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3rd PhD Symposium In Health Sciencies and Biomedicine

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Sociedad Española de Farmacología clínica

Universidad Autónoma de Madrid

3rd PhD Symposium in Health Sciences and Biomedicine

When?

June 8rd 2022

Where?

School of Medicine
Universidad Autonóma de Madrid

Arzobispo Morcillo, 4
28029 Madrid

Registration opens
April 2nd to May 20th

Abstract submission
April 2nd to May 6th

Organized by

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UAM Universidad Autónoma de Madrid

3rd PhD Symposium in Health Sciences and Biomedicine

June 3rd 2022

9:00-9:30: Registration @ Decanato Hall

9:30-10:00: Welcome and Opening by Academic Authorities @ Aula Magna

Amaya Mendikoetxea (UAM Rector) **and Miguel Remacha** (EDUAM Director)

10:00-11:30: Plenary sessions (10+5 min) @ Aula Magna

Chairpersons: Luis del Peso, Concha Peiró

1. **Cancer:** WNK1 modulates the multitarget drug rigosertib response in breast cancer cell lines.
   Ana Monfort-Vengut, PhD Programme in Molecular Biosciences
2. **Cardiovascular**: Resolvin D2 prevents cardiovascular damage in angiotensin II-induced hypertension.
   Lucía Serrano, PhD Programme in Pharmacology and Physiology
3. **Infection and inflammatory pathologies:** Extracellular microRNAs of Natural Killer cells modulate T-cell responses.
   Sara García Dosil, PhD Programme in Molecular Biosciences
4. **Metabolism and liver diseases**: Bone morphogenetic protein 2 is a new molecular target linked to nonalcoholic fatty liver disease with potential value as non-invasive screening tool.
   Patricia Marañón Barnusell, PhD Programme in Molecular Biosciences
5. **Neuroscience**: Regulation of β-amyloid clearance in APP/PS1 astrocytes by AMPK activation and MTORC1 inhibition.
   Marta García-Juan, PhD Programme in Molecular Biosciences
6. **Miscellaneous:** Hypoxia classifier for transcriptome datasets
   Laura Puente Santamaría, PhD Programme in Molecular Biosciences

11:30-12:00: Break

12:00-13:00: Keynote lecture @ Aula Magna

Chairperson: Isabel Sánchez

Guillermo Sánchez Prieto. More productivity and less stress in 5 principles

13:00-14:30: Plenary sessions (10+5 min) @ Aula Magna

Chairpersons: Nicolas Bayen, Isabel Sánchez

1. **Cancer:** Gasdermin B over-expression modulates HER2-targeted therapy resistance through LC3B/Rab7 interaction.
   Manuel Gámez Chiachio, PhD Programme in Molecular Biosciences
2. **Cardiovascular**: Activation of the mTORC1 and mitochondrial signaling under diabetic and hypertensive cardiomyopathy.
   Tianyu Hang, PhD Programme in Pharmacology and Physiology
3. **Infection and inflammatory pathologies**: Association of mutant spectra of SARS-CoV-2 with COVID-19 disease severity.
   Brenda Martínez González, PhD Programme in Molecular Biosciences
4. **Metabolism and liver diseases:** Cell tension controlling pathways and nutrient availability regulate plasma membrane ATP synthase trafficking.
   Laura Sotodosos Alonso, PhD Programme in Molecular Biosciences
5. **Neuroscience**: The sonic hedgehog agonist SAG attenuates mitochondrial dysfunction and decreases the neurotoxicity induced by frataxin-deficient astrocytes.
   Andrés Vicente Acosta, PhD Programme in Molecular Biosciences
6. **Miscellaneous**: Selective autophagy plays a protective role against acute and age-related retinal degeneration.
   Juan Ignacio Jiménez-Loygorri, PhD Programme in Molecular Biosciences

14:30-15:30: Agape-lunch @ Decanato Hall

15:30-16:30: 4 Parallel sessions (5 talks 7+3 min)

Session 1 @ Seminario II
Chairperson: Guillermo de Cárcer

1. **Cancer**: Brain Tumor Modelling Using the CRISPR-Cas9 Base Editing Technology.
   María del Mar Gardeazabal Bataller, PhD Programme in Molecular Biosciences
2. **Cancer**: Building insights into CDC37-RAF1 interaction: an opportunity for design new strategies to treat KRAS driven tumors.
   Laura de la Puente Ovejero, PhD Programme in Molecular Biosciences
3. **Cancer**: The secretome of highly metastatic cells as a source of biomarkers and metastatic effectors in colorectal cancer patients.
   Javier Robles Sebastián, PhD Programme in Biology
4. **Cancer**: Monitoring One-carbon metabolism by mass spectrometry for early diagnosis of cirrhosis and HCC.
   Laura Guerrero, PhD Programme in Molecular Biosciences
5. **Cancer**: Transcription factor NRF2 participates in cell cycle progression at the level of G1/S and mitotic checkpoints.
   Diego Lastra Martínez, PhD Programme in Molecular Biosciences

Session 2 @ Seminario I
Chairperson: Enrique Martín

6. **Infection and inflammatory pathologies:** Role of CD39 in COVID-19 Severity: Dysregulation of Purinergic Signaling and Thromboinflammation.
   Enrique Alfaro García, PhD Programme in Medicine and Surgery
7. **Infection and inflammatory pathologies:** Infections in Juvenile Idiopatic Arthritis, More Frequent than in Healthy Children? Prospective Multi Center observational Study
   Clara Udaondo, PhD Programme in Medicine and Surgery
8. **Infection and inflammatory pathologies:** Inflammasome Activation: A Keystone of Proinflammatory Response in Obstructive Sleep Apnea.
   Elena Díaz, PhD Programme in Medicine and Surgery
9. **Infection and inflammatory pathologies:** Prioritization and validation of asthma and respiratory allergy biomarkers using in silico analysis: Systems Biology.
   Lucía Cremades Jimeno, PhD Programme in Molecular Biosciences

Session 3 @ Aula Magna
Chairpersons: Javier Egea, Lucía Prensa

10. **Neuroscience**: At-home monitorization using artificial noses and multimodal sensors: a noninvasive approach to human routine certification.
    Carlos Garcia-Saura, PhD Programme in Computer and Telecommunication Engineering
11. **Neuroscience**: Regulation of the DNA damage response by E2F4 phosphorylation in its T249/T251 conserved motif and Alzheimer's disease.
    Aina Maria Llabrés Mas, PhD Programme in Molecular Biosciences
12. **Neuroscience**: In vivo nanotracer for the detection of brain thrombi in an AD mouse model.
    Carlos Cerón Hernández, PhD Programme in Molecular Biosciences
13. **Neuroscience**: Neuroimaging in individuals from the high-end of the general ability distribution: evidence from brain structural resilience and neocortical brain age.
    Javier Pérez Santonja, PhD Programme in Neuroscience
14. **Neuroscience**: Does MAPT have anything new to say? Discovery of novel non-aggregative Tau isoforms that are decreased in Alzheimer's disease.
    Daniel Ruiz Gabarre, PhD Programme in Neuroscience

Session 4 @ Seminario III
Chairperson: Konstantinos Stamatakis

15. **Infection and inflammatory pathologies:** Early use of corticosteroids and sarilumab in Sars-cov-2 pneumonia.
    Antonio F Caballero-Bermejo, PhD Programme in Medicine and Surgery
16. **Infection and inflammatory pathologies:** SARS-CoV-2 Membrane protein-specific antibodies from critically ill COVID-19-infected individuals are potent stimulators of NK cell activation.
    Daniel Fernández-Soto, PhD Programme in Molecular Biosciences
17. **Infection and inflammatory pathologies:** Neutralizing serum amyloid A1 as a therapeutic strategy for traumatic brain injury.
    Céline Decouty Pérez, PhD Programme in Pharmacology and Physiology
18. **Infection and inflammatory pathologies:** An inhibitor of the interaction between the transcription factor NRF2 and the E3 ligase adapter β-TrCP suppresses lipopolysaccharide-mediated inflammation.
    Raquel Fernández Ginés, PhD Programme in Molecular Biosciences
19. **Infection and inflammatory pathologies:** Clinical and Immunometabolic Patterns Determining Efficacy of DC-treatment reinvigorating HIV-1-specific CD8+ T cells in PLWH.
    Marta Calvet Mirabent, PhD Programme in Molecular Biosciences

16:30-17:30: 4 <u>Parallel sessions</u> (5 talks 7+3 min)

Session 1 @ Seminario II
Chairperson: Juan Arredondo

1. **Metabolism and liver diseases:** The Release of Exosomes from Astrocytes in Response to Fatty Acid Alters the Metabolism of Proopiomelanocortin (POMC) Neurons.
   Roberto Collado Pérez, PhD Programme in Medicine and Surgery
2. **Metabolism and liver diseases:** Obesity-associated renal alterations are mediated by Microsomal Prostaglandin E Synthase-1 (mPGES-1) in a sex-dependent manner.
   Constanza Ballesteros Martínez, PhD Programme in Pharmacology and Physiology
3. **Metabolism and liver diseases:** Regulation of BMP8A expression during hepatic fibrogenesis process.
   Stephania Chavez Isaza, PhD Programme in Molecular Biosciences
4. **Miscellaneous**: Mitophagy boosting protects cells against MNU toxicity.
   Juan Zapata Muñoz, PhD Programme in Neuroscience
5. **Miscellaneous**: Characterization of the mitochondrial Glutamyl-tRNAGln amidotransferase (GatCAB) as a new model for mitochondrial translation disorders.
   Sophie Fitch, PhD Programme in Molecular Biosciences

Session 2 @ Seminario I
Chairperson: Enrique Martín

6. **Cancer**: Multiplex Immunofluorescence reveals specific subsets of immune cell populations expressing CD137/TNFRSF9 as predictors of unfavorable outcomes in Hodgkin Lymphoma.
   Victoria Menéndez García, PhD Programme in Molecular Biosciences
7. **Cancer**: Volumetric parameters calculated with 2-[18F]FDG PET/CT and their correlation with biochemical analysis in patients with diffuse large B-cell lymphoma.
   Stefanía Guzman Ortiz, PhD Programme in Medicine and Surgery
8. **Cancer**: Looking for novel interactors of IL13Rα2 and tyrosine-protein phosphatase non-receptor type 1 (PTPN1/PTP1B) by using proximity-dependent biotinylation (BioID).
   Ángela Martín Regalado, PhD Programme in Biology**Cancer**: Use of anti-GD2 (Dinutuximab) as a target for CAR-T cells immunotherapy in neuroblastoma.
   Lorena García García, PhD Programme in Medicine and Surgery

9. **Cancer**: Adenine nucleotides transport across the inner mitochondrial membrane in cancer cells: role of ANTs and SCaMCs.
   Luis González Moreno, PhD Programme in Molecular Biosciences

Session 3 @ Aula Magna
Chairpersons: Isabel Lastres, Ana I. Rojo

10. **Neuroscience**: Generation and characterization of the adult neuron-specific Aralar/AGC1 knock-out mice
    Eduardo Herrada Soler, PhD Programme in Molecular Biosciences
11. **Neuroscience**: Dmrt5 beyond the cortex: early role in the sexual differentiation of the mouse limbic system.
    Rafael Casado-Navarro, PhD Programme in Molecular Biosciences
12. **Neuroscience**: Automatic synapse parameter exploration for the interaction of living neurons and models in hybrid circuits and hybrots.
    Manuel Reyes Sanchez, PhD Programme in Computer and Telecommunication Engineering
13. **Neuroscience**: Unravelling neuron-astrocyte communication in the dorsal raphe nucleus.
    Andrea Sánchez-Ruiz, PhD Programme in Neuroscience
14. **Neuroscience**: Astrocytes of Nucleus Accumbens control the impairments derived from chronic exposure of THC.
    Cristina Martín Monteagudo, PhD Programme in Neuroscience

Session 4 @ Seminario III
Chairperson: Fernando de la Cuesta

15. **Cardiovascular**: Resolvin E1 attenuates endothelial senescence induced by doxorubicin through the modulation of NLRP3 inflammasome activation.
    Licia Shamoon, PhD Programme in Pharmacology and Physiology
16. **Cardiovascular:** Role of caveolin-1 in heart extracellular matrix deposition and remodelling upon myocardial infarction.
    Marta Pulgarín Alfaro, PhD Programme in Molecular Biosciences
17. **Cardiovascular**: Targeted endothelial knockout of caveolin-1 in established atherosclerosis does not inhibit plaque progression.
    Rocío Muñiz Anquela, PhD Programme in Molecular Biosciences
18. **Cardiovascular**: Alteration of PGC1-α in the mitochondrial homeostasis of cardiomyocytes under hyperglycemia. Role of the GLP-1R activation.
    Jairo Lumpuy-Castillo, PhD Programme in Pharmacology and Physiology
19. **Cardiovascular**: A unique F-actin and junctional organization that maintains the corneal endothelial barrier.
    Gema Cerro Tello, PhD Programme in Molecular Biosciences

17:30-18:30: Awards and closing remarks
Daniel Jaque (UAM Research Vice-rector), Carlos Sánchez-Ferrer (School of Medicine Dean), María J Calzada and Óscar Lorenzo (Symposium Executive Committee)

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Abstract submission

PhD In Health Science and Biomedicine

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Role of CD39 in COVID-19 Severity: Dysregulation of Purinergic Signaling and Thromboinflammation

Elena Díaz-García [1 2], Sara García-Tovar [1], Enrique Alfaro 1, Ester Zamarrón [1 2], Alberto Mangas [1], Raúl Galera [1 2], José Juan Ruíz-Hernández [3], Jordi Solé-Violán [2 4], Carlos Rodríguez-Gallego [5 6], Ana Van-Den-Rym [7 8], Rebeca Pérez-de-Diego [7 8], Kapil Nanwani-Nanwani [9], Eduardo López- Collazo [10], Francisco García-Rio [1 2 11], Carolina Cubillos-Zapata [1 2 *].

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CD39/NTPDase1 has emerged as an important molecule that contributes to maintain inflammatory and coagulatory homeostasis. Various studies have hypothesized the possible role of CD39 in COVID-19 pathophysiology since no confirmatory data shed light in this regard. Therefore, we aimed to quantify CD39 expression on COVID-19 patients exploring its association with severity clinical parameters and ICU admission, while unraveling the role of purinergic signaling on thromboinflammation in COVID-19 patients. We selected a prospective cohort of patients hospitalized due to severe COVID-19 pneumonia (n=75), a historical cohort of Influenza A pneumonia patients (n=18) and sex/age-matched healthy controls (n=30). CD39 was overexpressed in COVID-19 patients' plasma and immune cell subsets and related to hypoxemia. Plasma soluble form of CD39 (sCD39) was related to length of hospital stay and independently associated with intensive care unit admission (adjusted odds ratio 1.04, 95%CI 1.0-1.08, p=0.038), with a net reclassification index of 0.229 (0.118-0.287; p=0.036). COVID-19 patients showed extracellular accumulation of adenosine nucleotides (ATP and ADP), resulting in systemic inflammation and pro-coagulant state, as a consequence of purinergic pathway dysregulation. Interestingly, we found that COVID-19 plasma caused platelet activation, which was successfully blocked by the P2Y12 receptor inhibitor, ticagrelor. Therefore, sCD39 is suggested as a promising biomarker for COVID-19 severity. As a conclusion, our study indicates that CD39 overexpression in COVID-19 patients could be indicating purinergic signaling dysregulation, which might be at the basis of COVID-19 thromboinflammation disorder

Keywords: CD39; COVID-19; hypoxia; purinergic dysregulation; thromboinflammation

Cite as: Díaz-García E., García-Tovar S., Alfaro E., Zamarrón E., Mangas A., Galera R., Ruíz-Hernández J.J., Sole-Violán J., Rodríguez-Gallego C., Van-Den-Rym A., Pérez-de-Diego R., Nanwani-Nanwani K., López-Collazo E., García-Río F. and Cubillos-Zapata C. Role of CD39 in COVID-19 Severity: Dysregulation of Purinergic Signaling and Thromboinflammatio. IBJ Plus 2022 1(s5) e00001. DOI: 10.24217/2531-0151.22v1s5.00001

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Competing Interests: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Obesity-associated renal alterations are mediated by Microsomal Prostaglandin E Synthase-1 (mPGES-1) in a sex-dependent manner.

Ballesteros-Martinez C[1], Rodrigues-Diez R[1,2,3], Salaices M[1,2,3], Briones AM[1,2,3]

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Introduction: Obesity is characterized by a chronic-low grade inflammation that causes pathophysiological alterations in different target organs, contributing, among others, to the development of renal damage. Microsomal Prostaglandin E Synthase 1 (mPGES-1) is an inducible isomerase responsible for the overexpression of prostaglandin E2 (PGE2) under inflammatory conditions, including obesity. Inhibition of PGE2 production by non-steroidal anti-inflammatory drugs, that block cyclooxygenase-2 (COX-2) cascade, limits inflammatory processes. However, it can be associated with side- effects as nephrotoxicity due to the inhibition of other prostaglandins production. New drugs targeting mPGES-1 have shown promising results in different experimental models. Nevertheless, to our knowledge, efficacy of inhibition downstream COXs in the renal impact of obesity remains unexplored.

Objective: To elucidate whether mPGES-1 depletion could prevent the development of renal alterations in an experimental model of obesity.

Methods: We have developed a model of HFD-induced obesity (60% fat, 13 weeks) in males and females mPGES-$1^{-/-}$ and their control littermates mPGES-$1^{+/+}$ mice. Renal structural alterations and changes in mRNA expression were studied by histological analysis and q-RT-PCR, respectively.

Results: IHFD decreased renal mPGES-1 gene expression in females but not in males. On the contrary, mPGES-1 expression was upregulated in perirenal adipose tissue (PRAT) of both sexes. In females, HFD induced kidney and PRAT hypertrophy that was prevented by the absence of mPGES-1; whereas in males, HFD did not modify kidney weight, while increasing PRAT weight significantly more in mPGES-$1^{+/+}$ than in mPGES-$1^{-/-}$ mice. Rt-qPCR studies revealed that HFD induced renal damage, inflammation and fibrosis in mPGES-$1^{+/+}$ male mice, which was not observed in mPGES-1 deficient mice. In agreement, histological studies showed that HFD induced glomerular hypertrophy and renal fibrosis only in mPGES-$1^{+/+}$ HFD males. These alterations were not likely mediated by oxidative stress. Of note, females were protected against renal inflammatory and fibrotic alterations induced by HFD. Additionally, mPGES-1 deficiency prevented HFD- induced PRAT inflammation, fibrosis and hypoxia in both males and females. HFD did not modify serum proteinuria in any sex.

Conclusion: mPGES-1 participates in the renal alterations associated with obesity in a sex-dependent manner.

Keywords: mPGES-1, kidney, renal, obesity, HFD.

Cite as: Ballesteros-Martinez C., Rodrigues-Diez R., Salaices M. and Briones AM. Obesity-associated renal alterations are mediated by Microsomal Prostaglandin E Synthase-1 (mPGES-1) in a sex-dependent manner. IBJ Plus 2022 1(s5) e00002. DOI: 10.24217/2531-0151.22v1s5.00002

Funding: The authors declare no fundings.
Competing Interests: The authors declare no competing interests.

Early use of corticosteroids and sarilumab in SARS-Cov-2 pneumonia.

Caballero-Bermejo AF[1], Sancho-López A[1], Ussetti-Gil P[2], Fernández-Cruz A[3], Avendaño-Solá C[1], Ruiz-Antorán B[1].

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The cytokine storm underlying the inflammatory phase of COVID19 has been suggested since the beginning of the pandemic as a target for treatment of the disease. The identification of the presence of elevated IL6 levels in patients with severe SARS-CoV-2 infection led to the hypothesis that the use of already marketed drugs whose mechanism of action is the inhibition of IL6 could result in clinical benefit for these patients. We hypothesised that the use of glucocorticoid-associated sarilumab in early stages of the proinflammatory phase of COVID19 could prevent progression to respiratory distress syndrome and death.

Hospitalised patients with SARS-CoV2 pneumonia, treated with nasal cannula and with analytical parameters of inflammation, were randomised (1:1 ratio) to receive sarilumab + standard therapy (sarilumab group) or standard therapy (control group). In all cases, standard therapy included glucocorticoid treatment for at least 3 days at a dose equivalent to methylprednisolone mg/kg/day. The primary endpoint was the percentage of patients who progressed to severe respiratory failure (defined as a BRESCIA-COVID score ≥3), intensive care unit (ICU) admission or death at any time up to day 15.

A total of 201 patients were included, of whom 99 were randomised to the sarilumab group and 102 to the control group. The percentage of patients who progressed to severe respiratory failure (BRESCIA≥3), ICU admission or death at any time up to day 15 was 16.6% in the sarilumab group and 15.69% in the control group (RR 1.03; 95% CI, 0.48 to 2.20). No differences in the safety profile between the two groups were detected. Mortality at day 28 was 2.02% in the sarilumab group vs. 1.96% in the control group (RR 1.03; 95% CI 0.14 - 7.46).

In conclusion, an early intervention with sarilumab and glucocorticoids did not demonstrate clinical benefit in our study population compared to standard therapy.

Cite as: Caballero-Bermejo AF., Sancho-López A., Ussetti-Gil P., Fernández-Cruz A., Avendaño-Solá C. and Ruiz-Antorán B. Early use of corticosteroids and sarilumab in SARS-Cov-2 pneumonia. IBJ Plus 2022 1(s5) e00003. DOI: 10.24217/2531-0151.22v1s5.00003.

Funding: The authors declare no fundings.
Competing Interests: The authors declare no competing interests.

Clinical and Immunometabolic Patterns Determining Efficacy of DC-treatment reinvigorating HIV-1-specific CD8+ T cells in PLWH

Marta Calvet-Mirabent[1,2], Ildefonso Sánchez-Cerrillo[1,2], Noa Martín-Cófreces[1,2,3], Hortensia de la Fuente[1,3], Ilya Tsukalov[2], Cristina Delgado-Arévalo[1,2], María José Calzada[2], Ignacio de los Santos[4,7], Jesús Sanz[4,7], Lucio García-Fraile[4,7], Francisco Sánchez-Madrid[1,2,3], Arantzazu Alfranca[1], María Ángeles Muñoz-Fernández[5], Maria J. Buzón[6], Enrique Martín-Gayo[1,2,7]

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Introduction: Heterogeneous dysfunctional states of CD8+ T cells in people living with HIV-1 (PWLH) has limited the efficacy of dendritic cell (DC)-based immunotherapies. Here, we studied associations between improved functional response to Gag-loaded adjuvant-primed DCs of CD8 T cells from PLWH with ART duration, memory subset distribution and exhaustion and metabolic profiles in these cells.

Methods: A cohort of n=49 PLWH on ART with undetectable plasma viremia and CD4+ T counts above 400cells/ml were recruited. Monocyte-derived DC were activated with Poly I:C and 2´3´cdiAM(PS)2 adjuvants in the presence of a pool of HIV-1 Gag peptides and co-cultured with autologous CD8+ T cells. Induction and polyfunctionality of HIV- 1 specific CD8+ T responses was evaluated by IFNγ and CD107a expression by FACS. Functionality of DC- stimulated CD8+ T cells was evaluated by co-culture with autologous CD4+ T cells and the ability to reduce proportions of p24+ CD4+ T cells. Individual or combined anti-PD1, TIGIT, TIM3 antibodies and Metformin

were used in some functional assays. Characterization of CD8+ T cell memory subset and exhaustion markers was analyzed by FACS. Metabolic profiles of CD8+ T cells were analyzed by Seahorse.

Results: Polyfunctionality and functional capacities to eliminate p24+ CD4+ T cells of HIV-1 specific CD8+ T cell responses from PLWH on ART for more than 10 years (LT-ARTp) significantly improved after activation with adjuvant-engineered DC in vitro (p=0.001 and p=0.0039; respectively). In contrast, CD8+ T cells from PLWH on ART for less than a decade (ST-ARTp) were less responsive to DC (p=0.0024) and unable to increase cytotoxic function (p=0.0156). This was associated with lower frequencies of central memory CD8+ T cells, increased co- expression of PD1 and TIGIT (p=0.0362) and reduced mitochondrial respiration and glycolytic induction after TCR activation (p=0.002). In contrast, enrichment on TIM3+ PD1- cells (p=0.001) and preserved glycolytic induction (p=0.0005) was observed in CD8+ T cells from LT-ARTp. Finally, combined treatment of anti-PD1, anti-TIGIT antibodies and metformin restored cytotoxic properties of dysfunctional CD8+ T cells from ST-ARTp (p=0.0156).

Conclusions: We identified new immunometabolic parameters potentially useful to personalize DC-based HIV-1 vaccines and improve specific CD8+ T cell response in different PLWH populations.

Keywords: HIV-1, immunometabolism, CD8+ T cells

Cite as: Calvet-Mirabent M., Sánchez-Cerrillo I., Martín-Cófreces N., De la Fuente H., Tsukalov I., Delgado-Arévalo C., Calzada MJ., De los Santos I., Sanz J., García-Fraile L., Sánchez-Madrid F., Alfranca A., Muñoz-Fernández MA., Buzón M.J. and Martín-Gayo E. Clinical and Immunometabolic Patterns Determining Efficacy of DC-treatment reinvigorating HIV-1-specific CD8+ T cells in PLWH IBJ Plus 2022 1(s5) e00004. DOI: 10.24217/2531-0151.22v1s5.00004.

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Competing Interests: The authors declare no competing interests.

Dmrt5 beyond the cortex: early role in the sexual differentiation of the mouse limbic system.

Rafael Casado-Navarro[1], Rodrigo Torrillas-de la Cal[1], Javier Macho Rendón[2], Juan Ramón Martínez-Morales[2], Esther Serrano-Saiz[1].

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The mammalian limbic system is sexually dimorphic and underlies sex-specific innate behaviors such as mating, maternal care, and aggression. However, the genetic programs involved in the sexual differentiation of the limbic system are poorly understood. Dmrt transcription factors have emerged as conserved regulators of sex-specific traits across animal kingdom. They have been studied mostly in invertebrates, where they play an important role in nervous system sexual differentiation. In mammals, Dmrt genes have been predominantly studied in the gonads, however, Dmrt5, controls cortex development in humans and mice. In fact, mutations in DMRTA2 (Dmrt5) lead to a recessive human condition characterized by microcephaly and lissencephaly. Surprisingly, there are no studies beyond the cortex nor comparing males and females. In this work we use Dmrt5 null mutant mice, RNA-seq and histological techniques to study Dmrt5 function in the developing mammalian nervous system beyond the cortex and comparing males and females. It is plausible that genetic factors involved in brain sexual differentiation, like Dmrt, may represent also etiologic causes for mental disorders, explaining the observed male-female sexual bias.

In this study we showed that Dmrt5 is broadly expressed in the mouse nervous system, including the vomeronasal organ, olfactory tubercle, or ventral tegmental area (all key nodes in the control of innate behaviors). Furthermore, we showed that Dmrt5 is necessary for the correct specification of the main olfactory epithelium, the olfactory bulb, and several hypothalamic nuclei. Additionally, we found a novel function for Dmrt5 as a suppressor of sex differences in gene expression in the posterior hypothalamic area and midbrain. In this region, Dmrt5 could be specifying and maintaining a neuronal subpopulation or a group of neurons only in males. Taken together, Dmrt5 comes out as a crucial transcription factor for the accurate differentiation of limbic regions, integrating sex information. By studying the genetic factors involved in sexual differentiation of the nervous system, we might find potential factors to either afford protection or generate vulnerability in one sex versus the other for sex-biased mental disorders.

Keywords: : Dmrt, limbic system, sexual differentiation, main olfactory epithelium, posterior hypothalamic area, midbrain.

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A unique F-actin and junctional organization that maintains the corneal endothelial barrier

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Introduction: The corneal endothelium is responsible for the correct hydration of the corneal stroma and for an adequate transport through this tissue. Corneal endothelial layer has a particular organization to maintain its curved shape and function. The corneal endothelium has low proliferative capacity and form a monolayer that is subjected to an even mechanical tension coming from the positive pressure of the aqueous humor. Preserving their barrier function under suboptimal conditions, such as corneal pathologies, age and transplantation, is essential for maintaining corneal transparency.

Material and methods: We have investigated the structure and the proteins in charge of maintaining corneal endothelial barrier function by confocal microscopy and time lapse spinning disk microscopy in murine corneas ex vivo, and hepatic organoids.

Results: We have characterized a novel filamentous actin network that organizes into radial structures arising from the center of the cell under the nucleus towards cell-cell junctions. This structure is also observed in spherical epithelial organoids whose cells are also exposed to positive luminal pressure.

Due to the simplicity of the model, it can be easily implemented in any clinic which leads to increasing ADR and preventing CRC, but requires validation in large multicenter trials.

Keywords: DCorneal endothelium, mechanical tension, confocal microscopy, cell junctions, organoids

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In vivo nanotracer for the detection of brain thrombi in an AD mouse model.

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Background: Alzheimer's disease (AD) is the most common cause of dementia. It is a multifactorial degenerative disease pathologically characterized by intracellular neurofibrillary tangles and extracellular deposition of amyloid. An early hemostatic dysregulation is also present and contributes to an increment in clot formation, leading to hypoperfusion, blood brain barrier disruption and neuronal loss. The detection of this prothrombotic state is of the upmost importance in diagnostic approaches to identify AD patients who would benefit from anticoagulation.

Method: The aim of this project is to use an in vivo nanotracer for the detection of brain thrombi in an AD mouse model by fast pre-targeted positron emission tomography (PET) imaging.

For that purpose, AD animals and their wild-type littermates were intravenously injected with the antiplatelet antibody against CD41 conjugated with transcyclooctene (TCO-antiCD41). Twenty-four hours later, [68Ga]core-doped iron oxide nanoparticles (NP) functionalized with tetrazine (TZ) were intravenously administered. TCO and TZ produce a rapid in vivo reaction by means of bioorthogonal chemistry, allowing to non-invasively evaluate platelets' levels by PET. Two hours after [68Ga]NP-TZ injection, a static PET study of each mouse was acquired with a scanner for small animals (nanoScan® PET/CT, Mediso, USA). Finally, biodistribution assays of different organs after the PET study were performed. All PET images were analyzed by regions of interest and voxel-wise analyses.

Conclusions: Our results provide a neuroimaging strategy to diagnose the prothrombotic state towards the personalization of anticoagulation treatment in AD patients.

Keywords: BMP8A, liver fibrosis, hepatic stellate cells.

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Regulation of BMP8A expression during hepatic fibrogenesis process

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Introduction: Hepatocellular injury is the main triggering event of wound healing response that leads to liver fibrosis. Hepatic stellate cell (HSC) activation is crucial in the progression of fibrogenic process since they represent the major source of extracellular matrix components and contribute to the inflammatory response by secreting proinflammatory cytokines. Bone morphogenetic proteins (BMPs) are soluble growth factors which exert pleiotropic effects in various tissues regulating different physiological processes of cellular homeostasis. Regarding BMP8A, its implication in liver damage has been poorly investigated. Therefore, the aim of this study was to determine BMP8A expression in different fibrosis-mediated liver damage scenarios.

Materials and methods: Histological study of livers was performed and hepatic BMP8A expression levels were determined by RT-qPCR in different experimental models of hepatic fibrosis: carbon tetrachloride injected mice, as a classic hepatic fibrosis model, mice subjected to bile duct ligation, as a model of cholestatic damage-derived hepatic fibrosis, and high fat diet fed mice, reproducing the progression of non-alcoholic fatty liver disease (NAFLD) which curses with variable states of concomitant fibrosis in advanced stages. Likewise, the same analysis was conducted in livers from 11 patients with biopsy proven NAFLD-derived fibrosis and 25 NAFLD patients without fibrosis. To reproduce the experimental conditions of the murine models, BMP8A levels were determined in hepatocytes stimulated with conditioned media derived from TGFbeta-stimulated hepatic stellate cells (LX2).

Results: Firstly, murine models were validated through a histological examination of the liver and a molecular analysis of fibrotic specific markers. Next, hepatic BMP8A mRNA expression was significant increased in all studied models of hepatic fibrosis comparing with the respective controls. In fact, there is a positive correlation between hepatic BMP8A levels and fibrosis stage as well as with markers of fibrosis. Accordingly, the clinical study revealed an elevated BMP8A expression in fibrotic livers, in comparison with those without any fibrotic sign. Furthermore, in vitro experiments also showed an increased BMP8A expression in Huh7 cells treated with conditioned media derived from TGFbeta-activated LX2 cells.

Conclusions: This study reveals for the first time an increased BMP8A hepatic expression in the context of hepatic fibrosis. Its detection in serum might be useful as a non-invasive tool for the diagnosis/prognosis of patients affected by this liver disease. Moreover, these results suggest that BMP8A is involved in hepatic fibrosis progression, being possibly relevant in its therapeutic manage.

Keywords: BMP8A, liver fibrosis, hepatic stellate cells.

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The Release of Exosomes from Astrocytes in Response to Fatty Acid Alters the Metabolism of Proopiomelanocortin (POMC) Neurons

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Exosomes are extracellular micro-vesicles with a complex content that include a vast heterogeneity of molecules such as growth factors, cytokines and RNAs, including micro-RNAs (mi-RNAs) that affect target cells. Neurons are influenced by neighboring astrocytes through the release of exosomes amongst other signals. We postulated that the metabolic status is communicated by hypothalamic astrocytes via exosomes to neighboring POMC neurons to modify their functions in the promotion of energy expenditure and satiety. With this aim, cultures of primary hypothalamic astrocytes were treated with both palmitic or oleic acid (PA or OA; 0.5 mM) for 24 hours. Exosomes were isolated and purified from the media culture and used to treat (1.25 or 2.50 µg/mL) a POMC neuronal cell line for 24 hours. Exosomes released in response to PA (E-PA) or (E-OA) did not change the expression of the markers of inflammation [interleukin (IL)-6] and Endoplasmic Reticle stress (CHOP) comparing to exosomes released in response to vehicle (E-V) or with no exosomes (control). Next-Generation miRNA sequencing analysis was performed to reveal modifications in miRNAs contained in exosomes. The content of miR-199a-3p and miR-145-5p was higher in E-PA compared to E-V. POMC neurons transfected with a mimetic of miR-199a-3p (1.5 pmol) increased insulin-like growth factor 1 receptor (IGF1r) protein levels ($p < 0.05$), as well as POMC expression (ns). Moreover, levels of mTOR and p70S6k, reported targets of miR-199a-3p, were decreased ($p < 0.05$, both). Mimetic overexpression of miR- 145-5p reduced POMC expression ($p < 0.001$) and protein levels of insulin receptor substrate 1 (IRS1; $p < 0.001$), which is a known target of this miRNA. To determine modifications in cellular metabolism in the POMC neurons in response to exosomal astrocytes, Seahorse Cell Mito Stress test was performed. The mitochondrial spare respiratory capacity of neurons was increased ($p < 0.0001$) in response to both doses (1.25 or 2.50 µg/mL) of E-PA and E-OA, with the maximal respiration ($p < 0.0001$) also increasing with E-PA or E-OA (both doses) compared to E-V or control. Our results suggest that astrocytes may communicate directly with neurons via exosomes, and that the exosomes content is modulated by the source cell in response to the nutritional environment. The messages contained in exosomes from astrocytes can directly modulate the levels of cellular receptors and factors involved in cell proliferation, protection, metabolism, and nutrient sensing. Specific miRNAs participate in these processes as well as on the neuropeptide expression in targeted neurons. In addition, cellular respiration is affected in POMC neurons treated with exosomes from astrocytes in response to fatty acid in a way that suggests that cells are preparing for a possible respiratory stress by increasing their spare respiratory capacity and maximal respiration.

Keywords: relevant keywords of your manuscript.

Cite as: Collado-Pérez R., Jiménez-Hernaiz M., García-Piqueras J., Guerra-Cantera S., Argente J., Fernández-Marcos PJ., Frago LM. and Chowen JA., The Release of Exosomes from Astrocytes in Response to Fatty Acid Alters the Metabolism of Proopiomelanocortin (POMC) Neurons. IBJ Plus 2022 1(s5) e00009. DOI: 10.24217/2531-0151.22v1s5.00009.

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Prioritization and validation of asthma and respiratory allergy biomarkers using in silico analysis: Systems Biology.

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Introduction. Asthma and respiratory allergy are chronic inflammatory diseases, with high prevalence and a wide clinical spectrum. Due to their heterogeneity, it is difficult to diagnose some patients and predict their response to treatments. Moreover, although allergic mechanisms have been implicated in most asthma diagnostics, there are still a 10-33% of patients with nonallergic asthma, less studied and understood. Thus, there is a need to define new biomarkers capable of classify patients correctly. At this respect, we defined a group of 94 potential biomarkers with the ability to differentiate clinical phenotypes and disease severity. Here, the objective was to theoretically prioritize those biomarkers using systems biology, based on their association with the studied diseases.

Methods. Anaxomics' TPMS technology (Therapeutic Performance Mapping System) was used to create one mathematical model, according to molecular motifs, per disease: respiratory allergy (RA), allergic asthma (AA) and nonallergic asthma (NA). The relationship of each candidate with the diseases was analyzed by artificial neural networks (ANNs) scores, according to their specificity. A validation of the theoretical results was performed through a study of their sensitivity and specificity, through ROC curve analysis, using gene expression data obtained from peripheral samples from healthy control subjects, RA patients, and asthmatic patients (AA and NA). Finally, a triggering analysis was performed, and possible pathways connecting triggering and specific proteins were created using Cytoscape program (Pathlinkers).

Results. First, two molecular motifs were defined for RA, shared with AA; three motifs were specific for AA; and two for NA. According to these molecular motifs, 21 from the 94 candidate biomarkers showed the highest specificity for at least one of the diseases studied: 7 for RA, 12 for AA and 2 for NA. Regarding the experimental validation, ROC curves analysis highlighted 13 genes with the potential to discriminate between phenotypes and severity according to the AUC (Area Under the Curve) obtained, confirming the correlation between theoretical and experimental specificity results in some of the genes analyzed. Finally, this study also revealed the ability of AKT1, STAT1 and MAPK13 to trigger the three conditions, along with TLR4 in asthma; and possible pathways connecting the 4 triggering proteins with the 21 specific proteins were built.

Conclusion. We theoretically prioritize 21 possible new molecular biomarkers according to their specificity with different respiratory diseases, and validated some of them. Also, 4 proteins were defined as triggers of the diseases, giving potential targets to future therapies.

Keywords: Allergy, artificial intelligence, asthma, biomarkers, respiratory diseases, systems biology.

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Neutralizing serum amyloid A1 as a therapeutic strategy for traumatic brain injury

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Traumatic brain injury (TBI) is the most common cause of disability for millions of people worldwide. It is not only a health problem, but also an economic problem, as there is no effective treatment yet and the sequelae last for the rest of the patient's life. After a TBI, acute phase proteins (APP) like serum amyloid A1 (SAA1) are released into the serum, promoting neuroinflammation among other things. It has been recently demonstrated that SAA1 binds to TLR4 and activates the production of proinflammatory cytokines, which in turn increases the production of SAA1.

TAK242 administration, an antagonist of TLR4, shows protective effects in mice models after a TBI. Therefore, we hypothesize that using a molecule to neutralize SAA1 in serum will also reduce the neurological severity score (NSS) after inducing the closed head injury (CHI). The results showed that the intraperitoneal administration of this molecule can reduce the neurological damage in animals after TBI. Regarding the effects of this molecule at the inflammatory level, we have seen that the administration of this inhibitory compound of the SAA1 pathway produces a decrease in the gene expression of proinflammatory cytokines (IL-1β, IL6 and TNFα). This study demonstrates that SAA1 blockade can reduce neurological damage and the inflammatory genetic profile after trauma in an animal model.

Keywords: traumatic brain injury, serum amyloid A1.

Cite as: Decouty Pérez C., Farré Alins V., Palomino Antolín A., Narros Fernández P., Calzada MJ., López Rodríguez AB. and Egea J., Neutralizing serum amyloid A1 as a therapeutic strategy for traumatic brain injury. IBJ Plus 2022 1(s5) e00011. DOI: 10.24217/2531-0151.22v1s5.00011.

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Inflammasome Activation: A Keystone of Proinflammatory Response in Obstructive Sleep Apnea

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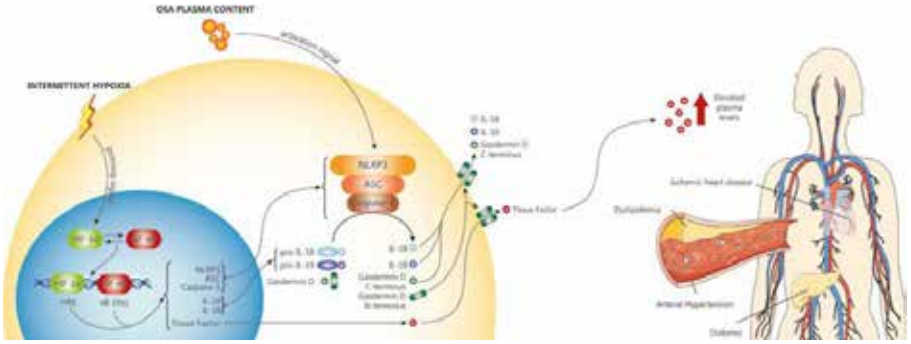
Introduction: As the mechanism that links obstructive sleep apnea (OSA) with the regulation of inflammatory response is not well known, it is important to understand the inflammasome activation, mainly of NLRP3 (nucleotide-binding oligomerization domain-like receptor 3).To assess the NLRP3 activity in patients with severe OSA and to identify its role in the systemic inflammatory response of patients with OSA.

Materials and Methods: We analyzed the NLRP3 activity as well as key components of the inflammasome cascade, such as adaptor molecule apoptosis-associated speck-like protein, caspase-1, Gasdermin D, IL-1b, IL-18, and tissue factor, in monocytes and plasma from patients with severe OSA and control subjects without sleep apnea. We explored the association of the different key markers with inflammatory comorbidities.

Results: Monocytes from patients with severe OSA presented higher NLRP3 activity than those from control subjects, which directly correlated with the apnea–hypopnea index and hypoxemic indices. NLRP3 overactivity triggered inflammatory cytokines (Il-1b and IL-18) via caspase-1 and increased Gasdermin D, allowing for tissue factor to be released. In vitro models confirmed that monocytes increase NLRP3 signaling under intermittent hypoxia in a hypoxia- inducible factor-1a–dependent manner, and/or in combination with plasma from patients with OSA. Plasma concentrations of tissue factor were higher in patients with OSA with systemic inflammatory comorbidities than in those without them.

Conclusions: In patients with severe OSA, NLRP3 activation might be a linking mechanism between intermittent hypoxia and other OSA-induced immediate changes with the development of systemic inflammatory response.



Keywords: obstructive sleep apnea, NLRP3, intermittent hypoxia, inflammasome.

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An inhibitor of the interaction between the transcription factor NRF2 and the E3 ligase adapter β-TrCP suppresses lipopolysaccharide- mediated inflammation.

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Inflammation plays a crucial role in the pathology of most chronic diseases, such as neurodegenerative and metabolic disorders. Transcription factor NRF2 has been proposed recently as a promising target to generate a beneficial therapeutic effect in most chronic diseases characterized by low-grade oxidative stress and inflammation. Most of the compounds identified as NRF2 activators are electrophiles that inhibit its main repressor: KEAP1. However, electrophiles display many off-target effects and elicit a supra-physiological NRF2 activation. As an alternative, we identified a small molecule that disrupts the interaction between NRF2 and its other repressor E3 ubiquitin ligase β- TrCP. In vitro and cell culture experiments demonstrated that our hit small molecule is a β-TrCP/NRF2 interaction inhibitor. This compound is specific for NRF2 and not for the other substrates described for β-TrCP, such as β-Catenin. Moreover, it attenuates the production of pro-inflammatory markers in cultured macrophages submitted to the endotoxin lipopolysaccharide (LPS). In vivo pharmacodynamics studies demonstrated selective exposure and NRF2 activation in liver. In mice submitted to LPS-induced acute liver inflammation, the compound greatly attenuated Kupffer cells activation and the NFkB-mediated inflammatory response. These findings report an innovative mechanism to activate NRF2 and it could be used as an alternative to conventional anti-inflammatory therapies.

Keywords: NRF2, β-TrCP, KEAP1, Protein-Protein Interaction (PPI) Inhibitor, Inflammation, LPS.

Cite as: Fernández-Ginés R., Rojo AI., Antonio Encinar J. and Cuadrado A. An inhibitor of the interaction between the transcription factor NRF2 and the E3 ligase adapter β-TrCP suppresses lipopolysaccharide- mediated inflammation. IBJ Plus 2022 1(s5) e00013. DOI: 10.24217/2531-0151.22v1s5.00013.

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SARS-CoV-2 Membrane protein-specific antibodies from critically ill COVID-19-infected individuals are potent stimulators of NK cell activation

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Introduction. The M glycoprotein is the most abundant structural protein of the SARS-CoV-2 particle and is one of the key components for virion assembly and morphogenesis. It is a major source of peptide antigens driving T-cell responses and previous studies have shown that most patients make antibodies against its N-terminal domain.Nevertheless, no studies of the function of M-specific antibodies have been reported.

Materials and Methods. We expressed the first 23 amino-acids of the M protein as a GST-fusion protein in E.coli. Sera from 37 COVID-19 patients (20 of them critically ill, admitted to ICU; 17 of them with mild disease) were assayed in a standard ELISA assay to detect reactive antibodies. The neutralization capacity of M-specific antibodies was assessed in neutralization experiments against SARS-CoV-2 virus using polyclonal antibodies purified by affinity chromatography from patient sera, while the ability of the antibodies in patients' sera to activate Natural Killer cells was measured in an ELISA-based assay in which antigen-bound antibodies were incubated with PBMC and NK cell activation was detected by flow cytometry.

Results. We could detect M-specific antibodies in most of the patients, and they could discriminate between infected and non-infected individuals with around 90% efficacy. No significant difference in the quantity of the antibody response was found between mild and critical patients. No polyclonal M-specific antibodies showed neutralization activity in the in vitro system used. Nevertheless, when PBMC from healthy donors were incubated with patients' M- specific antibodies bound to the GST-M fusion protein in an ELISA plate, NK cells were significantly activated, as measured by LAMP1+ degranulation and cytokine (MIP1β) production.

Conclusion. We show that most SARS-CoV-2 infected individuals produce specific antibodies against a surface-exposed epitope at the N-terminus of M glycoprotein. Although these antibodies do not appear to neutralize the virus efficiently, they are able to mediate Fc/FcγR interactions to drive the activation of NK cells.

Keywords: SARS-CoV-2, M glycoprotein, NK cells

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Competing Interests: No conflict of interest declared.

Characterization of the mitochondrial Glutamyl-tRNAGln amidotransferase (GatCAB) as a new model for mitochondrial translation disorders.

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Mitochondrial OXPHOS diseases are provoked by dysfunction of the OXPHOS system, showing heterogeneity from a genetic, biochemical and clinical perspective. This complexity is partly due to the involvement of proteins encoded in two genomes located in different cellular compartments, nuclear and mitochondrial DNA (mtDNA). mtDNA encodes part of the mtRNAs translation machinery, including 22 tRNAs. All these are charged by their corresponding aminoacyl-tRNA synthetases (ARS2), except mt-Gln-tRNAGln whose synthesis is carried out through an indirect pathway. The mt-tRNAGln is charged with glutamic acid (Glu) by a non-discriminating mitochondrial glutamyl-tRNA synthetase (EARS2), then the Glu is converted to Gln by a Glutamyl-tRNAGln amidotransferase, using free glutamine as an amide donor, yielding Gln- tRNAGln. This mitochondrial amidotransferase activity lies in the GatCAB complex, which is formed by three subunits: GatA (QSRL1), GatB (GATB), and GatC (GATC). Patients with mutations in these genes presented with severe cardiomyopathy and lactic acidosis, which underscores the importance of the GatCAB complex as an essential component in the translational machinery of mitochondrial protein synthesis.

We have generated Knockout (KO) lines for all subunits using the genomic editing system CRISPR/Cas9 in HEK293T cells. We measured the levels of the GatCAB subunits and mitochondrial proteins by western blot. We studied the oxygen consumption levels using a Clark electrode and detected the GatCAB complex by Blue Native-western blotting.

In all cases, KO cells show reduced levels of mitochondrial proteins, decreased oxygen consumption and an absence of the GatCAB complex compared to wildtype cells. In the QRSL1 and GATC KO cells the levels of all subunits diminish, but in GATB KO cells the levels of subunits A and C remain stable. Possibly, QRSL1 and GATC could form a stable but nonfunctional dimer, to which GATB would join.

GATB has two potential ATG codons at the start of its sequence separated by 12 nucleotides. From over 90 clones analyzed, we only obtained one KO, by generating an upstream ORF, and two cell lines with very low expression of GATB. The latter present a duplicated region at the beginning of GATB which changes the reading frame from the first ATG; suggesting the downstream ATG as an alternative translation start codon for those ribosome small subunits that do not assemble on the first one, yielding a GATB protein missing 4 amino acids.

Characterization of the molecular mechanisms that lead to the synthesis of mt-Gln-tRNAGln and its role in the physiology of the cell could allow us to comprehend the pathological manifestation of the defects in these genes and propose possible therapeutic avenues.

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Use of anti-GD2 (Dinutuximab) as a target for CAR-T cells immunotherapy in neuroblastoma

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Treating solid tumours with chimeric antigen receptors-modified T cells (CAR-T cells) has shown limited efficacy due to the lack of cancer-type specific antigens. Dinutuximab is a monoclonal antibody that recognizes a sphingolipid, disialoganglioside GD2, which has limited expression in normal tissues but is overexpressed in paediatric tumours, mainly neuroblastoma (NB) and diffuse intrinsic pontine glioma (DIPG) and other histone H3 K27M (H3K27M) mutated diffuse midline gliomas. Dinutuximab, an anti-GD2 antibody, is currently standard of care in the treatment of NB. We are contemplating the possibility of using Dinutuximab as target element for anti-NB-CAR-T cells. Our group have explored the feasibility of producing CAR-T cells from peripheral blood (PB), post-apheresis CD45RA+ fraction and cord blood (CB) using similar protocols of T cell transduction and expansion ability as a way to favour the persistence of CAR-T cells in the host. Afterward, we performed a deeply characterization of phenotype and functionality of anti-FITC-CAR-T cells derived from different sources, and the cytotoxic effect against anti-GD2-FITC- NB labelled cell line.

T cells purified from PB, CB and 45RA fraction after apheresis were cultured in vitro with cytokines IL-7+IL-15+IL-21 to maintain early memory phenotypes as Naïve T cells (TN). A second generation lentiviral expression vector (LV) was used to generate second generation CARs (FITC-L-BBz CAR). After 48h of activation in culture with anti-CD3/CD28 and cytokines, T cells were transduced with viral supernatants. Next, we performed a characterization of phenotype of anti-FITC-CAR-T cells by flow cytometry studying how the culture conditions enhanced a TN phenotype expressing CD45RA+ and CCR7+. Cytotoxic function of CAR-T cells was analysed in cocultures with LAN-1 NB cell line labelled with Dinutuximab conjugated with FITC.

Preliminary results showed that culture condition with IL-7/15/21 maintained more primitive phenotypes in 45RA (71% TN CD4+ and 66% TN CD8+) and CB (60% of TN CD4+ and 77% of TN CD8+) derived T cells than PB T cells (only a 20% of TN CD4+ and TN CD8+). PB and CB-derived CAR-T cells showed higher cytolitic function than 45RA fraction, resulting in a 30% of dead cells in both cases in a 24h-culture assay. Both, PB and CB derived CAR-T cells showed the best CAR-T activation capacity with 19,6% of cells from PB and 33,9% of cells from CB expressing early activation markers as CD25 and CD134. In vivo experiments are currently on-going to test the efficacy of CAR-T cells in combination with GD2 treatment.

Our strategy may complement the current use of Dinutuximab in the treatment of NB through its combination with a targeted CAR-T cell approach.

Keywords: CAR-T cells, neuroblastoma, dinutuximab, immunotherapy

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At-home monitorization using artificial noses and multimodal sensors: a noninvasive approach to human routine certification.

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Latest advances in e-health highlight the importance of gathering accurate data from patients, to allow for a better diagnosis, and in turn provide more effective treatments. The remote communication with patients however makes it difficult to gather accurate information, as it is often biased or limited to what the patient can communicate to the doctor. We have developed a noninvasive integrated system that can monitor human routines more effectively in a noninvasive manner using multimodal sensors.

The human body releases multiple substances into the air through its natural biological functions. Breathing, sweat, digestion, etc., all release chemical components (I.e., CO2, amines, methane) which we readily measure with

specialized olfactory sensors, effectively “artificial noses”. Our device registers the variability in these air parameters associated with cognitive activity and complements this data with other multimodal sensors (presence sensors, luminosity, loudness, etc.). In the integrated system proposed the data is then transmitted in real-time into a server where it can be securely stored, even for years, and quickly accessed and analyzed.

We have tested the use of the proposed noninvasive monitoring technology in university classrooms and in a primary school. Our results show that all routines in these environments are reflected in the sensor signals and that the artificial nose can be used to certify the corresponding cognitive activities. The noninvasive approach allows to obtain valuable data over long periods, and in the context of e-health this could allow a much better understanding of patient routines and the changes associated with health conditions. Also, the real time nature of this approach allows to implement “early warning” or notification strategies to quickly react upon changes in routines that signal a health problem.

Artificial noses and associated information systems to store and analyze the data online and offline can be used to noninvasively monitor human activity and, in particular, cognitive activity. We argue that this technology can be directly applied to monitor the elderly, people at early stages of neurogenerative diseases, any progress in cognitive disease development, and to ensure that patients correctly follow treatment.

Keywords: electronic noses, noninvasive human monitoring, cognitive characterization, cognitive certification

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Regulation of β-amyloid clearance in APP/PS1 astrocytes by AMPK activation and MTORC1 inhibition

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Alzheimer disease (AD) is the most common neurodegenerative dementia. It is characterized by the progressive accumulation of amyloid beta (Aβ) aggregates and hyperphosphorylated tau. This aberrant accumulation has been associated with autophagy dysfunction, occurring in early stages of the disease. AMPK and AKT-MTORC1 signaling pathways are central nodes in the balance between anabolism and catabolism. It is generally accepted that MTORC1 activation leads to the inhibition of autophagy, whereas AMPK activation is supposed to enhance this process. Some regulatory cross-talk between both pathways has been reported. Accordingly, autophagy inducers such as MTORC1 inhibitors may have beneficial effects in the clearance/prevention of protein aggregates in the brain, as we previously reported in the APP/PS1 AD mouse model. Until recently, most of the mechanisms that mediate protein clearance have been studied in neurons and the contribution of glial cells remains to be elucidated. The aim of this work is to determine the contribution of astrocytes to Aβ clearance through autophagy.

In an attempt to analyze the effect of autophagic flux modulation, we performed primary cultures of astrocytes form APP/PS1 mice and their wild type littermates. We treated them with different described drugs that act as autophagy inductors through the modulation of MTORC1 and AMPK pathways and evaluated the activation of these pathways and their effect in the autophagic flux by western blot. We found a slight increase of autophagy with rapamycin, a well-known MTORC1 inhibitor. Surprisingly, AMPK activation with metformin did not enhance autophagy, which could be due, at least in part, to an insufficient inhibition of MTORC1. Finally, we measured the direct effect of this modulation on autophagy-dependent amyloidosis through ELISA.

These results suggest that AMPK activation, in contrast to mTORC1 inhibition, is not sufficient to enhance autophagy in primary astrocytes, as we previously described in neurons. Thus, the protective mechanisms of autophagy against neurodegeneration must be further examined, with the final aim of describing mechanisms that allow an effective treatment for this neurodegenerative disorder.

Keywords: astrocytes; autophagy; APP/PS1; Alzheimer; amyloid accumulation; metformin; rapamycin.

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Brain Tumor Modelling Using the CRISPR-Cas9 Base Editing Technology

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Introduction: In common solid tumors, a plethora of genetic alterations can act as disease-driver mutation. Approximately, 95% of these variants are single-base substitutions, being cytidine-to-thymidine (C-to-T) conversion the most common among them. Cytosine base editors (CBEs) enable efficient C-to-T substitutions at targeted loci without double-stranded breaks, overcoming one of the main limitations in classical CRISPR-Cas9 technology.

A major application of base editing technology is the study or treatment of disease-associated point mutations. In this project, we will focus on the most common point mutations in different genes across paediatric and adult brain tumors: e.g. CTNNB1 and TP53, frequently altered in the medulloblastoma, and PIK3CA and TP53, commonly associated with adult gliomas.

Material and methods: To properly recapitulate some of the genetic alterations identified in brain tumors, we have combined the RCAS-TVA model and the CBE system for somatic genome editing. We have generated an animal model expressing TVA receptor under the control of specific promoters (GFAP or Nestin) together with an inducible CBE in order to exploit the base editing technology in the neural stem cells compartment in vivo. By intracranial injection of these mice with sgRNAs for specific genetic alterations, we will be able to recapitulate these point mutations in vivo.

Results: We have successfully generated a novel mouse model for generation of brain tumors driven by point mutations in several genes that are known to drive tumorigenesis in humans. Characterization of these tumors allowed the detection by Sanger sequencing of C-to-T conversion in all samples, as well as the activation of downstream signaling derived from each mutations. We observed a complete loss of TP53 expression upon Tp53Q97*, which translates into an early stop codon in Tp53 sequence. On the other hand, we also validated the overexpression of TP53 as a consequence of Tp53R270C mutations with no major changes in downstream effectors, as it has been previously described in the literature.

Conclusion: By integrating the RCAS-TVA system together with BE we have developed a more precise and flexible tool to better recapitulate in a more efficient way many point mutations identified in different brain tumor types. Using the RCAS-TVA-BE model we were able to model common point mutations in brain cancer and confirm their contribution in tumor formation.

Keywords: brain tumor, base editing, tumor modelling.

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Adenine nucleotides transport across the inner mitochondrial membrane in cancer cells: role of ANTs and SCaMCs

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Introduction: The regulation of adenine nucleotide levels in the mitochondria and the cytosol is essential for cell metabolism. To sustain mitochondrial respiration, ATP generated by ATP synthase must be exported to the cytosol, whereas mitochondria must be refilled with ADP and Pi. Adenine Nucleotide Translocases (ANTs), which catalyze the electrogenic ATP4-/ADP3- exchange, have been proposed to work together with ATP synthase and the mitochondrial phosphate carrier to support mitochondrial oxidative phosphorylation by forming the so-called ATP synthasome. Nevertheless, disruption of the main ANT isoform or pharmacological inhibition of ANTs in cancer cells does not decrease oxidative phosphorylation. Furthermore, the import of cytosolic ATP to the mitochondria when oxidative phosphorylation is inhibited (required, for example, for the maintenance of the ΔΨm) is neither mediated by ANTs in cancer cells. Here, we study the possibility that calcium-regulated mitochondrial ATP-Mg2+/Pi or ADP/Pi carriers, (also called SCaMCs), might participate in the mitochondrial transport of adenine nucleotides in cancer cells.

Material and methods: To study the function of SCaMC-1, the main isoform in cancer cells, we have generated SCaMC-1 KO HeLa cells using CRISPR-Cas9 genome editing. These cells have been characterized in terms of OCR (with Seahorse XF24), mitochondrial membrane potential (with TMRM) and mitochondrial ATP levels (with mito-GoATeam genetic probe) in basal conditions and in the presence of ETC inhibitors.

Results and conclusions: We show that SCaMC-1, the isoform that is abundant in tumor cells, mediates ATP import to the mitochondria after histamine stimulation of HeLa cells, probably to buffer calcium entry to the mitochondria. However, SCaMC-1 does not participate in the transport of cytosolic ATP for the maintenance of ΔΨm after OXPHOS inhibition, neither decreases mitochondrial respiration. Future studies based on the deletion of the other SCaMCs isoforms (SCaMC-2, SCaMC-3, SCaMC-3 like) together with simultaneous deletion of ANTs and SCaMCs will be required to shed light on the control of adenine nucleotides transport between the mitochondria and the cytosol in cancer cells.

Keywords: mitochondrial carriers, cancer cells, adenine nucleotides

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Monitoring One-carbon metabolism by mass spectrometry for early diagnosis of cirrhosis and HCC

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Introduction: Liver cancer represents one of the most frequent causes of death by cancer, ranked as the sixth most prevalent and the second in lethality. Hepatocellular carcinoma (HCC) is the predominant type of liver cancer and presents an increasing incidence during the last years. Late diagnosis is one of the reasons explaining the low survival rate of HCC patients (5 years survival after diagnosis below 20%). Remarkably, 80% of HCC cases develop in cirrhotic tissue (1). Main risk factors for HCC are well known and include hepatitis B and C viral infections or abusive alcohol consumption. However, the underlying molecular mechanisms remain unknown and its research will lead to the characterization of new biomolecular markers for the early diagnosis, prognosis and therapy of HCC.

Metabolic remodeling is a common feature among several hepatic disorders, from steatosis to HCC (2). Tumoral hepatocytes modify their metabolism to satisfy cancer's proliferative requirements. One-carbon metabolism (OCM) plays a fundamental role maintaining the differentiation and quiescent state of hepatocytes, and is recognized as the link between intermediate metabolism and epigenetic regulation. Owing to these reasons, it might be a potential source of biomarkers for early diagnosis and prognosis of HCC. Accordingly, it has been demonstrated that some OCM enzymes are differentially expressed in murine HCC models (3).

Materials and methods: We have developed a robust targeted mass spectrometry-based method, using SRM mode (Selected Reaction Monitoring), for the systematic quantification of 13 enzymes that participate in OCM. For this purpose, purified synthetic heavy standard peptides, as well as OCM recombinant proteins have been used. Sixty-four human liver samples, including 28 control samples, 21 tumoral samples and15 cirrhotic samples have been used.

Results and conclusions: We have demonstrated that there is a profound remodeling of the OCM cycle in HCC versus control samples, while cirrhotic samples tend to show intermediate expression levels between both physiological situations. Machine learning- based analysis of our results suggests that monitoring a panel of functionally related proteins might be useful for future clinical developments and improve the management of HCC patients. However, further experiments with larger cohorts are required to confirm these findings.

References

1) Ryerson, A. B., et al (2016). Annual Report to the Nation on the Status of Cancer, 1975-2012, featuring the increasing incidence of liver cancer. Cancer, 122(9), 1312-1337.

2) Vander Heiden, et al (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science, 324(5930), 1029-1033

3) Mora, M. I., Corrales, F. J., et al (2017). Prioritizing popular proteins in liver cancer: remodelling one-carbon metabolism. Journal of proteome research, 16(12), 4506-4514.

Keywords: Targeted proteomics , SRM, Biology and Disease Human Proteome Project (B/D-HPP), Liver cancer, One-carbon metabolism

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Volumetric parameters calculated with 2-[18F]FDG PET/CT and their correlation with biochemical analysis in patients with diffuse large B- cell lymphoma.

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Introduction/Aim: Diffuse large B-cell lymphoma, not otherwise specified (DLBCL-NOS) is a heterogeneous and aggressive group with a high mortality rate, thus, to improve risk stratification in terms of survival, several studies have been performed with 18F-Fluorodeoxyglucose positron emission tomography/computed tomography (2-[18F] FDG PET/CT) to assess the total tumor burden, through volumetric parameters such as tumor metabolic volume (MTV) and total lesion glycolysis (TLG). The aim is to evaluate the relationship of MTV and TLG with progression-free survival (PFS) and overall survival (OS), and their correlation with prognostic factors Beta-2-microglobulin (B2M) and lactate dehydrogenase (LDH).

Methodology: Retrospective study of 44 patients with LBDCG undergoing baseline 2-[18F]FDG PET/CT between January 2012 and December 2018. The calculation method for VMT and TLG was the SUV2.5 threshold. For the evaluation of VMT and TLG with PFS and OS, Harrell's C-index was used, after performing a Cox proportional hazards regression model. Pearson's correlation coefficient was used to correlate VMT and TLG with B2M and LDH.

Results: In relation to OS and PFS the VMT2.5 (p 0.006; p <0.001) showed statistically significant differences, while TLG2.5 (p 0.078; p <0.001) was statistically significant only in PFS. When comparing VMT2.5 and TLG2.5, VMT2.5 obtained a higher Harrell's C statistical concordance index for both OS (p 0.025) and PFS (p 0.008) showing great ability to discriminate between patients in whom the event does or does not occur. In the Pearson correlation analysis, VMT2.5 showed a good correlation with LDH (0.676) and a poor correlation with B2M (0.348). The TLG2.5 presented lower correlation with LDH (0.629) and poor correlation with B2M (0.274).

Table 1. Univariate analysis to evaluate the discriminatory capacity of each variable for both OS and PFS.

SG		
Volumetric Parameter	**Harrell's C**	**p-value**
MTV 2.5	0.7407	0.025
TLG 2.5	0.6990	
SLP		
Volumetric Parameter	**Harrell's C**	**p-value**
MTV 2.5	0.8010	0.008
TLG 2.5	0.7595	

Conclusion: The volumetric parameter MTV2.5 calculated with 2-[18F]FDG PET/CT can be a good prognostic indicator to predict PFS and OS in patients with DLBCL-NOS. In addition, it presents a good correlation with LDH.

Keywords: relevant keywords of your manuscript.

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Gasdermin B over-expression modulates HER2-targeted therapy resistance through LC3B/Rab7 interaction

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Tumor resistance to therapy is one of the most important challenges in current oncology. A clear example of this major issue is the HER2-positive tumors characterized by ERBB2 (HER2/neu) overexpression. HER2+ overexpression/amplification occurs in several tumors, especially in breast and gastric carcinomas that show an aggressive phenotype traditionally associated to poor prognosis. In fact, despite the clinical outcome of these tumors has gradually improved, due to the development of different antiHER2 therapies, many patients will not respond to these treatments or develop acquired resistance. Several potential resistance mechanisms to these targeted therapies have been described. Among them, our laboratory established, for the first time, that the amplification/overexpression of Gasdermin B (GSDMB), which occurs in more than 60% HER2+ breast cancer patients, is a marker of poor prognosis. Moreover, our data in HER2+ tumors revealed that GSDMB overexpression correlated to distant metastasis, poor clinical outcome, and reduced response to anti-HER2 therapies. In this context, we have attempted to elucidate the molecular mechanism whereby GSDMB collaborates in the resistance to anti-HER2 therapies.

To decipher the functional relevance of GSDMB in promoting resistance to HER2-targeted therapies we performed several molecular approaches (immunoblot, flow cytometry, immunoprecipitation, etc.) in different breast and gastric carcinoma cell models. Finally, we validated the efficacy of the identified targeted treatment using two complementary in vivo preclinical models (mice and zebrafish).

Our results confirmed that similarly to HER2 breast tumors, GSDMB is also over-expressing in more than 50% in HER2 gastric tumors. Furthermore, this over-expression renders HER2 breast and gastric cancer cells more resistant to anti- HER2 agents by promoting protective autophagy. Consistent with this, we proved that the combination of lapatinib with the autophagy inhibitor chloroquine increases the therapeutic response specifically in GSDMB-positive tumors in vitro and in vivo. Mechanistically, we also confirmed that GSDMB forms a complex with LC3B and Rab7. Finally, we validated these results in clinical samples of HER2 breast and gastric cancers, where GSDMB/LC3B/Rab7 co- expression associates significantly with relapse. In conclusion, our data decipher the molecular mechanism that underlies the relation between Gasdermin B and pro-survival autophagy with the final goal of finding a therapy that would be effective in overcoming resistance to anti-HER2 standard therapies in HER2/GSDMB+ tumors.

Keywords: tumor resistance, autophagy, HER2, GSDMB

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Activation of the mTORC1 and mitochondrial signaling under diabetic and hypertensive cardiomyopathy

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Introduction: Type 2 Diabetes Mellitus (T2DM) and hypertension (HTN) are correlated with cardiovascular disease (CVD). The presence of HTN in T2DM will lead to risk elevation in CVD. Inflammation, hypertrophy, apoptosis and fibrosis can be found in these patients. However, the mechanisms involved haven't been fully depicted.

Methodology: Cardiac biopsies from interventricular septum were isolated from patients with T2DM and/or HTN. Differential protein expression was evaluated by proteomics (hybrid trapped ion mobility spectrometry) and PEAKS software. Ingenuity Pathway Analysis (IPA, Qiagen) was used to predict the implication of molecular pathways. Cultured cardiomyocytes were used to reveal the alteration of relevant pathways under hyperglycemic, hyperlipidemic and pro-hypertensive milieu mimicked by high glucose, high fatty-acid (HF) and/or angiotensin-II.

Results: By proteomics, there is a huge difference between T2DM/HTN group and HTN in number of altered factors and its fold-change when compared with control. HTN induced a decrease of cardiac factors related to carbohydrate metabolism, mitochondrial homeostasis and respiration, while factors decreased in T2DM/HTN most related to metabolism and mitochondrial dysfunction. Also, T2DM/HTN increased fibrosis and apoptosis related factors. Interestingly, a potential regulation of mTOR complexes was suggested by bioinformatics. Thus, we confirmed the phosphorylation on Thr421/Ser424 of p70S6, a mTORC1 downstream mediator, under HF in cardiomyocytes. Also, we observed an enhanced phosphorylation on Thr172 of AMPKα, and a decreased expression of PGC1α and ACADm, in parallel to a lessening of ATP production. These alterations were abolished by rapamycin, an inhibitor of mTORC1 and metformin. Also, the silencing of a subunit of mTORC2, Rictor, induced a decrease of p-p70s6 under HF.

Conclusion: T2DM/ HTN can lead to dramatic protein changes in heart and induce a synergy cooperation when combined. The mitochondrial alteration may be major responsible of cardiac dysfunction. In particular, mTORC1 can be activated under hyperlipidemia to further lead to β-oxidation reduction and ATP synthesis. Therefore, the regulation of the mTOR-mitochondria signaling could be essential for prognosis of heart failure in T2DM and HTN patients.

Keywords: cardiomyopathy, Type 2 diabetes, hypertension, mTOR

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Generation and characterization of the adult neuron-specific Aralar/AGC1 knock-out mice.

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Aralar/AGC1/Slc25a12, the mitochondrial aspartate-glutamate carrier expressed in neurons, is the regulatory component of the NADH malate-aspartate shuttle. AGC1 deficiency is a neuropediatric rare disease (OMIM #612949, also named early infantile epileptic encephalopathy 39) characterized by hypomyelination, hypotonia, developmental arrest and epilepsy. In mice, the global aralar knockout shows hyperreactivity, growth retardation, motor discoordination, seizures and hypomyelination; thus, recapitulating the pathology of the human disease. Aralar brain expression is mainly restricted to neurons, while glial expression of Aralar has been demonstrated to be scarce. However, Aralar deficiency manifests alteration in fundamental glial functions such as glutamine and myelination synthesis by astrocytes and oligodendrocytes respectively. In order to dissect the role of neuronal Aralar in the disease mechanism of AGC1 deficiency, we have ablated the expression of Aralar in mature (from PND30 onward) excitatory cortical and hippocampal neurons using the CaMKIIα-driven Cre recombinase expression in Aralar$^{lox/lox}$ mice.

Here, we present the characterization of the mature neuron-specific aralar knock out mice. We demonstrated that the excision of exon 3 aralar starts from PND30 and continues to occur during mouse adulthood. Consequently, Aralar protein levels gradually decay across the 2nd to 6th month up to a 70- 75% decrease in the cortex and a 50% decrease in the hippocampus. The drop in Aralar levels drive an alteration in cortical metabolites causing a 25% and 50% decrease in aspartate and serine levels, the metabolites most affected in the brain of the global aralar-KO mice. This partial decreases in ARALAR and metabolites caused by cortical CaMKIIα-driven aralar ablation are not accompanied by growth or behavioral alterations in a battery of motor, memory and cognitive tests. The results suggest that the absence of Aralar in CaMKIIα expressing neurons (cortical and hippocampal mature excitatory neurons), does not mimic the alterations caused by global deficiency of Aralar/AGC1. However, since the levels of cortical ARALAR in this model resemble those of healthy aralar$^{+/-}$ mice, further experiments are being carried out to explore the origin (or location) of the ARALAR protein remnants and the possible metabolic compensation by other neurons and/or cell types in these mice. Without ruling out either that the ARALAR-MAS pathway could play a more critical role during neurodevelopment than adulthood in brain physiology and metabolism.

Keywords: Mitochondrial carrier, hypomyelination, mitochondrial disease, cortical metabolites , behavioral characterization

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Selective autophagy plays a protective role against acute and age- related retinal degeneration

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Objective. Age-related macular degeneration (AMD) is the leading cause of blindness in elderly people in the developed world, and the number of people affected is expected to almost double by 2040. The retina presents one of the highest metabolic demands that is partially or fully fulfilled by mitochondria in the neuroretina and retinal pigment epithelium (RPE), respectively. Together with its post-mitotic status, this context requires a tightly-regulated housekeeping system that includes selective mitochondrial autophagy. We want to assess the effects of selective autophagy deficit or induction in the retina given an AMD-like paradigm.

Materials and methods. Eyes from Ambra1$^{+/+}$, Ambra1$^{+/gt}$ and mito-QC mice were analysed using flatmount or cryosection immunostaining. Sodium iodate (SI) was used as a model of AMD-like damage and Urolithin A (UA) as a mitophagy inducer. ARPE-19 human cells were used as an in vitro model and analysed by immunostaining, flow cytometry and RT-qPCR. Bioinformatic analysis of public human datasets was also performed.

Results. Ambra1$^{+/gt}$ autophagy-deficient mice present alterations in the RPE, similar to those observed in human AMD patients, such as abnormal morphology or lipofuscin accumulation, which appear in an age-dependent manner. Furthermore, Ambra$^{1+/gt}$ mice are more sensitive to acute SI-induced retinal degeneration than their Ambra$^{+/+}$ littermates. UA induced mitophagy in vivo and prevented degeneration both in the neuroretina and RPE. This amelioration was also associated with decreased lipid peroxidation, gliosis and increased photoreceptor survival. In vitro, inhibition of mitophagy, or general macroautophagy, abolished this rescue.

Conclusions. Selective autophagy plays a protective role in the retina and can be exploited to preserve vision in physiological or pathological conditions.

Keywords: retina, neurodegeneration, autophagy.

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Transcription factor NRF2 participates in cell cycle progression at the level of G1/S and mitotic checkpoints.

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Transcription factor NRF2 is considered a master regulator of cell homeostasis, coordinating multiple cytoprotective responses that confer growth advantage to cells. Although its implication in redox homeostasis, detoxification or metabolic reprogramming has been widely studied, its participation in cell cycle division and the mechanisms governing this process has not been explored in detail.

In this study, we used several standard methods of synchronization of proliferating cells in G1, before S entry and during mitosis. Together with flow cytometry analysis, we monitored the participation of NRF2 along the cell cycle by knockdown of its gene expression. Our results showed that NRF2 levels are maximal at S phase entry, and minimal during mitosis. Besides, NRF2 absence caused both G1 and M arrest. A targeted transcriptomics analysis of cell cycle regulators showed that NRF2 depletion leads to changes in key cell cycle regulators, including *CDK2, TFDP1, CDK6, CDKN1A (p21), CDKN1B (p27), CCNG1 and RAD51.* This study gives a new dimension to NRF2 effects, showing its implication in cell cycle progression.

Keywords: Cell cycle, Restriction point, NRF2.

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Regulation of the DNA damage response by E2F4 phosphorylation in its T249/T251 conserved motif and Alzheimer's disease.

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Alzheimer's disease (AD) has a multifactorial etiology that includes DNA damage in neurons. The transcription factor E2F4, which can potentially regulate DNA repair, has two conserved threonines (T249/T251 in the mouse) that can be phosphorylated by p38MAPK (p38). The expression in vivo of the T249A/T251A E2F4 mutant form (E2F4DN) has been shown to be a multifactorial therapeutic agent against AD.

In this work, we have analyzed the effects of E2F4 phosphorylation in T249/T251 on the DNA repair response (DDR). To this aim, we used N2a mouse neuroblastoma cells treated with 10 µM camptothecin (CPT), a treatment known to induce Cited2 expression and subsequent cell death.

In this paradigm, the repression of Cited2 by E2F4 is abolished upon CPT treatment, thus allowing its E2F1-dependent expression followed by cell death. While E2F4 can be detected in both the nucleus and cytoplasm of N2a cells, phosphoT249-E2F4-specific immunoreactivity is specifically observed in the nucleus 4 h after treatment with CPT. Therefore, the known activation of p38 in response to CPT could lead to T249 phosphorylation of E2F4, thus suppressing its inhibition on E2F1 activity and allowing Cited2 expression. This hypothesis, is being tested in CPT- treated N2a cells co-transduced with adenoviral vectors expressing E2F1 together with either wild-type E2F4 or E2F4DN, in either the presence or absence of p38 inhibitors.

In summary, our work provides support for a novel mechanism used by E2F4 to regulate the response to DNA damage in pathological situations, which could participate in the therapeutic capacity of E2F4DN against AD.

Keywords: E2F4, E2F1, Cited2, Alzheimer's disease, DNA damage, p38, phosphorylation.

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Alteration of PGC1-α in the mitochondrial homeostasis of cardiomyocytes under hyperglycemia. Role of the GLP-1R activation

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Introduction: Cardiovascular disease is the major cause of morbidity and mortality in diabetic patients, and mitochondrial homeostasis can be a key organelle in cardiomyocytes. Peroxisome proliferator-activated receptor- gamma coactivator-alpha (PGC-1α), a master transcription factor of mitochondrial function, may be regulated under hyperglycemic stimulus and anti-diabetic drugs, such as Glucagon-like protein-1 receptor agonists (GLP-1RA).

Methodology: The mitochondrial protein expression of PGC-1α and Cytochrome-C was evaluated under 25 mM glucose (HG) and/or GLP-1RA stimulation in H9c2 cardiomyocytes after 18-48 hours. Western blotting was performed from cytosolic and enriched mitochondrial fractions. Also, PGC-1α related genes *(Cpt1α, Sdhb, Mfn1 and Nrf1)* were quantified by qPCR assay.

Results: Mitochondrial PGC-1α and Cytochrome-C, but not TFAM and PPARd, were increased only after 24h of HG. However, at this time, the levels of *Cpt1α, Sdhb, Mfn1 and Nrf1* were differentially regulated. Interestingly, co- incubation with GLP-1 and GLP-1RA attenuated expression of both PGC-1 and Cytochrome-C at the mitochondrial location.

Conclusions: Hyperglycemia may damage the myocardium by increase of cellular oxidation, but it also could enhance early-responses of cardioprotection by enhancing PGC-1α and Cytochrome-C at the mitochondria. Interestingly, activation of GLP-1RA may attenuate this effect, suggesting a new mechanism of action for GLP-1RA drugs.

Keywords: cardiomyopathy, Type 2 diabetes, PGC-1α, mitochondrial, Glucagon-like protein-1 receptor agonists
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Bone morphogenetic protein 2 is a new molecular target linked to nonalcoholic fatty liver disease with potential value as non-invasive screening tool.

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Background: Nonalcoholic fatty liver disease (NAFLD) is the commonest cause of chronic liver disease worldwide, being nonalcoholic steatohepatitis (NASH) its most clinically relevant form. Given the risks associated with taking a liver biopsy, the design of accurate non-invasive methods to identify NASH patients is of upmost importance. BMP2 plays a key role in metabolic homeostasis; however, little is known about its involvement in NAFLD onset and progression. This study aimed to elucidate the impact of BMP2 in NAFLD pathophysiology.

Methods: Hepatic and circulating levels of BMP2 were quantified in serum and liver specimens from 115 biopsy-proven NAFLD patients and 75 subjects with histologically normal liver (NL). In addition, BMP2 content and release was determined in cultured human hepatocytes upon palmitic acid (PA) overload.

Results: We found that BMP2 expression was abnormally increased in livers from NAFLD patients than in subjects with NL and this was reflected in higher serum BMP2 levels. Notably, we observed that PA upregulated BMP2 expression and secretion by human hepatocytes. An algorithm based on serum BMP2 levels and clinically relevant variables to NAFLD showed an AUROC of 0.886 (95%CI, 0.83–0.94) to discriminate NASH. We used this algorithm to develop SAN (Screening Algorithm for NASH): a SAN < 0.2 implied a low risk and a SAN ≥ 0.6 indicated high risk of NASH diagnosis.

Conclusion: This proof-of-concept study shows BMP2 as a new molecular target linked to NAFLD and introduces SAN as a simple and efficient algorithm to screen individuals at risk for NASH.

Keywords: nonalcoholic fatty liver disease, bone morphogenetic proteins, BMP2, non-invasive diagnosis, hepatocytes.

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Astrocytes of Nucleus Accumbens control the impairments derived from chronic exposure of THC.

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Drug use is a growing problem in actual society. Usually, the first experience with drugs takes place during adolescence, being cannabis the most used illicit drug. Although cannabis could be considered a harmless drug, we are beginning to appreciate its consequences. Chronic exposure to addictive drugs has shown to imbalance glutamate homeostasis in Nucleus Accumbens (NAc), altering plasticity mechanisms such as long-term depression. Therefore, it is crucial to elucidate the mechanisms underlying these alterations and how to reverse them. It is known the activation of cannabinoid receptors in astrocytes modulate synaptic plasticity and could be involved in glutamate homeostasis. However, the functional role of astrocytes in alterations derived from chronic drug exposure is not fully understood. In this study, we analyzed how astrocytes contribute to alterations produced by tetrahydrocannabinol (THC).

Using fiber photometry in vivo we analyzed astrocytic activity (Ca2+ and glutamate dynamics) in NAc after 1mg/kg THC chronic administration in wildtype and p38αMAPK-/- (Astrop38α) mice4 and we performed electrophysiology experiments to analyze synaptic plasticity. Moreover, we performed behavioral tests to assess whether THC had reinforcing properties or affected learning and memory. Furthermore, using a chemogenetic approaches (DREADDs) we activated NAc astrocytes to analyze their behavioral implications.
We observed: 1)THC increases astrocytic calcium activity; 2)THC induces glutamate release in NAc in wildtype, but not Astrop38α; 3)NAc astrocytes are involved in learning; 4)Removal of p38αMAPK in NAc astrocytes restores THC-related impairments.

Altogether, our results reveal astrocytes as critical elements for the maintenance of glutamate signaling, with a significant role in drug-use-related alterations.

Keywords: astrocytes, Nucleus Accumbens, THC, cannabinoid.

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Looking for novel interactors of IL-13 receptor α2 (IL13Rα2) and tyrosine-protein phosphatase non-receptor type 1 (PTPN1/ PTP1B) by using proximity-dependent biotinylation (BioID)

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Introduction: IL13Rα2 is a high affinity receptor for IL-13 that is re-expressed in late neoplastic stages. The IL- 13/IL-13Rα2 axis triggers invasion in colorectal cancer (CRC) and other cancers in a process mediated, among others, by the phosphatase PTPN1 (aka PTP1B), involved in the dephosphorylation of the autoinhibitory Src Tyr416, and further Src activation. PTPN1 takes part in IL13Rα2 internalization and associates with poor outcome in glioblastoma and CRC patients. Inhibition of IL13Rα2 pathway decreases invasion of multiple cancer cell lines (i.e., SKOV-3, PC-3 and KM12SM). To better define the oncogenic properties of IL13Rα2 and PTPN1, we aim to identify novel interactors by using proximity-dependent biotinylation coupled to mass spectrometry.

Materials and methods: PTPN1 and IL13Rα2 were cloned into pcDNA.5-pDEST-BirA-Flag-Ct by using LR clonase enzyme, obtaining the desired plasmids containing fusion genes PTPN1-BirA and IL13Rα2-BirA. Flp-In™ T-REx™ cells were co-transfected with pOG44 and pcDNA.5 vectors and selected with hygromycin. Then doxycycline and biotin were added to induce the expression of the fusion gene and proximity labelling. Biotinylated proteins were purified by affinity using streptavidin-sepharose, trypsin-digested and analysed in a Q-Exactive mass spectrometer. Raw data were analysed with MaxQuant, followed by Perseus for statistical analysis and SAINT for probabilistic scoring of protein-protein interaction data, which were subjected to gene ontology (GO) analysis.

Results: For IL13Rα2, 735 proteins were identified and 94 were scored as significant interactors by SAINT. GO enrichment analysis showed association with cadherin binding and response to reticulum stress, among others. 1009 proteins were identified and 217 were scored as significant PTPN1 interactors. GO enrichment analysis showed important association with mitotic cell cycle regulation, such as CDK1 (G2/M transition) or PCTN (centrosome component). Among the 19 IL13Rα2 and PTPN1 common interactors stand out those related to SNARE complex (YKT6, SNAP29 and VAMP3), which is involved in regulation of cancer invasion, drug resistance and kinase phosphorylation. Another interesting finding was the presence of VRK2, a kinase involved in MAPK pathway.

Conclusions: This study reveals novel individual interactors of IL13Rα2 and PTPN1, but also identifies common interactors of both proteins such as members of the SNARE complex and VRK2, opening new strategies for the inhibition of IL13Rα2 and PTPN1 in cancer metastasis.

Keywords: IL13Rα2, PTPN1, interactome, proximity biotynilation

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Association of mutant spectra of SARS-CoV-2 with COVID-19 disease severity

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Introduction: Replication of RNA virus, as SARS-CoV-2, leads to composition of sequences which are at different frequencies in the infected host. Minority variants present in mutant spectra can have an epidemiological importance generating more infectious variants, resistance to vaccines and drugs and affecting to the resolution of the disease. How different are the mutant spectra of virus isolated from patients with different disease outcome has not yet been studied.

Material and Methods: To approach this question, 30 patients infected with SARS-CoV-2 during the first pandemic wave in Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain were classified as mild, moderate and exitus according to the disease severity of COVID-19. RNA presents in the nasopharyngeal swabs of these patients was extracted. Four amplicons of nsp12 (polymerase) and two amplicons of spike (S) coding regions were amplified. PCR products were sequenced by ultra-deep sequencing (UDS) using MiSeq platform (Illumina). Sequences were analyzed with two pipelines and differences between the mutant spectra of each virus were determined. Possible structural and functional alterations of mutations detected have been studied.

Results: Most of the substitutions were found at frequencies between 0.5% to 30% in the mutant spectra. The number of mutations was significantly higher in patients with mild symptoms. The analysis of the mutant spectra resulted in a higher number of transitions and non-synonymous substitutions for in all COVID-19 categories. A major complexity of mutant spectra of virus isolated from mild patients were reflected in the study of several diversity indices, with significant statistical differences between COVID-19 categories. Structural analysis of nsp12 and spike substitutions, detected in the mutant spectra, showed possible alterations in the structure or function of the proteins.

Conclusion: The study of mutant spectra shows a positive association between the number of point mutations and the complexity of mutant spectra with a mild disease outcome. Possible models for this association are discussed. Structural and functional alterations are under study right now.

Keywords: COVID-19 severity; Mutant spectra; Ultra-deep sequencing

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Multiplex Immunofluorescence reveals specific subsets of immune cell populations expressing CD137/TNFRSF9 as predictors of unfavorable outcomes in Hodgkin Lymphoma

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The tumor microenvironment (TME) is a crucial determinant of tumor growth, progression, and resistance to chemotherapy in cancer, with classical Hodgkin's lymphoma (cHL) being one of the most representative examples. The major component of the tumor mass in cHL is a rich and vast immune microenvironment, comprising a diverse repertoire of cell populations, many of which remain unknown. The specific contribution of the different functional cell subsets in the TME, such as T lymphocytes, monocytic populations, or dendritic cells, is also undiscovered. Currently, around 30% of advanced-disease patients or some atypical cases of primary refractory tumors do not respond to treatment or relapse shortly afterwards. Response predicting features of TME have been recently identified, which suggest a rationale for alternative therapeutic strategies. All existing evidence indicates that HRS cells actively coordinate and remodel their complex TME and that its diverse components influence the therapeutic response in cHL patients.

The sensitivity of current technology enables analysis of specific functional phenotypes in the TME as diagnostic and predictive biomarkers. One example is the quantitative dissection of the TME with in situ multispectral imaging using formalin-fixed, paraffin-embedded (FFPE) tissues, with which we could analyze simultaneously CD68, CD137, CD30, CD3, PDL1, and PD1 markers to identify specific cell subsets.

Remarkably, some cell populations expressing CD137 were associated with unfavorable responses in cHL patients, such as the activated monocytic cells (CD68+ CD137+), the tumor-reactive T cells (CD3+ CD137+), and the tumor CD30+ CD137+ PDL1+ cell subset. By contrast, the CD3+ CD137- T-cell population was associated with better prognosis, suggesting that CD137 could be a new prognostic marker in cHL for poor outcomes. Moreover, some cell subsets that could not be studied until now due to the lack of appropriate technology were found to be more abundant in the first stages of the disease, whereas others were less abundant than in stage IV. For instance, we could study exhausted T cells (CD3+ PD-L1+ PD-1+) and other related cells that were largely unexplored in cHL. Finally, these findings were validated with independent series of patient samples, including immunohistochemical studies with specific antibodies and gene-expression analysis.

Keywords: classic Hodgkin Lymphoma; tumor microenvironment; immune cell phenotypes; CD137; TNFRSF9

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Targeted endothelial knockout of caveolin-1 in established atherosclerosis does not inhibit plaque progression

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Caveolae are small invaginations of the plasma membrane with functions in mechano-sensing and -adaption, membrane transport and transcytosis. Caveolin-1 (Cav1) is required for caveolae to form, and previous research has shown that global Cav1 knockout substantially reduces early atherosclerosis in mice – an effect suggested to be mediated by reduced endothelial LDL transcytosis. In the present study, we tested whether targeting caveolin 1 in endothelium of already established atherosclerosis in mice can be a potential therapy to limit plaque progression.

To allow the manipulation of endothelial Cav1 in atherosclerotic plaques, we bred mice that harbored a tamoxifen-inducible Cre recombinase transgene expressed under the control of the endothelium-specific cadherin 5 (Cdh5) promoter and were homozygous for either the wildtype (control, n= 32 males and n= 36 females) or a floxed Cav1 ($Cav1^{ECKO}$, n=31 males and n= 31 females) allele. Atherosclerosis was induced by injecting a single dose of adeno-associated virus vector encoding a gain of function mutant proprotein convertase subtilisin/kexin type 9 (PCSK9) followed by feeding high cholesterol diet for 20 weeks. At 8 and 4 weeks prior to study end, 5 doses of tamoxifen (20 mg/ml) were administered to control and $Cav1^{ECKO}$ mice. Recombination efficiency was assessed by immunofluorescence for Cav1. Plasma lipids were measured by standard techniques. Atherosclerotic plaque size, structure (necrotic core, lipid and collagen content), content of macrophages (CD68+) and smooth muscle cells (ACTA2+), and the expression level of fibronectin were studied by histological and immune fluorescence techniques in sections of the aortic root.

Caveolin 1 protein expression in plaque endothelium was reduced by 90% in $Cav1^{ECKO}$ compared with control mice by immunofluorescence microscopy (%area fraction of cav-1 signal in endothelium: 67.49 ± 16.87 $Cav1^{WT}$ vs 6.89 ± 8.66 $Cav1^{ECKO}$ in females and 59.85 ± 16.41 $Cav1^{WT}$ vs 5.47 ± 3.85 $Cav1^{ECKO}$ in males). In $Cav1^{ECKO}$ mice, plasma total and LDL cholesterol were slightly increased (15%) at endpoint (10.67 ± 2.82 for Cav1ECKO vs 9.6 ± 3.52 for $Cav1^{WT}$ in females and 14.31 ± 4.61 for Cav1ECKO and 11.48 ± 4.42 for Cav1WT mmol/L). No significant differences in plaque size, necrotic cores, lipid deposits, collagen composition, or content of macrophages and smooth muscle cells were observed. Fibronectin was reduced in $Cav1^{ECKO}$ males but not in females.

Efficient targeting of Cav-1 in the endothelium of murine plaques cannot stop the progression of already established atherosclerotic lesions.

Keywords: atherosclerosis, caveolin-1, endothelial cell

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Hypoxia classifier for transcriptome datasets

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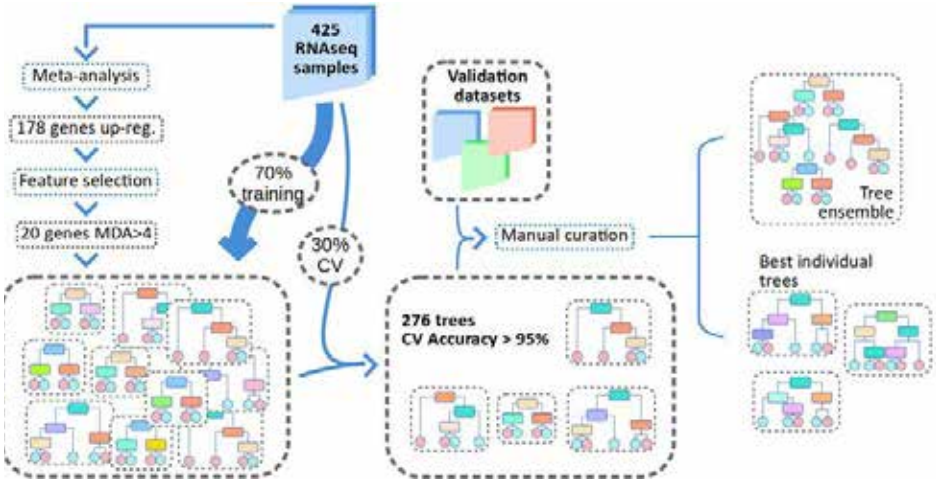
Molecular gene signatures are useful tools to characterize the physiological state of cell populations, but most have developed under a narrow range of conditions and cell types and are often restricted to a set of gene identities.

Focusing on the transcriptional response to hypoxia, we aimed to generate widely applicable classifiers sourced from the results of a meta-analysis of 69 differential expression datasets which included 425 individual RNA-seq experiments from 33 different human cell types exposed to different degrees of hypoxia (0.1-5\%O\textsubscript{2}) for 2-48h.

The resulting decision trees include both gene identities and quantitative boundaries, allowing for easy classification of individual samples without control or normoxic reference. Each tree is composed by 3-5 genes mostly drawn from a small set of just 8 genes (EGLN1, MIR210HG, NDRG1, ANKRD37, TCAF2, PFKFB3, BHLHE40, and MAFF). In spite of their simplicity, these classifiers achieve over 95\% accuracy in cross validation and over 80\% accuracy when applied to additional challenging datasets. Our results indicate that the classifiers are able to identify hypoxic tumor samples from bulk RNAseq and hypoxic regions within tumor from spatially resolved transcriptomics datasets. Moreover, application of the classifiers to histological sections from normal tissues suggest the presence of a hypoxic gene expression pattern in the kidney cortex not observed in other normoxic organs. Finally, tree classifiers described herein outperform traditional hypoxic gene signatures when compared against a wide range of datasets.

This work describes a set of hypoxic gene signatures, structured as simple decision tress, that identify hypoxic samples and regions with high accuracy and can be applied to a broad variety of gene expression datasets and formats.



Keywords: Transcriptome classification; Hypoxia; Gene expression; RNA-seq; Spatial transcriptomics

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Competing Interests: The authors declare that they have no competing interests.

Building insights into CDC37-RAF1 interaction: an opportunity for design new strategies to treat KRAS driven tumors.

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Introduction: genetic interrogation of the KRAS signaling pathway in genetically engineered mouse models harboring a $KRAS^{G12V}$ mutation has shown that RAF1 ablation promotes tumor regression, and is therefore highlighted as a promising therapeutic target. Recently, our group has been able to purify the full-length form of RAF1 in its native state and resolve part of its structure by Cryo-Electronic Microscopy. RAF1 is found as a component of a protein complex (RHC complex), including the chaperone HSP90 and its cochaperone CDC37, being this interaction crucial for RAF1 stability. In this work, we interrogate the role of CDC37-RAF1 interaction in the stability of RAF1 in order to identify effective approaches to degrade this kinase.

Materials and Methods: by site-directed mutagenesis we generated a plethora of RAF1 mutants, altering key residues located in its CDC37-binding region. Then, the relative levels of the RAF1 mutant proteins in the isolated RHC complexes were assessed by mass spectrometry (MS) and compared to those formed by the wild type protein.

To further evaluate the relevance of CDC37-RAF1 disruption, we designed small peptides covering the region of interaction. The binding affinity of these peptides was assessed by incubating the cochaperone with a nitrocellulose-bound dodecapeptide array (PepScan) displaying the corresponding RAF1 sequences. Then, to assess the phenotypic effect of the selected peptides, we functionalized them for *in vivo* delivery, using a TAT cell penetrating motif. After treating cells with RAF1 specific and non-specific peptides, culture proliferation was measured.

Results: according to MS results, some of the mutants resulted in RAF1 natural degradation, and same results were observed when the key residues involved in the interaction were mutated in CDC37 instead of RAF1. On the other hand, the treatment with CDC37-RAF1 interfering peptides selectively inhibited growth in lung cancer cell lines. These observations suggest that these RAF1-derived peptides may prevent RHC complex assembly, hindering RAF1 activity and thereby affecting cell proliferation.

Conclusions: altogether, our results highlight the importance of chaperon association for RAF1 stability, raising the possibility that the interface between RAF1 and CDC37 may represent a vulnerable region, which could be targeted to induce the degradation of RAF1. Our preliminary data point out to the use of peptidomimetics as a potential pharmacological approach capable of reproducing the therapeutic results obtained in experimental mouse models of lung cancer upon ablation of RAF1 expression, representing a potential strategy to treat KRAS driven tumors.

Keywords: RAF1, CDC37, lung adenocarcinoma, peptidomimetics, protein-protein interaction.

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Automatic synapse parameter exploration for the interaction of living neurons and models in hybrid circuits and hybrots

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Hybrid circuits that connect living and model neurons allow studying neural dynamics to assess the role of specific cells and synapses in emergent phenomena of neural computation (Reyes-Sanchez et al. 2020). In this work, we deal with the automatic adaptation and mapping of parameters in hybrid circuits and, in particular, those that yield dynamical invariants. Such invariants take the form of robust relationships between the intervals that build robust sequences arising from the cell interactions and have been recently unveiled in well-known CPGs (Elices et al. 2019).

In our methodology, we input biological time series with a characteristic temporal structure of spiking-bursting dynamics to different model neurons with monodirectional and bidirectional synapses implemented with dynamic clamp. To illustrate the protocol, we searched for dynamical invariants established between a living pyloric CPG cell and a Komendantov-Kononenko model neuron connected through a graded synapse model. The biological recordings were preprocessed to automatically adapt the corresponding time and amplitude scales to those of the synapse and neuron models employed. Our automatic experimental protocol then mapped the neuron and synapse parameters that yielded a predefined dynamical invariant. By using parallel computing, this approach readily achieved a full characterization of the parameter space that resulted in the predefined target dynamics. To search for dynamical invariants in real-time bidirectional connections, we also developed a genetic search that found valid set of parameters to reproduce the target dynamical invariant in a few iterations.

We illustrated this methodology in the study of the coordination generated by the dynamical invariants to balance flexibility and robustness in neural rhythms. Our results demonstrate that maps showing the presence of dynamical invariants can be built in a few minutes for monodirectional hybrid circuits and that the genetic algorithm can readily find dynamical invariants in bidirectional connections between living and model neurons. The proposed strategy can be generalized for any hybrid circuit and can also be used in the design of hybrots, i.e., robots whose locomotion is controlled by living neural circuits with feedback from the sensor robots.

Keywords: Electrophysiology, computational neuroscience, hybrid circuits

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The secretome of highly metastatic cells as a source of biomarkers and metastatic effectors in colorectal cancer patients.

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Introduction: Colorectal cancer (CRC) is the second leading cause of cancer death in developed countries, mainly as a consequence of metastatic spread. Secreted proteins are essential to communicate the cancer cells with the tumor- microenvironment, favoring tumor progression and metastasis. The conditioned medium or cellular secretome, including exosomes, has demonstrated to be a rich source of metastatic effectors and biomarkers of metastatization in different tumors. Our goal was to identify proteins altered in the secreted fraction of highly metastatic cells in order to determine altered biological pathways and potential biomarkers of prognosis.

Material and methods: A quantitative label-free proteomic analysis was carried out on the secretome of highly and poorly metastatic CRC cell lines from different genetic background. Proteomic results were validated using unbiased transcriptomic analyses. Hazard ratios and long-rank tests for the differentially-secreted proteins were determined in four different external datasets in order to select the proteins with clinical relevance for further analysis. Gene ontology was performed to determine the biological processes altered in highly metastatic cells.

Results: 221 differentially-secreted proteins were found to be significantly associated to metastasis. Some corresponding genes were able to predict the overall and progression free survival in stage II and stage III CRC patients. In addition, they showed higher expression in the stem cell-like and CMS4 subtypes of CRC, associated to worse prognosis. These genes were also associated to deficient mismatch repair, CpG-island methylator positive status and BRAF mutation. It is also remarkable that a significant number of altered proteins were involved in cholesterol metabolism, suggesting an enhancement of the LDL uptake and metabolism in highly metastatic cells. Extracellular matrix constituents involved in cell adhesion and migration were also overrepresented in the highly metastatic fraction.

Conclusion: Secretome of highly metastatic cells is enriched in proteins involved in essential pathways for metastasis such us adhesion, migration and lipid metabolism. In addition, several proteins with a robust and significant prognosis value were detected howing the value of the fraction as a source of biomarkers.

Keywords: Colorectal cancer, secretome, biomarkers, prognosis avier Robles was supported by an IND2019/BMD-17153

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Does MAPT have anything new to say? Discovery of novel non-aggregative Tau isoforms that are decreased in Alzheimer's disease.

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Introduction: Tauopathies, including Alzheimer's disease (AD), are a group of neurodegenerative disorders characterised by Tau hyperphosphorylation. Post-translational modifications of Tau such as phosphorylation and truncation have been demonstrated to be an essential step in the molecular pathogenesis of tauopathies. Alternative splicing is the process by which a single gene can produce multiple transcripts and, potentially, as many different proteins. Tau isoforms are generated by alternative splicing from a single gene, *MAPT*. In this work, we demonstrate the existence of a new, human- specific truncated form of Tau generated by intron 12 retention.

Materials and methods: Intron-12-retaining MAPT transcripts were detected using qPCR with SH-SY5Y neuroblastoma human cells and confirmed in human brain samples. Results were validated on a database of human RNA-seq samples (363 samples from three brain regions). Functional assays were carried out after cloning the isoform on the eukaryotic and prokariotic expression vectors pSG5 and pRK172. Functional analysis evaluated sarkosyl solubility, heparin-induced self-aggregation, microtubule stabilisation and binding affinity and epitope phosphorylation; all of them evaluated by Western blot. Heparin-induced aggregation and microtubule stabilisation were also analysed by electron microscopy. Protein levels on human samples were analysed by Western blot on Alzheimer's brain classified according to Braak stages I (n = 3), II (n = 6), III (n = 4), IV (n = 1), V (n = 10) and VI (n = 8), and non-demented control subjects (n = 10).

Results and conclusions: In this work, we demonstrated the existence of a previously undescribed, human-specific truncated tau isoform generated by intron 12 retention. RNA transcripts retaining intron 12 were detected in SH-SY5Y cells and, to a greater extent, in human brains, which were then further confirmed on a larger RNAseq public database. Functional analysis demonstrated that, while this new Tau isoform exhibits similar post-transcriptional modifications by phosphorylation and affinity for microtubule binding, it is less prone to aggregate than other Tau isoforms. Importantly, diminished protein levels of this new Tau isoform are found in Alzheimer's patients' brains with respect to non-demented control subjects, suggesting that the lack of this truncated isoform may play an important role in the pathology. Our results open up new research avenues focused on the exploration of *MAPT* alternative splicing and the striking characteristics of novel isoforms, that may help develop future therapies for Alzheimer's disease and other tauopathies.

Keywords: Alternative splicing; Alzheimer's disease; Intron retention; Tau; Tauopathies; Truncation.

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Unravelling neuron-astrocyte communication in the dorsal raphe nucleus.

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Introduction: Serotonin is a neuromodulator widely spread throughout the central nervous system and involved in a vast variety of behaviors, such as cognitive functions and emotional states. On this basis, serotonergic dysfunction has been related to several psychiatric disorders such as Major Depressive Disorder, schizophrenia, or autism. Despite serotonin's widespread distribution, the location of serotonergic neurons is restricted to the midbrain raphe nuclei. In this context, astrocytes are known to participate in synaptic transmission modulating neural circuits; however, their role in the serotonergic system remains largely unknown. My study aims to elucidate the neuron-astrocyte signaling at serotonergic nuclei, looking at the dorsal raphe nucleus (DRN).

Materials and methods: For the purpose of this project, we performed intracranial injections of viral constructs in adult mice to express the Ca2+ indicator GCaMP6 specifically in DRN astrocytes, Ca2+ imaging experiments to record astrocytic activity, electrophysiological recordings of neuronal activity using patch-clamp and immunohistochemistry techniques.

Results and conclusions: Preliminary data show that astrocytes from DRN respond to serotonin with an increase in the amplitude and frequency of intracellular Ca2+ events, which is partly mediated by serotonin type 2 receptors. As a consequence of triggering Ca2+ signaling, we found that serotonin induces gliotransmission, shown by an increased frequency of Slow Inward Currents (SICs) and modulation of excitatory synaptic transmission in dorsal raphe neurons. In conclusion, these data suggest that astrocytes might play a role in synaptic transmission and neuronal excitability in the DRN and, therefore, in the serotonergic-mediated actions of DRN.

Keywords: Astrocyte, serotonin, dorsal raphe, glia.

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Competing Interests: The authors declare no competing interests.

Resolvin D2 prevents cardiovascular damage in angiotensin II-induced hypertension

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Introduction: Vascular functional and structural alterations induced by hypertension are greatly influenced by low- grade chronic inflammation. Resolution of inflammation is orchestrated by specialized lipid pro-resolving mediators (SPMs), which derive from n3 fatty acids (PUFAs). Previous evidence suggest that SPM prevent vascular damage in several pathological situations including atherosclerosis or vascular restenosis. Among SPMs, resolvins (Rv) seem to have beneficial effects in some cardiovascular pathologies, but little is known about their effect on cardiovascular damage in hypertension.

Objective: The aim of this study was to evaluate the effects of resolvin D2 (RvD2) in blood pressure and cardiovascular damage associated with hypertension.

Material and methods: aorta, mesenteric resistance arteries (MRA), heart and peritoneal macrophages were taken from C57BL/6J mice, infused or not with angiotensin II (AngII; 1,44mg/kg/day; for 14 days) in presence or absence of RvD2 (100ng/mice, every second day, started before AngII infusion). Blood pressure was measured by tail-cuff plethysmography. Cardiac and vascular function and structure were studied with wire and pressure myographs, confocal microscopy, histological staining, and echocardiography. Circulating leucocyte were analyzed by flow cytometry and macrophages behavior by electrophysiology. Gene expression was analyzed with RT-PCR.

Results: Aorta or heart from AngII-infused mice showed altered expression of enzymes and receptors involved in SPMs biosynthesis and signaling. We also observed a downregulation of SPMs in heart tissues from these mice including 17R-RvD1 and RvE3. Treatment with RvD2 partially prevented the increase in blood pressure and in the content of circulating immune cells induced by AngII. RvD2 treatment also improved cardiac hypertrophy, fibrosis and dysfunction. Moreover, RvD2 treatment reduced vascular hypercontractility and endothelial dysfunction induced by AngII likely because of enhanced NO and PGI2 availability. RvD2 normalized AngII-induced vascular remodeling by decreasing media thickness and number of vascular smooth muscle cell, while it did not affect vascular stiffness. Finally, RvD2 reduced aortic and cardiac leukocyte infiltration and shifted macrophage phenotype towards a pro-resolving phenotype.

Conclusion: Our data shows that RvD2 treatment limits the cardiovascular alterations induced by hypertension. These findings highlight that activating resolution mechanisms by treatment with RvD2 may represent a novel therapeutic strategy for the treatment of cardiovascular alterations associated to hypertension.

Keywords: hypertension, cardiovascular damage, inflammation, proresolving lipid mediators.

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Resolvin E1 attenuates endothelial senescence induced by doxorubicin through the modulation of NLRP3 inflammasome activation.

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Introduction: Vascular aging is associated with endothelial cell senescence, favoring low-grade inflammation, endothelial dysfunction, and cardiovascular diseases. Cell senescence arises from a wide variety of endogenous and exogenous stressors including some anticancer agents such as doxorubicin. Recently, doxorubicin was linked to the innate immunity component NLRP3 inflammasome which is implicated in many vascular inflammatory disorders. There is a need for therapeutic tools to help cancer patients who have been exposed to cardiovascular toxic chemotherapy averting premature vascular complications. We investigated whether resolvin E1 (RvE1), an endogenous lipid mediator of the inflammation resolution phase, could prevent doxorubicin-induced senescence in cultured human umbilical veins endothelial cells (HUVEC) with focus on a potential involvement of the NLRP3 inflammasome.

Materials and Methods: Cell senescence was quantified by senescence-associated-β-galactosidase (SA-β-gal) staining. The expression of senescence markers (γH2AX, p21, p53) and inflammatory markers (pP65, NLRP3) was determined via Western blot. NLRP3 inflammasome activation was determined by visualizing the formation of ASC specks by indirect immunofluorescence.

Results: Doxorubicin (25 nmol/L) augmented the number of SA-β-gal positive HUVEC and the levels of γH2AX, p21 and p53 which were all reduced by RvE1 (10 nmol/L). In doxorubicin-treated cells, RvE1 further reduced the expression of pP65 and NLRP3 proteins and the formation of ASC specks as did the inflammasome assembly inhibitor MCC950 (1µmol/L). Additionally, both MCC950 and interleukin-1 receptor inhibitor anakinra diminished SA-β-gal positive staining induced by doxorubicin.

Conclusion: RvE1 offers a novel therapeutic approach against doxorubicin-induced cardiovascular toxicity and subsequent age-related vascular disorders by counteracting endothelial senescence through the modulation of NLRP3- inflammasome activation.

Keywords: Resolvin E1; Endothelial senescence; NLRP3 inflammasome; Doxorubicin; Interleukin 1β; Vascular aging

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Competing Interests: The authors declare no competing interests.

Infections in children with Juvenile Idiopathic Arthritis, more frequent than in healthy children? Prospective multi-center observational study.

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Abstract

Background

Children with JIA may have a higher risk of infection. Our objectives are to describe and compare infection rates in JIA patients vs non-JIA counterparts.

Methods A prospective, multicenter observational study was performed in Spain from 01/2017 to 06/2019. JIA patients from 7 participating hospitals and children without JIA (siblings of JIA patients, and non-JIA children from primary health centers) were followed up with quarterly questionnaires recording infection episodes. Tuberculosis, Herpes Zoster, and infections requiring hospital admission were considered severe infections. Rate of infection (episodes/patient/year) was compared using a Generalized Estimating Equations Model.

Results 371 children (181 JIA and 190 non-JIA) were included. Median age was 8.8years (IQR 5.5 – 11.3); 75% of JIA patients received immunosuppressive treatment (24% methotrexate, 22% biologic, 26% both). 667 infections were recorded,

15(2.2%) considered severe. Infection rate was 1.31 (95%CI 1.1 – 1.5) in JIA and 1.12 (95%CI 0.9 – 1.3) in non-JIA participants (p0.19). Age <4 years increased 2.5 times infection rate (2.51 vs. 0.98, p<0.001) in both groups. The most frequent infection sites were upper respiratory (62.6% vs. 74.5%) and gastrointestinal (18.8%vs 11.4%). There were no differences in severe infections (2.5%vs 2%, p0.65) among groups. In JIA children, younger age and higher disease activity (JADAS71) were associated with a higher infection rate.

Conclusions Despite being a potential risk group, we found no differences in infection rate, severity, type and antibiotic treatment between patients with and without JIA. Most infections were mild. Age below 4 years increased infection risk in both groups. Higher disease activity was associated with a higher infection rate in JIA patients.

Keywords: Juvenile Idiopathic Arthritis1, Safety2, Infections3

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THE SONIC HEDGEHOG AGONIST SAG ATTENUATES MITOCHONDRIAL DYSFUNCTION AND DECREASES THE NEUROTOXOCITY INDUCED BY FRATAXIN-DEFICIENT ASTROCYTES

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Friedreich's ataxia (FRDA) is predominantly a neurodegenerative disease caused by the deficiency of a protein called frataxin (FXN). Although the main pathological alterations are observed in neurons, it is becoming clear that other non-neuronal cells such as astrocytes may be actively involved in the neurodegenerative process associated with the disease.

Depending on the stimuli they respond to, astrocytes acquire different activation states in a process called astrogliosis. Neuroinflammatory stimuli induce the formation of A1 reactive astrocytes, which upregulate proinflammatory genes, being harmful for neurons. A1 astrocytes have been detected in post-mortem tissue of patients with different neurodegenerative disorders, being hypothesized that they might have deleterious effects on neurons, exacerbating the neurodegenerative process. Recent studies have demonstrated positive effects of Sonic Hedgehog (SHH) agonists in astrocyte viability and proliferation, astrocyte-mediated neuroprotection, and also positive effects in mitochondrial activity and dynamics. As mitochondrial changes are important components in the etiology of neurodegenerative disorders, the influence of SHH agonists in mitochondrial physiology could be of therapeutic relevance. In this work, we have thoroughly characterized astrocyte reactivity phenotype and mitochondrial status of FXN-deficient human astrocytes, evaluating as well the effect of SHH agonists on astrocyte reactivity, viability, and function.
We used an in vitro model based on a short hairpin RNA packaged in a lentiviral vector, which allowed us to decrease FXN levels in human cortical astrocytes, to similar levels as those observed in FRDA patients, and found that FXN-deficient cells had less cell viability and higher expression of several A1 reactive astrocyte markers, than control cells. Both phenomena were prevented by a chronic treatment with the smoothened agonist (SAG), a SHH signaling agonist. Moreover, FXN-deficient astrocytes showed defects in mitochondrial function and dynamics, which were partially rescued by SAG. Regarding the possible neuroprotective effects of SHH agonists, previous results showed that FXN-deficient astrocytes are able to induce neurodegeneration, and we have observed that the chronic treatment with SAG attenuated the neurotoxicity triggered by the treatment of mouse cortical neurons with conditioned medium of FXN-deficient astrocytes.

Overall, our results suggest that the treatment of FXN-deficient astrocytes with a SHH agonist like SAG, could be used as a possible target to reduce FRDA-associated neurodegeneration.

Keywords: Frataxin; Mitochondrial dysfunction; Neurotoxicity; Reactive astrocytes; Smoothened agonist; Sonic hedgehog.

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Competing Interests: The authors declare that no competing interest exists.

Mitophagy boosting protects cells against MNU toxicity

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Objective: A pharmacological model to study the human disease Retinitis Pigmentosa is N-methyl-N-nitrosourea (MNU) injection which results in retinal degeneration. Our objective is to study the autophagy and mitophagy status of the cells in this disease model.

Materials and methods: The mitophagy reporter (MitoQC) and autophagy reporter mice (mCherry-GFP-LC3) have been used to determine the mitophagic and autophagic flux in the retina after MNU injection. Retinal pigment epithelium cell line (ARPE-19) MitoQC has been used to elucidate the cellular and molecular mechanisms after MNU treatment.

Results: Neurodegeneration events appear one day after intraperitoneal MNU injection: reduction of photoreceptors thickness layer and increase of TUNEL and GFAP staining. Mitophagosomes and autophagosomes are less frequent in the retina of mice treated with MNU, but they are bigger and they tend to accumulate in the external limiting membrane. ARPE-19 cells are also vulnerable to MNU in a dose-dependent manner producing DNA damage, cytoplasm vacuolization, organelle alterations and cell death. Mitophagy levels depend on the MNU dose: an increase of the mitophagic flux is observed at low doses, whereas it seems to be blocked at high doses. We demonstrate this mitophagy is PINK-Parkin dependent and boosting this pathway makes cells more resistant to MNU. Finally, we achieve to protect MNU-treated retinae explants with the mitophagy inductor, DFP.

Conclusion: MNU treatment induces activation of PINK-Parkin dependent mitophagy. We propose that mitophagy could act as a defense mechanism in this disease model.

Keywords: Retinitis Pigmentosa, MNU and Mitophagy.

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