq approach. Sur8 KO affects oncogenic CRC transcription factors expression, as well as the modulation of some Wnt pathway regulators. In regard to miRNA data, we have observed deregulation of miRNAs related to CRC in Sur8 KO organoids. To determine the role that Sur8 plays in the development and progression of CRC, we have subjected our inducible conditional mice to chemical carcinogenesis and we have observed that Sur8 KO males display less and smaller tumors and do not present any adenocarcinoma. In addition, we have carried out Sur8 silencing in human CRC cell lines by infection with constitutive shRNA lentiviruses. We have observed that Sur8 silencing produces decreases of cell tumor proliferation, and reduction of p-ERK levels. Finally, we are evaluating the effects of putative therapeutic agents against Sur8 in human CRC cell lines. Concretely, we are testing Celastrol, which has been described that binds and blocks the action of Sur8 *in vitro*. We have observed that Celastrol treatment diminishes the cell tumor proliferation in this model. Altogether, our results indicate that Sur8 may have a determinant role in CRC progression and that Sur8 could be a potential molecular target for the design of novel strategies against CRC.

0493-R/M-P

NADPH oxidase 1 as a new regulator of the WNT pathway and the protective effect of vitamin D in colorectal cancer.

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Worldwide, colorectal cancer (CRC) is the third most common malignant neoplasm and the second leading cause of cancer-associated mortality, with an estimated increase in global prevalence of 60% by 2030 (1,2). Mutational inactivation of adenomatous polyposis coli (APC) is the hallmark of CRC and leads to an overactivation of WNT signaling that favors the development and progression of CRC (3). Large epidemiological studies suggest that the diabetic population is at increased risk for site-specific cancers, including CRC (4).

Our laboratory has shown that hyperglycemia induces the accumulation of ROS in CRC but not healthy cells, driving the activation of a newly described ROS/AMPK/EP300 axis that enhances Wnt/b-catenin signaling. Increased EP300 leads to increased acetylation of β-catenin at K354, a requirement for nuclear accumulation and transcriptional activation of WNT target genes (5,6).

The critical role driven by ROS suggest a possible involvement of the NADPH oxidases (NOX family, as a source of ROS. Specifically, NOX 1 and NOX 4 are expressed in colon epithelial cells, and their overexpression in CRC cells promotes cell proliferation and invasiveness (7,8,9,10). Our results indicate that hyperglycemia significantly increases NOX1 levels, in correlation with increased ROS production in CRC cells, suggesting a possible regulation of the ROS/AMPK/EP300 axis by NOX1.

Antioxidant mechanisms dealing with NOX1-induced ROS should be effective against CRC. Vitamin D (1α, 25-dihydroxyvitamin D3) is a powerful antioxidant that inhibits proliferation and promotes differentiation of CRC cells at least partially through inhibition of Wnt/β-catenin signalling. Consequently, vitamin D deficiency is associated with poor survival to CRC (11,12).

Our results indicate that vitamin D causes a reduction in the levels and / or activity of some members of the NOX family by turning off the ROS/AMPK/EP300/β-catenin axis and its proliferative and tumorigenic effects. The data suggest a new antitumor mechanism of vitamin D linked to its anti-oxidant action. Our results integrate independent epidemiological links between vitamin D deficiency, diabetes and cancer in one overarching and unifying mechanism.

1.Keum N & Giovannucci E. Nat Rev Gastroenterol Hepatol.2019 2.Arnold M.Gut.2017. 3.Schatoff EM. Curr Colorectal Cancer Rep.2017. 4.Ferguson RD. Vitam Horm. 2013. 5.García-Jiménez C. J Mol Endocrinol.2013. 6.Gutiérrez-Salmerón M, et al. PLoS Biol. 2020. 7.Stalin J. Life Sci Alliance. 2019. 8.Cho SY. Dig Dis Sci. 2018. 9.Laurent E. Int J Cancer. 2008. 10.Cho SY. Dig Dis Sci. 2018. 11.Tagliaferri S. Nutr Res Rev. 2019. 12.Ferrer-Mayorga G. J Steroid Biochem Mol Biol. 2019.

0496-R-P

Sur8 lipid rafts tethering impairs S338 C-Raf phosphorylation and consequent ERK activation.

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Sur8 protein is a positive modulator of RAS signaling and Sur8 mutants have been found in a group of pathologies called RASopathies, because they share a set of specific phenotypes found in patients with mutations in RAS and RAS-related signaling proteins. Nevertheless, the way of action of the best-characterized Sur8 mutation in this pathology, S2G, is controversial. Although this mutation places Sur8 at the plasma membrane, more concretely at the lipid rafts domain, authors differ on the ability of this mutated Sur8 version to influence on ERK activation. Sur8 can be found in different locations inside cells, being its membrane association an important factor for the RAS to RAF signal transduction in the RAS/RAF/MEK /ERK pathway. In this work, we aim to clarify the specific function of Sur8 at the concrete lipid rafts membrane location. For this purpose, we have directed Sur8 by its fusion with peptides of different proteins that favor their anchoring to lipid-rafts. We have observed that Sur8 lipid-raft tethering blocks ERK phosphorylation in different human cell lines, such as HEK 293T and HeLa; when concomitantly expressed with M-, H- or K-Ras V12, as well as upon EGF stimulation. Furthermore, this impairment can be explained by the inability of C-RAF to be phosphorylated at S338, as we do not observe changes in C-RAF S259 phosphorylation status when compared to non-directed Sur8. Concerning other pathways, we have observed that Sur8 lipid-raft tethering also blocks the phosphorylation of AKT at S473. In addition, we have explored C-RAF phosphorylation status in C260Y and E457K Sur8 mutants, described in *C. elegans*. Our data indicate that overexpression of Sur8 E457K mutant inhibits C-RAF S259 dephosphorylation and promotes C-RAF S338 phosphorylation. Lipid raft location of Sur8 E457K mutant did not affect C-Raf S259 dephosphorylation but impairs C-Raf S338 phosphorylation, which confirms that Sur8 needs to be out of this membrane location to favor C-Raf S338 phosphorylation. Finally, Sur8 lipid-raft attachment is able to repress proliferation and differentiation in different cell lines, accordingly to a reduction of p-ERK levels.

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Cámara Torres, Patricia	0306-P
Campillo, Isabel	0422-OS
Campins Machado, Francesc Miquel	0368-R/M-P
Campo, Elias	0185-R/M-P
Campos, Adrián	0183-P
Canalejo, Marta	0042-R/M-P, 0061-R-P
Canales Cortés, Saray	0278-R-P, 0308-R-P, 0311-R-P, 0100-R-P
Canals, Meritxell	0515-OI
Cancelas, Jose A.	0115-R-P

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Candiota, Ana Paula	0070-R-P, 0165-R-P, 0446-P
Canela, Enric	0515-OI, 0002-P
Canet, Francisco	0127-P, 0425-P
Cano Cano, Fátima	0065-R/M-P
Cano, Amparo	0531-OI
Cano, Javier	0219-P
Cano Marchante, G. Javier	0041-P, 0204-P
Cano Muñoz, Mario	0245-R/M-P
Cánovas Díaz, Manuel	0008-R-P, 0436-P
Cantó Santos, Judith	0013-R/M-P, 0026-R/M-P, 0187-R/M-P
Canudas, Silvia	0521-OI
Capó, Xavier	0091-P, 0130-P, 0218-P, 0287-P
Carballo, Jesús	0433-P
Carbonell, Teresa	0522-OI
Cardellach, Francesc	0013-R/M-P, 0026-R/M-P, 0187-R/M-P
Cardenas, Jose Miguel	0197-P
Cardoso, Teresa	0196-R/M-P
Carmeliet, Peter	0234-R/M-P, 0262-R/M-P
Carneiro, Carmen	0358-P, 0369-P
Carnero, Amancio	0257-R/M-P
Carpena, Xavi	0489-P
Carpintero Fernández, Paula	0079-R/M-OS
Carranza, Gerardo	0199-R/M-P
Carrasco Jimenez, Maria P.	0226-R/M-P, 0260-R/M-P
Carreño Tarragona, Gonzalo	0455-P
Carrera, Esther	0197-P, 0230-R-P
Carreras, Joaquim	0185-R/M-P
Carrillo, Paloma	0328-R-P
Carrión, Ángela	0292-R-P

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Carro Alvarellos, Marta	0069-R/M-P
Casado, Sabela	0372-R/M-P, 0382-R/M-P
Casado, Sabela	0382-R/M-P
Casado, Vicent	0515-OI, 0002-P
Casals, Cristina	0184-P, 0391-P
Casals, Núria	0155-R-P, 0186-R/M-OS, 0231-P, 0272-P
Casamayor, Antonio	0107-P, 0110-R-P
Casana, Estefanía	0005-R-P
Casares, Miguel	0160-P
Casas, Josefina	0457-R-P
Casas, Rosa	0026-R/M-P
Cascante, Marta	0191-R/M-P
Cases, Ildefonso	0111-P
Casillas Serra, Carlos	0124-P, 0363-P
Casino, Patricia	0046-P, 0058-OS
Castaño, Esther	0096-R-P, 0178-P
Castaño, Luis	0276-R/M-P
Castaños Mollor, Irene	0482-R/M-OS
Castell, Margarida	0004-R/M-OS
Castellano, Bernado	0038-P
Castellanos, Milagros	0309-R/M-OS
Castelo, Janire	0191-R/M-P
Castilla, Laura	0410-R-P
Castillejo, María Ángeles	0504-R/M-P
Castillo Mancho, Vicente	0148-P
Castro, Beatriz	0123-P
Castro, Cristian	0028-R-P
Castro Barquero, Sara	0026-R/M-P
Castro Osma, José A.	0122-R/M-P
Cavia, Mónica	0381-P, 0325-P
Cebrián Carmona, José	0321-P, 0323-P

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Cecone, Claudio	0259-P
Celaya, Adelaida M.	0086-R-P
Cenigaonandia Campillo, Aiora	0427-M-OS
Ceperuelo Mallafré, Victòria	0473-P
Cerdán, Maria Esperanza	0314-R/M-P, 0315-R/M-P, 0244-P, 0180-R/M-P
Cerrada Giménez, Marc	0038-P
Cervera, José	0214-P
Cesaro, Samuele	0245-R/M-P
Chakrabarti, Anob M.	0450-P
Chara, Juan Carlos	0265-P, 0312-P, 0313-OS
Chávez De Diego, Sebastián	0111-P, 0117-OS
Chichon, Francisco Javier	0375-P
Chiloeches, Antonio	0397-P
Chocarro Calvo, Ana	0317-P, 0333-P, 0355-R/M-P, 0493-R/M-P, 0374-R/M-P
Chojnacki, Jakub	0457-R-P
Chowen, Julie A	0152-P
Choya Foces, Carmen	0166-P, 0034-R/M-P
Cid, Maria C	0185-R/M-P
Cilleros, Dario	0397-P
Ciobu, Nicolae	0431-P
Clemente, Andrés	0183-P
Cobo, Fernando	0302-P
Codoñer Franch, Pilar	0299-P, 0301-P, 0383-P
Cogliati, Sara	0032-R/M-P
Colell, Anna	0471-R/M-P
Collado Pérez, Roberto	0152-P
Collinson, Lucy	0378-R-P
Coloma, Javier	0283-P
Colomer, Dolors	0185-R/M-P

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Colomina, Neus	0238-P
Comet, Jose P	0015-R/M-P
Compa, Montserrat	0091-P
Company, Sonia	0249-P
Conchillo Solé, Oscar	0367-P
Conejero Lara, Francisco	0245-R/M-P
Conesa, Irene	0037-R/M-P, 0051-R-P
Consiglio, Antonella	0155-R-P
Consortium, Gencode Orf	0240-OS
Constantinescu, Andrada	0446-P
Contestí, Joan	0175-R/M-P
Contreras, Asunción	0108-P, 0113-P
Contreras, F Xabier	0457-R-P, 0456-P, 0454-P
Corbí, Angel Luis	0330-R-P
Cordero Torres, Gustavo	0330-R-P
Cordoba, Octavi	0417-P
Córdoba Cañero, Dolores	0157-R/M-P
Cordomí, Arnau	0258-R-P
Cordova, Isabel	0494-R/M-P
Cornejo García, Jose Antonio	0481-R-P
Coronado, Montserrat	0492-OS
Corrales, Sagrario	0460-R/M-P
Cortés, Adriana	0025-M-OS
Cortes Canteli, Marta	0533-OI
Cortés Espinar, Antonio J.	0449-P
Costa, Andrea	0080-P
Costales Carrera, Alba	0467-R-P
Costell, Mercedes	0426-R/M-P
Costenaro, Lionel	0367-P
Costoya, Jose A.	0242-OS

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Cota, Ernesto	0248-OS
Couso, Juan Pablo	0240-OS
Cózar Castellano, Irene	0136-P
Crespo, Isidro	0489-P
Crespo, Javier	0083-R/M-P, 0209-R/M-P, 0215-R/M-P
Crespo, Maria	0081-P, 0463-R/M-P
Crispi, Fàtima	0026-R/M-P
Cruz, Antonio	0404-R/M-P
Cuadrado, Antonio	0509-OS
Cuellar, Virginia	0307-P
Cuerda, Erika	0101-R/M-P
Cuervo, Ana Maria	0023-R/M-P
Cuesta, Angel	0380-R-P, 0390-P, 0208-R/M-P
Cuevas, Dolors	0074-P
Culí, Joaquim	0148-P
Cuñarro, Juan	0372-R/M-P, 0382-R/M-P
Cursano, Giulia	0153-R/M-P
Cussó, Lorena	0075-P
Daiber, Andreas	0316-R/M-OS
Dames, Sibylle	0083-R/M-P
Daubon, Thomas	0207-R/M-OS
Daura, Xavier	0367-P
David Lluesma, Xavier	0363-P
Davila Ferreira, Nerea	0358-P
De Andrés Hernaiz, Raquel	0354-P, 0348-R-P
De Bragança, Sara	0164-P
De Diego Puente, Teresa	0008-R-P, 0436-P
De Dios, Cristina	0471-R/M-P

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De La Casa Esperon, Elena	0277-R-OS
De La Fuente, Lorena	0500-P
De La Gándara, Álvaro	0243-P
De La Peña, Gema	0156-R-P
De La Rosa, Enrique J.	0279-R/M-P
De La Rosa, Inés	0074-P
De La Rosa, Lucia	0071-R/M-P
De La Rosa, Miguel A	0198-P, 0090-P, 0118-P
De La Vieja, Antonio	0221-P, 0317-P, 0333-P, 0374-R/M-P
De Las Cuevas, Gemma	0477-P
De Las Rivas, Javier	0352-OS
De Llorens, Rafael	0014-R-P, 0015-R/M-P
De Lucas, María Pilar	0467-R-P, 0496-R-P, 0485-R-OS
De Marañón, Aranzazu M.	0127-P
De Marci, Ludovic	0358-P
De Miguel, Carlos	0025-M-OS, 0246-R/M-OS
De Oliveira Diz, Tadeu	0382-R/M-P, 0372-R/M-P
De Sancho, David	0392-R-P
De Smedt, Stefaan C	0476-OS
De Tapia, Lidia	0391-P
De Toro, María	0042-R/M-P
Deber, Alexandre	0210-R/M-P
Decastro, María Eugenia	0342-R-P
Deford, Christian	0393-P
Deglies Posti, Gianluca	0519-OI, 0225-R/M-OS
Degrave, Alisa	0394-R/M-P
Del Monte, Juan Pablo	0063-R/M-P

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Del Pozo Barriuso, Miguel Ángel	0059-P
Delgado, Igotz	0353-P
Delgado, Katia	0155-R-P
Delgado, Teresa C	0207-R/M-OS, 0261-R/M-P
Delgado Esteban, Maria	0322-R/M-P, 0373-R/M-P, 0478-R/M-P
Delgado Martín, Susana	0189-R-P
Delgado Moreno, Laura	0310-P
Delgado Ramos, Lidia	0117-OS
Delgado Román, Irene	0117-OS
Deudero, Salud	0091-P
Devida, Juan M	0369-P
Di Croce, Luciano	0512-OI
Dian, Cyril	0248-OS
Díaz, Lucía	0526-OI
Díaz, Noelia	0093-R/M-OS
Díaz, Pedro	0425-P
Diaz Grijuela, Elisa	0169-R/M-P
Diaz Guerra, María José Martinez	0341-P
Diaz Lobo, Mireia	0367-P
Diaz Morales, Noelia	0325-P
Díaz Moreno, Irene	0090-P, 0198-P, 0207-R/M-OS, 0118-P
Díaz Perales, Araceli	0235-R/M-P
Díaz Pozo, Pedro	0127-P
Díaz Quintana, Antonio	0090-P, 0198-P, 0207-R/M-OS, 0118-P
Diéguez, Carlos	0382-R/M-P, 0402-R/M-P, 0372-R/M-P
Dieguez Martínez, Nora	0281-P, 0252-P
Díez Guerra, Francisco Javier	0354-P
Diez Villegas, Paula Ariadna	0485-R-OS
Diez, Amalia	0492-OS
Diez, Ana María	0325-P
Díez, Jesús	0285-P
Díez, Paula	0352-OS
Díez, Rosana	0143-R-P
Diez Alarcia, Rebeca	0515-OI
Díez Guerra, F Javier	0348-R-P
Dillingham, Mark S	0151-R-OS, 0164-P
Dim De Oliveira, Guilherme	0078-P
Dinsdale, Elizabeth Ann	0342-R-P
Diop, Sokhna M.s Yakhine	0100-R-P
Dirany, Zeinab	0298-P
Doane, Michael P	0342-R-P
Doblado Bueno, Laura	0387-R/M-P
Dolcet, Xavier	0252-P
Dolz Edo, Laura	0181-R-P
Domb, Abraham J.	0097-R/M-P
Domínguez, Blanca	0369-P, 0358-P
Domínguez, Fernando	0358-P, 0369-P
Domínguez Jurado, Elena	0122-R/M-P
Domínguez Madrid, Nerea	0173-R/M-P
Domínguez Soto, Ángeles	0330-R-P
Dumesic, Phillip A.	0032-R/M-P
Durá, Lara María	0496-R-P
Durán Poveda, Manuel	0317-P
Duran, Adrià	0015-R/M-P
Durán, Adriana	0190-P
Duran, Jordi	0335-P
Efeyan, Alejo	0268-R/M-P
Egia Mendikute, Leire	0191-R/M-P
Eguileor, Álvaro	0257-R/M-P, 0261-R/M-P
Ehses, Janina	0450-P
El Dirany, Rima	0346-R-P
El Motiam, Ahmed	0400-P, 0419-P, 0488-OS
Eldering, Eric	0487-R-OS
El Dirany, Rima	0177-P, 0236-R-OS
Elguezabal, Natalia	0191-R/M-P, 0215-R/M-P
Elortza, Felix	0207-R/M-OS, 0460-R/M-P, 0209-R/M-P
Elowsson, Linda	0495-P
Emperador, Sonia	0060-R-P, 0486-R/M-P
Encinas, Mario	0241-P
Enrich Bengoa, Jennifer	0038-P
Enríquez, José Antonio	0032-R/M-P, 0034-R/M-P, 0470-OS
Errasti Murugarren, Erkaiz	0526-OI
Escalona Noguero, Carmen	0153-R/M-P, 0309-R/M-OS
Escasany, Elia	0253-R/M-P
Escribá, Pablo	0237-P
Escrivá Fernández, Jose P.	0288-P
Escudero Bravo, Paloma	0526-OI
Escudero Ibarz, Leire	0138-OS
Escuder Rodríguez, Juan José	0362-P, 0344-P
Espina Casado, Jorge	0039-R/M-P
Espino, Javier	0480-P, 0483-P
Espinosa Escudero, Ricardo	0202-P
Espinosa Gil, Sergio	0281-P, 0252-P
Espinosa López, Elisa María	0285-P
Espinoza, Vanessa	0426-R/M-P
Estarás, Matías	0125-P
Estarellas, Carolina	0049-P, 0286-P
Esteban, Alexandre	0291-R-P
Esteban, Jaime	0097-R/M-P, 0122-R/M-P
Esteban, Susana	0218-P
Estirado, Samuel	0480-P, 0483-P
Etxaniz, Asier	0407-P
Eugenia Lledó, Victoria	0188-P
Ezquerra, Artur	0275-R/M-P
Fabregat, Isabel	0305-R/M-P
Fabriás, Gemma	0457-R-P
Fafián Labora, Juan	0239-M-P
Fahey, Radka	0015-R/M-P
Fajardo, Ignacio	0389-R-P
Falcón, Rosa	0127-P, 0425-P
Famelis, Nikolaos	0519-OI
Farah, Shady	0097-R/M-P
Fariñas, Isabel	0181-R-P
Farràs, Rosa	0488-OS, 0211-R/M-P, 0500-P
Farrés, Jaume	0446-P
Fasiolo, Alberto	0226-R/M-P
Fathinajafabadi, Alihamze	0211-R/M-P, 0500-P
Favaro, Francesca	0487-R-OS
Fawzy, Nermeen	0231-P
Fedichev, Peter O.	0057-M-OS
Felipe, Raquel	0351-R-P
Ferandez Tussy, Pablo	0222-R-P
Fernández Aceñero, María Jesús	0128-R-P, 0429-R/M-P, 0467-R-P
Fernández Barral, Asunción	0467-R-P
Fernández Delgado, Elena	0480-P
Fernández González, Alfonso	0039-R/M-P
Fernández González, Ataulfo	0330-R-P
Fernández Justel, José Miguel	0497-OS
Fernandez Moya, Sandra Maria	0450-P
Fernández Novell, Josep M	0469-P
Fernández Parejo, Natalia	0485-R-OS
Fernández Pérez, Iván	0499-R/M-P
Fernández Rodríguez, Carmen	0196-R/M-P
Fernández Torres, Miguel Ángel	0188-P
Fernandez, Alba	0392-R-P
Fernández, Carmen	0257-R/M-P, 0261-R/M-P
Fernández, Emilio	0262-R/M-P, 0057-M-OS
Fernandez, Israel	0489-P
Fernandez, Pol	0472-R-P
Fernández Aceñero, María Jesús	0333-P
Fernandez Alvarez, Alfonso	0490-P
Fernández Ballester, Gregorio	0292-R-P
Fernandez Bermejo, Miguel	0125-P
Fernández Caldas, Enrique	0481-R-P
Fernández Calvo, Alba	0302-P
Fernández De Frutos, Mario	0149-R-P, 0156-R-P
Fernández Delgado, Elena	0483-P
Fernández Escamilla, Ana María	0292-R-P
Fernandez Fuentes, Narcis	0477-P
Fernández García, Paula	0237-P
Fernandez Gonzalez, Pol	0452-P
Fernández Justel, David	0439-P
Fernandez Leiro, Rafael	0275-R/M-P, 0519-OI, 0532-OS
Fernández Montes, Paula	0463-R/M-P
Fernandez Perez, Antonio	0263-R-P
Fernández Puente, Escarlata	0385-P
Fernández Ramos, David	0209-R/M-P, 0215-R/M-P
Fernández Recio, Juan	0430-OS
Fernández Rodríguez, Carmen	0207-R/M-OS
Fernández Rojo, Manuel A	0402-R/M-P
Fernández Rubio, Celia	0177-P, 0236-R-OS, 0346-R-P, 0408-P
Fernández Salguero, Pedro María	0043-R-P
Fernández Trasancos, Ángel	0045-R-P, 0075-P
Fernández Tresguerres, Jesús	0435-R-P
Fernández Veledo, Sonia	0473-P
Ferrando, Alejandro	0211-R/M-P
Ferré, Juan	0058-OS
Ferre, Marc	0281-P, 0252-P
Ferré, Sergi	0002-P, 0258-R-P
Ferreira, Patricia	0054-P
Ferreiro Vera, Carlos	0173-R/M-P
Ferrer Roig, Marta	0210-R/M-P
Ferrer, Miguel	0119-R/M-P
Ferrer, Miguel David	0287-P
Ferrera, Alberto	0497-OS
Ferreras, Mariola	0129-R-P
Ferrer Batallé, Montserrat	0015-R/M-P
Ferrer Navarro, Mario	0367-P
Ferrero, Eduardo	0494-R/M-P
Férriz Gordillo, Andrea	0254-R-P
Fillat, María F.	0228-P, 0270-R/M-P, 0331-R/M-P, 0339-R/M-P
Finger, Sebastian	0459-R/M-OS
Fiol, Miquel	0080-P
Fisher, Gemma Lm	0164-P
Fita, Ignacio	0526-OI

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Fiuza Marco, Carmen	0317-P, 0374-R/M-P, 0493-R/M-P
Flores De Mera, Mª Luz	0396-R/M-P
Flores Hernández, Raquel	0256-R-P
Folgueira, Cintia	0032-R/M-P
Fonseca, Danae	0488-OS
Fonseca, Eduardo	0079-R/M-OS
Fontalva, Sara	0111-P
Fontanil, Tania	0039-R/M-P
Fontova Palé, Pere	0096-R-P
Formisano, Joel	0178-P
Fornés, Amparo	0351-R-P
Fornés, Oriol	0477-P
Fort, Joana	0526-OI
Forte, Alessia	0049-P
Fosch, Anna	0186-R/M-OS, 0231-P
Fos Domènech, Júlia	0004-R/M-OS, 0263-R-P
Frago Fernández, Laura M	0152-P
Fraile Ágreda, Víctor	0184-P
Franco, David	0392-R-P
Franco, Rafael	0173-R/M-P, 0175-R/M-P, 0174-R/M-P
Franco Ezquerro, Laura	0132-R/M-P
Franco González, Juan Felipe	0233-R/M-OS
François, Patrice	0459-R/M-OS
Franco Losilla, Marta	0386-R-P
Freire, Raimundo	0238-P
Freitas, Joaquim	0377-P
Frenis, Katie	0316-R/M-OS
Fresquet Arnau, Vicente José	0288-P
Fuentes, Manuel	0278-R-P, 0311-R-P, 0100-R-P, 0352-OS, 0507-R/M

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Fuentes Fayos, Antonio Carlos	0468-R/M-P
Fuentes Rodríguez, José Manuel	0308-R-P
G. Marín, José J.	0250-P
G. Quiroga, Adoración	0464-R/M-P
Galán Moya, Eva María	0041-P, 0219-P, 0204-P
Galardini, Marco	0172-R-OS
Galindo, Antonio	0225-R/M-OS
Galindo Moreno, María	0126-P
Galindo Villardón, Purificación	0062-P
Gallardo, Nilda	0044-R/M-P
Gallego, Idoia	0422-OS
Gallego, Oriol	0438-R/M-P
Gallego Jara, Julia	0008-R-P, 0436-P
Gallego Mena, Mariona	0026-R/M-P
Galmés, Sebastià	0273-R/M-P, 0274-R/M-P, 0269-R/M-P
Galve Roperh, Ismael	0312-P, 0313-OS
Gálvez, Juan Antonio	0366-R-P
Gámez, Andres	0281-P
Gandía, Carolina	0211-R/M-P, 0500-P
García Cazorla, Yolanda	0199-R/M-P
García Delicado, Esmerilda	0112-P
García Fernández, Jose M.	0065-R/M-P
García García, Francesc Jose P.	0013-R/M-P
García Jiménez, Custodia	0317-P
García Martínez, Jose Manuel	0317-P
García Murria, Maria Jesus	0345-R-P
García Piqueras, Jorge	0152-P
García Rodríguez, Carmen	0482-R/M-OS
García Sosa, Alfonso T.	0236-R-OS
Garcia Valero, Juan	0185-R/M-P

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García, Celia	0127-P, 0425-P
García, Jesús	0272-P
García, Jordi	0272-P
Garcia, Jorge	0246-R/M-OS
García, Marcos D.	0242-OS
García, Raquel	0494-R/M-P
Garcia, Rosa Maria	0071-R/M-P
García Agulló, Juan	0444-P
García Arribas, Aritz B.	0465-P
García Bolufer, Pau	0181-R-P
Garcia Cano, Jesus	0216-R/M-P
García Cánovas, Francisco	0084-P
García Carmona, Francisco	0037-R/M-P, 0051-R-P
García Carrasco, Almudena	0253-R/M-P
García Chica, Jesús	0231-P
García Contreras, Consolación	0144-P
García Criado, Federico	0430-OS
García Del Portillo, Francisco	0046-P
García Fojeda, Belén	0391-P
Garcia Foncillas López, Jesus	0427-M-OS
Garcia Fontgivell, Joan Francesc	0133-R-P
García Garcés, Paula	0299-P, 0301-P
García García, Francesc Jose P.	0026-R/M-P, 0187-R/M-P
García García, María	0048-R-P
García García, Miguel Eduardo	0468-R/M-P
García Giménez, José Luís	0247-P
Garcia Gimeno, Maria Adelaida	0092-R/M-P
García Heredia, José Manuel	0257-R/M-P
García Jiménez, Custodia	0333-P, 0355-R/M-P, 0493-R/M-P, 0374-R/M-P
García Jiménez, Mª Concepción	0486-R/M-P

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García Laínez, Guillermo	0393-P
García Macia, Marina	0262-R/M-P, 0057-M-OS
García Mañas, Celia	0321-P, 0323-P
García Marín, Luis J.	0437-P, 0502-P, 0350-R-P
García Maroto, Federico	0321-P, 0323-P
García Martín, Carmen	0526-OI
García Martínez, José Manuel	0333-P, 0355-R/M-P, 0374-R/M-P, 0493-R/M-P
García Mato, Ángela	0086-R-P
García Molina, Francisco	0084-P
García Molina, Pablo	0084-P, 0260-R/M-P
García Mouton, Cristina	0404-R/M-P
García Navas, Rósula	0289-R/M-P
García Otero, Laura	0026-R/M-P
García Pérez, Miguel Ángel	0383-P, 0299-P, 0301-P
García Ponce, Ángel Luis	0389-R-P
García Puga, Mikel	0360-R/M-P
García Ramirez, José Javier	0341-P
García Rodríguez, Néstor	0511-OI
García Romero, Noemí	0132-R/M-P
García Ruiz, Pedro Antonio	0084-P
Garcia Saez, Ana J.	0518-OI
García Sáez, Juan	0305-R/M-P
García Sastre, Adolfo	0488-OS
García Silva, Susana	0444-P
García Trevijano, Elena R	0247-P, 0249-P
García Tuñón, Ignacio	0098-R/M-P
Garcia Vaquero, Marina L.	0507-R/M-P
García Vicente, Roberto	0455-P
García Vicente, Laura	0256-R-P
García Villalón, Ángel Luis	0387-R/M-P

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Garranzo Asensio, María	0082-R-P, 0128-R-P, 0171-R-P
Garrido Cárdenas, José Antonio	0321-P, 0323-P
Garrido García, Vanesa	0455-P
Garrido Godino, Ana Isabel	0142-R/M-P
Garrido Perez, Nuria	0060-R-P, 0064-R-P
Garriga, Damia	0489-P
Garriga, Pere	0452-P, 0472-R-P
Garrigós, Víctor	0370-P
Gaudó, Paula	0060-R-P, 0064-R-P
Gavaldà Navarro, Aleix	0409-OS, 0443-P
Gavilan, María P.	0090-P
Gayoso, Manuel J.	0136-P
Geibel, Sebastian	0519-OI
Geier, Andreas	0202-P
Geiger, Otto	0307-P
Gerardi, Gisela	0325-P, 0381-P
Gereñu, Gorka	0384-R/M-P, 0360-R/M-P
Gianotti, Magdalena	0466-P, 0474-P
Gianzo, Marta	0024-R/M-P
Gibert, Isidre	0367-P
Giglione, Carmela	0248-OS
Gil Pitarch, Clàudia	0196-R/M-P
Gil, Fernando	0489-P
Gilabert, Joan F	0526-OI
Gil Bea, Francisco	0384-R/M-P
Gil Cartón, David	0459-R/M-OS

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Gil Ortega, Marta	0230-R-P
Gil Ortiz, Fernando	0459-R/M-OS
Gil Pitarch, Clàudia	0222-R-P, 0257-R/M-P, 0261-R/M-P
Gil Sanz, Cristina	0501-R-P
Giménez, Alberto	0100-R-P
Giménez Bejarano, Alberto	0308-R-P, 0311-R-P, 0278-R-P
Giménez Bonafé, Pepita	0220-R/M-P
Giner Lamia, Joaquín	0046-P
Giovanetti, Lisandro Jose	0369-P
Giralt, Marta	0443-P
Giroud Gerbetant, Judith	0406-R/M-P
Gmurczyk, Karolina N.	0283-P
Goding, Colin	0145-P, 0355-R/M-P, 0374-R/M-P
Godoy, José M.	0437-P, 0502-P
Goikoetxea Usandizaga, Naroa	0083-R/M-P, 0191-R/M-P, 0196-R/M-P, 0215-R/M-P, 0222-R-P, 0257-R/M-P, 0261-R/M-P, 0276-R/M-P, 0209-R/M-P
Gomar Alba, Mercè	0102-R/M-P
Gómez De Cedrón, Marta	0069-R/M-P
Gómez Jaramillo, Laura	0065-R/M-P
Gómez Villafuertes, Rosa	0112-P
Gómez, Carmela	0289-R/M-P
Gómez, Cristina	0160-P, 0482-R/M-OS
Gomez, Manuel Jose	0140-R-OS
Gómez, María	0497-OS
Gómez Baena, Guadalupe	0285-P
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Gómez Coronado, Diego	0156-R-P
Gómez Rodríguez, Pilar	0267-R/M-P
Gómez Santos, Beatriz	0276-R/M-P, 0353-P
Gomez Vicente, Beatriz	0203-R/M-P
Gomez Villafuertes, Rosa	0076-P
Gomila, Silvia	0440-R/M-OS
Gongora, Rafael	0507-R/M-P
Gonzáles Fernández, Lauro	0350-R-P
Gonzalez Bullón, David	0227-P
González Castaño, Diego	0358-P
González De Pablos, Ignacio	0267-R/M-P
González Gaitano, Gustavo	0298-P
Gonzalez Menendez, Pedro	0179-R-P
Gonzalez Morena, Juan M.	0095-P
González Moreno, Mónica	0396-R/M-P
González Recio, Irene	0196-R/M-P
González Rodríguez, Nayim	0303-P
González Romero, Elisa	0214-P
González, Andrés	0228-P
Gonzalez, Antonio	0125-P
González, Mar	0512-OI
González, María Isabel	0329-P
González, Nayim	0283-P
González, Rosa Ana	0100-R-P
Gonzalez, Sonia	0099-R/M-OS
González Arzola, Katiuska	0090-P
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Gonzalez Guerrero, Rebeca	0145-P
Gonzalez Magaña, Amaia	0129-R-P
Gónzalez Miguel, Javier	0282-R/M-P
González Polo, Rosa Ana	0308-R-P, 0311-R-P, 0278-R-P
González Recio, Irene	0222-R-P, 0257-R/M-P, 0261-R/M-P
González Rellan, Maria J.	0209-R/M-P
González Romero, Francisco	0353-P, 0276-R/M-P
González Santamaría, José	0488-OS
González Siso, María Isabel	0342-R-P, 0344-P, 0362-P
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González Vicens, Agustí	0466-P, 0474-P
Goñi, Felix M	0407-P, 0465-P
Gorospe, Myriam	0207-R/M-OS
Gotor Martínez, Cecilia	0412-R/M-OS
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Gracía, Hermenegildo	0503-P
Granado, Miriam	0387-R/M-P
Graña, Osvaldo	0001-R-P
Grasa, Laura	0159-P
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Gratacós Mulleras, Anna	0015-R/M-P
Grau, Josep Maria	0013-R/M-P, 0187-R/M-P, 0026-R/M-P
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Guerrero, José Peña	0346-R-P
Guerrero, Pedro Enrique	0014-R-P
Guerrero Hernandez, Martina	0185-R/M-P
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Guerrero Sánchez, Victor M.	0504-R/M-P
Guevara, Rommel	0349-R/M-P
Guevara Hoyer, Kissy	0330-R-P
Guigó, Roderic	0291-R-P
Guillén, Alba	0290-R/M-P
Guillen, Natalia	0205-P
Guinovart, Joan	0335-P, 0469-P
Guío, Jorge	0228-P, 0270-R/M-P, 0331-R/M-P, 0339-R/M-P
Guisantes Batan, Eduardo	0044-R/M-P
Guitart Mampel, Mariona	0026-R/M-P
Guitián Caamaño, Amanda	0079-R/M-OS
Gutierrez Uzquiza, Alvaro	0208-R/M-P
Gutiérrez, Fernanda	0349-R/M-P
Gutiérrez Merino, Carlos	0324-P
Gutiérrez Salazar, Mónica	0337-OS
Gutiérrez Salmerón, María	0493-R/M-P, 0317-P, 0333-P, 0355-R/M-P, 0374-R/M-P
Gutiérrez Santiago, Francisco	0142-R/M-P
Gutiérrez Uzquiza, Álvaro	0380-R-P, 0390-P
Guzmán Aránguez, Ana	0082-R-P, 0128-R-P, 0188-P
Guzmán, Ana	0171-R-P
Guzmán, Manuel	0312-P, 0313-OS, 0515-OI, 0304-R/M-P

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Gygi, Steven P.	0032-R/M-P
H Bouzaher, Yanis	0419-P
Hang, Tania	0460-R/M-P
Hardisson, David	0429-R/M-P
Haro, Diego	0521-OI
Hendrix, Jelle	0429-R/M-P
Hermanns, Heike M.	0202-P
Hermenegildo, Carlos	0066-P
Hermosin, Jesus M	0322-R/M-P, 0478-R/M-P
Hernandez Baixauli, Julia	0162-P
Hernández, Alberto	0444-P
Hernández, Ana	0109-P
Hernández, Ana Sandra	0026-R/M-P
Hernández, Ángela Patricia	0507-R/M-P
Hernández, Félix	0033-R/M-P
Hernandez, Lluis	0185-R/M-P
Hernández Ainsa, Carmen	0486-R/M-P
Hernández Álvarez, María Isabel	0335-P
Hernández Caselles, Trinidad	0145-P
Hernández Hernández, Ángel	0062-P, 0069-R/M-P, 0098-R/M-P
Hernández Lao, Tamara	0504-R/M-P
Hernández Madrigal, Miguel	0487-R-OS
Hernández Sánchez, Catalina	0279-R/M-P
Hernansanz Agustín, Pablo	0034-R/M-P, 0470-OS, 0166-P
Herráez, Elisa	0202-P, 0250-P, 0223-P
Herrera, Alejandro	0104-R-P
Herrera, Blanca	0305-R/M-P
Herrera, Maria Guadalupe	0472-R-P
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Herrero Ruíz, Joaquín	0126-P
Herruzo, Esther	0433-P
Hichem Bouzaher, Yanis	0488-OS
Hidalgo, Alberto	0404-R/M-P
Hofkens, Johan	0429-R/M-P
Homar Ruano, Patricia	0515-OI, 0002-P
Hormeño, Silvia	0151-R-OS, 0283-P
Hoti, Gjylije	0037-R/M-P, 0259-P
Hubner, Norbert	0240-OS
Huecas, Sonia	0129-R-P
Huerga Gómez, Alba	0312-P, 0313-OS
Huerta Lopez, Carla	0045-R-P
Huertas Lárez, Raquel	0379-P
Huesa, Juanjo	0046-P, 0058-OS
Hugo, Martín	0189-R-P
Hurtado De Lera, Ana	0350-R-P
Ibañez, Borja	0432-P
Ibañez, Mariam	0214-P
Ibeas, Kevin	0004-R/M-OS, 0005-R-P, 0155-R-P
Iglesias, Guillermo R	0226-R/M-P
Iglesias Ara, Ainhoa	0276-R/M-P
Igual, J.carlos	0181-R-P
Igual, Juan Carlos	0102-R/M-P, 0181-R-P
Ijurko, Carla	0062-P
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Izquierdo, Isabel	0143-R-P
Izquierdo Lahuerta, Adriana	0253-R/M-P
Jabalera Ruz, Ylenia María	0302-P, 0226-R/M-P
Jahn, Andreas	0495-P
Jamin, Annette	0481-R-P
Japón, Miguel Ángel	0396-R/M-P, 0126-P
Jawed, Areeb	0172-R-OS
Jedrychowski, Mark	0032-R/M-P
Jerez, Carmen	0113-P
Jernborm, August	0171-R-P
Jiménez Carretero, Daniel	0059-P
Jiménez Guerrero, Rocío	0396-R/M-P
Jiménez Jiménez, Víctor	0059-P
Jiménez, Esperanza	0351-R-P
Jiménez, Nelia	0143-R-P, 0146-R-P
Jiménez, Rafael	0446-P
Jimenez, Verónica	0005-R-P
Jimenez Blasco, Daniel	0057-M-OS, 0262-R/M-P
Jimenez Chillaron, Jose P. C.	0263-R-P
Jiménez García, Carlos	0330-R-P
Jiménez García, Manuel	0218-P
Jiménez Hernaiz, María	0152-P
Jiménez López, Concepción	0226-R/M-P, 0302-P
Jiménez López, José M	0260-R/M-P
Jimenez Martinez, Marina	0471-R/M-P

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Jiménez Rojo, Noemi	0465-P
Jiménez Ruiz, Jesús	0063-R/M-P
Jiménez Salvador, Irene	0486-R/M-P
Jiménez Villegas, José	0509-OS
Jimeno González, Silvia	0111-P
Johansson, Jan	0391-P
Johnson, Rory	0291-R-P
Jordan, Albert	0497-OS
Jordano Raya, Marina	0157-R/M-P
Jorrín Novo, Jesús V.	0504-R/M-P
Jover, Ana	0425-P
Juan P., Bolaños	0057-M-OS
Juanes Velasco, Pablo	0352-OS, 0507-R/M-P
Juanhuix, Judith	0489-P
Juez Castillo, Graciela	0195-R/M-P
Jurado, Juan	0376-P
K. Dhakar, Nilesh	0359-R/M-P
Khazaei Monfared, Yousef	0259-P, 0359-R/M-P
Kiebler, Michael A.	0450-P
Kirstein, Martina	0501-R-P
Kleinekathöfer, Ulrich	0459-R/M-OS
Koller, Dora	0387-R/M-P
Konieczna, Jadwiga	0080-P
Kozik, Patrycja	0138-OS
Kratchmarova, Irina	0024-R/M-P
Kräusslich, Hans Georg	0457-R-P
Kreir, Mohamed	0459-R/M-OS
Kröller Schön, Swenja	0316-R/M-OS
Kronqvist, Nina	0391-P
Kumarasinghe, Lorena	0092-R/M-P
Kuntic, Marin	0316-R/M-OS
Kvandova, Miroslava	0316-R/M-OS
L. Cortajarena, Aitziber	0361-R/M-OS
Labella, Jose I.	0113-P, 0108-P
Lachiondo Ortega, Sofía	0196-R/M-P, 0191-R/M-P, 0207-R/M-OS, 0215-R/M-P, 0261-R/M-P, 0257-R/M-P, 0222-R-P
Ladero, Iraia	0191-R/M-P
Lafuente Gómez, Nuria	0153-R/M-P
Lago Maciel, Ana	0433-P
Lalinde, Elena	0420-P
Lamas Maceiras, Mónica	0180-R/M-P, 0244-P, 0314-R/M-P, 0315-R/M-P
Landeira Viñuela, Alicia	0352-OS, 0507-R/M-P
Langegger, Maria	0527-M-OI
Lanzas Olsina, Laura	0263-R-P
Lanzón, Borja	0253-R/M-P, 0267-R/M-P
La-Presa, Rebeca	0094-R/M-P, 0099-R/M-OS, 0203-R/M-P
Lara, Alberto	0428-P
Lara, Eliana	0349-R/M-P
Lara, Esther	0111-P
Lara, Rebeca	0420-P
Lara Astiaso, Ester	0194-P
Larrañaga, Maite	0186-R/M-OS
Larráyoz Ilundáin, Marta	0288-P
Larráyoz, Ignacio M.	0420-P
Larrea, Esther	0346-R-P, 0408-P
Larriba, María Jesús	0493-R/M-P
Lasa, Marina	0397-P
Latorre, Adriel	0290-R/M-P
Latorre, Ana	0153-R/M-P
Latorre, Eva	0119-R/M-P, 0159-P
Martínez Escardó, Laura	0070-R-P
Lázaro Carot, Laura	0501-R-P
Lazcanoiturburu, Nerea	0305-R/M-P
Lécrevisse, Quentin	0352-OS
Lecue Costas, Elena	0361-R/M-OS
Lee, Flora C.y.	0450-P
Leiva, Diego	0424-P
Leiva, Magdalena	0463-R/M-P
Leiva Vega, Luis	0032-R/M-P, 0463-R/M-P
León Navarro, David Agustin	0081-P
León, Eduardo Andrés	0195-R/M-P
León, Gonzalo	0496-R-P
Leon, Raquel	0197-P
Lerin, Carles	0263-R-P
Li, Ying	0445-P
Lidholm, Jonas	0481-R-P
Liekkinen, Juho	0476-OS
Lilie, Hauke	0459-R/M-OS
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Lillo Márquez, Alejandro	0174-R/M-P, 0173-R/M-P
Lillo, Jaume	0173-R/M-P
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Limón Mortés, M. Cristina	0126-P
Linares, María	0455-P
Liquori, Alessandro	0214-P
Lizcano, Jose Miguel	0252-P, 0281-P
Lladó, Isabel	0466-P, 0474-P
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Llamas González, Yessica Y	0488-OS
Llanes, Julia	0190-P
Llinàs, Pere	0251-R-P
Lloberas, Núria	0096-R-P
Llop, Antonio	0108-P, 0113-P
Llop, Esther	0014-R-P, 0015-R/M-P
Llop, Marta	0214-P
Llorca, Oscar	0275-R/M-P, 0283-P, 0519-OI, 0526-OI
Lluís, Carme	0258-R-P
Lohner, Karl	0177-P
López Alonso, Victoria	0467-R-P, 0485-R-OS
López Briones, Tania	0467-R-P, 0485-R-OS
Lopez Cortajarena, Aitziber	0394-R/M-P
López De Munain, Adolfo	0360-R/M-P
López López, Susana	0401-R-P
López Molina, Mari Paz	0221-P
López Quintela, Manuel Arturo	0358-P
López, Icíar P.	0061-R-P, 0420-P, 0042-R/M-P
López, Javier	0482-R/M-OS
López, Juan Antonio	0032-R/M-P
López, Judith	0017-R/M-P
López, Raquel	0219-P
López Briones, Tania	0496-R-P
López Cara, Luisa C	0226-R/M-P, 0260-R/M-P
López Corcuera, Beatriz	0351-R-P
López Domènech, Sandra	0127-P, 0425-P
Lopez Fabuel, Irene	0057-M-OS
López Gallardo, Ester	0486-R/M-P
López García, Marta	0282-R/M-P
Lopez Guerra, Diego	0125-P
Lopez Guillermo, Armando	0185-R/M-P
López Hoyos, Marcos	0215-R/M-P
López Lara, Isabel Mª	0307-P
López López, Susana	0341-P
López Malo, Maria	0107-P
López Martín, Estrella	0064-R-P
López Miranda, Santiago	0280-R/M-P
López Morente, Miriam	0239-M-P
López Nicolás, José Manuel	0037-R/M-P, 0051-R-P
López Oliva, Mª Elvira	0056-R/M-P
López Quintela, Arturo	0369-P
López Rivas, Abelardo	0040-R-P
López Rodriguez, Elena	0335-P
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López Rosa, Raquel	0041-P, 0204-P
López Valls, Maria	0309-R/M-OS
Lopitz Otsoa, Fernando	0209-R/M-P
Lorenz Fonfria, Victor A.	0337-OS
Lorenzo Gotor, Nieves	0139-R-P
Lorenzo Martín, Luis Francisco	0485-R-OS
Lorenzo, Julia	0446-P
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Lorenzo Catoira, Lidia	0180-R/M-P, 0244-P, 0314-R/M-P
Lorizate, Maier	0454-P, 0456-P, 0457-R-P
Lozano Terol, Gema	0008-R-P, 0436-P
Lozano, Elisa	0223-P, 0250-P
Lozano Ureña, Anna	0501-R-P
Lu, Lu	0077-P
Lucas, Julie	0245-R/M-P
Lucas, María	0505-R/M-P
Lucea, Susana	0205-P
Lucena, M Isabel	0196-R/M-P
Lucendo, Estefanía	0424-P
Luciano, Fedra	0487-R-OS
Luders, Jens	0275-R/M-P
Lue, Neal F.	0283-P
Luengo, Yurena	0153-R/M-P
Luis Pedraz, José	0422-OS
Luis Lima, Sergio	0253-R/M-P, 0267-R/M-P
Lumpuy Castillo, Jairo	0460-R/M-P
Luna Giles, Francisco	0480-P, 0483-P
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Luque Garriga, Francisco Javier	0286-P
Luque, F. Javier	0049-P
Luque Huertas, Raúl	0468-R/M-P
Luque Navarro, Pilar M	0226-R/M-P, 0260-R/M-P
Lutz, Beat	0313-OS
Lutz, Susanne	0394-R/M-P
M. Herrero, Jorge	0464-R/M-P
M. Mielu, Lidia	0221-P
Mabe, Jon	0222-R-P
Macià, Anna	0074-P
Macian, Fernando	0023-R/M-P
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Madeira, Ana	0473-P
Madrid Valiente, Alejandro	0114-P
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Manich, Gemma	0038-P
Manjarrés Raza, Israel	0304-R/M-P
Manocchio, Francesca	0067-M-P
Manrique, Inmaculada	0410-R-P
Manso, Luis	0515-OI
Manso, Yasmina	0443-P
Manzano Cuesta, Anna	0220-R/M-P, 0096-R-P, 0178-P, 0216-R/M-P
Manzano Figueroa, Sara	0208-R/M-P
Manzano, Sara	0390-P
Marcilla, Antonio	0122-R/M-P
Marco, Carmen	0260-R/M-P
Marcon, Luciano	0527-M-OI
Marcos Prado, Teresa	0071-R/M-P
Marente, Julia	0357-R-P
Marfany, Gemma	0093-R/M-OS, 0254-R-P
Marí, Miquel	0181-R-P
Maria Del Bas, Josep	0162-P
María Herranz, Jose	0222-R-P
Marín, José J.g.	0202-P, 0223-P, 0209-R/M-P, 0215-R/M-P
Marin, Julia V	0349-R/M-P
Marín, M. Pilar	0434-P
Marin, Silvia	0191-R/M-P
Marina, Alberto	0046-P
Marina Zarate, Ester	0140-R-OS
Marín Baquero, Mikel	0295-P, 0423-R/M-P
Maroto Blasco, Alba	0453-P

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Marqués, Javier	0025-M-OS
Marquina, Maribel	0190-P
Marrero, Ana Dácil	0365-R/M-P, 0403-R-P
Marrero, Pedro F.	0521-OI
Marsal Beltran, Anna	0473-P
Martí, Eulàlia	0087-OI
Martí, Rosa Mª	0074-P
Martí Díaz, Román	0145-P
Martín García, José Manuel	0036-R/M-P
Martín López, Gerardo	0399-P
Martin Nieto, Jose	0137-R-P
Martín Orozco, Rosa M	0317-P
Martín, Aina	0495-P
Martín, César	0227-P, 0196-R/M-P, 0207-R/M-OS
Martin, Edgar	0190-P
Martín, Franz	0083-R/M-P
Martin, Mª Angeles	0056-R/M-P
Martín, Mairena	0081-P, 0121-P
Martín, Marian	0080-P
Martin, Silvia	0185-R/M-P
Martín Bernardo, Belén	0194-P
Martín Clar, Es-Perança	0218-P
Martin Cofreces, Noa	0375-P
Martín Colom, Julia	0468-R/M-P
Martín Cuevas, Eva M.	0295-P, 0423-R/M-P
Martín Expósito, Manuel	0142-R/M-P
Martínez Amores, Brezo	0317-P
Martínez Blanco, Elena	0354-P
Martínez Chantar, Mª Luz	0196-R/M-P
Martínez De La Cruz, Luis Alfonso	0196-R/M-P

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Martínez De La Torre, Margaret	0076-P
Martinez De Marañon, Aranzazu	0425-P
Martinez De Tejada, Guillermo	0346-R-P, 0177-P
Martínez Férriz, Arantxa	0211-R/M-P, 0500-P
Martínez Máñez, Ramón	0422-OS
Martínez Poveda, Beatriz	0365-R/M-P
Martínez Ruiz, Antonio	0166-P
Martinez Valverde, Angela	0065-R/M-P
Martínez, Alfonso	0261-R/M-P
Martínez, Almudena	0428-P
Martínez, Cristina	0214-P
Martinez, Guillermo	0147-R-P
Martínez, J. Alfredo	0160-P
Martínez, Mª Isabel	0158-P
Martínez, Pau Alfonso	0257-R/M-P
Martinez, Salvador	0023-R/M-P
Martínez, Sergio	0123-P
Martinez, Sonia	0444-P
Martínez, Vicente	0428-P
Martínez Barba, Enrique	0145-P
Martinez Bernabe, Toni	0417-P, 0431-P, 0453-P
Martínez Blanco, Elena	0348-R-P
Martinez Calle, Marta	0476-OS
Martínez Chacón, Guadalu-Pe	0278-R-P, 0308-R-P, 0311-R-P
Martínez Chantar, María Luz	0083-R/M-P, 0207-R/M-OS, 0215-R/M-P, 0460-R/M-P, 0209-R/M-P, 0222-R-P, 0257-R/M-P, 0261-R/M-P, 0276-R/M-P, 0191-R/M-P
Martínez Cignoni, Melanie Raquel	0474-P, 0466-P
Martínez Climent, Jose Ángel	0288-P
Martínez Cruz, Luis Alfonso	0083-R/M-P, 0207-R/M-OS

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Martínez Delgado, Beatriz	0064-R-P
Martínez Garay, Carlos	0309-R/M-OS
Martínez Garza, Úrsula	0521-OI
Martínez Gil, Luis	0114-P
Martínez González, Salomé	0133-R-P
Martínez Irujo, Juan José	0025-M-OS
Martínez Junquera, Mónica	0420-P
Martínez Lombardia, Eva	0505-R/M-P
Martínez López, Joaquín	0455-P
Martínez Macías, María Isabel	0157-R/M-P
Martinez Martinez, Arturo	0210-R/M-P
Martínez Martínez, Desirée	0397-P
Martinez Morales, Juan	0141-OS
Martínez Moreno, Álvaro	0282-R/M-P
Martinez Peula, Oihane	0169-R/M-P
Martínez Poveda, Beatriz	0328-R-P, 0366-R-P, 0403-R-P, 0410-R-P
Martínez Ruiz, Antonio	0034-R/M-P, 0189-R-P
Martínez Teipel, Blanca	0452-P
Martínez Useros, Javier	0333-P
Martínez Vigara, Maria	0418-P
Martínez Vivancos, Adrián	0436-P
Martín González, Aurora	0505-R/M-P
Martín Hidalgo, David	0350-R-P, 0502-P, 0437-P
Martín Martín, Yolanda	0149-R-P, 0156-R-P
Martín Orozco, Rosa María	0493-R/M-P, 0333-P, 0355-R/M-P, 0374-R/M-P
Martín Pedraza, Laura	0481-R-P
Martín Prieto, Eva	0385-P
Martins, Filipa	0022-R/M-OS
Martín Santamaría, Sonsoles	0233-R/M-OS
Martín Taboada, Marina	0267-R/M-P

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Marzese, Diego	0251-R-P
Marzo, Isabel	0143-R-P, 0146-R-P
Mas, Aina	0472-R-P
Mascaró, Catalina M.	0160-P
Masiá, Jesús	0097-R/M-P, 0101-R/M-P
Masiá Adalid, Susana	0078-P
Maslon, Magdalena	0497-OS
Massaguer, Anna	0014-R-P
Mas Sanchez, Aina	0452-P
Mata Martínez, Pablo	0467-R-P, 0485-R-OS, 0496-R-P
Mata, Ana M.	0324-P
Matallana, Emilia	0370-P
Matamoros Recio, Alejandra	0233-R/M-OS
Matarredona, Esperanza R	0104-R-P
Matas Cespedes, Alba	0185-R/M-P
Matencio Durán, Adrián	0259-P
Matencio, Adrián	0037-R/M-P, 0051-R-P, 0359-R/M-P
Mateo García, Lorena	0363-P
Mateos, David	0130-P, 0160-P
Mateos, Jose M	0125-P
Mateos White, Isabel	0501-R-P
Mateus, Andre	0172-R-OS
Matheu, Ander	0360-R/M-P
Matias Guiu, Xavier	0074-P
Matínez, Guadalupe	0100-R-P
Mato, Susana	0312-P, 0313-OS
Mato Basalo, Rocío	0239-M-P
Matorras, Roberto	0024-R/M-P

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Matthews Palmer, Teige	0303-P
Matute, Carlos	0265-P, 0312-P, 0313-OS
Mayán Santos, María D.	0079-R/M-OS, 0217-R/M-P
Mayo, Juan C.	0179-R-P
Mayor, Ugo	0092-R/M-P
Mazariegos, Marina	0444-P
Mazuecos, Lorena	0044-R/M-P
Mckay, Tristan R.	0057-M-OS
Meana, Javier	0169-R/M-P
Medina Torres, Miguel Ángel	0365-R/M-P
Medina, Diego L.	0057-M-OS
Medina, José M.	0256-R-P, 0104-R-P
Medina, Miguel Ángel	0328-R-P, 0389-R-P, 0403-R-P, 0410-R-P
Medina, Milagros	0054-P
Medina Gómez, Gema	0253-R/M-P, 0267-R/M-P
Medrano, Francisco J.	0129-R-P
Megias, Elisabet	0281-P
Megías Roda, Elisabet	0252-P
Meinnel, Thierry	0248-OS
Melero, Beatriz	0325-P
Memon, Danish	0172-R-OS
Mena, Adriana	0426-R/M-P
Mena Morlans, Ingrid	0238-P
Méndez, Ester	0181-R-P
Méndez Gúzman, Iván	0505-R/M-P
Mendez Villamil, Sara	0230-R-P
Mendia, Javier	0407-P
Mendiola, Marta	0429-R/M-P

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Menéndez Mendez, Aida	0112-P
Menendez Gutierrez, Maria Piedad	0115-R-P
Mengual, Regina	0373-R/M-P, 0478-R/M-P
Menguiano Vázquez, Tamara	0199-R/M-P
Mera, Paula	0004-R/M-OS, 0005-R-P, 0155-R-P
Mercader, Josep	0495-P, 0503-P
Mercado Gómez, María	0222-R-P, 0257-R/M-P, 0196-R/M-P, 0261-R/M-P
Merchante, Catharina	0366-R-P
Merckx, Pieterjan	0476-OS
Merfort, Irmgard	0393-P
Merhi, Faten	0346-R-P
Merino, Nekane	0129-R-P
Mesa Valle, Concepción M.	0321-P, 0323-P
Mesonero, José Emilio	0159-P, 0119-R/M-P
Mestre, Daniela	0276-R/M-P
Mietrach, Nicole	0519-OI
Milagro, Fermín	0246-R/M-OS
Milisenda, José César	0013-R/M-P, 0187-R/M-P
Milkiewicz, Malgorzata	0215-R/M-P
Milkiewicz, Piotr	0215-R/M-P
Millán, Gonzalo	0420-P
Millán, José María	0214-P
Mingarro, Ismael	0114-P, 0345-R-P
Mingo Casas, Patricia	0221-P
Miralles, Marc	0237-P
Miranda Quintana, María Del Pilar	0239-M-P
Miras Portugal, María Teresa	0112-P, 0076-P
Miró, Laura	0014-R-P
Mirra, Serena	0443-P
Mocavini, Ivano	0512-OI
Mole, Sara E.	0057-M-OS
Molina, Alexis	0477-P
Molina, Elena	0191-R/M-P
Molina, Maria Luisa	0023-R/M-P
Molina Alarcón, Milagros	0097-R/M-P, 0101-R/M-P, 0122-R/M-P
Molina Fernandez, Ruben	0477-P
Molina Hernández, Verónica	0282-R/M-P
Moltó, María Dolores	0170-R-P, 0363-P
Monasterio, Bingen G.	0465-P
Monedero, Carlota	0109-P
Moneriz, Carlos	0028-R-P
Monfared, Yousef Khazaei	0037-R/M-P
Monjo, Marta	0495-P
Monory, Krisztina	0313-OS
Monsalve, Eva María	0341-P, 0494-R/M-P, 0387-R/M-P
Monserrat Mesquida, Margalida	0130-P, 0160-P
Montalvillo, Enrique	0507-R/M-P
Monte, María Jesús	0202-P, 0215-R/M-P, 0223-P
Montemayor, Sofía	0160-P
Montenegro, María F.	0145-P
Montero Calle, Ana	0082-R-P, 0128-R-P, 0429-R/M-P
Montero, Ana	0171-R-P
Montero, Juan Carlos	0041-P
Montero, Nuria	0085-P
Montes, Lorena	0197-P, 0230-R-P
Montes, Ruth L	0407-P
Montesinos, Pau	0214-P
Montironi, Chiara	0487-R-OS
Montoya, Julio	0060-R-P, 0064-R-P, 0486-R/M-P
Moog, Christiane	0245-R/M-P
Mora, Alfonso	0032-R/M-P, 0463-R/M-P
Mora, Vicente	0127-P, 0425-P
Morales Fernández, María Luz	0455-P
Morales, Enrique	0267-R/M-P
Morales, José Manuel	0383-P
Morales Vidal, Carmen	0470-OS
Mora Molina, Rocio	0040-R-P
Moran Alvarez, Alba	0179-R-P
Morán Costoya, Andrea	0466-P, 0474-P
Moranta, David	0218-P
Morante Redolat, J.manuel	0181-R-P
Morant Ferrando, Brenda	0057-M-OS, 0262-R/M-P
Mora Santos, Mar	0126-P
Morel, Bertrand	0245-R/M-P
Morén, Constanza	0013-R/M-P, 0026-R/M-P, 0187-R/M-P
Moreno Herrero, Fernando	0164-P
Moreno, Estefanía	0515-OI, 0002-P
Moreno, Fermín	0384-R/M-P
Moreno, Jose Carlos	0221-P
Moreno, M. Teresa	0420-P
Moreno, Miguel	0206-OS
Moreno, Nerea	0190-P
Moreno, Pedro	0013-R/M-P, 0187-R/M-P
Moreno Bueno, Gema	0515-OI
Moreno Caceres, Joaquim	0487-R-OS
Moreno García, Leticia	0509-OS
Moreno Herrero, Fernando	0151-R-OS, 0283-P, 0295-P, 0423-R/M-P
Moreno Recio, María Dolores	0157-R/M-P
Moreno Yruela, Carlos	0451-P
Morillas, Carlos	0127-P, 0425-P
Morillo, Margarita	0472-R-P
Morón Ros, Samantha	0409-OS
Morote, Mireya	0214-P
Mosteiro, Lorena	0276-R/M-P
Mota Brea, Manuel	0442-R-P
Mudge, Jonathan	0240-OS
Mueller, Patrick	0527-M-OI
Muguerza, Begoña	0067-M-P, 0449-P
Mulero, Miquel	0162-P, 0449-P
Munar Gelabert, Margalida	0453-P
Munarriz Cuezva, Eva	0169-R/M-P
Munro, Sean	0225-R/M-OS
Münzel, Thomas	0316-R/M-OS
Muñiz, Pilar	0325-P, 0381-P
Muñoa Hoyos, Iraia	0024-R/M-P
Muñoz, Alberto	0467-R-P, 0493-R/M-P
Muñoz, Pau	0281-P
Muñoz, Sergio	0005-R-P
Muñoz Centeno, Mari Cruz	0111-P, 0117-OS
Muñoz Fontela, César	0419-P
Muñoz López, Sonia	0121-P
Muñoz Moreno, Laura	0379-P
Muñoz Muñoz, José Luis	0084-P
Muñoz Pinedo, Cristina	0487-R-OS
Muratore, Veronica	0193-R/M-P
Murillo Cuesta, Silvia	0194-P
N. Iriondo, Marina	0407-P
Nadal, Xavier	0173-R/M-P
Nadal Serrano, Mercedes	0508-P
Nam Cha, Syong Hyun	0277-R-OS
Naroa Goikoetxea Usandizaga, Naroa	0207-R/M-OS
Naval, Javier	0143-R-P, 0146-R-P
Navaridas, Raul	0252-P
Navarro Brugal, Gemma	0174-R/M-P, 0258-R-P
Navarro Ramírez, Eliezer	0317-P
Navarro, Enrique	0101-R/M-P
Navarro, Francisco	0142-R/M-P
Navarro, Gemma	0173-R/M-P, 0175-R/M-P
Navarro, Juan Antonio	0124-P, 0170-R-P
Navarro, Pablo	0292-R-P
Navarro Masi-P, Èlia	0067-M-P
Navarro Orcajada, Silvia	0037-R/M-P, 0051-R-P
Navarro Ramírez, Eliezer	0333-P, 0355-R/M-P, 0493-R/M-P, 0374-R/M-P
Navarro Sabaté, Àurea	0096-R-P, 0220-R/M-P, 0178-P
Nayak, Ramesh	0115-R-P
Nesme, Joseph	0310-P
Nguewa, Paul A.	0236-R-OS, 0408-P, 0298-P, 0346-R-P, 0177-P
Nicola Llorente, Mariano	0521-OI
Nicolás, Francisco Jose	0280-R/M-P
Nieto Garai, Jon Ander	0454-P, 0456-P, 0457-R-P
Nieto Jimenez, Cristina	0204-P, 0219-P, 0041-P
Nieva Zuluaga, Ane	0353-P
Nieves, Manuel	0428-P
Nikolic, Ivana	0463-R/M-P
Nilsson, Peter	0171-R-P
Niso Santano, Mireia	0278-R-P, 0308-R-P, 0311-R-P, 0100-R-P
Noblejas López, María Del Mar	0041-P, 0219-P, 0204-P
Nogales, Rogelio	0310-P
Nogueiras, Rubén	0032-R/M-P, 0209-R/M-P, 0261-R/M-P, 0402-R/M-P
Nogués, Laura	0444-P
Nourshargh, Sussan	0378-R-P
Novella, Susana	0066-P
Novellasdemunt, Laura	0178-P
Novelle, Marta G.	0402-R/M-P
Novillo Quirola, Danielle	0361-R/M-OS
Novo, Nerea	0054-P
Novo, Paula	0242-OS
Nuncia Cantarero, Miriam	0204-P, 0219-P
Núñez, Enrique	0351-R-P
Nuñez, Vanessa	0115-R-P
Núñez Ramírez, Rafael	0058-OS
Núñez Roa, Cati	0473-P
Nurtdinov, Ramil	0291-R-P
O´Connor, José Enrique	0383-P
O´Rourke, Colm J.	0276-R/M-P
O'connor, José Enrique	0299-P, 0301-P, 0383-P
Obaya, Álvaro J.	0039-R/M-P
Oberoi, Pranav	0217-R/M-P
Ocaña, Alberto	0041-P
Ocaña, Ana V.	0097-R/M-P, 0101-R/M-P, 0122-R/M-P
Ochoa, David	0172-R-OS
Ochoa Bueno, Blanca Isabel	0265-P
Ochoa Grullón, Juliana	0330-R-P
Odriozola Gil, Yosu	0386-R-P, 0111-P

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Oelze, Matthias	0316-R/M-OS
Olarte, Andrea	0289-R/M-P
Olazar Intxausti, June	0456-P
Oliva, Baldo	0438-R/M-P, 0477-P
Oliva, José Luis	0467-R-P, 0496-R-P
Oliva, Marian	0510-OI
Oliván, Irene	0228-P
Olivares González, Lorena	0422-OS
Oliveira, Marvin	0377-P
Olivencia, Lorena	0302-P
Oliver, Eduardo	0432-P
Oliver, Jordi	0416-P, 0417-P, 0418-P, 0431-P, 0447-R/M-P, 0453-P, 0458-P
Oliver, Paula	0080-P, 0395-R/M-P
Oliver Pons, Carla	0503-P
Olivos Oré, Luis Alcides	0112-P, 0076-P
Olloqui Sariego, José Luis	0198-P
Olmeda, Bárbara	0335-P, 0476-OS
Olmo, Rosa	0085-P
Olsen, Christian A.	0451-P
Ordinas, Margarita	0237-P
Orellana, Guillermo	0404-R/M-P
Orfao, Alberto	0352-OS
Oria Muriel, Manuel A	0045-R-P
Orquín González, Miguel Ángel	0299-P, 0301-P, 0383-P
Orrego, Lina M.	0195-R/M-P
Ortega Rodríguez, Judith	0485-R-OS, 0467-R-P, 0496-R-P
Ortega, Esperanza	0123-P
Ortega, Felipe	0076-P, 0112-P
Ortega, Gabriel	0495-P
Ortega, Natividad	0284-P
Ortega, Sagrario	0001-R-P
Ortega Vidal, Juan	0365-R/M-P
Ortiz Mellet, Carmen	0065-R/M-P
Ortiz Mateu, Juan	0114-P
Ortiz Ruiz, Alejandra	0455-P
Ortuño, Joaquin A.	0084-P
Orzáez, Mar	0393-P, 0424-P
Osinalde, Nerea	0024-R/M-P
Osta, Rosario	0509-OS
Ostolaza, Helena	0227-P
Otaegi, Sara	0457-R-P
P. Devos, Damien	0438-R/M-P
P. Xirodimas, Dimitris	0209-R/M-P
Pablo Torres Jiménez, Carmela	0235-R/M-P
Paes, Ana Belén	0066-P
Pajares, Maria A.	0095-P
Pajuelo Lozano, Natalia	0464-R/M-P
Palacín, Manuel	0526-OI, 0406-R/M-P
Palacín Aliana, Irina	0132-R/M-P
Palacios, David	0284-P
Palacios, Ivonne	0004-R/M-OS
Palacios, Nuria	0317-P
Palacios Marin, Ivonne	0263-R-P
Palao Treviño, Nerea	0208-R/M-P, 0380-R-P, 0390-P
Palazon, Asis	0191-R/M-P, 0207-R/M-OS
Palazuelos, Javier	0312-P, 0313-OS
Pallardó, Federico V	0247-P, 0170-R-P
Pallarés, Pilar	0429-R/M-P
Pallàs, Mercè	0004-R/M-OS
Palmero, Mercedes	0137-R-P
Palomera, Luis	0143-R-P
Palomero, Jesús	0385-P
Palou, Andreu	0080-P, 0105-P, 0274-R/M-P, 0343-P, 0479-R/M-P
Palou, Mariona	0105-P, 0269-R/M-P
Pamblanco, Merce	0349-R/M-P
Pandiella, Atanasio	0041-P
Paniagua Herranz, Lucía	0112-P
Panisello, Mar	0004-R/M-OS
Paraíso, West Kristian D.	0272-P
Pardal, Ricardo	0442-R-P
Pardo Sánchez, José Miguel	0211-R/M-P
Pardo, Demian	0309-R/M-OS
Pardo, Jose M.	0428-P
Pardo, Julián	0393-P
Pardo, Leonardo	0258-R-P
Pardo Díaz, Rosario	0031-R/M-P, 0030-R/M-OS
Pardo Marqués, Virginia	0149-R-P, 0156-R-P
Paredes, Ana	0115-R-P
Paredes, Marta	0100-R-P
Paredes, Sergio	0435-R-P
Paredes Barquero, Marta	0278-R-P, 0308-R-P, 0311-R-P
Paredes Martínez, Francisco	0046-P
Pareja Sánchez, Yerma	0111-P
Parés, Xavier	0446-P
Parets, Sebastián	0237-P
Paricio, Nuria	0415-P, 0147-R-P
Pariente, José Antonio	0483-P, 0480-P
Parisini, Emilio	0226-R/M-P, 0260-R/M-P
Parra Izquierdo, Iván	0482-R/M-OS
Parra Vargas, Marcela	0263-R-P
Parrilla, Manuel	0496-R-P
Pastrana, Cesar L	0164-P
Patel, Gaurangkumar	0387-R/M-P
Pavón Trujillo, Dácil María	0059-P
Payán, Laura	0111-P
Pazos, Elena	0242-OS
Pedrazza, Leonardo	0210-R/M-P
Pedrosa, María	0504-R/M-P
Peinado, Héctor	0444-P
Peinado, José	0285-P
Peinador, Carlos	0242-OS
Pejenaute, Álvaro	0025-M-OS
Peláez García, Alberto	0082-R-P, 0128-R-P, 0429-R/M-P
Peleato, Mª Luisa	0270-R/M-P, 0339-R/M-P, 0331-R/M-P
Pelegrín, Pablo	0393-P
Peña Jiménez, Daniel	0467-R-P, 0485-R-OS
Peña, Jorge	0421-P
Peña Guerrero, José	0177-P, 0236-R-OS, 0408-P
Peñas, Ana	0494-R/M-P
Peñate, Xenia	0111-P
Pequerul, Raquel	0446-P
Peracaula, Rosa	0014-R-P, 0015-R/M-P
Peral, Belén	0387-R/M-P
Perálvarez Marín, Alex	0038-P
Perdomo, Germán	0136-P
Pérez Valderrama, Begoña	0396-R/M-P
Perez Gonzalez, Nataly EmPeratriz	0361-R/M-OS
Perez Inestrosa, Ezequiel	0095-P
Pérez López, Cristina	0330-R-P
Pérez Sen, Raquel	0112-P
Pérez, Beatriz	0500-P
Pérez, Catalina	0175-R/M-P
Pérez, Elena	0085-P
Pérez Alea, Mileidys	0446-P
Pérez Álvarez, María José	0399-P
Pérez Arévalo, José	0282-R/M-P
Pérez Aso, Miguel	0452-P
Pérez Cremades, Daniel	0066-P
Pérez Dorado, Inmaculada	0248-OS
Pérez Fernández, Alejandro	0098-R/M-P
Pérez Fernández, Jorge	0142-R/M-P
Perez Galan, Patricia	0185-R/M-P
Pérez García, Ana	0149-R-P, 0156-R-P
Pérez Gil, Jesús	0335-P, 0335-P, 0404-R/M-P, 0476-OS
Pérez Gómez, Eduardo	0515-OI
Pérez Gutiérrez, Lorena	0378-R-P
Perez Jimenez, Raul	0392-R-P, 0302-P
Pérez Lluch, Sílvia	0291-R-P
Pérez Lorite, Neus	0238-P
Pérez Martínez, Francisco Carlos	0122-R/M-P, 0097-R/M-P, 0101-R/M-P
Pérez Mejías, Gonzalo	0198-P
Pérez Sala, Dolores	0095-P
Pérez Sánchez, Natalia	0481-R-P
Pérez Silva, Laura	0223-P, 0250-P
Pérez Tanoira, Ramón	0097-R/M-P, 0122-R/M-P
Pérez Victoria, José M.	0195-R/M-P
Pertusa, Clara	0299-P, 0301-P
Perugorria, Maria J.	0276-R/M-P
Péter, Mária	0237-P
Petrov, Petar	0222-R-P, 0257-R/M-P, 0261-R/M-P
Picazo, Cecilia	0370-P
Pichel, José G	0061-R-P, 0420-P, 0042-R/M-P
Picher, Carmen	0247-P
Picó, Catalina	0269-R/M-P
Pilar Izquierdo, María C.	0284-P
Pimenta Lopes, Carolina	0210-R/M-P, 0216-R/M-P
Pineda Sanatella, Alberto	0490-P
Pintado, Cristina	0044-R/M-P
Pintor, Aránzazu	0463-R/M-P
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Planelles Herreros, Vicente J.	0225-R/M-OS
Plata Gómez, Ana Belén	0268-R/M-P
Platero Luengo, Aida	0442-R-P
Poch, Enric	0288-P
Polvillo, Rocio	0141-OS
Pomar, Catalina A.	0269-R/M-P, 0273-R/M-P, 0479-R/M-P
Pons, Antoni	0287-P
Pons, Daniel G	0416-P, 0447-R/M-P, 0458-P, 0418-P, 0417-P, 0431-P, 0453-P, 0508-P
Porcar, Manuel	0290-R/M-P
Porcuna Doncel, Jesus	0115-R-P
Porras Gallo, Almudena	0208-R/M-P, 0380-R-P, 0390-P
Porras, Marta	0413-P
Porrini, Esteban	0253-R/M-P, 0267-R/M-P
Porto, Vanesa	0358-P, 0369-P

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Poveda, Ana	0349-R/M-P
Poves, Carmen	0128-R-P
Prat, Norbert	0176-P
Prats, Neus	0335-P
Prats Ejarque, Guillem	0077-P
Prensner, Jon	0240-OS
Prescott, Mark	0285-P
Prezado, Yolanda	0358-P
Pricolo, María Rosaria	0045-R-P, 0075-P
Prieto, Ignacio	0494-R/M-P
Prieto Álamo, María José	0376-P
Prieto Bermejo, Rodrigo	0098-R/M-P
Proenza, Ana María	0474-P, 0466-P
Prósper, Felipe	0025-M-OS
Pucciarelli, M. Graciella	0046-P
Puelles, Luis	0076-P
Puglia, Michele	0024-R/M-P
Puig, Ángela	0435-R-P
Pulido Gómez, Nerea	0321-P, 0323-P
Pulido Quetglas, Carlos	0291-R-P
Puras, Gustavo	0422-OS
Puyet, Antonio	0492-OS
Quader, Sabina	0272-P
Quarantotti, Valentina	0138-OS
Quereda, Cristina	0137-R-P
Quesada, Ana R.	0328-R-P, 0389-R-P
Quetglas, María Magdalena	0287-P
Quetglas Llabrés, Maria Magdalena	0130-P, 0160-P
Quilis, Inma	0181-R-P
Quintana Cabrera, Rubén	0304-R/M-P, 0516-OI, 0057-M-OS
Quintela, Cesar	0494-R/M-P
Quintero, Francisco J.	0428-P
Quiñones, Mar	0366-R-P
R. Artalejo, Antonio	0076-P
R. Ferrón, Sacri	0501-R-P
R. Macías, Rocío I	0250-P
R. Quesada, Ana	0365-R/M-P, 0403-R-P, 0410-R-P
R. Varela, Yaiza	0407-P
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Raïch, Iu	0173-R/M-P, 0175-R/M-P
Ramírez, Cristina M	0149-R-P, 0156-R-P
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Ramírez, Manel	0015-R/M-P
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Rancan, Lisa	0435-R-P
Raote, Ishier	0528-OI
Raspopovic, Jelena	0527-M-OI
Razzaghi, Neda	0472-R-P
Rebassa, Joan Biel	0175-R/M-P
Redón, Josep	0124-P
Reglero Real, Natalia	0378-R-P
Reguera, Ana Cristina	0231-P, 0186-R/M-OS
Reifs, Antonio	0302-P, 0392-R-P
Reina Aibar, Marc	0004-R/M-OS
Rejano Gordillo, Claudia María	0043-R-P
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Requejo, Felix	0369-P
Resel, Eva	0312-P
Reyes, José C.	0111-P
Reyes, Marjorie	0004-R/M-OS
Reyes, Melissa	0028-R-P
Reyes Palomares, Armando	0455-P, 0492-OS
Reynés, Bàrbara	0080-P, 0105-P, 0395-R/M-P
Reynés, Clara	0287-P
Rey Souto, Cora	0315-R/M-P
Ribas Aulinas, Francesc	0263-R-P
Ribot, Joan	0105-P, 0269-R/M-P, 0273-R/M-P, 0343-P
Ricote, Mercedes	0115-R-P, 0253-R/M-P
Riera Begué, Ainhoa	0339-R/M-P
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Rincón, Mercedes	0215-R/M-P
Río, Carlos	0495-P
Ríos, Antonio	0260-R/M-P
Ríos, Rosa M.	0090-P
Ríos Marco, Pablo	0260-R/M-P
Ritzefeld, Markus	0248-OS
Rivas, Carmen	0400-P, 0419-P, 0488-OS
Rivas Delgado, Alfredo	0185-R/M-P
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Rivera Hernandez, Geovanny	0307-P
Rivero, Guadalup e	0169-R/M-P
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Rivière, Frédéric	0248-OS
Robles Guirado, Jose Angel	0176-P
Roca, Angela	0452-P
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Roca Agujetas, Vicente	0471-R/M-P
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Rodal Bravo, Lucia	0221-P
Rodenas, Reyes	0428-P
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Rodrigo, Alberto	0074-P
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Rodríguez Moratinos, Ana Beatriz	0480-P
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Rodriguez, Cristina	0373-R/M-P
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Rodríguez, Manuel S	0207-R/M-OS, 0400-P
Rodríguez, María Elena	0032-R/M-P, 0463-R/M-P
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Rodríguez, Rosalía	0272-P
Rodriguez, Vanina	0185-R/M-P
Rodríguez Agudo, Rubén	0222-R-P, 0257-R/M-P, 0261-R/M-P
Rodríguez Belmonte, Esther	0314-R/M-P, 0315-R/M-P, 0342-R-P
Rodriguez Candela Mateos, Marina	0217-R/M-P
Rodriguez Carvallo, Eddie	0210-R/M-P
Rodríguez Cuellar, Elías	0267-R/M-P
Rodríguez Cumbreras, Pablo	0442-R-P
Rodríguez De La Rosa, Lourdes	0055-P, 0086-R-P
Rodriguez Diaz, Ciro	0309-R/M-OS
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Rodríguez García, Alba	0455-P
Rodríguez García, Ana	0178-P
Rodriguez Iruretagoyena, Begoña	0191-R/M-P
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Rodríguez Peña, M Mar	0473-P
Rodríguez Pérez, Rosa	0504-R/M-P
Rodríguez Rodríguez, Ana Elena	0253-R/M-P, 0267-R/M-P
Rodríguez Rodríguez, Rosalía	0186-R/M-OS, 0231-P
Rodriguez Ronchel, Ana	0140-R-OS
Rodriguez Vigil, Carmen	0486-R/M-P
Roeder, Jasmin	0217-R/M-P
Rojas Cabañeros, José María	0467-R-P
Rojas Cabañeros, José María	0485-R-OS, 0496-R-P
Rojo, Ana I	0509-OS
Rolas, Loic	0378-R-P
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Roldán Torres, Ildefonso	0127-P
Romaguera, Dora	0080-P
Roman Fernández, Jose Luis	0317-P, 0333-P, 0355-R/M-P, 0374-R/M-P, 0493-R/M-P
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Romero, Antonio	0129-R-P
Romero, Esperanza	0310-P
Romero, Francisco	0126-P, 0396-R/M-P
Romero Albillo, María V.	0097-R/M-P
Romero Picó, Amparo	0402-R/M-P
Romero Sánchez, Carlos Manuel	0341-P
Romo González, Marta	0069-R/M-P, 0098-R/M-P
Roncero, Cesáreo	0305-R/M-P
Roncero, Vicente	0125-P
Ros, Manuel	0253-R/M-P
Roscales, Mikel	0167-R-OS
Ros Carrero, Cristina	0102-R/M-P
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Rosón Burgo, Beatriz	0214-P
Ross Thriepland, Douglas	0138-OS
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Roy, Rosa	0271-R/M-P
Rubin Pedrazzo, Alberto	0259-P, 0359-R/M-P

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Ruiz De Adana, Juan Carlos	0197-P
Ruiz De Gauna, Mikel	0353-P
Ruiz Del Rio, Jorge	0361-R/M-OS
Ruiz, María Ángeles	0405-P
Ruiz Gómez, Mar	0148-P
Ruiz León, Ana María	0026-R/M-P
Ruíz Martínez, Carlos	0061-R-P
Ruiz Orera, Jorge	0240-OS
Ruiz Pesini, Eduardo	0486-R/M-P, 0060-R-P, 0064-R-P
Ruiz Plazas, Xavier	0133-R-P
S Rodríguez, Manuel	0419-P
Saavedra, Almudena	0329-P
Sabido, Eduard	0512-OI
Sabio, Guadalupe	0032-R/M-P, 0083-R/M-P, 0463-R/M-P
Sacristán Santos, Marta	0239-M-P
Sáenz De Urturi, Diego	0276-R/M-P
Saenz Antoñanzas, Ander	0360-R/M-P
Sáez, Carmen	0126-P, 0396-R/M-P
Saéz, Laura	0284-P
Sagarovi, Uri	0444-P
Sainz, Rosa M.	0179-R-P
Saiz Baggetto, Sara	0181-R-P
Sala, Ernest	0495-P, 0503-P
Salaberry, Eduardo	0287-P
Salamini Montemurri, Martín	0315-R/M-P, 0180-R/M-P, 0244-P
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Salazar, Juan Diego	0127-P
Salazar Mardones, Gonzalo	0325-P, 0381-P
Salazar Roa, María	0001-R-P, 0022-R/M-OS
Salgado, Jesús	0337-OS
Salido, Gines M	0125-P
Salido, Sofía	0365-R/M-P
Salomonis, Nathan	0115-R-P
Sampedro, Diego	0337-OS
San Martín Pérez, Estefanía	0277-R-OS
San Román, J. Alberto	0482-R/M-OS
San Sebastian, Eider	0392-R-P
San Segundo Acosta, Pablo	0082-R-P, 0171-R-P
Sanabria Reinoso, Estefania	0141-OS
Sancha, Leticia	0264-R/M-P
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Sánchez Crespo, Mariano	0482-R/M-OS
Sánchez De Diego, Cristina	0210-R/M-P, 0220-R/M-P
Sanchez De Groot, Natalia	0139-R-P
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Sánchez De Medina, Veronica	0173-R/M-P
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Sanchez Gomez, Francisco J.	0095-P
Sánchez Martínez, Maricruz	0429-R/M-P
Sánchez Pérez, Ana María	0499-R/M-P
Sánchez, Aránzazu	0305-R/M-P, 0380-R-P
Sánchez, Cristina	0494-R/M-P, 0515-OI
Sánchez, Esther	0524-OI
Sánchez, Juana	0105-P, 0395-R/M-P, 0479-R/M-P
Sánchez, Lourdes	0159-P
Sánchez, Pilar	0123-P
Sánchez, Ricardo	0455-P
Sánchez Bayuela, Tania	0482-R/M-OS
Sánchez Bellver, Laura	0254-R-P
Sanchez Cabo, Fatima	0140-R-OS
Sánchez Cruz, Alonso	0279-R/M-P
Sánchez De La Cruz, Alberto	0374-R/M-P, 0333-P, 0355-R/M-P, 0493-R/M-P
Sánchez De La Torre, Aníbal	0312-P, 0313-OS
Sanchez De Diego, Cristina	0216-R/M-P
Sánchez Del Campo, Luis	0145-P
Sánchez Gómez, María Victoria	0265-P
Sánchez López, Christian M	0122-R/M-P
Sanchez Madrid, Francisco	0375-P
Sánchez Martel, David	0285-P
Sánchez Martín, Manuel	0098-R/M-P
Sánchez Melgar, Alejandro	0121-P
Sanchez Moran, Irene	0264-R/M-P
Sanchez Muñoz, Isabel	0197-P
Sánchez Ortiz, David	0075-P
Sánchez Pérez, Isabel	0464-R/M-P
Sánchez Ramón, Silvia	0330-R-P
Sanchís Bonet, Ángeles	0379-P
Sancho, María Del Carmen	0101-R/M-P
Sancho, Mónica	0393-P, 0424-P
Sancho Bru, Pau	0222-R-P
Sanchon Sanchez, Paula	0223-P
Sancho Vaello, Enea	0459-R/M-OS
Sandin, Daniel	0201-R/M-P
Sandoval, Andrés	0228-P
Sandoval, Viviana	0521-OI
Sanjuan, Alejandra	0214-P
San Segundo, Pedro	0433-P
Santalucía, Tomàs	0087-OI
Santa María, Cristina	0497-OS
Santamaría, David	0079-R/M-OS
Santamaría, Eva	0191-R/M-P
Santamaría, Javier	0349-R/M-P
Santamaría Aranda, Eduardo	0337-OS
Santas, Jon Ander	0413-P
Santiago Freijanes, Paz	0217-R/M-P
Santín Montanyá, María Inés	0063-R/M-P
Santolaria, Carlos	0110-R-P
Santos, Beatriz	0433-P
Santos, Eugenio	0289-R/M-P
Santos, Gabriela	0394-R/M-P
Santos, Nagore	0167-R-OS
Santos, Vanesa	0444-P
Santos Moreno, Javier	0035-R-P
Sanz, Alba	0268-R/M-P
Sanz, Francisco José	0415-P, 0147-R-P
Sanz, Guillermo	0214-P
Sanz, Miguel Ángel	0214-P
Sanz, Pascual	0092-R/M-P
Sanz Lammora, Héctor	0521-OI
Sarasa Buisan, Cristina	0331-R/M-P
Sargas, Claudia	0214-P
Sastre Serra, Jorge	0416-P, 0417-P, 0418-P, 0431-P, 0447-R/M-P, 0453-P, 0458-P, 0508-P
Satari, Leila	0290-R/M-P
Saura, Josep	0087-OI
Savitski, Mikhail	0172-R-OS
Schaar, Louise	0337-OS
Schaeper, Ute	0083-R/M-P, 0196-R/M-P
Schaerli, Yolanda	0035-R-P
Scheurer, Stephan	0481-R-P
Schieweck, Rico	0450-P
Schlosser, Andreas	0519-OI
Schönfelder, Jörg	0392-R-P
Schulz, Jana Felicitas	0240-OS
Segarra Tomás, José	0133-R-P
Segués, Nerea	0250-P
Seguí Moya, Pedro	0097-R/M-P, 0101-R/M-P, 0122-R/M-P
Segura, Tomás	0341-P
Seijo Vila, Marta	0515-OI
Selinger, Leticia	0387-R/M-P
Selma Soriano, Estela	0124-P, 0170-R-P
Sen Martín, Laura	0075-P
Seoane Abelenda, Rocío	0488-OS, 0400-P, 0419-P
Sequera, Celia	0380-R-P, 0390-P
Serna, Marina	0275-R/M-P
Serra, Dolors	0004-R/M-OS, 0005-R-P, 0155-R-P, 0263-R-P, 0272-P
Serra, Francisca	0274-R/M-P, 0395-R/M-P, 0479-R/M-P
Serrano, Alba	0343-P
Serrano, Alfonso	0146-R-P, 0143-R-P
Serrano, Antoni	0027-P, 0332-P
Serrano, Malena	0271-R/M-P
Serrano, Manuel	0001-R-P, 0086-R-P
Serrano, Rebeca	0437-P, 0502-P
Serrano Heras, Gemma	0341-P
Serrano Maciá, Marina	0083-R/M-P, 0191-R/M-P, 0209-R/M-P, 0215-R/M-P, 0222-R-P, 0257-R/M-P, 0261-R/M-P, 0196-R/M-P
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Serrat, Judit	0282-R/M-P
Serrat, Neus	0185-R/M-P
Sesé, Borja	0251-R-P
Sevilla, Emma	0228-P, 0270-R/M-P, 0331-R/M-P, 0339-R/M-P
Shahraz, Mohammed	0172-R-OS
Shaw, Pamela J	0509-OS
Shen, Jessica	0248-OS
Siles Lucas, Mar	0282-R/M-P
Silva, André	0377-P
Simon Molas, Helga	0220-R/M-P, 0096-R-P, 0178-P
Simón, Fernando	0282-R/M-P
Simón, Jorge	0083-R/M-P, 0196-R/M-P, 0207-R/M-OS, 0209-R/M-P, 0215-R/M-P, 0222-R-P, 0257-R/M-P, 0261-R/M-P
Singh, Nadia	0277-R-OS
Sisó, Pol	0074-P
Skehel, J. Mark	0519-OI
Sohlenkamp, Christian	0307-P
Sola, Eva	0425-P
Sola Leyva, Alberto	0226-R/M-P, 0260-R/M-P
Sola Martínez, Rosa Alba	0436-P
Solana Manrique, Cristina	0147-R-P, 0415-P
Solar Málaga, Soraya	0350-R-P
Soler Valls, Ana Josefa	0277-R-OS

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Solivera, Juan	0468-R/M-P
Soliz Rueda, Jorge R.	0449-P
Solomando, Antònia	0091-P
Somoza, Álvaro	0153-R/M-P, 0309-R/M-OS
Somoza, Beatriz	0230-R-P
Somoza, María Luisa	0481-R-P
Sorensen, Soren	0310-P
Soria, Xavier	0074-P
Soriano, Eduardo	0443-P
Soriano, María Eugenia	0069-R/M-P
Soriano Teruel, Paula M	0393-P
Sorribas, Victor	0205-P
Sosa, Cecilia	0205-P
Sot, Begoña	0153-R/M-P, 0309-R/M-OS
Sotillo, Javier	0282-R/M-P
Soto, Maria Jose	0307-P
Sousa Ortega, Ana	0141-OS
Spaczynksa, Monika	0432-P
Sparavier, Aleksandra	0512-OI
Spiegelman, Bruce M.	0032-R/M-P
Spínola Amilibia, Mercedes	0243-P
Spiridon Bodi, Mihai	0102-R/M-P
Stamm, Paul	0316-R/M-OS
Stein, Frank	0172-R-OS
Steinmetz, Lars	0172-R-OS
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