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1st PhD Research Symposium in Health Sciences and Biomedicine.

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Madrid, May 18th 2018

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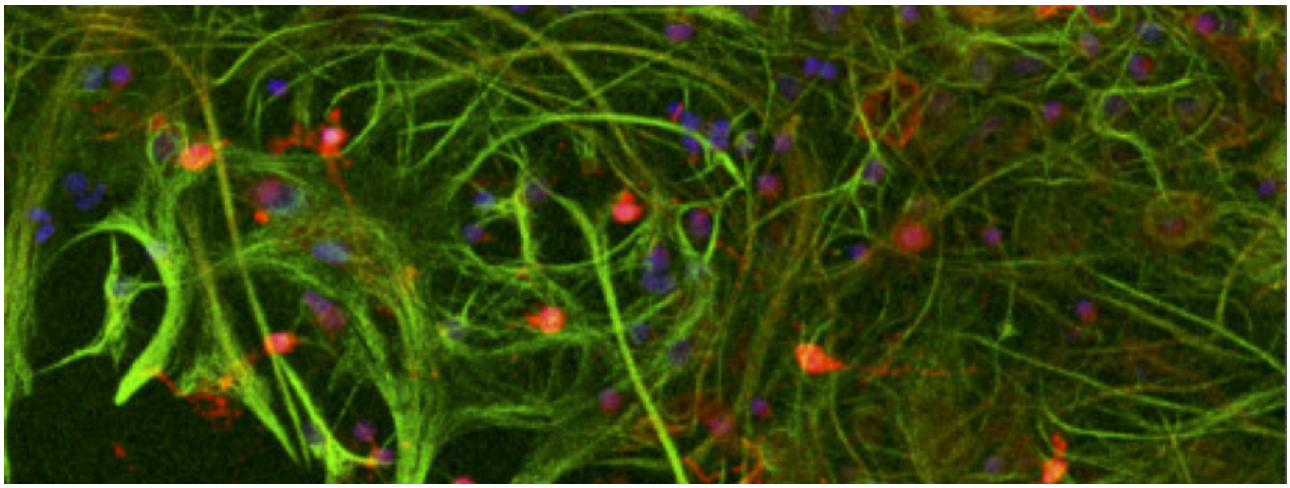

1st PhD Research Symposium in Health Sciences and Biomedicine

Abstract Book

School of Medicine
Universidad Autónoma de Madrid
18 May 2018

UAM UNIVERSIDAD AUTÓNOMA DE MADRID

Facultad de Medicina

Welcome and presentation

On behalf of the organizing committee, we welcome you to the first **1st PhD Research Symposium in Health Sciences and Biomedicine** organized by the Faculty of Medicine of Universidad Autónoma de Madrid (UAM), Madrid.

The Symposium will take place at the **Medicine Campus UAM** on **May 18th, 2018**. Doctoral Programs in Health Sciences and Biomedicine (Medicine and Surgery, Epidemiology and Public Health, Molecular Biosciences, Microbiology, Neuroscience, Pharmacology and Physiology, Clinical and Health Psychology) with merging common interests in this area will celebrate a one-day Symposium to strengthen their commitment to broaden collaborative strategies in education and research.

The meeting will be a stimulating gathering of advanced graduate students, where **those at least in their second academic tutelage**, are invited to present a scientific communication including the main hypothesis, objectives and results of their Doctoral Theses. PhD students in their first year are welcome for registration and meeting attendance.

This innovative event will be an optimal forum to showcase their research and foster communication and potential collaborations between PhD students from different laboratories. The Symposium will consist of both moderated **poster sessions and oral communication sessions by PhD students**, providing ample time for discussion both within and outside the scientific sessions. In addition to the regular presentations, we have scheduled an **invited keynote lecture** on professional scientific skills and career guidance that will be inspirational for young researchers. The meeting will be held in **English**.

The Symposium has the major aim of preparing our future PhDs for an excellent performance in critical exposition and data discussion both for their thesis defense and future scientific meetings. We expect original research and lively discussions on a broad range of topics within Health Sciences and Biomedicine between clinical and basic researchers from our faculty, hospitals and associated centers of research.

We hope you enjoy the Symposium,

Juan Antonio Vargas Núñez

Dean of the Faculty of Medicine (UAM)

Honorary President of the Symposium

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Programme

8:30-9:00 **Registration**
Main Hall

9:00-9:15 **Welcome & Opening**
Aula Magna

9:15-10:45 **Oral Session 1 Neuroscience Aula Magna**
Chairpersons:
María García-Amado (Dpto. AHyN, UAM)
Ana. I Rojo (Dpto. Bioquímica, UAM)

1.1. NEURO01 Noradrenaline innervation in the primate thalamus: similarities and differences in macaques and human. ***I. Pérez Santos***

1.2. BIO12 The role of p27kip1 in the development, differentiation and maturation of mesencephalic dopaminergic neurons. ***C. Palmer***

1.3. BIO27 The role of PKD1 in brain injury: ROS detoxification and neuroprotection. ***J. Pose***

1.4. MED19 The response to a high fat diet in a mouse model of Alzheimer's disease is sexually dimorphic. ***A. Freire-Regatillo***

1.5. MED23 Exosomes as predictive biomarkers in acute ischemic stroke patients: a traslational research approach. ***E. Alonso López***

1.6. PHAR08 Effect of antidepressants of clinical use on neuronal nicotinic acetylcholine receptors. ***I. Gameiro-Ros***

9:15-10:45 **Oral Session 2 Endocrinology, Metabolism and Immunoinflammation**
Seminario 1
Chairpersons:
Juan Antonio Moreno (IIS-Fundación Jiménez Díaz)
Julián Aragonés (Dpto. Medicina, UAM)

2.1. MED06 Study of genetic basis of idiopathic central precocious puberty. ***N.V.Ortiz-Cabrera***

2.2. BIO07 A novel role for the Hippo pathway mediator TAZ in thyroid differentiation. ***C. Fernández-Méndez***

2.3. BIO19 The Role of Liver X Receptors in the homeostasis of Splenic Red Pulp Macrophages and Iron Metabolism. ***M. C. Orizaola***

2.4. BIO26 Tofacitinib restores the inhibition of reverse cholesterol transport induced by inflammation: understanding the lipid paradox associated with rheumatoid athritis. ***S. Pérez-Baos***

2.5. PHAR04 Anakinra prevents interleukin-1β-dependent endothelial damage and vascular smooth muscle inflammation in diabetic vasculopathy. ***A. San Hipólito Luengo***

10:45-11:15 **Coffee & Poster Viewing**
Main Hall

11:15-12:45 **Oral Session 3 Cardiovascular and Renal**
Aula Magna
Chairpersons:
José Luis López Sendón (Dpto. de Medicina, UAM)
Rafael Selgas (Dpto. de Medicina, UAM)

3.1. PHAR10 Oxidative stress is linked to lifetime cardiovascular risk stratification in young/middle age individuals. ***E. Rodríguez-Sánchez***

3.2. MED17 Adequacy of antithrombotic treatment, characteristics and in-hospital mortality of elderly patients with non-valvular atrial fibrillation: results of the NONAVASC registry. ***A. Gullón***

3.3. PHAR02 The noncanonical Notch ligand DLK1 regulates renal inflammation. ***L. Márquez Expósito***

3.4. PHAR05 Pharmacogenetic algorithm for acenocumarol dosing improve anticoagulation control: a multicenter randomized clinical trial. ***H.Y. Tong***

3.5. MED07 Comparison of pharmacological treament alone versus treatment combined with implantable cardioverter-defibrillator therapy in patients over 75 years. ***J.A. Palfy***

3.6. MED04 A stardard AV fistula in animal model. ***C. Arriagada***

3.7. PHAR11 Loss of NLRP6 expression increases the severity of kidney injury. ***L. Valiño-Rivas***

Oral Session 4 Oncology
Seminario 1

11:15-12:45

Chairpersons:
Ricardo Sánchez (IIBm)
Antonio Pérez Martínez (Dpto. de Pedriatría, UAM)

4.1. BIO10 S100A9 mediates resistance of brain metastasis to radiation therapy. ***C. Monteiro***

4.2. BIO29 Study of the role of microRNAs in T-cell lymphoblastic lymphoma development through the regulation of expression of FBXW7 gene. ***I. Vázquez-Domínguez***

4.3. BIO48 Functional analysis of Mastl mutations in cancer**.** ***M. Maroto***

4.4. BIO04 RNA-sequencing analysis identifies downstream genes of TCF4 involved in the development of non-small cell lung cancer. ***O. Vera***

4.5. OTHER04 Mitochondrial activity plays a critical role in multiple myeloma resistance. ***A. Ortiz***

Plenary Lecture "5 Ways to Keep Attention When Presenting"

12:45-13:45

Aula Magna
G. Sánchez Prieto
Socio Director Grupo BLU. Profesor de Habilidades ICADE

13:45-15:00 **Lunch**

15:00-16:30 **Moderated Poster Session**
Main Hall

Oral Session 5 Novel Therapies
Aula Magna

16:30-18:00

Chairpersons:
T. Romacho (Dpto. Farmacología, UAM)
M. Granado (Dpto. Fisiología, UAM)

5.1. BIO24 Preclinical Safety and Efficacy Evaluation of Lentivirally transduced Hematopoietic Stem Cells for the treatment of Leukocyte Adhesion Deficiency type ***I. C Mesa Nuñez***

5.2. BIO22 Functional characterization of CNS2 DNA regulatory element of the mouse Tyr gene by CRISPR-Cas9 mutagenesis. ***S. Josa***

5.3. BIO30 Protection against Middle East respiratory syndrome coronavirus infection by immunization with genetically engineered live-attenuated viruses. ***F. Gutiérrez-Álvarez***

5.4. BIO11 NRF2 controls proteostasis through the transcriptional regulation of autophagy. ***M. Pajares***

5.5. MED11 Curcumin treatment decreases acute kidney injury associated with rhabdomyolysis. ***M. Guerrero-Hue***

5.6. MED05 Posterior components Separation with double mesh for abdominal's wall incisional hernia: surgical technique and results. ***E. Jiménez Cubedo***

Oral Session 6 Senescence and Aging
Seminario 1

16:30-18:00

Chairpersons:
Guillermo de Cárcer (CNIO)
Javier Egea (IIS La Princesa)

6.1. BIO28 Generation and characterization of a reversible HGPS mouse model to design potential future therapies. ***A Sánchez Lopez***

6.2. PHAR07 Generation and potential applications of an X-linked dyskeratosis congenita model in human hematopoietic stem cells. ***C. Carrascosa-Rubio***

6.3. PHAR03 Angiotensin-(1-7)/Mas axis attenuates endothelial cell senescence by Nrf2/ activation. ***A. Romero***

6.4. NEURO03 Gait evaluation of patients with mild cognitive decline and mild Alzheimer's disease. ***J.A. Martín Gonzalo***

6.5. PSYCHO02 Physical activity as a potential protective factor in people at Increased Genetic Risk for Alzheimer's Disease. ***J. de Frutos-Lucas***

6.6. PHAR09 Multigarget compounds for the treatment of neurodegenerative diseases. Nrf2-EpRE pathway as key target. ***P. Michalska***

18:00-18:30 **Awards and Closing**
Aula Magna

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Abstracts

PhD Programme in Clinical and Health Psychology

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Assessing individual change without knowing the test properties: Item bootstrapping.

Juan Botella[1], Desirée Blázquez[1], Javier Revuelta[1], Manuel Suero[1].

[1]Department of Social Psychology and Methodology, Universidad Autónoma de Madrid, Madrid, Spain.

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Introduction: Botella et al. (2018) proposed to use the 'non parametric bootstrap' method (Efron & Tibshirani, 1994) to the responses given by an individual to the items of a test in order to create confidence intervals for an individual's true test score for situations in which classical procedures cannot be used. In six databases containing the responses to several psychological scales, two procedures were applied to create the confidence intervals; a classical one, Estimating the True Score (ETS; Gulliksen, 1950), and the Bootstrap of items (BSI). When there was an expected change in the criterion of interest after an intervention and when there was not, the rates of significant change obtained with both procedures were very similar. These results suggested that BSI was a promising solution when other methods could not be applied. However, evidence is needed from different research contexts to assess the performance of BSI.

Material and Method: On the basis of the Partial Credit Model (Masters, 1982), a IRT model for polytomous response data, two simulation studies were programmed in R to examine the performance of the BSI technique in comparison to the classical ETS method. In study 1, focused on no change scenarios, we examine the influence of several test features on the width of the BSI confidence interval and its coverage rates of the true score. The factors assessed are the subjects' trait level, skewness of the trait distribution, number of items' categories, number of items, and internal consistency reliability (Cronbach's alpha). Study 2 was carried out to analyze the significant change rates of BSI and ETS given a change in the subjects' trait level.

Results: Study 1 shows that the BSI confidence interval is narrower as the subject's trait level gets more extreme and the test internal consistency is higher. The BSI coverage rates of the true score reach appropriate values when the test is made up of at least 20-25 items. Results from study 2 reveal that BSI has lesser statistical power to detect a significant change than ETS as the change in the subject's trait level is bigger.

Conclusion: The classical procedure ETS seems to be yet the best option. Nevertheless, the classical procedures for elaborating confidence intervals involve knowing several properties of the test given a fixed sample and, sometimes, these properties are unknown or are not trustworthy. Given the differences in the performance of the methods examined, BSI is a good option to create confidence intervals for an individual's true test score for situations in which classical procedures cannot be used.

Keywords: Bootstrap, Individual change, Reliable change, Significant change, Psychometric properties.
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Editor: Name of the editor here.
Cite as: Juan Botella, Desirée Blázquez, Javier Revuelta, Manuel Suero. Assessing individual change without knowing the test properties: Item bootstrapping. IBJ Plus 2018 (S2):e00120 doi: 10.24217/2531-0151.18v1s2.00120.
Funding: The research was carried out without funding support.
Competing Interests: The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of competing interests.

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Physical Activity as a Potential Protective Factor in People at Increased Genetic Risk for Alzheimer's Disease.

Jaisalmer de Frutos-Lucas[1, 2], Fernando Maestú Unturbe[2, 3], Juan Manuel Serrano[1], Simon Laws[4]

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[2]Center for Biomedical Technology
[3]Psicología Experimental, Procesos Cognitivos y Logopedia, Universidad Complutense de Madrid
[4]School of Medical and Health Sciences, Edith Cowan University

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Introduction: Alzheimer's disease (AD) is the most common cause of dementia. Unfortunately, no cure has been found to prevent the advance of this devastating neurodegenerative disease. The AD neuropathological process begins up to 20 years before we are able to detect the first clinical manifestations of the disease. Thus, it remains possible that pharmacological and non-pharmacological interventions are more effective in preclinical stages of AD. On the other hand, physical activity (PA) has been thought to improve health and cognition since ancient times. However, it has not been until recently that we have started to understand the mechanisms that underlie this relationship. Therefore, the aim of my studies is to investigate how PA influences different biomarkers of AD (grey matter volumes, functional connectivity, blood markers of neuroinflammation, brain amyloid deposition, etc.) in different samples of healthy elders who are at increased risk for AD, either because they are carriers of different genetic risk factors or because they are relatives in first degree of AD patients.

Methods: For these studies I have been able to have access to two main sources of data. These are the Australian imaging, biomarkers & lifestyle flag study of ageing (aibl) dataset and that of the longitudinal studies in healthy and pathological aging carried out at the Laboratory of Cognitive and Computational Neuroscience (Center for Biomedical Technology). The set of assays that compose these protocols include: magnetoencephalography, magnetic resonance imaging, genotyping, neuroinflammation assays, nutritional assessment, physical activity measurement, neuropsychological assessment, ophthalmological assessment among others.

Results: Preliminary results suggest that although healthy older adults do in general benefit from PA, such benefit is larger or exclusive to those who are not at increased genetic risk for AD. Genetic risk for poor dopamine transmission has also been identified as a risk factor for low PA engagement and cognitive decline.

Conclusion: Higher levels of PA are associated to lower levels of different early markers of AD. However, this effect tends to be more moderate or non-existent carriers of certain genetic risk factors. Nevertheless, physical inactivity increases the risk of AD even further in those who are already at increased risk for AD.

Keywords: Physical Activity, Genetics, Alzheimer's Disease, healthy Aging
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Funding: La Caixa Foundation PhD Scholarship to Jaisalmer de Frutos
Competing Interests: No Conflict of interests.

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The impact of subjective well-being on mortality.

Natalia Martín-María MS[1,2,3], Marta Miret PhD[1,2,3*], Francisco Félix Caballero PhD[1,2,3], Josep Maria Haro MD[2,4], José Luis Ayuso-Mateos PhD-MD[1,2,3]

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Introduction: Subjective well-being has been recognized as an important global health issue. The aims of the present study are: 1) to review the existing evidence on whether subjective well-being is a protective factor for mortality in the general population, analyzing the differential impact of evaluative, experienced, and eudaimonic well-being, and 2) to disentangle the differential influence that positive affect, negative affect, and evaluative well-being might have on mortality, after adjusting for health and other lifestyle factors and to analyze whether this association is different in people with and without depression.

Methods: A systematic review of longitudinal studies on the general population was carried out in the PsycINFO, Web of Science, and PubMed databases. Data on the studies' characteristics, quality, and the effects of variables were extracted. A meta-analysis was conducted on the studies included in the systematic review. In the empirical part of the study, a nationally representative sample of 4,753 people from the general population in Spain was followed up after 3 years. Analyses were performed with Cox regression models among the total sample and separately in people with and without depression.

Results: A total of 62 articles that investigated mortality in general populations, involving 1,259,949 participants, were found. The meta-analysis showed that subjective well-being was a protective factor for mortality [pooled HR= 0.920; 95% CI = (0.905, 0.934)]. The impact of subjective well-being on survival was significant in both men and women. The three aspects of subjective well-being were significant protective factors for mortality. The same results were found in the longitudinal study, in which all three well-being variables showed separately a statistically significant association with mortality, after adjusting for age, sex, and years of education. However, after adjusting for health status and the other well-being components, only positive affect remained as marginally associated with a decreased risk of mortality in the overall sample [HR = 0.87; 95% CI = 0.73–1.03], and in particular among individuals without depression [HR = 0.82; 95% CI = 0.68–0.99].

Conclusion: Our results from the meta-analysis suggest that evaluative, experienced, and eudaimonic well-being are associated with a decreased risk of mortality. However, our analysis adjusting for health status and the other well-being components, showed that only positive affect is inversely associated with mortality in individuals without depression. Future research should focus on assessing interventions associated with a higher level of positive affect in order to produce longevity gains.

Keywords: Subjective well-being longitudinal study, meta-analysis, mortality, depression.
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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.

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Abstracts

PhD Programme in Medicine and Surgery

Control of the professional exposure to sevoflurane waste in sedation of pediatric patients for magnetic resonance.

Alonso Prieto M[1*], Alonso Calderón JL[2], Sanabria Carretero P[3].

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Introduction: Volatile anesthetics must be used in compliance with the recommended set of control strategies, institutional policies and laws in order to keep the occupational exposure in safe limits. Operating theatres are provided with the mandatory gas scavenging and ventilation systems, which have demonstrated to be effective reducing the volatile anesthetic environmental pollution. Unfortunately, these measures are often unavailable outside the operating room. There are also ecological concerns about the possible ecological impact of volatile anesthetics. Research in these matters is very scarce.

Material and methods: We developed a prospective observational study to compare air concentration of sevoflurane during sessions of sedation in pediatric patients in the magnetic resonance room in three different conditions: basal group: without any intervention to reduce gas pollution; second group, using a gas scavenger connected to the breathing circuit; third group: using the scavenging system and a supplementary ventilation of the room.

Results: The preliminary analysis shows a statistically significant reduction in air waste sevoflurane concentration between basal group and the second group: mean reduction 22,89 (CI 95%: 12,74-33,03) parts per million (ppm) ($p<0,001$) and also between the basal and the third group: mean reduction 28,57 (CI 95%: 19,51-37,63) ppm ($p<0,001$). We didn´t find differences between second and third group.

Regarding the legal limits of professional exposure, expressed as “Valor límite ambiental-exposición diaria” (VLA-ED®), which represents the mean concentration considered as safe for a standard 8 hours a day and 40 hours working week, the study shows that only in the third group, the limit stays under the limit considered as safe (2 ppm).

Conclusions: Using sedation with sevoflurane of pediatric patients in the magnetic resonance room might result in professional overexposure to volatile anesthetic waste if the proper conditions of gas scavenging and ventilation are not provided. These conditions that guarantee occupational safety can be achieved easily with simple and inexpensive strategies.

Keywords: sevoflurane, anesthetic waste, occupational exposure.
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Editor:
Cite as: Alonso Prieto M, Alonso Calderón J, Sanabria Carretero P. Control of the professional exposure to sevoflurane waste in sedation of pediatric patients for magnetic resonance. IBJ Plus 2018 (S2):e00050 doi: 10.24217/2531-0151.18v1s2.00050.
Funding: This study has been partially funded by Abbvie S.L.
Competing Interests: No conflict of interest declared.

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Late-onset cardiotoxicity in Anthracyclinebased chemotherapy for breast cancer patients.

R Mata Caballero[1], J M Serrano Antolin[2], IA Gonzalez Garcia[2], J Muniz Garcia[3], A Curcio Ruigomez[2], JJ Alonso Martín[4].

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Background: Anthracycline cardiotoxicity (AC) represents a mayor limitation for the treatment of breast cancer patients (pts) and it may manifest years after treatment (lateonset cardiotoxicity). The aim of the study is to establish incidence and predictors of lateonset cardiotoxicity in a cohort of these type of pts.

Methods: 100 consecutive pts receiving Anthracyclinebased chemotherapy (CHT) were included in this prospective study. All pts underwent evaluation at baseline, at the end of CHT, 3 months after the end of CHT and 1 and 4 years after the beginning of CHT. Clinical data, systolic and diastolic echo parameters and cardiac biomarkers including high-sensitivity Troponin T (TnT), NTproBNP and Hearttype fatty acid binding protein (HFABP) were assessed.

Results: Mean doxorubicin dose was 243 mg/m2. Mean followup was 51.8±8.2 months. At one year incidence of AC was 4% and at the end of followup was 18% (15 pts asymptomatic left ventricular systolic dysfunction, 1 pt heart failure and 2 pts a sudden cardiac death). Forty nine pts developed diastolic dysfunction (DD) during first year. In the univariate analysis DD during first year was the only parameter associated with AC (Table). In the logistic regression model DD was independently related with the development of AC, with an odds ratio value of 7.5 (95% CI 1.5935.3).

Conclusions: Incidence of lateonset cardiotoxicity is high but mostly subclinical. Diastolic dysfunction early after chemotherapy is a strong predictor of anthraciclyne cardiotoxicity.

	AC+	AC-	P value
N	18	82	
Age	50.4±9.2	51±9.1	0.78
DD (%)	88	41	0.005
Hypertension	33.3	28.0	0.77
Diabetes	11.1	9.8	1
Hyperlipidemia	11.1	14.6	1
Smoking status	38.9	32.9	0.84
Anthracycline dose (mg/m2)	234±4.8	242±4.6	0.73
Radiotherapy (%)	38.9	42.7	0.79
TnT end of CHT (ng/L)	13.2±5.8	12.0±5.6	0.44
TnT 3 months after CHT (ng/L)	14.1±8.2	12.1±5.5	0.20
H-FABP end of CHT (ng/mL)	2.8±1.4	3.2±2.0	0.20
H-FABP 3 months after CHT (ng/mL)	3.5±2.2	3.3±1.8	0.65
NTproBNP end of CHT (pg/mL)	86±103	60±47	0.10
NTproBNP 3 months after CHT (pg/mL)	78±95	56±62	0.28
Abbreviations: AC+, pts developing AC; AC, pts not developing AC; rest abstract text			

Keywords: cardiotoxicity, anthracycline, chemotherapy, breast cancer, diastolic dysfunction.
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IBJ Plus IberoAmerican Journals

Predictive factors of 1-year mortality after a hip fracture. A literature review.

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Introduction: Fragility hip fracture is a frecquent event in elderly people associated with high mortality. For reasons not yet fully understood, 1-year mortality after a hip fracture varies between 12.1% and 35%, which signifies an excess of mortality of 8% to 18% per year compared to the population of the same age without hip fracture. This study aimed to review the factors most frequently described to predict 1-year mortality after a hip fracture

Methods: Studies published in Pubmed were reviewed by dividing them into systematic reviews and meta-analysis, studies performed in orthogeriatric co-managed units, studies performed in Orthopaedic Services and studies that used prognostic risk scores.

Results: Seven systematic reviews and meta-analysis including 741,247 patients, 11 co-managed studies including 5,829 patients, 5 studies that used risk scores including 12,820 patients and 34 studies performed in Orthopaedic Services including 452,842 patients were included. The most frequently described factors associated with one year mortality were age and male sex (34 studies); prior functional status, such as impaired mobility and dependence for basic or instrumental activities of daily living (16 studies); mental problems, such as the presence of cognitive impairment or dementia (16 studies); clinical factors, such as the number of comorbidities (14 studies); malnutrition (14 studies); health-care related factors, such as delayed surgery (14 studies) ; high ASA grade (13 studies); the presence of postoperative complications (7 studies) and social factors, such as living in residential care (12).

Conclusions: Many studies including big samples of patients have been published about this topic. Described factors are from very different nature; there are demographic, functional, cognitive, clinical, severity of illness and social factors. Unfortunately only a few of them are modifiable factors.

Keywords: Mortality, Hip fracture, Orthogeriatrics.
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IBJ Plus IberoAmerican Journals

A Standard AV Fistula in Animal Model.

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Introduction:
The number of patients with chronic kidney disease in renal replacement therapy is steadily increasing. Among the different substitution therapies, **hemodialysis** is the most used. The Arteriovenous Fistulae (AVF) is the Gold Standard.
Complications are the main disadvantage of AVF. Overall (primary and secondary) patency of AVF at one year is 55% and 62% respectively, which in the long term is of crucial importance for the morbidity and mortality of these patients. Understand biology and complications requires as a first step to create AV fistulas in biological (animal) models, previous to any intervention. There are few experiences with animal models of AV fistula and no standard model.

Objectives:
1. Create a standard animal AV fistula model.
2. Maintain an AV fistula

Material and Methods:
We performed 4 AV fistulas in pigs (Specimen Suido), of 2 months old, 20 kg weight at implant, gender female.

Step 1: No oral intake the night before after midnight. Pre-anesthesia: Ketamine 10 mg/kg IM.
Anesthesia Induction: 5% sevoflurane in oxygen through face mask. Prophylactic antibiotic: Cefazolin 1 g/iv.
Step 2: The surgical procedure:

a. Groin shave.
b. Ultrasound identification of femoral vessels.
c. Sterile draping preparation and material technique.
d. Inguinal longitudinal incision 5 cm below groin, to localize common femoral vein, and femoral artery.
e. Control of proximal and distal arteries with clamps bulldog atraumatic.
f. Anticoagulant intraop (2.000 heparin units).
g. Anastomosis with latero-lateral venotomy-arteriotomy (7 mm). Poplypropilene 7-0 continuous suture.
h. Fascial and skin closure, silk suture separated.

Step 3: Controls:

i. Ultrasound control after AV creation and bi-weekly.
j. Aspirin 300 mg daily.
k. Use of antibiotic: amoxicil/clavulanic in case of local infection.

Results:
1. Duration of controls: 8 weeks.
2. Maturation of AV fistula occurred at 4 weeks.
3. Infections occurred in 25%. Heal after antibiotic treatment: 100%.
4. Spontaneous thrombosis was avoided using antiplatelet prophylactic, without complications.
5. Caliber fistula increased the first 4 weeks, with patency of 100%.
6. Caliber reduction was after 4 weeks, complete occlusion at 8 weeks.
7. Doppler Turbulence flow interpretation: good patency.
8. Doppler laminar flow interpretation: pre-stenotic.

In conclusion, we created a feasible AVF animal model, with a key close ultrasound observation for period of 8 weeks, with optimal prevention of early thrombosis and optimal management of early infection. Patency after 4 weeks was 100%. With this model, we can proceed with minimally invasive interventions to increase the patency of AVF fistulas before the total occlusion period (8 weeks).

Keywords: AV fistula, animal model, minimally invasive techniques.
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Posterior Components Separation with double mesh for abdominal's wall incisional hernia: surgical technique and results.

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INTRODUCTION: The complex incisional hernia of the abdominal wall is a challenge for wall surgeons. For years, posterior component separation surgery has been used as a safe and reliable technique for the treatment of most incisional hernias, including midline, subcostal and lateral. In addition, the use of two meshes is proposed, which increase the benefits of this technique. The aim of this study is to present the results of the posterior components separation technique with two meshes, for the repair of complex abdominal wall hernias, including lateral and midline.

MATERIAL AND METHODS: A prospective and descriptive multicentre study was carried out that including patients from Spanish and foreign centers, who underwent repair of incisional hernias of the abdominal wall, medial and lateral, using the technique of Posterior Components Separation (with or whithout *transversus abdominis* release) placing a double mesh (reabsorbable and non-absorbable) in extraperitoneal position, from May 2010 to December 2016. Data related to patient, surgical technique, as well as complications and long-term follow-up, were collected in a specific database.

RESULTS: 169 patients were included, 99 men (58.6%) and 70 women (41.4%), mean age 60.9 years (range 32-86 years). 47.3% had midline hernias, of which 49% were type M1-M5, while 35% had lateral hernias, being the most frequent L3 type of the European Hernia Society (EHS). According to the classification of the Ventral Hernia Working Group (VHWG), 50% had grade II hernias. The mean of the maximum size of the defect was 12.7 cm (range 4-40 cm). Regarding the surgical technique, 3 patients underwent posterior components separation over *transversus abdominis* muscle or Carbonell technique (2%), while 166 patients underwent posterior components separation with *transversus abdominis* muscle release (SPC -TAR) (98%), with a mean duration of surgical procedure of 219 min (range 65-490 min). As local complications there were 33 subcutaneous seromas (19.5%), 23 deep haematoma (13.6%) and 21 surgical site infections (12.4%). After a mean follow-up of 21,4 months (range 12-54 months), we only found 2 hernia recurrence (1,2%) and 3 symptomatic bulging (1,7%). The timing of recurrence was between 10 and 21 months.

CONCLUSIONS: The posterior components separation with double mesh placement improves the results in the repair of the complex incisional hernias of the abdominal wall, midline and lateral or subcostal, with an acceptable incidence of local and systemic complications, and a low recurrence rate.

Keywords: Hernia. Posterior components separation. Incisional hernia.
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Study of genetic basis of Idiopathic Central Precocious Puberty.

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Idiopathic central precocious puberty (ICPP) is the premature activation of the hypothalamic-pituitary-gonadal axis in the absence of organic disease. Up to now, loss-of-function variants of the maternally imprinted gene MKRN3 (MIM:616346) are most common genetic cause of ICPP, in 3 patients gain-of-function mutations of KISS1/KISS1R have been described. Recently, DLK1, another maternally imprinted gene have been implicated in the origin of ICPP, Methods: The sample group: 28 patients with ICPP, we gathered blood samples from them, their parents and affected relatives. Control group: 46 female European adults with known menarche age. We studied the clinical exome of 20 patients from the sample group using the Illumina platform. Variants were filtered using a list of 7 genes related to the gonadotropin-releasing hormone pathway: GNRHR, LIN28B, KISS1, KISSR1, MKRN3, TAC3, TACR3. Variants were validated using sanger sequencing, in both the family and control group. Study of chromosome 14 haplotypes using STRs around DLK1 in the 28 families. Results: In the primary analysis we found 1 likely pathogenic variants in 1 patients, 2 variants of unknown significance (VOUS) in 2 patients listed in table 1, we also found polymorphisms (not shown) in the genes listed. We found none Uniparental Disomy in the sample group.

Table 1. List of variants.

Gene	Probably pathogenic variants	N	VOUS	N
MKRN3	LRG_1045:c.[203G>A];[203=] NP_005655.1:p.[Arg68His];[Arg68=]	1		
KISS1			NM_002256.3:c.[58G>A];[58=; NP_002247.3:p.Glu20Lys	1
			NM_002256.3: c.[268C>G] NP_002247.3:p.His90Asp	1

Conclusion: variants in MKRN3 are the most frequent genetic cause of familial ICPP, so it is wise to screen for MKRN3 mutations in all patients with ICPP. The burden of DLK1 in the pathogeny of ICPP is yet to be assessed. Besides, because multiple studies, including this one, failed to demonstrate the presence of pathogenic variants in the coding regions of the genes related to this disease, we assume that it must be caused by alterations of epigenetic or regulatory factors. Further studies are needed to probe this hypothesis.

Keywords: Central Precocious Puberty, variants .
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IBJ Plus IberoAmerican Journals

Comparison of pharmacological treatment alone versus treatment combined with implantable cardioverter-defibrillator therapy in patients over 75 years.

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BACKGROUND: The implantable cardioverter defibrillator (ICD) reduces mortality in selected patients. The role of ICD in patients over 75 years is not well established.

MATERIAL AND METHODS: Between January 2008 and July 2014, we assessed patients aged ≥75 years with left ventricular ejection fraction ≤35%. We identified 385 patients with a class I or IIa recommendation for an ICD implantation. Based on the patients or attending cardiologists' decisions, 92 patients received an ICD. In order to avoid potential confounding factors, we performed a propensity-score matched analysis.

RESULTS: Finally, 126 patients were included (63 with ICD). The mean age was 79.1±3.1 years (86.5% males). As compared with the medical therapy group, the ICD patients had a lower percentage of chronic obstructive pulmonary disease (19.0% vs 38.1%, p<0.05), with higher use of beta-blockers (BB) (85.7 vs 70.0%, p<0.05). Other treatments were otherwise similar in both groups. There were not differences in relation with age, etiology or other comorbidities. During follow-up (39.2±22.4 months), total mortality was 46.0% and cardiovascular events (death or hospitalization) occurred in 66.7% of the patients. After a multivariate analysis, only BB therapy was shown to be an independent protective variable with respect to mortality [HR 0.4 (0.2-0.7)]. ICD therapy did not reduce overall mortality or cardiovascular event rate.

	OR	CI 95 %
Previous HF		NS
Cerebrovascular disease	2.188	1.182-4.049
ICD		NS
Betablockers	0.425	0.243-0.743
LVEF	0.939	0.903-0.976

CONCLUSION: According to our results, the use of ICD did not demonstrate any benefit as compared with medical therapy. Well-designed randomized controlled studies in patients over 75 years are needed to ascertain the value of ICD therapy.

Keywords: elderly, implantable cardioverter defibrillator, heart failure
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Check Mate of ADHD-II: A pilot study.

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Introduction: Attention deficit hyperactivity disorder (ADHD) is a major public health issue. ADHD is the most prevalent psychiatric disorder diagnosed in youth people, affecting between 4% and 8% of youth people worldwide. The multimodal treatment -drugs, psychological treatment, and psychoeducation- is the most effective treatment. Pharmacological intervention is the most frequent and convenient treatment for ADHD in developed countries. Unfortunately, many parents are reticent to putting their child on drugs. Furthermore, psychotherapy is usually expensive. On the other hand, there are preliminary reports suggesting that chess, a traditional, simple board game that requires the use of complex cognitive strategies could be used in different mental disorders, including ADHD. In this context, testing the putative therapeutic effect of chess training is worthwhile. The objective of the present study is to test if chess is useful in children with ADHD within the context of the "Check mate of ADHD" Project (www.jaquemate-tdah.com).

Material y Methods: The HUPH Institutional Review Board committee approved the study (n12, 16, July 11th 2016). After a complete description of the study, all children and their parents signed the written consent.

Thirty-two children aged 7 to 14 years with ADHD were included between July 1st 2016 and December 31st 2017. All children were randomized to either the experimental group (CG) -received chess classes during one month- or the control group -treatment as usual, TAU-. The following scales were used for psychological testing: The Spanish Version of the Swanson, Nolan and Pelham Scale for parents (SNAP-IV), the Abbreviated Conner's Rating Scales for parents (CPRS-HI), The Behavior Rating Inventory of Executive Function (BRIEF), The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) and The Conner's' Continuous Performance Test II. Mann-Whitney correlation analyses were used to ascertain differences between both groups. Statistical significance was set as $p<.05$.

Results: There weren´t significant relationship between children played chess against those who didn´t play ($p<.05$). We found a slight improvement in the CG group as compared with the TAU group in the CPT-II that reached no statistical significance.

Conclusions: One month of chess training is not useful for the treatment of ADHD. This is not surprising, given that cognitive rehabilitation –cognitive-behavioral therapy; neurofeedback- usually requires a minimum of three months to find some improvements. This pilot project highlights the importance of carrying out larger studies and during a longer period using a case-control randomized design.

Keywords: Chess, ADHD, treatment
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Levels of fecal calprotectin in healthy children (0 to 18 years). The importance of age.

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Introduction: Calprotectin is a calcium binding protein of neutrophil granulocytes that correlates strongly with neutrophil infiltration of the intestinal mucosa when measured in faeces. However, fecal calprotectin (FC) is a non-specific inflammation marker and can be potentially influenced by factors like diet and age. In fact, previous studies report that children tend to have higher FC levels than adults. However, only a few trials have been specifically designed to establish the normal FC levels in healthy children, and in each case analyzed small and/or heterogeneous samples. Our primary aims in this study were to (i) establish normal levels of FC in healthy children living in a modern Spanish urban environment; and (ii) analyze their correlation with age.

Material and methods: A multicenter, cross-sectional and observational study was conducted between January 2015 and December 2016. We enrolled healthy voluntary donors from 0 to 18 years of age who attended one of the four participating primary health centers for routine growth monitoring or vaccination. The exclusion criteria were: (i) immunodeficiency; (ii) gastrointestinal or autoimmune disease; (iii) intake of drugs or gastrointestinal symptoms in the previous 15 or 30 days respectively; or (iv) positive finding in the accompanying microbiological study. We determined the FC levels using the Quantum Blue® Calprotectin test, and we perfomed stool cultures, including parasites, rotavirus and adenovirus detection. All data was analysed using SPSS® version 20.

Age group	No. of Subjects	Mean FC (µg/g)	10 thP (µg/g)	50 thP (µg/g) (median)	90thP (µg/g)
< 1 month	43	344,3	156	303	620
1-6 months	64	424	76	325,5	993
6-12 months	46	167,7	30	63	488
12-24 months	42	217,7	30	97	533
2-4 years	45	116,1	30	71	271
4-8 years	64	89,1	30	46	163
8-12 years	46	85,4	30	34,5	143
12-18 years	45	45,2	30	30	75
Total (0-18 years)	395	191,6	30	77	508,4

Table 1: FC levels in each age group. FC: fecal calprotectin. 10thP: 10th percentile. 50thP: 50th percentile. 90thP: 90th percentile.

Results: We included 395 subjects from 3 days to 16.9 years old (mean 4.2 years, SD 4.7 years), distributed in 8 age groups. Among them, 204 were boys (51.6%) and 191 were girls (48.4%). The FC levels in each age group are shown table 1. The FC values did not show a normal distribution, with higher figures in children under six months old. The correlation between age (days) and FC (µg/g) was analyzed using the Spearman correlation coefficient, with a bilateral significance of 0.00.

Conclusions: normal FC values in healthy children were higher than those considered as pathological in adults. This was particularly the case in children under four years old, and especially in those under 12 months. A negative correlation with age was observed. Based on this, it seems necessary to reconsider the levels of FC considered pathological in pediatric patients by age group.

Keywords: fecal calprotectin, children, healthy.
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Competing Interests: All authors have declared no conflicts of interest.

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Satisfaction, quality of life and perception of patients regarding burdens and benefits of vitamin K antagonists compared with direct oral anticoagulants in patients with nonvalvular atrial fibrillation. ALADIN Study.

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Introduction: Atrial fibrillation (AF) is associated with a fivefold excess risk of stroke. Except when contraindicated, chronic oral anticoagulants, with vitamin K antagonist (VKAs) or direct oral anticoagulants (DOACs), are the therapy of choice for reducing the risk of thromboembolic complications. Few data are available on the impact of anticoagulation therapy on satisfaction and quality of life in patients with AF. ALADIN was designed to validate the Anti-Clot Treatment Scale (ACTS) questionnaire in outpatients with AF treated with oral anticoagulants, attended in internal medicine and neurology departments in Spain. **The objectives** of the present study were to analyze the impact of treatment with oral anticoagulants on quality of life, satisfaction and patients' perception of the burdens and benefits of anticoagulation therapy and to identify the factors associated with these variables.

Material and Methods: ALADIN was an observational cross-sectional study carried out in neurology and internal medicine departments in Spain. The study population comprised patients with non valvular AF (NVAF), treated with oral anticoagulants who had been seen in those departments in Spain, from September 2014 to March 2015. The patients completed the ACTS, SAT-Q (Satisfaction Questionnaire) and EQ-5D-3L (EuroQol 5 dimensions questionnaire, 3 level version) questionnaires. The statistical analysis was performed using SPSS for Windows.

Results: The study population comprised 1337 patients, of whom 587 were taking DOACs and 750 VKAs. Compared with VKAs, DOACs were more commonly prescribed in patients with a history of stroke and in patients with a higher thromboembolic risk. The ACTS Burdens score (54.83±6.11 vs 49.50±9.15, $p<0.001$) and ACTS Benefits score (12.36±2.34 vs 11.48±2.46, $p<0.001$) were higher with DOACs than with VKAs. The logistic regression analysis performed with data from the SAT-Q is shown in the table.

Conclusions: NVAF patients treated with oral anticoagulants had many comorbidities and a high thromboembolic risk. Satisfaction and quality of life with oral anticoagulants were high, although they were both better with DOACs than with VKAs.

Keywords: atrial fibrillation; oral anticoagulation; satisfaction; ACTS.
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Curcumin treatment decreases acute kidney injury associated with rhabdomyolysis.

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The release of myoglobin into the bloodstream due to a muscle damage (rhabdomyolysis) can induce acute kidney injury (AKI). Myoglobin causes tubular cell death, oxidative stress and nitric oxide decrease, promoting vasoconstriction and endothelial damage in the kidney. Curcumin, a compound from the Curcuma longa, has different antioxidant effects because it is an inductor of Nrf2 (Nuclear factor (erythroid-derived 2) -like 2). In this study we analyzed the possible beneficial effect of curcumin in the prevention of AKI associated with rhabdomyolysis.

We performed an experimental model of AKI associated with rhabdomyolysis by a single intramuscular injection of 50% glycerol (10ml/kg body weight) in each thigh caudal muscle in male C57BL/6 mice at 12 weeks of age. Curcumin was injected intraperitoneally (1mg/kg) the day before and the same day of the injection of glycerol. Animals were sacrificed 24 hours post glycerol injection. Blood and kidney samples were collected to perform gene expression studies by Real Time-PCR and protein expression by western blot and immunohistochemistry. In addition, we performed in vitro studies with murine tubular cell (MCT) to study the molecular mechanisms involved in the protection of curcumin against the adverse effects of myoglobin.

Mice with rhabdomyolysis had increased serum levels of urea and creatinine, as well as increased histological damage (tubular death, tubular dilatation, loss of brose border and oedema). In line with these results, we observed an increase in gene expression of tubular damage (NGAL and KIM-1) and endothelial activation markers (ICAM-1 and endothelin), as well as increased expression of proinflammatory cytokines (CCL2 and TNF-α), oxidative stress (production of MDA and decreased GSH content), induction of catabolism of the heme group (HO-1 and ferritin), and cell death (TUNEL). All these effects were partially reversed with curcumin treatment. In tubular cells, the administration of curcumin induced the activation of Nrf2 and HO-1. In line with our in vivo data, administration of curcumin to the MCTs decreased cell death and oxidative stress induced by myoglobin.

Our results suggest that renal damage associated with rhabdomyolysis decreases with curcumin treatment, so this compound could be a possible therapeutic approach in patients with this pathology.

Keywords: curcumin, Nrf2, rhabdomyolysis, AKI, tubular cells.
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Non-celiac gluten sensitivity and chronic low back pain. Retrospective study of response to gluten-free diet in non-celiac patients with spondyloarthritis features.

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Objective: To study non-celiac gluten sensitivity in patients with chronic low back pain and spondyloarthritis features.

Methods: This is a retrospective study with 110 non-celiac patients with refractory low back pain and spondyloarthritis features, who underwent a gluten-free diet.

Demanding improvement was defined as reaching at least one of the following objectives: Be asymptomatic, remission of chronic low back pain, normal life recovery, return to work, change from being limited in bed or wheelchair to be able to walk, recovery of independence for personal care, withdrawal of opioids.

Results: The average age at onset of low back pain was 30 years. The average duration was 15 years. 62% of the patients achieved a demanding improvement. The average duration of gluten-free diet in patients with demanding improvement was 60 months. 96% of the patients with demanding improvement who ingested gluten had clinical worsening. . Oral aphthae and having a celiac relative were associated with a good response to a gluten-free diet. Out of 28 patients with axial spondyloarthritis, 23 had demanding improvement. Out of 16 patients with uveitis, 13 had demanding improvement.

Conclusions: Gluten-free diet may be beneficial in non-celiac patients with chronic low back pain and spondyloarthritis features, and in patients with axial spondyloarthritis. Information about clinical variables to consider in predictive models of response to a gluten-free diet is provided.

Keywords: Gluten, sensitivity, spondyloarthritis
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Conceptualization and use of Physical Restranit in ICU from the experience of Physicians and Nursing Assistant: looking for an interdisciplinary reading.

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Introduction: In spite of all current international evidence, Critical Care Units (ICU) are one of the clinical settings were physical restraint are used excessively. Theoretical frameworks developed in other caring contexts indicate that nurses are the main actors in physical restraint management. However, other members of ICU staff such as physicians and nursing assistants can wisely contribute in nursing decision making about physical restraint use.

The aim of this study was to explore physicians and nursing assistant experience on ICU physical restraint management and to identify the way this experience can influence nurses' decision making.

Material and methods: A multicenter phenomenological research was carried out in 17 ICUs in Madrid (Spain). The ICUs were stratified according to physical restraint "common use" versus "lacking use". Three focus groups were developed. The first one was composed by nursing assistants from "common use" ICUs, the second one by nursing assistants from "lacking use" ICUs and the last by physicians of both types of ICUs. Sampling method: purposeful. Data analysis: thematic content analysis. Data saturation was achieved.

Results: Four main themes emerge from data analysis: 1) safety and risk meanings (patient safety versus staff safety), 2) restraint types, 3) professional responsibilities (prescription and professional roles) and 4) "zero restraint" paradigm. Conceptualization about the use of physical restraint shows differences in some themes depending the type of ICU policies about physical restraint use and management (common use versus lacking use).

Conclusions: A real reduction of physical restraint use in ICUs should have a key starting point: the acceptance of the complexity of the phenomenon. The differences in the use of physical restraints among UCIs are influenced by individual, collective and organizational factors. These factors determine the understanding of safety and risk, the focus of caring (patient or clinician based care), the concept of restrain, professional responsibilities and interventions, and team interactions and leadership.

Keywords: Restraint, Physical; Critical Care; Qualitative Research; Hermeneutics; Focus Groups.
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The malnutrition screening aimed at excess diabetes mellitus, hypertension and chronic kidney disease in a sample population from the city of Portoviejo, Manabí in 2017.

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The screening carried out between November 1st and on December 15th, 2017, a field, exploratory, descriptive and correlation investigation was carried out, based on an opportunistic and selective strategy with a sample of 398 adults malnourished by excess a total of 190 overweight and 208 obese from the parish Andrés de Vera, in the city of Portoviejo, the province Manabí; possible through capillary glucometry evidencing 106 cases of prediabetes and 61 of diabetes mellitus; 99 in pre-hypertensive stage and 199 with arterial hypertension. Although no case of chronic kidney disease was identified, with the application of the albumin urine test in 24-hour, 7 patients with different degrees of albuminuria were identified. Given the effectiveness and simplicity of the procedure used and its low cost, we propose its generalization in the province and the country.

Keywords: Adults, malnutrition, hypertension, diabetes.
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Quality of life of patients with chronic pain and frailty criteria in a primary care center.

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Frailty is a clinical state in which there is an increase in an individual's vulnerability for developing increased dependency or mortality when exposed to a stressor. It is a common state in older adults and it has consequences in physical activity limitation, depression and poor quality of life. Chronic pain is also a frequent condition in older adults. Its consequences are similar to the consequences of frailty. Frailty can be prevented and managed by physical activity encouragement; our aim is to examine the effectiveness of a physical exercise program to manage chronic pain and frailty.

Objectives: This investigation is divided into two phases: the first phase of the investigation is a cross-sectional study whose objective is to analyze a population of older adults with chronic pain to determine their frailty statement, physical activity, pain characteristics and quality of life. The second phase of the investigation consists on a randomized controlled trial whose objective is to study the effectiveness of a physical exercise program for patients identified as pre-frail older adults in the phase 1 of the investigation.

Methods: Participants in the first phase of the investigation will be recruited by the consultation of the electronic registers of the Madrid Health Service. Patients older than 65 years with chronic pain will be searched and their frailty status will be assessed and related to pain characteristics, quality of life and physical activity. Pre-frail patients will be identified and they will be randomized into a control group and an intervention group. Control group will receive ordinary recommendations for physical activity, and intervention group participants will be included in a physical exercise program for 8 weeks. The physical exercise program consists on warm-up exercises, muscle strengthening and stretching, balancing and self-administered massage After the intervention, and after 6 months follow-up, frailty statement, physical activity, quality of life, depression and intensity and location of pain will be assessed in both groups.

Keywords: frail elderly, chronic pain, exercise therapy, motor activity, quality of life.
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Evaluation of a computerized system for the monitoring of procedures performed in a general surgery service.

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Background: Surgical research is faced with difficulties not encountered in clinical research. The variety of surgical interventions, their undefined nature, the patient´s previous physiopathological status, the surgeon´s expertise, and the variety of adverse events, their severity, and occurrence in time – all of which makes some of them go unnoticed – cause surgical research to be considered as highly complex. Structured hospital databases are not producing the evidence decision makers arguably need in surgical departments, as those databases gather administrative information and exclude information related to surgical care. Therefore we must develop an easily manageable database integrating all the clinical and surgical data (Real World Data) aimed at monitoring and assessing surgical results from a third-level Hospital´s General Surgery Department.

Methods: A prospective observational study, was carried out from 2011-2013, based on RWD of a 4,572 surgical interventions record cohort from 4,248 patients. This RWD was developed by refining and structuring all the information contained in the following, but not exclusive, list of hospital databases: BMDS, HP-HCIS, HP-DOCTOR. Furthermore, we have incorporated information that is not readily available in hospital databases: adverse events severity ranking, patient comorbidity, surgical complexity level etc. To demonstrate this system´s versatility, twelve surgical scenarios were developed in two anatomical areas (colon and rectal) owing not only to the type of cases per se but also to the diversity and complexity of adverse events.

Results: Mortality in colon surgery was not recognized by existing databases as surgery-related in 50% of the cases, with the number going up to 83.33% in rectal surgery. The analysis reveals that 3.2% of those patients requiring re-intervention following right hemicolectomy surgery went unnoticed by the hospital databases on their first admission. Through the analysis of the same cohort over a five-year span, it was found that 18.58% additional patients were not included in hospital databases. The number of patients undergoing low anterior resection surgery requiring re-interventions in the first admission and going unnoticed to hospital databases was 6.38% and 31.91% over a 5-year period.

Conclusion: The information extracted from current hospital databases does not necessarily allow the surgeon to take the best decision based on prior surgical evidence. It is mandatory that the surgeon define all the clinical and surgical data. All the data must be structured in order to build and develop a surgical RWD thereby transforming this data into information instrumental in the decision making process.

Keywords: Real World Data, Basic minimum data ser, Hewlett Packard Healthcare Information System, Hewlett Packard Doctor.
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Adequacy of antithrombotic treatment, characteristics and in-hospital mortality of elderly patients with non-valvular atrial fibrillation: results of the NONAVASC registry.

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Background and objectives: The prevalence of non-valvular atrial fibrillation (NVAF) increases with age and is associated with high morbi-mortality rates. The main goals of this study were to describe the characteristics of elderly patients hospitalised with NVAF, to evaluate the adequacy of the antithrombotic strategies used and to identify the predictors of in-hospital mortality.

Patients and methods: Observational, prospective, multicentre study carried out on patients with NVAF over the age of 75, who had been admitted for any medical condition to Internal Medicine departments in Spain.

Results: We evaluated 804 patients with a mean age of 85±5.1 years, of which 53.9% were females. The prevalence of risk factors and cardiovascular disease was high: hypertension (87.6%), heart failure (65.4%), ischemic cardiomyopathy (24.4%), cerebrovascular disease (22.4%) and chronic kidney disease (45%). Antithrombotic treatment was prescribed in 86.2% of patients at the admission: anticoagulants (59.7%), antiplatelet (AP) medication (17.8%) and double therapy (8.7%). Older age, AF diagnosed within <1 year, higher HAS-BLED scores and severe cognitive impairment were associated with the use of antiplatelet therapy. Permanent AF favoured the use of anticoagulants. During the hospitalization 10.1% (n=81) of the patients died. The strongest determinants of in-hospital mortality were the baseline functional status (Barthel Index) (OR for total dependency 4.73, 95% CI 2.32-9.63), and admissions for stroke (OR 3.55, 95% CI 1.41-8.90) and acute renal failure (OR 1.93, 95% CI 1.12-3.32).

Conclusions: elderly patients hospitalized with NVAF showed numerous comorbidities and their in-hospital mortality was high. The anticoagulation rates were low and 18% received only AP drugs. The patient's age and the severity of the cognitive impairment were determinant for the choice of the antithrombotic strategy. The baseline functional status was the strongest predictor for in-hospital mortality, ahead of comorbidities and pharmacological therapies. These results enhance the importance of incorporating a global geriatric assessment in order to guide and adjust our therapeutic efforts during the management of these complex patients.

Keywords: atrial fibrillation, elderly, antithrombotic treatment, functional status.
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Surgical treatment of type A acute aortic dissection with "Frozen Elephant Trunk" technique.

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Background: Acute type A aortic dissection (AAAD) is a surgical emergency. In patients with arch and descending aorta involvement (DeBakey type I), a total aortic arch replacement with frozen elephant trunk (FET) could favour false lumen thrombosis and improve long-term results. We hereby present our experience with this technique in a low-volume centre, to assess if the technique is feasible to treat such disease.

Methods: From January 2011 to December 2016, 43 patients with acute type A aortic dissection were operated on in our Institution, which carries out 300-350 annual procedures. Among these, 12 patients with an intimal tear in the aortic arch and/or proximal descending aorta received a FET procedure (10 males, age 57 years). Concomitants procedures were aortic valve replacement (42%), Bentall (25%) and aortic valve repair (17%).

Results: Cardiopulmonary bypass, cross-clamp and circulatory arrest times were 235±43 min, 171±33 min and 75±20 min, respectively. The operative mortality was 16,7% (n=2). Stroke and rethoracotomy for bleeding occurred in 8% (n=1) and 8% (n=1), respectively. There was not spinal cord injury. Follow up was 36,1 months. During follow-up, no patients died or required a reoperation on the downstream aorta.

Conclusion: Although all patients have been operated on in a low-volume centre, our results with FET in AAAD are acceptable. Even though this technique demands high technical skills, the learning curve has not had impact on mortality or morbidity.

Keywords: Aortic Dissection, Frozen Elephant Trunk.
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The response to a high fat diet in a mouse model of Alzheimer's disease is sexually dimorphic.

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High fat diet (HFD)-induced metabolic alterations, including neuroinflammation and gliosis, are risk factors for Alzheimer's Disease (AD), with males and females possibly responding differently. Transgenic amyloid protein mice (TgAPP) are used as a model of AD and HFD-induced metabolic alterations are a risk factor for AD. We analyzed the response of male and female TgAPP and wild type (WT) mice to a HFD.

Male and female (7-8 months old) WT C57BL/6 mice (controls) and TgAPP mice were put on either a HFD or a low-fat diet (LFD) for four months before sacrifice. Circulating metabolic parameters were measured. The mRNA expression levels of orexigenic neuropeptides Agouti-Related Protein (AgRP) and neuropeptide Y (NPY), the anorexigenic neuropeptide pro-opiomelanocortin (POMC) and interleukins (IL) 1-beta and -6 were analyzed in the hypothalamus and leptin receptor (LepR) and cytokines in visceral and subcutaneous adipose tissues by RT-PCR. Intracellular signaling pathways were analyzed by Western-blotting.

All females significantly increased their body weight ($p<0.0001$) on the HFD, while males did not. Triglycerides were elevated in all APP mice compared to their controls ($p<0.03$). Circulating insulin levels were lower in WT female mice than in WT males. There was a tendency to increase circulating insulin with HFD, but it was only significant in APP mice of both sexes ($p<0.02$). Circulating levels of monocyte chemoattractant protein (MCP) -1 were higher in LFD male mice than in LFD females and HFD decreased MCP-1 levels in males, but not in females. Circulating levels of plasminogen activator inhibitor (PAI) -1 were higher in males than in females. In males, HFD lowered the mRNA levels of AgRP ($p<0.005$) and NPY ($p<0.005$), regardless of genotype, and phosphorylated protein kinase B (pAkt) levels only in hypothalami of APP mice ($p<0.05$). In females, HFD increased POMC mRNA levels ($p<0.05$) in both genotypes. WT males had higher POMC mRNA levels than APP males regardless of diet ($p<0.01$) and tended to have higher hypothalamic IL1beta expression s.

LepR expression in visceral adipose tissue was higher in APP males than in WT males ($p<0.04$). However, APP females had lower LepR expression than WT females ($p<0.03$), which was significant in those on a HFD ($p<0.05$). Also, WT males had lower LepR expression than WT females ($p<0.003$).

In conclusion, mice from this AD model exhibit different metabolic and hypothalamic responses to HFD compared to WT mice, with these responses also depending on sex. Thus, these data further support the concept that dietary intake and the sex of an individual are factors that should be taken into consideration in the study and treatment of Alzheimer's Disease.

Keywords: high-fat diet, Alzheimer's Disease, sex differences.
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IBJ Plus IberoAmerican Journals

Initial evaluation of a real-time Prostatic Fusion Biopsy system: starting the programme.

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Development of Multiparametric Magnetic Resonance Imaging (mpMRI) of the prostate has allow for the use of a variety of ultrasound-MRI fusion systems, drawing the prostatic biopsy in last two years. We present here our initial results of a series of transperineal prostatic biopsies using an image fusion system.

Between november-2015 and december-2016, we selected 85 out of 121 evaluated subjects candidates to prostate biopsy. We used a fusion image software (Biopsee® and the stepper EX^3 with estabilizer Micro-Touch®(Civco®). All cases were submitted to a prior prostatic mpMRI in wich was described, at least one suspicious lesion. The procedure was performed under general anaesthesia and the patients were discharged the same day. The aim of this study is to estimate the diagnostic powerful of this test and to correlate images and pathologic finds.

We performed 85 transperineal fusion biopsies (112 targets). Mean age 67.5 y, median prostate volume 72.97cc. 69 p were submitted to a prior transrectal ultrasound prostate biopsy (TRUSB) (which is consider as the standart so far) and 55 of them had a negative result.

Median number of cores token per patient was 18 (target + randomized). 63 /85 biopsied cases had an elevated suspicious lesion in their MRI, resulting in final diagnostic of Prostate Cancer (PCa) 66.6% (table1). If the MRI describes an elevated suspicious lesion, 55% of patients have a clinically significant PCa.

We conclude that implementation of a real-time Prostatic Fusion Biopsy system in clinical practice has shown as an effective tool to improve detection rates of PCa in our institution. Diagnostic output of this procedure is superior to TRUSB detection rates published so far. We observed good correlation between MRI and presence of a clinically significant PCa. An adequate selection of candidates for this procedure is required, taking into account radiological report.

Table 1:

SOSPECHA RM	BIOPSIA +	BIOPSIA -	TOTAL
ELEVADA PIRADS 4-5	42(66,6%) 23 Gleason≥ 3+4 19 Gleason 3+3	21	63
INTERMEDIA PIRADS 3	4	18	22
	44	39	85

Keywords: Prostate Fusion Biopsy. Multiparametric MRI. Prostate Biopsy.
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Competing Interests: Competing interest explanation.

Facial bradykinesia in Parkinson´s disease and its correlation with motor and non-motor symptoms.

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Introduction: Reduced facial expression or amimia is one of the most typical features of Parkinson´s disease (PD). Despite being described in classic texts, its significance, physiopathology and correlation with motor and non-motor symptoms, including depression and cognitive impairment, is largely unknown.

Material and Methods: We studied facial bradykinesia in a group of 60 PD patients.

Clinical assessment was performed with the (on) Unified Parkinson´s Disease Rating Scale (UPDRS) and Hohen-Yahr scale (on). Cognitive study was assessed by Parkinson Disease cognitive Rating scale (PD- CRS). Depression by the 16-Item Quick Inventory of Depressive Symptomatology (QIDS-SR16) and Facial bradykinesia was rated according to item 19 of UPDRS III.

Results: Facial bradykinesia statistically correlated with motor UPDRS (r: 0.54 Spearman), Hoehn-Yahr scale (r: 0.3865) and also with cognitive status (r: 0.35).

Conclusion: Our study suggests that facial bradykinesia correlates with motor and cognitive situation in PD.

Keywords: Facial bradykinesia, Amimia, Parkinson´s disease
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IBJ Plus IberoAmerican Journals

Functional outcomes of the renal transplants obtained from controlled donation following cardiopulmonary death (DCD) with compartmental normothermic extracorporeal membrane oxygenation support (NECMO).

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Introduction: Since the implementation of the DCD protocol at our institution in 2012, some modifications have taken place in order to improve the organ viability. As for this, the NECMO has proven to be an effective method and to help reduce the isquemic organ damages.

Objetives: To analyse the functional outcomes of the transplanted kidneys (TK) obtained from DCD using NECMO.

Methods: We have reviewed the results from 15 kidney transplants (KT), whose organs where obtained from 9 DCD using NECMO as the preservation method, from 2015 to 2017 at our institution.
Currently, in our protocol, arterial and venous femoral canulae and the aortic occlusion balloon catheter are placed in the operating room, on a premortem and setup.
We have assessed the donors and recipient characteristics and also the TK outcomes, using a descriptive method from all our data available.

Results: From the 9 DCD, we have obtained 15 valid kidney grafts, who were transplanted at our institution, just one was not suitable due to anatomic alterations. The other 2 allografts were offered to other Hospitals.
Donor age averaged 52 years and allografts recipients averaged 48 years.
Four patients (26%) underwent previous kidney transplant, with an overall kidney allograft survival of 13.4 years.
Prior to KT, 13% were on a pre-dyalisis basis and the remaining 86% were on renal replacement therapy for an average of 14 months.
Cardiopulmonary death occurred within an average of 12.2 minutes following withdrawal of life support.
From the 15 KT, 13 (83%) continue to function, 1 had arterial thrombosis which led to transplantectomy and the other suffered acute rejection.
The 6 month average Cr was 1.6mg/dL.
Delayed graft function, defined as at least one postoperative episode of dialysis, occurred in 40% of kidneys (6/15).

Conclusions:
1- The organ quality from DCD is comparable to brain death donors.
2- ECMO supported DCD is feasible and easily implemented if necessary resources are available
3- Helps optimizing the donation-renal transplant process as the medical team responsible is involved.

Keywords: Renal transplant. ECMO. DCD.
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Exosomes as predictive biomarkers in acute ischemic stroke patients: A translational research approach.

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Main objective: to study the prognostic predictor potential of exosomes as biomarkers in acute ischemic stroke patients.

Specific objectives:
-To determine whether exosome serum levels are specific or not to cerebral ischemic injury.
-To correlate exosomes serum levels with functional outcome as well as brain repair markers detected in serum in patients and in an experimental animal model. In addition, in the animal model we aim to correlate the above variables with damage/repair markers in brain histological sections.
-To identify an exosome level cut-off associated with functional outcome that could be of utility as a predictor of prognosis.
-To evaluate the composition of exosomes released into serum in relation to functional outcome and to investigate possible differences in the composition in terms of various diseases in patients and the animal model.

Methodology:
-Clinical, observational study on 200 subjects (N=50 in each group) distributed as follows: Healthy; Non-lacunar acute ischemic stroke (middle cerebral artery); Lacunar acute ischemic stroke; Acute myocardial infarct
-Animal model study on 70 rats (N=10 in each group) distributed as follows: Healthy; Cerebral infarct (middle cerebral artery); subcortical cerebral infarct; Acute myocardial infarct and 3 Sham groups for each animal group.
Study variables: functional outcome by various scales; neuroimaging (Magnetic Resonance Imaging); exosome levels (ExoQuickELISA); exosome composition assessment by the proteomic technique (Orbitrap); Brain repair markers in serum by ELISA in subjects and animals. In the animal model, repair markers in brain tissue will also be analyzed using immunofluorescence and Western blot techniques. Exosomes will be isolated from serum. They will be measured in subjects at 24/72hours and at 3months and in the animals at 24hours and 14days.

Preliminary data: Study is open for recruitment since March 2017.125patients have been included(62.5% of total).Age range 32-90years:50healthy volunteers(21men,29 women;mean age: 62.6), 35patients with non-lacunar acute ischemic stroke(15men,20women; mean age: 71);17patients with lacunar acute ischemic stroke(11men,6women; mean age 66) and 23patients with acute myocardial infarct (9women,14men; mean age:52.5).At this rate, recruitment could be finished in ten months.In the animal model 61 rats (87%)have been included: 10healthy rats(5males,5females),10rats in the group subcortical cerebral infarct(5males,5females) and their 10 shams rats(5males,5females),8rats in the group cerebral infarct(middle cerebral artery)(3males,5females)and their 10shams rats(5males,5females)and 8rats in the group myocardial infarct(5males,3females) and their shams rats (5males).

Keywords: exosome, stroke, biomarkers
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IBJ Plus IberoAmerican Journals

Adaptation of the dimensional anhedonia rating scale (DARS).

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Introduction: Anhedonia is described as “pleasure desensitization”. Only two of the nine available scales for measuring anhedonia have been adapted to be used in a Spanish population. The Dimensional Anhedonia Rating Scale (DARS) is a 17-item self-report scale that has demonstrated reliability and validity in the measurement of anhedonia. The aim of this work was to translate the DARS into Spanish and to determine its reliability and validity in a group of patients with different psychiatric diagnoses.

Methods: The original DARS scale (English version) was translated and back-translated from Spanish to English and subsequently validated 134 psychiatric outpatients older than 18 years recruited from Psychiatry Department of the Fundación Jiménez Díaz University Hospital, Madrid, Spain, with a range of psychiatric diagnoses. The Spanish version of the Snaith Hamilton Pleasure Scale (SHAPS) was also administered to obtain the convergent validity of the DARS scale. Sociodemographic characteristics and diagnosis were also collected in all patients. Statistical analysis was performed by using a factor analysis. Internal reliability was assessed by calculating the Cronbach’s alpha index and the convergent validity was obtained by means of the Pearson’s correlation among total DARS and its subscales and SHAPS score.

Results: Our results show that the Spanish version of the DARS maintains the psychometric characteristics of the original English questionnaire. A strong internal consistency was observed (Cronbach alpha=0.92 for total scale score and 0.91-092 for subscale scores). In addition, a strong significant correlation was found between the total scores of the DARS and the SHAPS (r=0.51, p<.01).

Conclusions: The results show that the Spanish DARS maintains the psychometric characteristics of the original questionnaire, with a strong internal consistency and adequate validity. Particularly, the addition of items reflecting motivation, interest and effort brings potential advantages to the current measure over other anhedonia scales. Its structure seems to be suitable to differentiate anhedonia state of anhedonia trait in different diagnoses; which leaves open the possibility to future studies addressing this issue. Additionally, a future line of research to be consider is the transdiagnostic study of anhedonia, comparing DARS in patients with different psychiatric diagnoses.

Keywords: Anhedonia, Psychiatric Status Rating Scales, Validity, Reliability, Scale Development
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IBJ Plus IberoAmerican Journals

Predicting response to standard first line treatment in high grade serous ovarian cancer by angiogenesis-related genes.

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Introduction: Predicting response to treatment in High Grade Serous Ovarian Carcinoma (HGSOC) still remains a scientific challenge. The standard of care for first line treatment, based on a combination of carboplatin and paclitaxel, achieves high response rate, but the development of drug-resistance is one of the major limitations of efficacy. Therefore, the identification of biomarkers able to stratify patients is a critical step for prognosis and treatment of disease. Emerging and consolidated evidences suggest that angiogenesis is an important process in the development of ovarian carcinoma and chemoresistance phenomena.

Material and Methods: The aim of this study is to identify selected genes or a combination of them related to this process as biomarkers for response in HGSOC. We include thirty-nine patients diagnosed with HGSOC, with formalin-fixed and paraffin-embedded (FFPE) biopsy tissue and clinical data available and we examined the baseline tumor expression of 82 angiogenesis-related genes.

Results: Univariate analysis identified 5 statistically significant genes (ANGPT1, ARNT, CD34, EGF and MMP3) associated to response to treatment. Also, a multivariate analysis by Lasso-penalized Cox regression generated a model of 7 genes combined expression (AGT, CD34, EGF, EPOR, IL8, MMP3 y MMP7). Area under the curve (AUC) and Cross-validated Kaplan-Meier were generated to estimate the predictive accuracy of these predictors, that need of further validation in order to confirm these results. Our data support the important role of angiogenesis in ovarian carcinoma.

Conclusion: In conclusion, we have identified a reduced marker profile that could have a role in the prediction to standard chemotherapy response in HGSOC, but further investigation for validating it is needed.

Keywords: ovarian cancer, angiogenesis, response prediction.
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IBJ Plus IberoAmerican Journals

Levels of molecular biomarkers of angiogenesis and growth (Intraplatelet) in patients with systemic sclerosis with pulmonary involvement.

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Introduction: Systemic sclerosis is a serious disease of the connective tissue, characterized mainly by dysfunction of the microcirculation, dysregulation of the immune system and fibrosis in various organs. Angiogenesis is a complex process regulated by both angiogenic and angiostatic factors. Normally the functions of these factors are under an adequate balance, however, under certain conditions these factors can be induced, initiating disorganized angiogenic phenomena. Among the angiogenic factors with high levels it is VEGF-α, PDGF BB, HGF, TFG-β1, FGF2, G-CSF, Ang-2, leptin, IL 8, IL 6, IL1. However, these levels have been done in serum and never intraplatelet as in this work.

Methods: We have consecutively included 24 patients who met criteria for systemic sclerosis proposed by ACR / EULAR 2013 and who had been assessed in the different services, during the follow-up, at least one videocapillaroscopic study was carried out, respiratory function tests, echocardiogram and high resolution tomography. Additionally, patient records were accessed to obtain all clinical, serological and demographic parameters. The patient was referred to the Unit of Non-Transfusion Hemotherapy to collect platelet-poor plasma and platelet-rich plasma using an apheresis procedure and thus obtained the platelet concentrate sample (PCS) and the plasma sample (PS) in which the desired angiogenic factors will be measured (Currently samples are frozen at -80 c, pending financial support from two relevant entities to purchase the kits).

Sixteen healthy individuals (controls) were recruited. The platelet samples will be lysed through the freeze-thaw system and subsequently the growth factors (GF) will be measured in said sample, together with the plasma GF levels will be measured (VEGF-α, PDGF BB, HGF, TFG-β1, FGF2, G-CSF, IGF-1, IL 8, IL 6, IL1).

Results and Conclusions: A descriptive summary has been made of the data collected from the patients without finding statistically significant associations (multivariable study), however, this is not the objective of the study, since the main objective is to assess intraplatelet factor levels. We hope that the day of the symposium we will have the preliminary results since in recent days we have received the money for the purchase of the kit of growth factors that we will to measure.

Keywords: Systemic Sclerosis, Intraplatelet-angyogenesis factors, Growth factors
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IBJ Plus IberoAmerican Journals

How does self-efficacy influence pain perception, postural stability and range of motion in individuals with chronic low back pain?

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Introduction: Low back pain (LBP) is the most prevalent musculoskeletal problem among adults, its a great socioeconomic impact and one of the most frequent cause of work absenteeism and disability. Subjects with chronic LBP (CLBP) can present an alteration in psychological factors and a lack of self-efficacy of pain. This study aimed to compare the process of summation induced by the repetition of pain related to activity, as well as the lumbar range of motion and postural stability in patients with non-specific DLC (NSLBP) based on the level of self-efficacy.

Material and Methods: Sixty patients with NSCLBP were classified into two groups based on a median split of score on the Chronic Pain Self-Efficacy Scale; High self-efficacy group (n=30) with a mean age of 38.17 ±12.24 and low self-efficacy group with a mean age of 36.53±13.83. After consenting to participate, all the recruited participants received a sociodemographic questionnaire, a set of self-report measures and completed the Temporal Summation Lifting Task, Lumbar Range of Motion and Multi-Directional Functional Reach Test (MDFRT).

Results: Our results indicated that low self-efficacy group presented less lumbar range of motion and postural stability, in addition to more pain intensity in temporal summation lifting task compared to patients in high self-efficacy group. The strongest correlations were found in the analysis for the high self-efficacy group where it was found that there was a positive relation between the fear of movement and temporal summation lifting task ($r = .711$ $p < .01$) and MDFRT forward ($r = .738$ $p < .01$).

Conclusions: In conclusion, high of self-efficacy group has less intensity of pain in the temporal summation lifting task, more range of movement and a greater functional range, in addition to a less influence of psychological factors.

Keywords: self-efficacy; Non-specific chronic low back pain; physical variables; psychological variables.
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Mortality associated factors of patients admitted with flu during 2015-2016 and 2016-2017.

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OBJECTIVES: The aim of this study is to analyze demographic and clinic factors related to mortality of patients admitted due to flu during 2015-2016 and 2016-2017 periods, at La Paz University Hospital of the Community of Madrid, Spain.

MATERIAL AND METHODS: This is an unicentric, transversal and retrospective study of patients admitted due to infection by influenza virus at La Paz University Hospital, during 2015-2016 and 2016-2017 periods. Demographic, clinical, microbiological, therapeutic and comorbidities characteristics were collected for this group of patients. Univariate and multivariate analysis were performed in order to evaluate the objective of the study. Ethic guidelines were followed.

RESULTS: 33 out of 429 patients included in the study died (2015-2016 period: 4 patients; 2016-2017: 29 patients). Mortality risk factors observed: development of secondary bacterial pneumonia (OR 3.3 [1.6-7.1]), acute respiratory distress syndrome (ARDS) (OR 7.1 [2.8-18.1]), multiorgan failure (MOF) (OR 16.4 [7.1-38.1]). On the other hand, oseltamivir antiviral treatment proved to be a protective factor (OR 0.4 [0.1-0.8]). In the multivariate analysis, age (OR 1.067 [1.02-1.11]) and multiorgan failure (MOF) (OR 43.2 [13.1-142.8]) were independently associated to the mortality of the analyzed patients; while antiviral treatment was a protective factor itself (OR 0.3 [0.1-0.7]).

CONCLUSION: Mortality associated to patients admitted with flu would be related to old age and to multiple organ failure. Moreover, the oseltamivir treatment is a protective factor towards mortality.

	B	S.E.	Wald	Sig.	Exp (β)	95.0% C.I. for Exp (β)	
						Lower	Upper
AGE	.065	.022	8.992	.003	1.067	1.023	1.114
MULTIORGAN FAILURE	3.765	.610	38.073	.000	43.170	13.055	142.755
ANTIVIRAL TREATMENT	-1.377	.545	6.384	.012	0.252	.087	.734
Constant	-7.161	1.878	14.549	.000	.001		

Keywords: flu, mortality, oseltamivir
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Inhibitor of differentiation-1 (ID1) expression correlates with epithelial-mesenchymal-transition (EMT)-related proteins in epithelial ovarian cancer (EOC) and constitutes a novel prognostic factor.

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Introduction: Ovarian cancer is the most lethal gynecological cancer, and most patients present with advanced stage disease. To date, outcome prediction after multimodal therapy remains one of the most important challenges for patient's stratification according to effective treatments.

Epithelial to mesenchymal transition (EMT) is now recognized as a potential mechanism for tumor progression. One of the main hallmarks of this process is the loss of E-cadherin (ECAD) by the activation of transcriptional repressors such as Snail and Slug.

Objectives: To investigate the expression of inhibitors of differentiation (ID) proteins in Advanced Epithelial Ovarian Cancer (AEOC) and their relation with EMT-related markers. To analyze IDs expression in relation with clinical and pathological features in order to establish their role as prognostic factors.

Material and Methods: 69 patients with AEOC (stages III-IV) were included in this study. All patients underwent surgical cytoreduction and received a combined chemotherapy with carboplatin and paclitaxel. Immunohistochemistry (IHC) with specific antibodies for ID1, ID2, ID3 and ID4 was performed on tissue microarray sections, as well as for some EMT regulators, including ECAD, ZEB1, ZEB2, SNAIL, SLUG, LOX, and LOXL2.

Expression levels were scored for intensity (range 0-3) multiplied by the percentage of positive cells (H-score). Survival curves were calculated using the Kaplan–Meier method, and hazard ratios were estimated using the Cox proportional hazards model. IDs expression was correlated with appropriate clinical and pathological variables.

Results: ID2 and ID3 proteins were uniformly expressed in our series of EOC. ID1 and ID4 were overexpressed in different proportions (41% and 88% of cases, respectively). No correlation between increased ID proteins expression and histological subtype, tumor grading, debulking surgery status or treatment response was detected. ID1 overexpression correlated with prognosis, specifically with Time to Treatment Failure (TTF) in both, uni- and multivariate analysis adjusted to clinical factors (HR: 1.06; 95% CI: 1.02 - 1.09), and with Overall Survival (OS) [HR: 1.03 (95% CI: 1.00 - 1.05)] in the univariate analysis. This finding was confirmed in the group of high-grade serous carcinomas. ID1 is known to be associated with more invasive features of cancer, and with EMT. In our cohort, ID1 expression was correlated with some EMT-regulators, such as ECAD and SLUG ($p < 0.05$).

Conclusions: ID proteins expression is frequently deregulated in EOC patients and it seems to influence clinical prognosis, particularly, ID1. Their usefulness as prognostic biomarkers should be further investigated in larger series.

Keywords: Ovarian cancer, ID1, biomarker.
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IBJ Plus IberoAmerican Journals

Radio as an instrument of rehabilitation and recovery in patients diagnosed with severe and chronic mental disorders in community settings.

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Introduction: The purpose of this research is to study how the performance of a radio workshop affects the rehabilitation (psychiatric and psychosocial) and recovery of patients diagnosed with severe and chronic mental disorder in three Centers for Psychosocial Rehabilitation (CRPS) of the Community of Madrid that carry out radio programs on community radio stations.

Method: It is an exploratory, applied, transversal, descriptive, explanatory and comprehensive study. We have used a comprehensive multi-method model based on the integral theory that has combined methodological pluralism, where three investigative moments have been established, a first analytical empirical moment, a second hermeneutical phenomenological moment and a third investigative moment where participant observation has been used. The sample is constituted by 23 users of the CRPS of Los Cármenes, Villaverde and Getafe who have participated in the radio programs Ábrete Camino, Mejor Imposible, and Frecuencia favorable; seven coordinators-therapists of the radio workshops; three directors of the CRPS of Los Cármenes, Villaverde and Getafe; and three directors of the community radio stations Radio Onda Merlín Comunitaria, Radio Getafe and Radio Ágora Sol.

Results: Radio, as a mass media, is an excellent instrument that enables recovery and psychosocial rehabilitation, since it allows the radio workshop participants to establish new interpersonal relationships, increase motivation, enhance their autonomy, strengthen decision-making and responsibility, commitment to the activity and colleagues, facilitate free expression, creativity and spontaneity, encourage and increase the critical spirit, promote involvement throughout the recovery process, work awareness of the population on the reality of the person with mental disorder, and develop positive attitudes towards group work. On the other hand, the scores in the Brief Psychiatric Rating Scale, BPRS, at the time of the start of radio activity and at the present time (time to do the research), show that psychiatric symptoms improve with radio activity, except in the case of hallucinations and delusional thinking, which got worse.

Conclusions: The participation in a radio workshop in a community setting by patients with a diagnosis of severe and chronic mental disorder allows to promote autonomy, stimulate social relationships, the development of new roles, support the overcoming and remission of symptoms, and favoring the community integration.

Keywords: radio, psychosocial rehabilitation, recovery in mental health, severe and chronic mental disorder.
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IBJ Plus IberoAmerican Journals

Characteristics of cystic fibrosis (CF) patients with troubled evolution after suffering an atelectasis.

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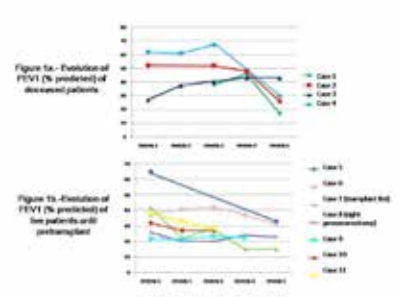
Introduction: The objective of this study was to analyze atelectasis as a factor of poor prognosis in CF.

Material and methods: A retrospective study was conducted in CF units nationwide. Up to December 2017, 46 CF cases that had suffered atelectasis have been included. We have studied: demographic data, respiratory infections, pulmonary complications and comorbidities, lung function and exacerbations, diagnosis through imaging tests, medical and bronchoscopic treatment, clinical and radiological improvement and final evolution. They were collected in different follow-up periods, following the ethical regulations of each center.

Results: in our series, atelectasis accounted for 4% of the CF population studied. 11/46 cases (23.9%) had a torpid evolution, of which six were women (54.5%)

- Microbiology: At the time of atelectasis, Pseudomonas aeruginosa was found in 8/11 patients (72.7%), followed by Staphylococcus aureus methicillin-sensitive in six of them (54.5%).
- Mutations: six patients were heterozygous, two were homozygous, and two had an unknown allele for the Phe.508 mutation. One patient had a different mutation.
- Location: five patients presented it in RUL (of which, one of them had an episode of bilobar atelectasis together with the lingula), three patients in LUL and one in LLL, one segmental patient in the left lung and finally, one patient suffered two episodes of atelectasis that affected the LUL, lingula and ML.
- Pulmonary complications /comorbidities: one patient presented ABPA and another had a pneumothorax. Three patients suffered an hemoptysis, of which two required embolization. Of the total of eleven patients, only two of them did not present pancreatic insufficiency.
- 4 patients died (36.4%): 1 patient died in the transplant, another included in the list and the last two, without having even entered the list, at the ages of 26, 31, 33 and 39 years, respectively, between 1 and 9 years after the diagnosis of atelectasis.
- 7 live patients (63.6%): 5 patients received a lung transplant, 1 patient was in the transplant waiting list, 1 patient required the realization of a right pneumonectomy.



Conclusions:
Risk factors could be established for the appearance of atelectasis with torpid evolution: carrier of the Phe.508del mutation, recurrent hemoptysis, localization in upper lung lobes, pancreatic insufficiency and polymicrobial chronic bronchial infection (P. aeruginosa and SAMS)
A fall in FEV1 is observed after atelectasis, causing death, entry into the transplant list or finally the transplant, and motivating, in the current study, to know if in our cohort, this decline is due to the evolution of the underlying disease or the appearance of pulmonary complication

Keywords: atelectasis, cystic fibrosis, prognosis
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T cell exhaustion profile (CD4+/CD28- CD8+/CD28-) in stem cell transplantation (SCT) and respiratory viral infection (RVI). Clinico-epidemiological findings.

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Background: Recipients of allogeneic stem cells grafts have clonally expanded CD8+/CD28- and CD4+/CD28- T lymphocytes during the early period after SCT, this cellular dynamic is probably associated with the acquisition of a toxic phenotype who overproduce granzime and perforin. This scenario predisposes to continuous inflammation, increase Tcell cytotoxic, NK activity (NKPer+Gran+) and favor the appearance of senescent lymphocytes. The direct consequences of respiratory viral infection (RVI),could also initiate a cascade of immunologic events (T cell exhaustion, inflammation)

Methods: In a prospective study, peripheral blood samples were obtained previous SCT and at day +100 post SCT from 34 patients (11 autologous, 23 allogeneic).To evaluate the expression of CD4+/CD28-Per+/Gran+, CD8+/CD28-Per+/Gran+, flow cytometry analysis was performed:100µL of PB was labeled, with a panel of 8 monoclonal antibodies: PERFORIN FITC, GRANZIME PE, CD4 PerCP, CD28 APC, CD8 APCH7, CD16/56 V450 and CD45 V500.The molecular detection of RV were tested with the CLART® Pneumovir assay based on the principle of multiplex PCR and DNA microarray.Statistical analysis: IBM SPSS v24

Results: 34 patients were evaluated from November 16 to December 17 at the HULP.The median percentages of baseline CD8+/CD28- cell line was 9,09% (range, 4,1-14,7) and the median percentages of CD8+/CD28-cell line at +100d were 29,2% (range, 13,3 -38,9). Likewise, significant differences were found (p=0.001). We compared between the group of patients who had a RVI and the group without RVI: We found differences between patients with VRI and those who did not have VRI in the CD8+/CD28- +100d cell line, the median percentages of the RVI patients were 58.43% (range, 42.72-68.43) and the median percentages in the uninfected were 29.26% (range, 13.26-38.94) (p=0.002).

Conclusion: This is the first data publication of this project, we show a dynamic change in the exhausted phenotype of T cell lymphocytes CD8+/CD28- throughout the SCT. There is a statistically significant difference when analyzing patients infected with RV vs non infected patients (baseline vs + 100d). In agreement with previous reports, there was a marked increased od CD8+/CD28- cell fraction early after SCT. Exposure to certain VR in the first 100d after STC may contribute the appearance of populations of exhausted T lymphocytes CD8+/CD28- favoring a sustained inflammatory environment and probably works as a trigger in certain immune-dysregulation

Keywords: Stem cell transplantation, CD4+ T cells, CD8+ T cells, T cell exhaustion
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Relevance of nocturnal hypoxemia and alterations of REM sleep in carbohydrate metabolism disorders.

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Introduction. Several evidences suggest a possible relationship between respiratory sleep disorders and the development of insulin resistance (IR) and later, diabetes.

Objective: To compare sleep characteristics among people without IR, with IR or with established diabetes.

Material and methods. We selected 96 consecutive subjects referred to a multidisciplinary unit of sleep breathing disorders for suspected obstructive sleep apnea. The anthropometric and clinical characteristics were collected, including comorbidities and usual treatment. A supervised video-polysomnography was performed, according to guidelines, and the next morning, an extraction of venous blood was carried out to determine the HOMA and QUICKI indexes. IR was considered when HOMA> 2 or QUICKI <0.339.

Results: 30 subjects had a normal carbohydrate metabolism, so they were considered as a control group, while 48 had IR and 18 had diabetes. There was no significant difference in the anthropometric characteristics or in the association with other metabolic or cardiovascular comorbidities when the three groups were compared. A progressive increase in nocturnal hypoxemia was noted between the control group, with IR and diabetes, based either on the desaturation index (18.5 ± 12.8 vs. 27.8 ± 18.2 vs. 41.1 ± 13.3 h-1, p = 0.045) or in the nighttime with SatO2 < 90% (12.5 ± 12.5 vs. 21.9 ± 17.0 vs. 35.8 ± 18.1%, p = 0.042). Likewise, control groups, with IR and with diabetes showed a progressive increase in the hypopnea index (18.6 ± 14.0 vs. 21.6 ± 22.5 vs. 33.6 ± 23.8 h-1, p = 0.033) and in the RDI (25.4 ± 12.7 vs. 27.4 ± 18.8 vs. 45.2 ± 19.8 h-1, p = 0.048). Strikingly, differences between the study groups were only evident in the REM sleep, both for the hypopnea index (15.2 ± 14.2 vs. 17.5 ± 20.0 vs. 32.0 ± 28, 7 h-1, p = 0.021) as for the RDI (11.4 ± 18.8 vs. 24.5 ± 16.3 ± 37.1 ± 17.5 h-1, p = 0.036).

Conclusion: There is a progression in nocturnal hypoxemia and in the presence of respiratory events among patients suffering from IR and diabetes. It is likely that the contribution of these alterations to the dysregulation of carbohydrate metabolism has a particular relevance during the REM sleep.

Keywords: REM, carbohydrate metabolism, nocturnal hypoxemia.
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Correlation between metacarpal cortical bone mineral density measured by dual x-ray densitometry and radiogrammetry on early arthritis patients.

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Introduction: Low bone mass at metacarpal (MC) diaphysis measured by radiogrammetry (DXR) has been described as a poor prognostic factor in rheumatoid arthritis (RA). This technique is not available in our environment. Our group has previously described the measurement of bone mineral density (BMD) at metacarpophalangeal joints (MCP) by dual X-ray absorptiometry (DXA). This measurement showed an acceptable correlation with the DXR at MC bones. DXR at MC mainly assesses cortical bone, whereas DXA at MCP mainly analyzes trabecular bone. We have developed a procedure to evaluate MC's (2nd to 4th) bone mass of the nondominant hand through DXA.

Objectives: To study the correlation between BMD at 2nd to 4th MC of the nondominant hand measured by DXA with data obtained by DXR.

Methods: 171 patients belonging to the Princesa Early Arthritis Reginster Longitudinal (PEARL) (84% women, 55.4 years at symptoms onset; 56.7% fulfilled RA 2010 criteria; 52% RF+ and 43.5% ACPA+). Demographic, clinical and laboratory data were collected per protocol. Hand X-rays were performed at baseline and after one year of follow-up, as well as nondominant hand BMD assessment by DXA (Hologic © QDR4500, Elite). The standard Hologic© software allows to design regions of interest (ROI) similar to that in DXR. The BMD by DXA was the average of 3 successive measurements. The BMD by DXR was measured with standardized software Sectra (Linköping, Sweden) on hand digital X-ray (GE © DX Definium 8000).

Statistical analysis was performed using Stata 12 for Windows, including linear correlations according to the Spearman test between the BMD values of MC by DXA and DXR and BMD by DXA at global hand and MCP joints. A multivariate analysis was performed to determine which variables account for the differences between MC bone mass measured by DXR and DXA.

Results: 248 BMD measurements (154 at baseline and 94 at second visit) of the 3 regions described whose values BMD 0.529±0.074 g/cm2 (MC-DXR); 0.427±0.060 g/cm2 (MC-DXA), 0.327±0.041 g/cm2 (Total Hand DXA), 0.265±0.040 g/cm2 (MCP DXA). Difference with MC-DXR: 0.104±0.074 (MC-DXA; p<0.0001), 0.206±0.060 (Total Hand DXA; p<0.0001), 0.268±0.053 (MCP DXA; p<0.0001). Correlation with MC-DXR: 0.865 (MC-DXA; p<0.0001), 0.824 (Total Hand DXA; p<0.0001), 0.717 (MCP DXA; p<0.0001).

Conclusions: MC bone mass measured by DXA shows the lowest absolute difference and the best correlation with MC bone mass by DXR. Female gender (beta coefficient = 0.013; p = 0.039), patients older than 65 year old (beta coefficient = 0.014; p = 0.019) and patients with higher body mass index (beta coefficient = 0.002 by kg/m2; p = 0.019) were significantly associated with lower differences between the values of MC by DXA and DXR.

Keywords: bone mineral density, rheumatoid arthritis, DXA.
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Is HCV-RNA in Chronic Hepatitis C Recipients of Kidney Transplantation detected by Ultracentrifugation or in Peripheral Blood Mononuclear Cells, after antiviral treatment and Sustained Viral Response?

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Background and aim: Presence of HCV- RNA after ultracentrifugation of serum samples during the follow-up of chronic hepatitis C patients with a sustained virological response (SVR) has previously described to predict reactivation of hepatitis C virus (HCV) infection, in patients treated with conventional antivirals. The aim of this study is to analyse if HCV- RNA is detected after serum ultracentrifugation or by peripheral blood mononuclear cells (PBMC) in chronic hepatitis C recipients of kidney transplantation (KT) with a SVR (defined as serum HCV-RNA negativity by conventional assays 6 months after the end of therapy) to antiviral therapy.

Patients and methods: We tested 13 adults recipients of KT, whose HCV infection had been treated with antiviral therapy. HCV –RNA was tested by real-time RT- PCR in PBMC and in 2 ml of plasma after ultracentrifugation.

Results: Only in one male, genotype 1b, previously treated with interferon, HCV- RNA was detected in plasma by ultracentrifugation. HCV- RNA was no detected in any case by RT- PCR in PBMC. We included 9 males, (69.2%), whose etiology of renal insufficiency was mostly a glomerular disease (38.5%) followed by hereditary (23,1%), or systemic (15.4%). All the patients had previously received dialysis, (hemodialysis in 58.3%, peritoneal dialysis in 16.7%, and both in 20%). 7 patients (53.7%), had their first KT, and in the others were the second one. The predominant HCV genotype was 1b (53.8%), followed by 3a (46.2%). 6 patients had been treated with DAA. 3 of them had been previously treated with interferon, and had a reactivation of HCV infection, then treated with direct-acting antiviral (DAA). HCV- RNA was no detected in PBMC or in plasma after ultracentrifugation in any case in those patients, treated with DAA.

Conclusions: Only in one male, genotype 1b, previously treated with interferon in monotherapy, HCV- RNA was detected in plasma by ultracentrifugation. HCV- RNA was no detected in any case by RT- PCR in PBMC and either in the 6 recipients of KT treated with DAA by RT- PCR in PBMC and in plasma after ultracentrifugation. Although more patients and longer follow up, are need, to make definitive conclusions, it seems that DAA are more effective, than conventional antivirals.

Keywords: Occult Hepatitis C Virus, Kidney Transplantation
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Role of HIF2α oxygen sensing pathway in bronchial epithelium biology.

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Introduction: Pulmonary epithelium is the first contact line with oxygen but the role of hypoxia-inducible factors (HIFs) in the airway's primary response to oxygen fluctuations as well as cigarette smoke remains largely unknown. The lung exhibits the highest levels of HIF2α, which is the main regulator of renal erythropoietin (Epo) in response to hypoxia.

Objectives: Here, we first explored whether HIF2α isoform could also induce Epo in the lung as a compensatory response to hypoxia and second, whether the HIF-dependent program could also be induced in other scenarios different from hypoxia such as cigarette smoke exposure.

Methods: We used mouse models of loss and gain of function of HIF2α pathway as well as mice exposure to atmospheric hypoxia and cigarette smoke. Lung tissues from these mice were subjected to protein and RNA analysis as well as immunohistochemistry analysis.

Results: Our novel data shows that lung induces Epo expression in response to hypoxia as well as upon constitutive activation of the HIF pathway. Importantly, this extra-renal source of erythropoietin is completely dependent on the HIF2α isoform and mainly confined in the bronchial epithelium. Regarding the relevance of HIF2α in bronchial epithelium, we previously showed that HIF2a-dependent genes such as Resistin-like molecular-alpha (RELMα) are induced in Club cells in response to hypoxia. Significantly our latest data also shows that RELMα and other HIF-dependent genes are also remarkably induced in cigarette smoke-exposed mice.

Conclusions: Our findings have unveiled that bronchial epithelium acts as an extra-renal source of Epo and suggests the role of airway HIF expression in response to cigarette smoke exposure.

Keywords: Hypoxia, Molecular pathology, Animal models.
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Abstracts

PhD Programme in Microbiology

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ISG15 governs mitochondrial function in macrophages following Vaccinia virus infection.

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Introduction: Protein modification by ubiquitin and ubiquitin-like proteins is a key regulatory process of the innate and adaptive immune response. In turn, many viruses, including poxviruses, have evolved strategies to antagonize the antiviral and inflammatory effects of the innate immune response in order to keep infected cells alive, until virus replication is complete. The interferon (IFN)-stimulated gene 15 (*ISG15*) encodes the protein ISG15, an ubiquitin-like modifier. It is one of the most abundant proteins induced by interferon, and its expression is associated with antiviral immunity.

Material and Methods: To identify protein components implicated in IFN and ISG15 signaling, we compared the proteomes of murine *ISG15*$^{-/-}$ and *ISG15*$^{+/+}$ bone marrow derived macrophages (BMDM) after Vaccinia virus (VACV) infection. We focused on the mitochondria, as the levels of several mitochondrial proteins were different. Mitochondria were isolated from BMDM stimulated or not with IFN, and were treated to determine the localization of ISGylated mitochondrial proteins by *western-blot*. Also, using the Seahorse technology, we performed different metabolic assays to analyze the respiratory metabolism in presence or not of ISG15, and *western-blot* to evaluate mitophagy-related proteins. Furthermore, analysis of iNOS production and Arginase-1 activity was performed to explore the different macrophage polarization patterns.

Results: These analyses revealed that several mitochondrial pathways were altered in *ISG15*$^{-/-}$ BMDM treated with IFN. Oxidative phosphorylation (OXPHOS), Adenosine triphosphate (ATP) and reactive oxygen species (ROS) production was higher in *ISG15*$^{+/+}$ BMDM than in *ISG15*$^{-/-}$BMDM following IFN treatment, indicating the involvement of ISG15-dependent mechanisms. An additional consequence of *ISG15* depletion was an impaired mitophagy and a significant change in macrophage polarization. Although infected *ISG15*$^{-/-}$ macrophages showed a robust proinflammatory cytokine expression pattern typical of an M1 phenotype, a clear blockade of nitric oxide (NO) production and Arginase-1 activation was detected. Accordingly, following IFN treatment, NO release was higher in *ISG15*$^{+/+}$ BMDM than in *ISG15*$^{-/-}$ BMDM concomitant with a decrease in viral titer. Thus, *ISG15*$^{-/-}$ macrophages were permissive for VACV replication following IFN treatment.

Conclusion: Our results describe a novel role for ISG15 controlling the dynamic functionality of mitochondria, specifically, OXPHOS and mitophagy, broadening its physiological role as an antiviral agent. These findings are clinically relevant since mitochondrial dysfunction is seen in many pathologies, underscoring the importance of the relationship between cellular metabolism and immune response.

Keywords: ISG15, mitochondria, OXPHOS, mitophagy, macrophages, Vaccinia virus
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Competing Interests: The authors have declared that no competing interests exist.

An undercover bacterium for health applications.

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Pseudomonas putida KT2440 is a non-pathogenic soil bacterium (GRAS-Generally Regarded As Safe)[1,2,3]. High stress-tolerating capacity especially towards organic compounds has put it into the center of attention in biotechnology applications. It has been exploited from industrial applications i.e. the Pseudomonas 2.011, to environmental applications i.e. bioindicators of toxics[12] in details. Here, our purpose is to refactor that species for health related areas.

Such deep genome reprogramming requires precise genetic tools to use. Moreover, having a model organism that can intuitively endure harsh conditions and to disturb least the harmony where it is introduced to is an advantage. In this aspect, the enhanced genomic manipulation methods[8,9,10] recently introduced for *P. putida* in addition to robust central metabolism together with being orthogonal to the microbiome of humans, we propose to use a new chassis of *P. putida* specially engineered for health related applications for the first time to our knowledge.

In this work, we are showing our endeavor and methodology for a proof-of-concept towards using *P. putida* as a chassis organism for Human Health fields. The idea is to keep the homeostasis of the host organism and avoid interfering the microbiome as much possible. As it is shown in many studies, there is a relationship between disrupted microbiome and certain diseases such as allergies, obesity, cancer, asthma, inflammations and infections[4,5,7]. Taking this into account, the platform strain we are developing is designed to make emphasis on non-invasive and non-immune response creating conditions.

We are in the process of performing the deletions to make this strain invisible to the immune system. To do that, the genomic manipulation techniques i.e. homologous recombination[8], or ssDNA based deletion[9] enforced with CRISPR/Cas[9] system[10] are used. For the circuitry designs, we are developing an automated platform in *P. putida* based on the Cello system[6] converted into SEVA format[13] (Standard European Vector Architecture). Horseshoe crab immunity testing platform will be used to assay the immune response of this Invisible Putida chassis.

We have managed to make important deletions from the genome of *P. putida*, in order to suppress biofilm formation capacity and avoid immune response in the recipient. These deletions correspond to the operons of flagella, biofilm formation responsible operons (*lapA, lapF*) and other genes responsible for creating immune response in the host. The planned deletions correspond to 131 kb of the genome, and 69 kb of it is already deleted. A deeper understanding may be possible with upcoming deletions and immune response tests.

Keywords: non-pathogenic, probiotic, chassis, invisible, therapeutic bacteria, orthogonal, biosensors, synthetic biology, P. putida.
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Abstracts

PhD Programme in Molecular Biosciences

Role of Lipid Transfer Proteins at ER-Golgi Membrane Contact Sites during Bunyavirus Infection.

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Introduction. Bunyavirales order compromises a large group of enveloped, negative-stranded RNA viruses which includes serious emerging and re-emerging pathogens for animal and plants, and are responsible of encephalitis and haemorrhagic fevers in humans. However, there are currently no vaccines or antiviral drugs to treat their infection. Bunyaviruses assemble a complex viral factory involving Golgi membranes where virus particles bud and mature. To build their membranous scaffolds, virus interfere cellular lipid metabolism and transport. Recent evidence suggests that viruses usurp lipid transfer proteins (LTPs) from their hosts to provide lipids for their replication complexes. For that reason, LTPs are expected to be potential targets for developing new therapeutic antiviral drugs. We are studying bunyavirus factories in HEK293 cells and the role of four different LTPs localised at membrane contact sites between endoplasmic reticulum and Golgi (LTP1-4) to identify which ones are subverted by bunyaviruses to their replication sites.

Materials and Methods. We have used HEK293 cell lines expressing tagged variants of human LTPs by tetracycline induction. Cells have been infected with the ATCC Br-87 strain of Bunyamwera virus (BUNV) or a fluorescent recombinant Gc-GFP-BUNV virus. Intracellular viral protein accumulation, viral titter and percentage of infected cells have been evaluated by western botting, plaque-based assay and flow cytometry, respectively. LTPs location after infection have been visualized by confocal microscopy. For high-resolution imaging of bunyavirus factories, cells have been visualized by electron microscopy.

Results. Confocal and electron microscopy have shown that BUNV induces morphological changes at rough endoplasmic reticulum. Changes are visualized as condensed and randomly folded membranes covered by ribosomes at the perinuclear region, which resembles convoluted membranes described in other viral infections. Regarding the role of first selected LTP1 and LTP2 in viral infection, no significant viral titter differences have been found when overexpressing any of them. LTP1 has been imaged by confocal microscopy at Golgi-derived viral factories and it increases the intracellular production of viral proteins during replication. LTP1 inhibition partially reduces BUNV intracellular protein accumulation.

Conclusions. BUNV infection of HEK293 cells modifies the architecture of rough endoplasmic reticulum. Preliminary results support that LTP1 and LTP2 are involved in bunyavirus infection cycle.

Keywords: bunyavirus, viral factories, lipid transfer protein, microscopy.
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Competing Interests: The authors declare that no competing interests exit.

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Exploring the regulation of frataxin expression by neurotrophic factors in the mouse cerebellum after physical exercise.

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Friedreich's ataxia is a predominantly neurodegenerative disease caused by recessive mutations that ultimately lead to a deficiency of frataxin (FXN) protein. It mainly affects the spinocerebellar system, thus leading to lack of motor coordination and loss of balance. However, little is known about the regulation of frataxin gene expression under different physiopathological situations. Our group has recently shown the prominent role of neurotrophic factors (specifically the Brain-derived Neurotrophic Factor; BDNF) to elicit neuroprotection against frataxin deficiency both *in vitro* and *in vivo*. Compelling evidence has pointed out a link between physical activity and the expression of neurotrophic factors in the nervous system through several molecular mechanisms In this work, we aim to explore the link between physical exercise, neurotrophic factors and frataxin gene expression in the mouse cerebellum. To achieve this, we subjected our mice to a spontaneous exercise protocol lasting 8 weeks, allowing us to identify, between the "runners" a sub group of "high runners" in order to check for "activity-amount" specific effects. We tested, through qPCRs, the levels of mRNAs of the factors we have previously demonstrated are involved in frataxin regulation such as BDNF, Neurotrophin 3 (NT3) and Sonic Hedgehog (SHH) as well as FXN itself. Our results show an increment in the levels of mRNAs for NT3 and SHH, while we were not able to detect significant changes in FXN or BDNF levels. Then we checked FXN protein levels by performing an ELISA assay, showing a significant increment in FXN protein in an amount directly related to the physical activity performed by the mouse. Similar, but not significant, results for BDNF protein levels were found. To search for more possible mediators of the effect of physical exercise on FXN protein level, we analyzed the levels of multiple cytokines using a protein array, which have led to the identification of several potential candidates for FXN upregulation. In view of these data, we suggest that physical exercise up-regulated FXN protein possibly through a posttranscriptional mechanism. A more thorough knowledge of the mediators and molecular mechanisms underlying FXN up-regulation may provide some clues for new therapeutic approaches to curb neurodegeneration in Friedreich's ataxia.

Keywords: Friedreich's ataxia, frataxin, physical exercise, neurotrophic factors, neurodegeneration.
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Undesired sample preparation artifacts can hamper the immuno-purification of lysine acetylation in proteomic studies.

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Introduction: Large-scale analysis of post-translational modifications by mass spectrometry relies on highly selective affinity tools directed against specific chemical moieties. In a set of lysine acetylation (K-ac) immuno-purifications, we noticed a fraction of high-quality spectra not assigned to peptide sequences. A mass-tolerant search revealed a K-ac peptide distribution at +42.0103 Da and an unexpected distribution at +43.00543 Da. The presence of CHNO neutral losses in the spectra unequivocally confirmed the identity of carbamylation.

Methods: We used a mass-tolerant search to identify the chemical modification that was copurified during immune-purification (IP) of acetylated peptides. Afterwards, we performed the lysis and protein extraction for subsequent IP under four different conditions: (1) Urea-Hepes and heat, (2) Urea-Hepes and room temperature, (3) Urea-Tris and room temperature and (4) sodium deoxycholate.

Results: Urea-based buffers typically used in proteomics can artifactually induce carbamylation at the side chains of lysine (K-cam) and/or peptide N-termini (Nter-cam). Thus, we examined the "basal levels" of carbamylation in the experimental conditions frequently used in K-ac protocols (*i.e.* HEPES and 56 ºC reduction). We performed fractionation of the total proteome and found 5% Nter-cam and 0.8% K-cam. The higher proportion of Nter-cam over K-cam peptides (1731/286) agrees with previous reports and other published data sets that we re-analysed. Then, we searched again our immuno-purified samples and found the inverted Nter-cam/K-cam proportion (462/3005). This suggested that the antibody co-purified, in addition to K-ac, K-cam peptides in a specific manner. This could be explained by their similar structures which only differ in the methyl (acetylation) and amino (carbamylation) groups.

We devised a strategy to minimize carbamylation in order to improve the selectivity of K-ac immuno-purifications. Either protein reduction at room temperature or replacement of HEPES for a primary amine Tris buffer decreased carbamylation levels ~6-fold which produced a ~2-fold improvement of K-ac peptides. However, substitution of urea for sodium deoxycholate increased K-ac almost 4-fold, with 96% of the modified peptides being acetylated.

Conclusions: We recommend the use of urea-free buffers for lysis and digestion prior purification of K-ac. The use of an ionic detergent improves the selectivity of the IP. Our findings suggest that the tools used for the enrichment of modifications in proteins/peptides should be carefully evaluated.

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Keywords: Lysine acetylation, carbamylation, immune-purification, urea.
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RNA-sequencing analysis identifies downstream genes of TCF4 involved in the development of non-small cell lung cancer.

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Introduction: The standard treatment for non-small cell lung cancer (NSCLC) and ovarian cancer is a Platinum-based chemotherapy, although the main clinical problem associated is the progression of the disease to a platinum-resistant state. This fact has limited its efficacy in these tumor types, which is one of the first causes of cancer deaths in developed countries. Thus, it is of great interest to identify predictive molecular biomarkers that could help in the patient treatment selection.

Material and methods: In this study we used array-CGH to analyze the cytogenetic alterations that arise in NSCLC after cisplatin treatment, by using four paired sensitive (S) and resistant (R) cell lines: H23S/R and H460S/R, A2780S/R and OVCAR3S/R. Validation of the results was performed through qRT-PCR in the same cells. Finally, our translational approach included the analysis of the expression in a total of 22 lung primary tumors and 7 adjacent non-tumor tissues, followed by the RNA-sequencing of a group of these samples and the validation by RT-PCR to confirm the results.

Results: Our experimental approach revealed the presence of a common deletion of the gene TCF4 in a mosaic manner in at least 50% of the resistant cells in both tumor types, while a decrease in *TCF4* expression was confirmed through qRT-PCR in the same cells. As *TCF4* is a transcription factor of Wnt signaling, we analyzed the expression of the downstream effector *DKK1* that is involved in the Wnt pathway. The translational analysis of the tumor and control samples showed that *TCF4* expression is frequently downregulated in these tumor types. Moreover, RNA-sequencing and Wnt-pathway analysis in a subgroup of these patients allowed us to identify four genes with potential role in the development of this malignancy.

Conclusions: Our results indicate that TCF4 is regulating downstream effectors of the Wnt-Signaling pathway that can be involved in the tumor establishment and progression in NSCLC. Altogether, we present a novel role for Wnt signaling pathway in the response to CDDP-based chemotherapy, which could be use as a potential therapeutic target for lung cancer.

Keywords: TCF4, Cisplatin (CDDP), cancer
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Competing Interests: The authors have no conflicts of interest to declare.

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NDUFS2 Mitochondrial Complex I deficiency induces adipose tissue degeneration via IIS in a Drosophila melanogaster model.

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Cardiac diseases are the foremost cause of death due to health problems in the western society. Amongst the causes of cardiac disease, those caused by metabolic defects, especially mitochondrial inborn errors, have difficult treatment and prognosis. This organelle provides energy trough oxidative phosphorylation, abbreviated as OXPHOS, and participates in apoptotic pathways. Permanent OXPHOS metabolism decay due to deficiencies in electron transport chain genes activates cell-signalling routes, leading to tissue wasting and disease. To study these diseases, we decide to develop a Drosophila melanogaster model in which nuclear encoded respiratory chain gene NDUFS2 has been specifically interfered in indirect flight skeletal-like muscle using UAS-GAL4 system. NDUFS2 is a structural subunit of the complex I core reported to cause hypertrophic cardiomyopathy among other symptoms. NDUFS2 IFM specific interference causes muscle and fat bodies degeneration, although the last ones are not NDUFS2 interfered and present healthy mitochondria. Gene expression assays indicate that the phenotype of wasted skeletal muscle leads to the increased expression of Impl2 gene in this tissue. Impl2 is an inhibitor of insulin/ insulin-like growth factor signalling. We suggest Impl2 overexpression induces lipid and glucose mobilization, and therefore, the observed fat bodies reduction leading to a phenotype reminiscent to insulin resistance and lipodystrophy in mammals. These results highlight the importance of knowing the routes triggered by mitochondrial malfunction and, thereafter, the tissue metabolic interdependence involved.

Keywords: mitochondria, Drosophila, NDUFS2, cardiomyopathy.
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Induction of antiviral CD8+ T lymphocyte-mediated protective memory responses.

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CD8+ T lymphocyte-based vaccines are necessary to provide effective protection against pathogens that cannot be controlled by humoral immunity. For this purpose, cytomegaloviruses (CMVs) are one of the most attractive vaccine vector candidates due to their induction of a strong and long-lasting CD8+ T lymphocyte response. Previously in our team we have characterized the role of Nras in the generation of protective memory CD8+ T lymphocytes. We found that Nras-deficient mice have impaired memory CD8+ T lymphocyte responses caused by a marked deficiency of the antigen-mediated early induction of T-box transcription factor Eomesodermin (Eomes) (Iborra *et al*, J Exp Med 2013).

We took advantage of a murine CMV (MCMV) vector developed by Dr S. Jonjić that conferred strong and long-lasting CD8+ T-lymphocyte-based protection against bacterial challenge in mice (Tršan *et al.*, PNAS 2013), and analyzed the capacity of MCMV vectors to improve CD8+ T lymphocyte memory responses in Nras-deficient mice. By doing this, we aimed to assess the potential of MCMV to overcome situations of deficient immune memory, providing helpful information for further vaccine design by describing the detailed mechanisms underlying the superior protective capacity of MCMV vaccine vectors.

Keywords: memory CD8+ T lymphocytes, murine cytomegalovirus, vaccines.
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A novel role for the Hippo pathway mediator TAZ in thyroid differentiation.

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Introduction. The Hippo signalling pathway plays a key role in the control of cell proliferation and its dysregulation has been associated with tumourigenesis. However, little is known about the regulatory mechanisms of the Hippo pathway in the thyroid gland. TAZ, a transcriptional coactivator in Hippo signalling, has been described to interact with Pax8, a main driver of thyroid differentiation, increasing thyroglobulin transcription. Pax8 is the main regulator of sodium iodide symporter (NIS), an important protein not only for the correct function of the thyroid gland, but also for radioiodide treatment of thyroid cancer.
The aim of this work was to study the role of the Hippo pathway and its mediator TAZ in NIS regulation, and hence in thyroid differentiation.

Material and Methods. We assessed the levels and the location of Hippo pathway components in thyroid follicular cells by RT-qPCR, Western Blotting and Immunofluorescence. The involvement of TAZ in NIS regulation was evaluated using chromatin immunoprecipitation (ChIP) and luciferase reporter assays. Cell-surface biotinylation and radioiodide uptake assays were used to analyse NIS membrane location and function.

Results. TSH the main inductor of NIS expression activates the Hippo pathway, thereby increasing pYAP and promoting TAZ translocation to the cytoplasm where it is marked for degradation by the proteasome machinery. On the other hand, a known negative regulator of NIS function such as TGFβ increases TAZ nuclear levels. On the contrary to what has been described for the thyroglobulin gene, TAZ is a negative regulator of NIS expression. This effect is due to a decreased Pax8 binding to the NIS enhancer. Accordingly, TAZ silencing partially impairs TGFβ-induced NIS repression and allows NIS membrane location, improving iodine uptake.
Conclusion. Altogether these data establish a novel role of the Hippo pathway, and particularly the cofactor TAZ, in the regulation of NIS expression in thyroid cells by the crosstalk with the main pathways playing a role in thyroid differentiation.

Keywords: Hippo pathway, TAZ, thyroid, NIS.
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Neuroprotective strategies against circadian alterations in a glioma model.

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Introduction: Glioblastoma multiforme (GBM) is the most common and lethal tumor from the central nervous system, and it is resistant to all current treatments. GMB causes neurological symptoms in patients such as sleep disturbances and memory loss. We propose that neurodegeneration plays a central role in GBM progression and death. Our results indicate that GBM induces synapse loss in the neuromuscular junction in a Drosophila melanogaster model of glioma, one of the first events leading to neurodegeneration. Our goal is to study the signals from the glioma that cause degeneration in neighbouring neurons.

Materials and methods: Using RT-qPCR, the results show that glioma brains upregulate ImpL2, an antagonist of the insulin pathway. The downregulation of ImpL2 using RNAi meanwhile a glioma is developed is able to rescue the synapse number in neuromuscular junctions and the glial cell number.

Currently, we focus our study on the effect of GMB in circadian rhythms analysing the circadian locomotor activity in adult flies. Preliminary results show that, as GBM advances, circadian rhythmicity is progressively disturbed. Moreover, we have shown that, in the absence of GBM, a genetically driven decrease of the number of synapses in clock neurons is sufficient to cause circadian disruptions. Using neuroprotective strategies like the overexpression of Rheb, a target of insulin pathway, we can improve the circadian performance, and increase the lifespan.

Results and conclusions: The upregulation of ImpL2 in glioma brains leads the neurodegenerative process disrupting the insulin pathway in neurons. One of the functional effects is the circadian activity distribution. However, the protection of this pathway allows us to increase the quality of life and lifespan. This may open an opportunity to develop a novel therapy based on the treatment of the neurological symptoms in glioma patients.

Keywords: Glioma, neurodegeneration, circadian rhythms.
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Mechanisms of resistance of basal cell carcinoma to Photodynamic Therapy.

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Basal cell carcinoma is the most common cancer worldwide. One of the non-invasive treatments for BCC is Photodynamic Therapy that, like many other anticancer therapies, in occasions, may cause recurrences. So, in this work, there were evaluated possible PDT- resistance mechanisms in three murine basal cell carcinoma cells lines (ASZ, BSZ and CSZ), comparing original populations with cells resistant to 10 cycles of PDT (10thG). Also, after the generation of 10thG populations, they were inoculated in mice; and then, developed tumors were cultured by explant to isolate two new populations (P T and 10thG T). In general, resistant populations acquired fusiform morphologies, diminished their proliferation capacity and their size and presented higher tumorigenic capacity than original cells. Besides, their resistance depend on location or production of protoporphyrin IX, the photosensitizer employed for PDT. Finally, it was observed that p53 determined the characteristics acquired by resistant populations: resistant cell line with p53 expression presented less proliferation rate than those without p53, and inhibition of Wnt/β-catenin pathway by increasing Gsk3β and increased expression of N-cadherin compared to original cells and. On the other hand, resistant cells without p53 had their Wnt/β-catenin signaling pathway activated by diminishing Gsk3β and presented less adhesion capacity by decreasing E-cadherin and β-catenin expression on the membrane. All these results put light on the different mechanisms of basal cell carcinoma resistance to PDT, what may help to improve its use, for example, by combining it with other co-adjuvant treatments.

Keywords: skin cancer, photodynamic therapy, resistance
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S100A9 mediates resistance of brain metastasis to radiation therapy.

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Background: Brain metastasis affects 10-30% of cancer patients. Standard of care is still based on palliative treatments including surgery and radiation. In order to understand why current therapies fail and whether there are ways to improve them we have developed an experimental model that incorporates whole brain radiation therapy (WBRT). With this new model we aim to identify critical mediators of radiation resistance to explore novel ways to radiosensitize brain metastasis.

Material and methods: We analyzed the effects of radiation in different brain metastasis models using *in vitro*, *ex vivo* and *in vivo* experimental approaches. *In vivo* WBRT was applied to experimental mouse models harboring established brain metastases using several hypofractionated protocols mimicking those given in the clinic. Additionally, we performed RNAseq comparing experimental conditions that correlate with different sensitivities to radiation to identify potential mediators of radiation resistance.

Results and discussion: We found no impairment in the growth rate and thus no impact in overall survival when three different WBRT protocols were applied to mice harboring brain metastasis. However, *in vitro,* brain tropic cell lines from lung and breast cancer with different oncogenomic profiles were highly sensitive to radiation. Interestingly, when we used culture methods that promote metastasis initiation capabilities or incorporate the brain microenvironment, we observed significantly decreased sensitivity to radiation. We then compared gene expression between BrM cell lines under conditions inducing resistance versus sensitivity to radiation, and identified *S100A9* as a **top candidate.** Although absence of S100A9 does not influence the incidence of brain metastasis, when combined with WBRT a dramatic decrease in brain colonization is evident by histology. There exists a blood-brain barrier-permeable inhibitor targeting potential receptors of S100A9-mediated activation of NFκB. We have shown that this inhibitor is effective at impairing radiation resistance in organotypic brain cultures, and we are currently testing its potential as novel radiosensitizer in vivo. Analysis of S100A9 levels in human brain metastasis samples reflects inter-patient heterogeneity, suggesting that this molecule could be used as a biomarker to rationalize the use of WBRT.

Conclusions: Our results support that in spite of not affecting metastatic colonization per se, targeting S100A9 in BrM models, combined with WBRT, reduces tumor burden *ex vivo* and *in vivo*. Further, we have identified an inhibitor blocking S100A9 mediated activation of NFkb capable of reproducing this result, suggesting a potential therapeutic application of our findings.

Keywords: Brain Metastasis/ S100A9/ Radiation therapy
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NRF2 controls proteostasis through the transcriptional regulation of autophagy.

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Introduction: Cells control the abundance and quality of the proteome through a wide network that integrates signaling pathways, gene expression and protein degradation systems. Degradation of cytosolic components inside lysosomes is carried out by specific types of autophagy in mammals, including macroautophagy and chaperone mediated autophagy (CMA). Considering autophagy as a proteostatic and defensive mechanism, we sought to determine if this process could be regulated by the transcription factor NRF2, classically considered the master regulator of the antioxidant cell response.

Material and methods: We performed a bioinformatics analysis to identify putative NRF2 binding sequences, termed antioxidant response elements (AREs) among autophagy related genes. Some of them were further validated by ChIP analysis. Messenger RNA and protein levels of cells under basal levels or submitted to different treatments were assessed by quantitative PCR and immunoblot. CMA activity in intact cells was determined with a photoswitchable reporter. The impact of NRF2 deficiency *in vivo* was determined by immunofluorescence and immunoblot.

Results: The bioinformatics analysis allowed us to identify putative NRF2 binding sequences, termed antioxidant response elements (AREs), in many genes whose products participate in macroautophagy and CMA. Several were further validated as NRF2-regulated genes by ChIP assays and quantitative PCR in *Nrf2*-deficient cells. Interestingly, *Nrf2*-knockout cells exhibited impaired macroautophagy flux in response to oxidative stress. *Nrf2*-knockout cells also showed reduced LAMP2A lysosomal levels (the limiting step for CMA) and, consequently, impaired CMA activity. Treatment with pro-oxidants up-regulated CMA through the transcriptional induction of *Lamp2a*, but to a lesser extent in *Nrf2*-deficient cells. Interestingly, pharmacological activation of NRF2 led to increased expression of many of these autophagy-related genes. This novel role of NRF2 in the regulation of autophagy may have an impact on proteinopathies, such as Alzheimer's Disease (AD). Indeed, NRF2 deficiency results in reduced neuronal expression of some of these markers as well as increased intracellular APP/Aβ and insoluble TAU in a mouse model of AD.

Conclusion: Our results point to a novel role of NRF2 in the regulation of autophagy and suggest a new strategy to combat proteinopathies.

Keywords: NRF2, autophagy, oxidative stress.
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The role of p27^{kip1} in the development, differentiation and maturation of mesencephalic dopaminergic neurons.

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Parkinson's Disease (PD) is one of the most common neurodegenerative disorders, generally characterized by the loss of specific dopaminergic neurons (DAn) in the midbrain. Current treatment options are available to help relieve primary motor symptoms, but their long-term effectiveness is limited. For this reason, alternative treatment options are being sought in the form of stem cell replacement therapies. Pluripotent stem cells, known for their ability to self-renew and differentiate into any cell lineage of the three germ layers, are a popular source of cells for the differentiation of DAn. In our lab, we are investigating the role of the protein p27^{Kip1} (p27) in the differentiation process of these types of neurons. p27 is a cyclin/cyclin dependent kinase inhibitor (CKI) belonging to the Cip/Kip family of proteins, best known for its function in the cell cycle. In this work, we investigate and analyze the effects of p27 on the development, differentiation and maturation of dopaminergic neurons *in vivo* and *in vitro,* using standard techniques of immunocyto- and histochemistry, western blot and quantitative-PCR. Our preliminary results show that mouse induced pluripotent stem cells that lack the protein p27 has led to a significant decrease in the production of TH+ (tyrosine hydroxylase, the limiting enzyme in dopamine production) cells, while the opposite effect was seen in mouse embryonic stem cells nucleofected to overexpress p27. In vivo, we have seen that at embryonic age E13.5, the production of TH in p27 knock-out mice was greatly reduced compared to wild type controls. We are currently doing a deeper analysis of other markers important for proper dopaminergic neuron development to decipher the mechanistic effects of p27. This would allow us to better apply the use of this protein to improve current differentiation protocols of dopaminergic neurons for stem cell replacement therapies in Parkinson's Disease.

Keywords: Parkinson's, DAn, DAn development, iPSCs, p27Kip1.
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Role of the transcription factor NRF2 in hippocampal neurogenesis and in a mouse model of Alzheimer´s Disease.

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Introduction: During adulthood, new hippocampal granule neurons are generated in the hippocampus by differentiation of neural stem/progenitor cells (NSPCs) in the subgranular zone (SGZ). The implication of hippocampal neurogenesis in learning and memory functions point it as a therapeutic strategy to face the cognitive deficits related with aging and neurodegenerative diseases. Hippocampal neurogenesis can be modulated by oxidative stress, neuroinflammation and proteinopathy. Here, we hypothesized that Nuclear Factor-Erythroid 2-Related Factor 2 (NRF2), as a master regulator of cellular homeostasis, might modulate the fate of NSPCs at the hippocampus.

Material and methods: immunohistochemistry analysis of hippocampal coronal sections of WT, NRF2-/-, APP/TAU/WT and APP/TAU/NRF2-/- mice at indicated age points. Long term potentiation (LTP) and Morris water maze test assays in 6 month- old mice of indicated genotypes. Immunocytochemistry of primary cultures of NSPCs of mice in postnatal day 0 to 4 (P0-P4) and 3 months of age in proliferation and differentiation conditions and lentiviral silence and overexpression of NRF2.

Results: NRF2-/- mice showed an impairment in LTP, correlating with an exacerbated reduction in hippocampal NSPCs from birth to adulthood. In vitro analysis using neurosphere assay corroborated this data, showing a reduced proliferative capacity of SGZ-derived NSPCs from newborn and 3-month-old NRF2-/- mice. Differentiation analysis pointed that NRF2-deficiency alters proper differentiation profile, favouring an abnormal rate between glial and neuronal differentiation. Ectopic expression of NRF2 in Nrf2-deficiency NSPCs attenuated the impact in their clonogenic, proliferative and differentiating capacity. Furthermore, when we performed the knockdown of the NRF2 gene in wild type NSPCs, data showed the alterations described previously for NRF2-/- NSPCs. Subsequently, to further analyse NRF2 implication in pathology, we used mice that express human mutated forms of TAU(P301L) and the amyloid protein precursor APP(V717I), in the presence or absence of NRF2. We report cognitive deficits in APP/TAU/NRF2-deficient mice considering the registered decrease in hippocampal LTP and poor performance in the Morris water maze test. Immunohistochemistry analysis of SGZ evidenced the detriment of NSPCs pool and neuronal differentiation in APP/TAU/NRF2-deficient mice at different age points.

Conclusions: The data support that NRF2 is important in the maintenance of proper proliferation and differentiation rates of hippocampal NSPCs. Our findings highlight the importance of NRF2 pharmacological upregulation to preserve the neurogenic functionality of the hippocampus and improve cognitive functions in AD.

Keywords: neurogenesis, NRF2, NSPCs.
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Competing Interests: The authors declare that they have no conflict of interest relating to the publication of this manuscript.

In vitro enhancement of collagen matrix deposition and cross-linking by coupling lysyl oxidase (LOX) with bone morphogenetic protein-1 (BMP-1) and its application in tissue engineering.

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Tissue engineering is emerging as a powerful therapeutic strategy to treat injured or degenerated tissues by implanting natural, synthetic, or semisynthetic tissue and organ mimics. The use of native ECM substrates is favored, as, theoretically, they should provide the structural and functional cues required for better preservation and growing conditions. However, standard cell culture conditions are far from ideal given the fact that the diluted microenvironment does not favor the production of ECM components. This is particularly true for collagen, the most important structural biomolecule, as its synthesis and deposition onto matrix is enzymatically rate-limited. An incomplete conversion of procollagen by C-proteinase/bone morphogenetic protein-1 (BMP-1) has been proposed to severely limit *in vitro* collagen deposition. BMP-1 also catalyzes the proteolytic activation of the precursor of the collagen cross-linking enzyme, lysyl oxidase (LOX) to yield the active form, suggesting a deficit in cross-linking activity under standard conditions. We hypothesized that the addition of LOX and BMP-1 may represent a strategy to boost *in vitro* deposition of collagen.

For that purpose we have generated HEK293-based cell systems that produced supernatants enriched with LOX and BMP-1 enzymes which, when combined together, recapitulated *in vitro* the proteolytic activation of LOX. Then we have implemented fibroblast cultures with these supernatants enriched in LOX and BMP-1 that strongly increased the deposition of collagen onto the insoluble matrix at the expense of the soluble fraction in the extracellular medium. Using decellularization protocols, we also provide evidence that fibroblast-derived matrices are able to regulate the adipogenic and osteogenic differentiation of human mesenchymal stem cells (MSC), a powerful cell tool in regenerative medicine, and these actions were modulated by LOX/BMP-1-modified matrices. These results demonstrate that we have developed a convenient protocol to enhance the capacity of *in vitro* cell cultures to deposit collagen in the ECM, and that this technology represents a promising approach for application in tissue engineering.

Keywords: Extracellular matrix, collagen deposition, lysyl oxidase, bone morphogenetic protein-1, tissue engineering.
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Competing Interests: The authors declare no competing interest.

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Vesicular trafficking and polarity of ICAM-1 in inflamed liver tissue.

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Introduction: During the inflammatory response, cellular barriers are transiently disrupted to let immune cells reach the inflammatory focus. The liver regulates immune tolerance by regulating leukocyte trafficking and exposure to immune receptors. Hepatocytes are polarized epithelial cells with an apical domain facing the bile canaliculus (BC) and a basolateral domain in contact with the liver parenchyma. Polarized hepatocytes confine ICAM-1, the ligand of leukocyte β2 integrins, in the BC so it cannot access infiltrated immune cells. Upon loss of polarity, ICAM-1 is exposed and mediates leukocyte adhesion to dysfunctional cells. We study mechanisms of intracellular trafficking that determine ICAM-1 polarization in hepatic cells and how inflammation modulates it. We are also generating an in vitro co-culture system based on adult liver 3D organoids in which we will mimic liver parenchyma to study the crosstalk between lymphocytes, endothelial and parenchymal cell barriers.

Material and methods: *Cells and Hepatic Organoids.* HepG2 human liver hepatocellular carcinoma cell lines were culture in DMEM supplemented with 10% fetal bovine serum. Human liver organoids from EpCAM+ ductal cells have been generated in collaboration with Dr. M. Huch, at the Gurdon Institute (Broutier et al., 2016). *Intracellular trafficking assays and confocal microscopy.* Transcytosis assays, cell line and liver organoid immunostaining were performed as described (Reglero-Real et al., 2014, Broutier et al., 2016). *Mass spectrometry and validation of the interactome of ICAM-1-BirA**. HepG2 cells stably expressing ICAM-1-BirA* were grown for 24 h in 50 µm biotin supplemented medium and pull down assays were performed. Extracts were analyzed by quantitative proteomics.

Results and Conclusions: Hepatic basolateral ICAM-1 relocates to the tight junctions domains where is accumulated and probably mediates the recruitment of vesicular trafficking that translocate it to the microvilli at the BC. The basolateral translocation of ICAM-1 is partially prevented by endocytosis inhibitors. Taking advance of BioID assays, we performed mass spectrometry analysis of ICAM-1-BirA* cells and proteomics revealed trafficking machinery in proximity of ICAM-1. We validated by immunoprecipitation and colocalization assays the direct interaction of ICAM-1 with some members of the SNARE and MAL family, both implicated in the regulation of apicobasal polarity in epithelial cells. Giving the limitations of culturing primary hepatocytes, we have introduce the new technology for growing adult human liver organoids and our aim is to co-culture them with endothelial cells to analyze the mechanisms mediating ICAM-1 trafficking and its relevant function in hepatic cells.

Keywords: Hepatocyte, apicobasal polarity, ICAM-1.
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Competing Interests: None of the authors declared a conflict of interest.

Lamin A/C enhances APC capacity to stimulate CD4 T cell responses.

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Introduction:
Antigen presentation by dendritic cells (DCs) stimulates naïve $CD4^+$ T cells, triggering T cell activation and adaptive immune responses. Several pathological processes are linked to abnormal Antigen Presenting Cells (APC) antigen presentation that alters the subsequence T cell response. Nuclear envelope lamin A/C controls chromatin organization, gene transcription, DNA replication and damage responses, cell differentiation and polarization during migration. Moreover, lamin A/C, when expressed in T cells, enhances $CD4^+$ T cell responses. However lamin A/C role in APC function has not been determined. Our objective is to analyze the role of lamin A/C in APC capacity to trigger adaptive immune responses.

Material and methods:
We compared by flow cytometry and live imaging microscopy, the capacity of WT and lamin A/C deficient ($Lmna^{-/-}$) DCs to promote activation, proliferation and differentiation of naïve $CD4^+$ T cells in both *in vitro* and *in vivo* experiments. DCs were obtained from $LysMcre^{+/+}$ x LmnaFl/Fl and their control mice.

Results:
Comparing $Lmna^{-/-}$ GM-CSF bone marrow derived-DCs with WT DCs in *in vitro* experiments, we observed a reduced capacity of $Lmna^{-/-}$ DCs to form conjugates with naïve $CD4^+$ T cells and to promote $CD4^+$ T cells activation and proliferation. We also observed a significant decreased production of $IFN\gamma^+$ $CD4^+$ T cells after T Cell Receptor (TCR) stimulation accompanied by less induction of the T helper 1 (Th1)-specific transcription factor T-bet. Lamin A/C deficiency in DCs also facilitates a greater differentiation towards a T regulatory (Treg) phenotype measured by the expression of Foxp3 and CD25. This effect was also corroborated *in vivo* since $Lmna^{-/-}$ mice in myeloid LysM-expressing cells exhibited less differentiation towards a Th1 phenotype and increased Treg phenotype upon vaccinia virus infection.

Conclusion:
Lamin A/C in APCs is a critical regulator of the activation, proliferation and differentiation of naïve $CD4^+$ T cells.

Keywords: Lamin A/C, CD4 T cell, APC, adaptive immunity.
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Effectiveness and molecular basis of CDK4/6 inhibition in combination with taxanes in pancreatic cancer.

Beatriz Salvador[1], Mónica Álvarez[1], Camino Menéndez[1], Pedro P. López-Casas[1], David Shields[2,3], Manuel Hidalgo[4,*], Marcos Malumbres[1,*]

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Introduction: Pancreatic Ductal Adenocarcinoma (PDAC) is among the deadliest human cancers with a 5-year survival rate of less than 5% using the standard of care gemcitabine/nab-paclitaxel. A very frequently disrupted gene in PDACs is CDKN2A (>90%), which encodes the cyclin-dependent kinase (CDK)4/6 inhibitor p16. Recently, CDK4/6 inhibitors have been approved for breast cancer treatment, and preclinical assays for PDAC are giving promising results.

Material and methods: PDAC Patient-Derived Xenografts (PDX) models and PDX-derived cell lines were used for in vivo and in vitro studies, respectively. Cellular studies were performed using proliferation and cell cycle assays in combination with flow cytometry, immunoblotting, fluorescence microscopy and live cell imaging techniques. Drug treatments were performed with the CDK4/6 inhibitor PD-0332991 (Palbociclib®), and with Paclitaxel (Taxol®) or Nab-Paclitaxel (Abraxane®) for in vitro and in vivo studies, respectively.

Results: Treatment of different PDX-derived cell lines with the combination of taxanes and CDK4/6 inhibitors resulted in a higher anti-proliferative effect than both drugs used as single agent. Cell cycle studies showed that inhibition of CDK4/6 prevented recovery from treatment with taxol. At the molecular level we found that the combined treatment induced a clear interruption in retinoblastoma pathway, even higher than CDK4/6 inhibition in monotherapy. Gene expression profiles comparing single versus combined treatment are currently being performed to further understand the molecular basis underlying the effectiveness of this type of treatment. Moreover, to assess the efficacy of this new combined treatment in vivo, we treated nine PDAC PDX models with PD-0332991 and nab-paclitaxel, following the same schedule. Importantly, eight of them presented an increased tumor growth inhibition in the combination with respect to the monotherapies.

Conclusions: Although the molecular mechanism underlying the effectiveness of this treatment is not completely understood yet, our data suggest a good therapeutic value for the combination of CDK4/6 inhibitors and taxanes in PDAC treatment.

Keywords: CDK4/6 inhibitor, taxol, Pancreatic Ductal Adenocarcinoma
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Relevance of coronavirus viroporins and pdz-binding- motifs in virus replication and virulence.

Carlos Castaño-Rodriguez[1], Jose M. Honrubia[1], Javier Gutierrez-Alvarez[1], Raul Fernandez-Delgado[1], Carmina Verdia-Baguena[2], Maria Queralt-Martin[2], Vicente Aguilella[2], Eric Bailly[3], Pascale Zimmermann[3], Jean-Paul Borg[3], Stanley Perlman[4], Isabel Sola[1], Luis Enjuanes[1]

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Introduction: Coronaviruses (CoVs) are pathogens responsible for a wide range of existing and emerging diseases in humans and domestic and companion animals. A CoV causing the severe acute respiratory syndrome (SARS-CoV) was identified in Southeast China in 2002, infecting more than 8,000 people, with mortality in approximately 10% of cases Viroporins are viral proteins with ion channel (IC) activity that play an important role in several processes including virus replication and pathogenesis. SARS-CoV encodes three: proteins 3a, E and 8a. Additionally, 3a and E proteins have a PDZ-binding motif (PBM), which may bind over 400 cellular proteins containing a PDZ domain, making them relevant for the control of cell function. In the present work, a comparative study of the functional motifs included within the SARS-CoV viroporins was performed focusing on the roles of the IC and PBM of E and 3a proteins.

Material and methods: Mutant viruses deleting each of the viroporins or affecting E and 3a proteins IC activities and PBMs were engineered from a mouse adapted infectious cDNA clone. Virus replication and virulence was assessed in vivo in BALB/c mice. 3a protein gene and its mutant variants were introduced in a BaculoVirus vector, produced in insect cells and purified by affinity chromatography. Then, their IC activity was measured in planar lipid bilayers. The interaction between CoVs PBMs and each of the cellular PDZ domains was studied using yeast-two hybrid.

Results and Discussion: Our results showed that both the full-length E and 3a proteins were required for optimum SARS-CoV replication and virulence whereas viroporin 8a had a minor impact on these activities. However, IC and PBM activities of E, but not 3a protein, were necessary for virulence in mice. Interdependence between E and 3a was identified. A virus missing both proteins was not viable, whereas the presence of either protein with a functional PBM restored virus viability, indicating functional redundancy between the PBMs of these proteins. Given the relevance of SARS-CoV PBMs, the presence of these motifs was studied in MERS-CoV, another highly pathogenic human CoV which includes a PBM in both E and 5 proteins. The interaction between the 256 known human PDZ domains and the PBMs of SARS-CoV and MERS-CoV was studied and more than 20 cellular proteins mostly involved in virus-cell and cell-cell interaction were identified. Some of these proteins are involved in immune response or in the infectivity of other viruses. Collectively, these results demonstrate redundant roles for the IC and PBMs for optimal virus replication and pathogenesis suggesting that they are potential targets for antiviral therapy.

Keywords: Coronavirus, SARS-CoV, viroporins, PDZ, PBM.
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The Role of Liver X Receptors in the homeostasis of Splenic Red Pulp Macrophages and Iron Metabolism.

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Introduction: The liver X receptors (LXRα and LXRβ) are members of the nuclear receptor superfamily of transcription factors. In macrophages, LXRs play essential roles in the coordination of both metabolic and immune responses, such as the transcriptional control of lipid metabolism or the modulation of innate and adaptive immune responses. Tissue resident macrophages are professional phagocytes that orchestrate innate immune responses and have considerable phenotypic diversity at different anatomical locations. In the spleen, there are different macrophage subpopulations, including red pulp and marginal zone macrophages, which play specific roles in homeostasis and disease. Red pulp macrophages (RPMs, identified as CD45+F4/80hiCD11blo by flow cytometry) are specialized cells that are important for the maintenance of Red Blood Cells (RBC) homeostasis, by actively phagocytosing injured and senescent erythrocytes, and thus being critical for the recycling of hemoglobin iron. We have previously reported that LXRs are crucial for the differentiation of splenic marginal zone (MZ) macrophages. Here we now show these nuclear receptors importance in the correct functioning of the red pulp of the spleen.

Material and methods: We used C57Bl/6 LXRαβ-/-, LXRα-/- and wild type mice, to compare the RPM and monocyte subpopulations both in the red pulp of the spleen and bone marrow using Flow Cytometry. Cell Sorting technique allowed us to perform transcriptional profiling, and quantitative PCR to monitor specific gene expression in these cell populations. Iron and hemoglobin concentration was analyzed through nephelometry, and iron distribution in the spleen by Prussian Blue histological staining.

Results: LXRαβ null mice present marked defects in splenic RPM subpopulation, despite elevated proportion of monocytes in the spleen. Presumably as a result of these alterations, iron handling is impaired in LXRαβ-deficient mice, that accumulate excessive iron in the splenic red pulp. In addition, LXRαβ-deficient mice also present defects in F4/80hiCD11blo iron-recycling bone-marrow resident macrophages. Strickingly, transcriptional analysis of RPM population in LXR-null mice showed defective expression of CD163, the hemoglobin scavenger receptor, which results in increased hemoglobin concentrations in the tissue.

Discussion: These results indicate a new role for LXR nuclear receptors in the regulation of iron homeostasis, possibly in part through the generation of an appropriate splenic RPM compartment.

Keywords: LXR, RPM, iron, CD163, Hemoglobin.
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Unravelling transformation of Follicular Lymphoma to Diffuse Large B- Cell Lymphoma.

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Introduction: Follicular lymphoma (FL) is an indolent but typically incurable disease with a long natural history. Disease outcome is variable: some patients do not need treatment, while others follow a more aggressive course characterized by interspersed episodes of remission and relapse, associated with lower sensitivity to therapy . The reported frequency of histological transformation (HT) to more aggressive lymphomas (transformed FL, tFL), most commonly to diffuse large B-cell lymphoma (DLBCL), varies markedly, from 30% to 60% of patients, is associated with poor prognosis.

Material and methods: We conducted targeted massive parallel sequencing of 22 FL/transformed diffuse large B-cell lymphoma (tFL) pairs and 20 FL samples from non-transformed patients.

Results: We have identified genes more frequently mutated in tFL than in matched FL samples. The most relevant were SOCS1 (FL=4.5% vs. tFL=22.7%), GNA13 (9.1% / 22.7%), B2M (9.1% / 22.7%), POU2AF1 (18.2% / 31.8%) and LRP1B (18.2% / 27.2%). The POU2AF1 gene was found recurrently mutated in tFL sampels (31.8%). All the mutations were in the 3 bp exon 1 splice donor site. Additionally, two of the POU2AF1-mutated tFL cases had mutations in the POU2F2 gene. We examined whether there was any difference in FL diagnosis samples from patients who transformed compared with those from non-transformed (ntFL) patients. We sequenced 20 samples from ntFL patients (without evidence of transformation after a follow-up of at least 5 years) with the same sequencing panel. When comparing FL and ntFL, although a similar number of genes were recurrently mutated in ntFL as in FL from patients who transformed (5 in ntFL, 6 in FL), the heterogeneity in the patterns of mutations was greater in FLs of transformed patients: 48 genes were recurrently mutated in FL biopsies from transformed patients while only 23 genes were recurrently mutated in ntFL samples. Finally, we looked for genes that were more frequently mutated in FL than in ntFL samples. We found a group of eight such genes: UBE2A, POU2F2, DSP, TAGAP, PCLO, LRP1B, NOTCH2 and CSMD. We found that a mutation in any of these genes was strongly associated with transformation (Fisher exact test P=0.0005).

Conclusions: We identified recurrently mutated genes that may be involved in transformation, the most relevant of them (POU2AF1, GNA13 and LRP1B) having roles in B-cell differentiation, GC architecture and migration. We also found genetic alterations in FL samples that differ in patients who did or did not transform, and so could be used to predict transformation at the time of FL diagnosis. This information might be useful for following up patients at higher risk of transformation.

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Adipose tissue and liver crosstalk: new insights into gender differences in hepatocellular carcinoma incidence.

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Hepatocellular carcinoma (HCC) is the third most common cancer type and the second leading cause of cancer-related death. The incidence of HCC is rising worldwide due to the increased prevalence of obesity. Therapeutic options for this malignancy are limited, being survival after diagnosis very poor. Better preventive, diagnostic and therapeutic tools are therefore urgently needed, particularly in view of the important contribution of obesity to HCC incidence worldwide.

Obesity multiplies the risk of developing liver cancer, probably because it can result in fatty liver disease, inflammation and cirrhosis, a well-known predisposing factor for this malignancy. In addition, epidemiological studies have shown a higher incidence of HCC in men than in women, a dimorphism also observed in mouse models. However, the specific mechanisms underlying the correlation between obesity, gender and HCC are unknown.

Adipose tissue is one of the most important contributors to the adaptation to obesity, through the regulation of fuel metabolism storage, the release of nutrients and, indirectly, through the production of circulating adipokines. Importantly, this organ shows evident gender disparities in the production of these secreted factors, but their role in HCC is controversial and requires further investigation.

Here, we used allograft experiments and diethylnitrosamine (DEN)-induced HCC mouse models to understand the connection between adipokine´s secretion and liver cancer regarding gender differences. We demonstrate that decreased levels of an adipokine account for the increased hepatic cancer risk in males. We found that higher activation of a protein kinase is responsible for the inhibition of white adipose tissue adipokine secretion in males, therefore promoting tumor development. This increased hepatic cancer cells proliferation is a consequence of the reduced activation of relevant signaling pathways in this organ, which exert a protective effect against tumor development in females.

This study shows that gender differences in adipocytes are important players in HCC development and can contribute to the increased incidence of HCC in males. Our results unravel a clear adipose tissue and liver crosstalk, clarifying a new mechanism underlying gender disparity in liver cancer development, and suggest its potential to guide new strategies for cancer therapeutics.

Keywords: hepatocellular carcinoma, liver, adipose tissue, adipokines, gender.
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Competing Interests: The authors report no conflict of interest.

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Functional characterization of CNS2 DNA regulatory element of the mouse *Tyr* gene by CRISPR-Cas9 mutagenesis.

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Introduction: Tyrosinase (Tyr) is the main enzyme of the melanin biosynthetic pathway. The Tyr gene is only expressed in pigment cells, of which there are two different known types: melanocytes from neural crest and retinal pigment epithelium (RPE) cells from optic cup. Tyr mutations cause cell hypopigmentation or albino (null) phenotype, with severe associated visual deficits. In human, mutations in TYR are responsible of a rare disease called Oculocutaneous Albinism Type 1 (OCA1). Previous works using standard and artificial chromosome-type BACs determined that promoter and proximal regulatory elements where sufficient to drive Tyr expression. Later on, a major regulatory region located at -15kb was found, containing enhancer and boundary elements, which our lab has been able to evaluate in melanocytes using CRISPR/Cas9 technique. However, Tyr expression in RPE cells was not driven by this element. Some years ago, an element called CNS2 (Conserved Non-coding Sequence) was proposed to be responsible of Tyr expression in RPE. We have analyzed this region to assess its relevance and functional implications.

Methods: Using CRISPR/Cas9 technique, we have designed sgRNAs at each side of the CNS2 element to produce both Double Strand Breaks (DSB) aiming to achieve a deletion. This has allowed us to obtain several alleles, among which is our desired deletion. Once the deletion was well established in a mouse colony, its function has been assessed by histology analysis of skin and eye, expression of Tyr and adjacent genes by RT-qPCR, and melanin content.

Results: We have been able to obtain 34 mice out of 81 (42%) with a mutation in any of the DSB, of which 9 out of 81 had a deletion (11%). This includes deletions of the desired region of interest. We have also obtained alleles with point mutations and an inversion. We decided to maintain four of the mouse lines: one with the CNS2 complete deletion, two with different partial deletions and an inversion. When analyzed by histological techniques or melanin content assay, we have not observed differences between any of our mice and wild type. However, when Tyr gene expression was evaluated in eye, we observed a decrease of expression in CNS2 complete deletion mice versus wild type animals

Conclusions: In this project, we have been able to achieve a 42% of mice with a mutation, and 11% with a deletion, which demonstrate the robustness and efficiency of CRISPR/Cas9 system. When CNS2 relevance has been evaluated, we have observed that this element regulates Tyr expression in RPE but it is not completely necessary for its proper function, probably because of the presence of other regulatory elements in Tyr locus.

Keywords: CRISPR/Cas9, albinism, tyrosinase.
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CDCA7 regulates lymphoma cells migration and invasion through reorganization of the tubulin and the actomyosin cytoskeleton.

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Introduction: Cancer cells accumulate numerous molecular alterations relative to their normal counterparts that confer them tumor formation capacities including indefinite proliferation activity (immortality), cell death resistance, or the capacity to invade the surrounding tissue. Great efforts have been made to uncover genes involved specifically in tumor formation, but most of the identified genes participate in processes related with cell proliferation. Accordingly, therapies targeting these genes also impair the proliferation of normal cells. Although immortality is a characteristic of tumor cells, it is not sufficient for tumor formation. Indeed, some non-tumor cells are capable to divide indefinitely, such as Lymphoblastoid B-Cell Lines (LCLs). To identify genes involved in tumor traits independent of limitless proliferation, we compared gene expression profiles of B-cell lymphomas with those of LCLs and identified >1,600 differentially expressed genes. One of the most significantly up-regulated genes was CDCA7, whose knockdown in lymphoma cells decreased their capacity to form tumors in immunodeficient mice (manuscript under revision).

Methods and Results: Histological examination of xenotransplanted lymphoma tumors showed that while CDCA7-silenced lymphoma cells did not infiltrate the surrounding muscle and adipose tissues, CDCA7-competent lymphoma cells massively infiltrated them, suggesting that CDCA7 might be a critical mediator of lymphoma invasiveness. Lentivirus encoding a control shRNA or various CDCA7-specific shRNAs were used to knockdown (KD) CDCA7 in DG-75, BL-2 and Toledo lymphoma cells and assess the invasion and migration capacity of these cells in vitro and in vivo and the molecular mechanisms involved. We found that CDCA7 KD sharply decreased invasion and migration of DG-75, BL-2 and Toledo cells in matrigel- and fibronectin-coated transwells, respectively. Moreover, CDCA7 KD also impaired invasion/migration of DG-75 cells in zebrafish embryos. CDCA7 KD did not affect cell adhesion to fibronectin or fibronectin receptors expression, but markedly altered the actomyosin and the tubulin cytoskeleton activation and polarization.

Conclusions: Our results strongly suggest that CDCA7 is a critical mediator of lymphoma cells invasion through its capacity to regulate the dynamics of both the tubulin and the actomyosin cytoskeleton.

Keywords: CDCA7, lymphoma, invasion, migration.
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Preclinical Safety and Efficacy Evaluation of Lentivirally transduced Hematopoietic Stem Cells for the treatment of Leukocyte Adhesion Deficiency type I.

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Introduction: Leukocyte Adhesion Deficiency Type I (LAD-I) is a primary immunodeficiency characterized by recurrent and life-threatening bacterial and fungal infections. It is caused by mutations in the ITGB2 gene, encoding the CD18 subunit of β2 integrins. These mutations lead to defective or absent expression of β2 integrins on the leukocytes' surface, limiting their adhesion to the endothelium and therefore their extravasation to infection sites. As it is the case with other monogenic immunodeficiencies, LAD-I is a good candidate for ex vivo gene therapy. In a previous work, a therapeutic Chim.hCD18 lentiviral vector (LV) was generated in which the human CD18 protein was expressed under the control of a chimeric promoter preferentially active in myeloid cells. This vector obtained the Orphan Drug Designation by the EMA (EU/3/16/1753) and FDA (DRU-2016-5430) Agencies.

Materials and Methods: We have tested the efficacy of the LV.Chim.hCD18 vector in mouse CD18 KO cells that resemble the severe phenotype of the disease. Bio-distribution studies have been performed in wild type mice transplanted with LV.Chim.hCD18 transduced Lin- BM cells. The regulation of the hCD18 expression was also evaluated by transducing healthy donor CD34+ cells from cord blood with the lentiviral vector.

Results: Ex vivo gene therapy experiments performed in CD18 KO mice have shown that even transductions leading to low vector copy numbers in the transduced cells are able to show restoration of the membrane expression of leukocyte's integrins. Bio-distribution studies demonstrated that the presence of the LV was restricted to the hematopoietic tissue and that no changes in the hematopoietic reconstitution were observed. Also, neither histopathology alterations in the organs of these mice, nor unexpected deaths or weight changes were observed in these mice, indicating the absence of toxicity of the gene therapy approach. Moreover, studies conducted with human hematopoietic cells showed that the integration of the therapeutic LV.Chim.hCD18, even when used at high multiplicities of infection, did not result in supra-physiological expression levels of CD18 in the membrane of transduced cells.

Conclusions: Altogether, these results evidence the preclinical efficacy and safety of the proposed LAD-I gene therapy approach.

Keywords: Primary immunodeficiencies; LAD-I, hCD18, Integrins, Gene Therapy.
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Tofacitinib restores the inhibition of reverse cholesterol transport induced by inflammation: understanding the lipid paradox associated with rheumatoid arthritis.

Pérez-Baos Sandra[1], Barrasa Juan Ignacio[1, 2], Gratal Paula[1], Larrañaga-Vera Ane[1], Prieto-Potin Iván[1], Herrero-Beaumont Gabriel[1*], Largo Raquel[1]

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Introduction: Rheumatoid arthritis (RA) patients have increased cardiovascular mortality, paradoxically associated with reduced serum lipid levels. In fact, an inverse relationship between C-reactive protein (CRP) and circulating lipid levels has been observed. The JAK inhibitor tofacitinib (TOFA) ameliorates RA systemic and joint inflammation with a concomitant increase in serum lipids. Our aim was to analyze the effect of TOFA on the lipid and inflammatory profile of hyperlipidemic rabbits with chronic arthritis (CA), and on the regulation of reverse cholesterol transport (RCT) during chronic inflammation.

Methods: CA was induced in 18 high fat diet (HFD)-fed rabbits. Four weekly intra-articular injections of ovalbumin were given to previously immunized animals. Nine CA rabbits were treated with TOFA (10mg/kg/day) for two weeks. Six healthy HFD-fed rabbits were used as controls. Fully differentiated THP-1 cells were exposed to HFD rabbit serum or ox-LDL to become foam cells. Thereafter, cells were stimulated with IFNγ in presence or absence of TOFA for 24 hours. Protein was collected for western blot studies and cholesterol accumulation was assessed by an Oil Red-O staining.

Results: CA rabbits showed lower levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) than controls (p=0.001 and p=0.012). C-reactive protein (CRP) levels were dramatically increased in all CA animals, although a reduction was observed with the treatment (p=0.006). We also found an inverse correlation between serum TC and CRP (R=-0.454, p=0.029). TOFA tended to increase serum TC and LCL-C and was able to reduce the lipid content within synovial macrophages up to a 58% (p=0.041), without modifying synovial macrophage density. In foam macrophages in culture, IFNγ further stimulated the intracellular lipid accumulation (p=0.041) along with a decrease in the protein levels of the nuclear factor liver X receptor alfa (LXRα) and the cholesterol transporter ATP-binding cassette transporter 1 (ABCA1) (p=0.002 and p=0.0047, respectively). TOFA prevented the lipid accumulation within macrophages (p=0.029) by increasing LXRα and ABCA1 synthesis in a JAK/STAT-dependent manner (p=0.047 and p=0.004, respectively), while it was unable to reduce lipid accumulation in ABCA1 silenced macrophages.

Conclusions: Our results suggest that active inflammation could be favouring lipid accumulation within tissue macrophages, thus inducing a decrease in serum lipid levels. TOFA may prevent this phenomenon, at least partially, by restoring ABCA1-mediated cholesterol efflux in macrophages. These findings further explain how serum lipid levels are diminished in RA and partially justify the effect of TOFA on the lipid profile of RA patients.

Keywords: Rheumatoid Arthritis, Inflammation, Cholesterol, Macrophages, ABCA1
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Competing Interests: The authors declare no conflicts of interest.

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The role of PKD1 in brain injury: ROS detoxification and neuroprotection.

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Introduction: Oxidative stress is a major pathophysiological mediator of degenerative processes in many neurodegenerative diseases. It is an early event produced during excitotoxicity and is one of the main causes of neuronal damage. It has been described that Protein Kinase D1 (PKD1) is activated by oxidative stress and regulate detoxification of free radicals in tumor cells. However, the role of PKD1 in brain injury associated with excitotoxicity and oxidative stress damage has not yet been explored.

Methods: We analyze the activity of PKD1 using in vitro and in vivo models of neuronal damage, as well as human ischemic stroke samples. In addition, and through the use of pharmacological inhibitors, lentiviral silencing and neuronal conditional knockout mice we study the role of this kinase in the pro-survival oxidative stress detoxification pathway and the regulation of their activity in these processes.

Results: We find that excitotoxicity provokes an inactivation of neuronal PKD1 and as a consequence there is a decline of IKK/NF-κB survival cascade and an increase in reactive oxygen species. We identify the first molecular mechanism involved in PKD1 inactivation, caused in excitotoxicity by the action of phosphatases not identified so far. Consistent with these results, we demonstrate that the elimination of PKD1 in murine models increases neuronal damage after ischemic stroke and the neurospecific expression of dephosphorylation-resistant active PKD1 prevents kainic acid-induced neuronal death in vitro and in vivo.

Conclusions: Our data support that the loss of neuronal PKD1 activity is detrimental for neuronal survival and ROS-detoxification and suggest that this kinase could be a promising target for treatment of excitotoxic brain damage associated to acute and chronic neurodegeneration.

Keywords: Excitotoxicity, oxidative stress, neuronal death, stroke, PKD1
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Generation and characterization of a reversible HGPS mouse model to design potential future therapies.

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare fatal genetic disorder characterized by premature aging and early death (average life span: 14.6 years). Classical HGPS is caused by a de novo dominant point mutation in the LMNA gene (encoding mainly lamin A and C). This LMNA mutation (typically c.1824 C>T, p.G608G) gives rise to a lamin A variant called progerin, a mutant protein that remains permanently farnesylated due to incomplete maturation and that exerts a dominant-negative effect.

HGPS patients appear healthy at birth but develop several clinical features during the first and second year of life. Taking into account that there is still no definitive cure for HGPS and that HGPS cannot be diagnosed right after birth, it is critically important to know if this disease is reversible or if, at least, it is possible to stop its progression. In addition, it is necessary to investigate what is the contribution of tissue-specific and systemic factors to the development of the HGPS phenotype in order to ascertain whether a therapy delivered in a tissue-specific manner would be enough to restore normal body condition in HGPS patients.

Using the CRISPR-Cas9 technology, we have generated for the first time a “reversible” transgenic mouse model called LmnaHGPSrev which expresses progerin ubiquitously and recapitulates the HGPS phenotype. Importantly, our design allows the elimination of progerin expression and the restoration of lamin A expression upon activation of the Cre-recombinase.

We have demonstrated that LmnaHGPSrev mice recapitule HGPS features, and that it is possible to remove progerin and restore lamin A expression after tamoxifen-inducible Cre expression. This HGPS model will allow us to control progerin suppression and lamin A restoration at different times during disease progression, either ubiquitously or in a tissue-specific manner. We therefore plan to investigate the potential reversibility of the damage caused by progerin, and assess the effectiveness of possible future therapies designed to suppress progerin expression in specific tissues.

As shown in the figure, our new mouse model expresses only progerin and lamin C but not lamin A. However, after tamoxifen induction to activate Cre, progerin expression is progressively suppressed while lamin A protein expression is restored.

Keywords: progeria, mouse model, reversion
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Competing Interests: The authors declare no conflict of interest.

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Study of the role of microRNAs in T-cell lymphoblastic lymphoma development through the regulation of expression of FBXW7 gene.

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Introduction: T-cell lymphoblastic leukaemia/lymphoma (T-ALL/T-LBL) are agressive hematological cancers prevalent in the childhood. Different genes have been related with the development of this disease including the tumor suppressor gene FBXW7, which plays an important role acting through the proteasome degradation of key oncoproteins. In this work, we showed that up-regulation of a pool of microRNAs contributes significantly to the down-regulation of FBXW7 in T-LBL development.

Material and methods: In order to elucidate the mutational status of FBXW7, we performed targeted-deep-sequencing analyses in a sample series of human T-LBL. Massive sequencing by RNA-Seq was accomplished to study the expression levels of FBXW7 isoforms and to select a pool of miRNAs candidates to be involved in a FBXW7 regulation: hsa-miR-223-3p, hsa-miR-195-5p and has-miR-101-3p. Transfecting SUP-T1 cell line with miRVana-miRNA mimics and inhibitors assessed the experimental validation of the effect of miRNA up-regulation over FBXW7 levels and its specific targets. We completed the studies of mRNA and protein expression with proliferation assays. Finally, we performed rescue experiments in order to define the role of each isoform in the T-LBL development.

Results: Despite we did not observe any mutation in FBXW7, we noticed a significant reduction in the amount of mRNA of two of the three FBXW7 isoforms (α and β isoforms), indicating that down-regulation of FBXW7 is a common feature in the pathogenesis of these diseases. Two of the selected miRNAs hsa-miR-101-3p and has-miR-195-5p are able to cause a significant reduction of FBXW7 protein levels acting alone or in combination, respectively. This drop in FBXW7 levels is also related with a reduction of cell survival and proliferation mainly due to the up-regulation of c-MYC and CCNE1. According to our data, this relationship between FBXW7 and its targets are dependent on the reduction of α and β isoforms reduction.

Conclusions: In our disease context, up-regulation of a pool of microRNAs portrays a highly dynamic landscape in the regulation of FBXW7 expression. Besides this, each FBXW7 isoforms has a different role on its targets regulation and consequently in the T-LBL development.

Keywords: T-LBL development, Regulation of FBXW7 expression by microRNAs, FBXW7-isoform specific functions.
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Protection against Middle East respiratory syndrome coronavirus infection by immunization with genetically engineered live-attenuated viruses.

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Introduction: Middle East respiratory syndrome (MERS) coronavirus (CoV) is a life threatening human CoV, against which there are no approved treatments or vaccines. The development of vaccines against MERS-CoV is mainly based on the Spike (S), which is highly immunogenic. Nonetheless, vectored or subunit vaccines against MERS-CoV have been demonstrated to carry out side effects. Despite the concerns about it safety, live-attenuated vaccines (LAV) have proven it potential to induce strong and lasting immune responses. Our laboratory demonstrated that the Envelope (E) protein of severe acute respiratory syndrome CoV (SARS-CoV) is a virulent factor. Lack of E gene attenuated the virus and provided full protection against virulent SARS-CoV challenge.

Material and methods: Two types of LAV have been engineered using a MERS-CoV reverse genetic system. The first one is based on the full deletion of the envelope (E) protein (rMERS-CoV-ΔE), which resulted in a replication-competent and propagation-defective one-cycle virus. The second type, a replication-competent and propagation-competent virus, initially consisted of a collection of six rMERS-CoVs carrying partial deletions of 9-11 amino acids spanning the C-terminal domain of the E protein (rMERS-CoV-E*); of them, three deletion mutants (E*-Δ1, E*-Δ2, and E*-mutPBM) were selected for further analysis. E*-Δ1 and E*-mutPBM mutants were viable, while E*-Δ2 was stabilized by introducing five amino acids within the engineered deletion (E*-Δ2ins). In order to produce ΔE virus, wild type (wt) E protein was provided in trans. Since E protein induced cell death, the complementation was performed using an inducible system optimized for transient expression.

Results: In the presence of E protein provided in trans, titers of the ΔE virus were raised ten-fold (>105 pfu/ml), whereas titers of E* and wt viruses were not significantly increased. Attenuation of rMERS ΔE, E*-Δ1, E*-Δ2ins, and E*-mutPBM viruses was evaluated in vivo in a transgenic mouse model susceptible to MERS-CoV infection. All E*-Δ1 infected animals lost weight and died after 8 days post-infection (dpi). Interestingly, ΔE, E*-Δ2ins, and E*-mutPBM infected animals recovered from the infection and survived, indicating that these viruses were attenuated. ΔE, E*-Δ2ins, and E*-mutPBM immunized animals were then challenged with virulent MERS-CoV. All the animals survived and no weight loses were observed.

Conclusions: Three LAV candidates have been obtained against MERS-CoV infection. ΔE seems to be the most promising due to its capacity to replicate but not to propagate. However, a lot of work is still to be done concerning the evaluation of the immune response, the genetic stability, and the safety.

Keywords: MERS, coronavirus, vaccine, live-attenuated, Envelope
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Competing Interests: The authors did not declare any competing interests.

Mechanisms controlling the cilia localization of INPP5E, a phosphoinositide 5-phosphatase mutated in MORM and Joubert syndromes.

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Introduction: INPP5E is a ciliary phosphoinositide 5-phosphatase whose mutations are the cause of two human ciliopathies, Joubert (JBTS) and MORM syndromes, and whose activity supports Hedgehog-dependent tumor progression. We previously showed that INPP5E is a critical regulator of ciliary phosphoinositide levels, which in turn control ciliary protein composition and Hedgehog signaling (Garcia-Gonzalo et al. 2015 Dev Cell). However, the mechanisms controlling INPP5E ciliary localization remain unclear.

Material and methods: We have used site-directed mutagenesis to examine how INPP5E cilia localization is controlled.

Results: We find that INPP5E cilia localization depends on two separate regions located at the beginning and end of its catalytic domain. These two regions, albeit separated by circa 300 residues, come together as a concave surface on the folded catalytic domain. Several residues on this surface are needed for INPP5E to bind ARL13B, a JBTS-causative ciliary protein known to be required for INPP5E to localize to cilia. Since INPP5E is reported to leave cilia in response to mitogenic signals, we also studied whether INPP5E ciliary targeting is regulated by phosphorylation. We find that INPP5E undergoes tyrosine phosphorylation, and that a mutant mimicking phosphorylation of a tyrosine on the above-mentioned surface prevents INPP5E cilia localization.

Conclusions: We conclude that INPP5E cilia localization is controlled by a ciliary targeting signal (CTS) present in its folded catalytic domain. This CTS acts, at least partly, by allowing INPP5E to interact with ARL13B. Moreover, our preliminary data suggest that CTS phosphorylation can interfere with INPP5E cilia localization.

Keywords: INPP5E, Joubert syndrome, MORM, ARL13B, cilia, phosphorylation.
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Competing Interests: The authors declare no competing interests.

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Generation of "mini-brains" from pluripotent stem cells to study brain development.

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Due to the complexity of the human brain, it is difficult to study many brain disorders in model organisms. The results that we can obtain nowadays are all made in monolayer cell cultures (2D), and, although highly valuable, those methods are devoid of a tridimensional component necessary for normal organ development. Therefore, the ability to model human brain development in vitro represents an important step in our study of developmental processes and neurological disorders.

It has recently been described that pluripotent stem cells (Embryonic Stem Cells (ES) or induced Pluripotent Stem Cells (iPS)) in a suitable environment are capable of generating three-dimensional (3D) structures called "cerebral organoids or mini-brains". They recapitulate different stages of human cortical development, generating a variety of regional identities organized in discrete domains able to connect with each other.

We are setting up a human and mouse ES cells three dimensional organoids culture system. The differentiation in organoids (3D) is being carried out according to the protocol recently published by Lancaster and Knoblich (2014) Nature Protocols 9(10):2329-40. It is based on a first phase of generation of floating embryoid bodies, followed by a second phase of transferring these embryoid bodies to plates for their neural induction and subsequent differentiation. The characterization of cultures and identification of different neural structures and phenotypes are being performed at cellular and molecular level by immunocytochemistry and quantitative-RT-PCR.

Together, these studies would indicate that three-dimensional organoids can recapitulate human or mouse neurodevelopment and it can be useful to study the pathogenesis of neurological diseases.

Keywords: Embryonic stem cells, pluripotency, organoids.
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Competing Interests: The authors indicate that they have no potential conflict of interest with this work.

Lysyl oxidase-like 3 in melanoma progression.

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Malignant melanoma is the skin cancer with highest mortality rate, characterized by its heterogeneity, aggressiveness and resistance to treatment. Melanoma is characterized by a high mutation burden. The common BRAFV600E oncogenic mutation is already present in nevi, and further genetic alterations, both in vitro and in animal models, are required for malignant transformation.

LOXL3 has been described as one of the most highly expressed proteins in melanoma cell lines compared to normal melanocytes. Our previous results indicate that LOXL3 is overexpressed in human melanoma cell lines and primary and metastatic human melanoma samples, whereas in vitro studies suggest that LOXL3 downregulation is detrimental for their proliferation and survival but the specific role played by LOXL3 in vivo in melanomagenesis is still ill defined.

We thus decided to characterize the involvement of LOXL3 in melanoma initiation, progression and/or dissemination and the underlying molecular mechanisms involved using a combination of in vitro and in vivo approaches.

For that purpose, we have depleted Loxl3 expression in B16 F1 and F10 mouse melanoma cell lines using lentiviral shRNAs to analyze the effects of Loxl3 silencing on cell proliferation and survival. We have also performed in vivo tumorigenesis and metastasis assays to study the role of Loxl3 after orthotopical or tail vein injection of Loxl3-silenced cells. Our initial results suggest that Loxl3 depletion negatively affects melanoma cell proliferation.

Besides, we have generated a genetically engineered melanoma mouse model with Loxl3 conditional loss-of-function. In these conditional mice, upon 4-hydroxytamoxifen (4-HT) treatment, the Tyr::CreERT2 transgene allows melanocyte specific Cre expression promoting constitutive active mutant BRAFV600E expression and PTEN loss and concomitant Loxl3 deletion. Topical application of 4-HT results in the development of pigmented skin lesions which progress to malignant melanoma whereas metastasis is detected in lymph nodes and lungs. In order to evaluate the contribution of Loxl3 to melanoma initiation and/or progression we have performed a pilot experiment using the generated BRAFV600E/+/PTENlox/lox/Loxl3loxl/lox and corresponding controls BRAFV600E/+/PTENlox/lox/Loxl3+/+. Upon evaluating the timing and incidence of nevi and melanomas as well as overall survival after 4-HT treatment, our preliminary results show that, in the absence of Loxl3, the onset of pigmented lesions is delayed while overall survival is increased compared to control animals. Altogether, the present data support a key role for Loxl3 in melanomagenesis.

Keywords: LOXL3, $BRAF^{V600E}$, PTEN, melanoma.
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Activation of brown adipose tissue (BAT) might play a major and beneficial role against insulin resistance associated to inflammation. Beneficial effects of modulating SIRT1 activity.

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Introduction: Activation of brown adipose tissue (BAT) plays a promising role against metabolic diseases such as obesity or type 2 diabetes mellitus (T2DM). These conditions are associated with chronic low-grade systemic inflammation, which is considered a critical underlying factor in the development of insulin resistance.

Sirtuin 1 (SIRT1), a NAD+-dependent protein deacetylase, has emerged as a key metabolic sensor in various metabolic tissues that modulates a variety of cellular processes like energy metabolism or stress response. Although SIRT1 overexpression is protective against diverse metabolic complications, little is known about the etiology of these benefits.

Material and methods: To identify the mechanisms implicated in the potential therapeutic benefit of targeting SIRT1 in BAT to ameliorate inflammation-mediated insulin resistance we have used an in vivo model of lean mice with or without moderate SIRT1 overexpression. We performed an in vitro model of differentiated brown adipocytes obtained from these mice in order to study the role of this protein in thermogenesis and insulin signaling. The impact of SIRT1 in BAT inflammation was studied by an acute treatment with bacterial lipopolysaccharide (LPS). All these processes were analyzed by western blot and RT-PCR.

Results: Our results indicated that SIRT1 overexpression enhanced insulin sensitivity in BAT, and after an acute treatment with LPS, the induction of the proinflammatory cascades were attenuated in these cells.

Conclusions: Our results suggest that activation of SIRT1 in brown adipocytes might play a major and beneficial role against insulin resistance associated to inflammation.

Keywords: BAT, SIRT1, INFFLAMATION.
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Therapeutic activity of GSE4 peptide in cellular models of idiopathic pulmonary fibrosis.

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Idiopathic pulmonary fibrosis (IPF) is considered a rare disease but also the most common manifestation within telomeropathies, mainly in adulthood. Although 80% of families with IPF have not been genetically characterized, some mutations have been described in hTERT, hTERC and other genes of the telomerase complex, causing telomerase activity failure, telomeric shortening, DNA damage, senescence, inflammation and oxidative stress. GSE4-peptide, corresponding to an internal domain of dyskerin, has proved to reverse these effects in dyskeratosis congenita (DC) cells, another telomeropathy. Taking into account the therapeutic role of this peptide in DC, the objective of this work was to verify if GSE4 is also effective reversing some of these parameters in different cellular models of IPF.

In the VA13 cell line, lung fibroblasts deficient in telomerase, we compared by TRAP assay the differences that occur in telomerase activity by transiently expressing the hTERT/hTERC WT genes or different mutations associated with IPF in these genes. In addition, we expressed GSE4 in these cells to check if the peptide corrects the deficiency in telomerase activity generated by these mutations. In the same way, we expressed these mutations in a human fibroblast cell line called FN1iib to compare the differences that occur in DNA damage, senescence and inflammation, and to check if GSE4 reduces some of these parameters delivering it by nanoparticles. DNA damage was analyzed by detection of γ-H2A.X expression by western blot, and senescence and inflammation by measuring CDKN1A/CDKN2A and IL6 expression, respectively, by q-RT-PCR. Moreover, we treated a rat alveolar cell line called RLE-6TN with bleomycin to induce fibrosis, and we expressed GSE4 on it to check if the peptide reduces DNA damage, senescence and inflammation and if it corrects the deficiency in telomerase activity.

We observed that all mutants show a deficiency in telomerase activity, being more severe in a hTERC mutant. In addition, GSE4 manages to correct this deficiency in the most severe mutations, suggesting that it acts when telomerase activity is lower. We verified that all mutants exhibit DNA damage and senescence, being higher in the hTERC mutants. Besides, GSE4 is able to reduce DNA damage in all the mutants. These results agree with the fact that hTERC plays a main role in the maintenance of the structure and function of the telomerase complex since mutations in this gene produce the most severe alterations. Finally, in the rat cells model we saw that GSE4 reduces the levels of cellular DNA damage, senescence and inflammation and increases telomerase activity, suggesting that this peptide could be a good therapeutic tool in IPF.

Keywords: idiopathic pulmonary fibrosis, telomeropathy, GSE4, TERT, TERC.
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Evaluation of the physiopathology of the allan-herndon-dudley syndrome. A characterization of double knock-out mice model of the disease.

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Thyroid Hormones (THs, T4 and T3) are essential in the development of the brain, regulating processes such as differentiation of neural cells and synaptogenesis. THs are secreted into the blood from the thyroid gland, mainly as T4, which is converted into T3, the nuclear active form, by the enzyme deiodinase 2 (DIO2), to exert genomic actions. Allan-Herndon-Dudley Syndrome (AHDS) is associated to a X-linked condition caused by mutations in monocarboxilate transporter 8 (MCT8), a transmembrane transporter highly specific for THs. AHDS is characterized by an endocrine and a neurological syndrome with congenital hypotonia that progresses to spasticity with severe psychomotor impairment. Evidences strongly suggest that the neurological syndrome in MCT8 deficiency is mainly due to a brain hypothyroidism, since the access of THs across brain barriers is impaired.

We analyzed the expression of synaptic scaffold proteins and the expression of different proteins in different populations of nerve cells in MCT8-deficient brain samples from an 11-year-old subject, in comparison to a control subject of the same age. In parallel, we studied the synaptogenesis markers distribution in neurons and the expression of different proteins in nerve cells in double knock-out mice for Mct8 and Dio2 brains, as a model for the disease. Protein expression was analyzed by immunohistochemistry. Our results showed a decreased expression of most of the synaptic proteins in the MCT8-deficient human brain in comparison to the control brain. To characterize glial cells, we evaluated the expression of GFAP, and we observed an increase in the number of astrocytes. Additionally, to evaluate microglial cells, we analyzed the expression of Iba-1. Our results revealed an increase in the number of Iba-1 immunopositive cells in the MCT8-deficient human brain. We also observed an increase in the number of GFAP immunopositive astrocytes in the cerebral cortex of Mct8/Dio2 knock-out mice. Regarding microglial cells, our results revealed an increase in the number of Iba-1 positive cells, in the cerebral cortex and hippocampus of Mct8/Dio2 knock-out mice. To differentiate resting and reactive microglial cells, we studied CD68 expression, a membrane protein much more abundant in reactive microglia cells. Our results showed a significant increase in the expression of this protein in the brain of the double knock-out in comparison to the wild-type mice. Our results suggest that synaptic transmission could be altered in patients with MCT8 deficiency, and the characterization of nerve cells suggests an inflammatory response due to MCT8 deficiency, what could be related to the psychomotor delay.

Keywords: Allan-Herndon-Dudley syndrome, hypothyroidism, synaptogenesis, microglia, astrocyte.
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Studying resistance to antivirals in single cells with Correlative Light and Electron microscopy.

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Introduction: Development of highly effective, broad-spectrum antivirals is the major objective shared by the fields of virology and pharmaceutics. High throughput screening of molecules has identified promising candidates for developing optimal broad-spectrum antiviral agents. In our lab we study the replication factories of several pathogenic RNA viruses. In the last few years we have developed new imaging techniques to study viruses in cells and applied these methods to study the factories assembled by bunyaviruses. Several bunyaviruses are important pathogens for humans. Climate change together with increased global trade and travel is causing a rapid dissemination of the vectors of bunyaviruses and some other important arboviruses such as Dengue, Zika and Chikungunya. These viruses constitute a global threat because there are currently no vaccines or specific antiviral drugs for many of them.

Material and methods: We have recently started to study viral infections at the level of single cells. With fluorescent recombinant bunyaviruses and Correlative Light and Electron Microscopy (CLEM), we have studied the heterogeneous response of cell populations to bunyavirus infection and to treatment with antivirals. The response of cells to the antiviral treatment was studied by live cell video microscopy and confocal microscopy. Production of infectious viruses was quantified by plaque assay and western blot.

Results: Vero cells infected with a fluorescent bunyavirus (Gc-GFP-BUNV) at different MOI (1, 5 and 10 PFU/cell) and times post infection (0, 4, 6, 10, 15, 24 and 36 hp.i.) were treated with different concentrations of Ribavirin (10, 20, 32, 50 and 150 µg/mL). As expected, Ribavirin was non-toxic and efficient at 20, 32 and 50 µg/mL. Infection was blocked in ~ 90% of cells when the antiviral was added at 0 h p.i. Those cells where the virus escaped from the antiviral treatment were selected and processed by CLEM and ultrastructural analysis. A small percentage of cells (~3%) presented a peculiar phenotype. These cells expressed fluorescent viral proteins but did not exhibit the characteristic morphological changes associated to infection, such as Golgi fragmentation.

Conclusions: The principal target site of Ribavirin in Vero cells infected by BUNV seems to reside in the RNA-dependent RNA-polymerase (RdRp). Nucleus and nucleolus are shown as good markers of the infective status of cells. Further studies to identify the role of the nucleolus in bunyavirus infection are in progress. Ribavirin inhibits BUNV replication and cytopathic effect in Vero cells. CLEM in combination with cell sorting and biochemical analysis is revealing how antiviral resistance is generated in cells.

Keywords: Bunyavirus, Ribavirin, Correlative Light and Electron Microscopy (CLEM), Antivirals, Drug Resistance.
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Are the exosomes involved in the response to chemotherapy in cancer?

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Introduction: Non-small cell lung cancer accounts for the highest number of cancer-related deaths worldwide, so does ovarian cancer when referring to gynecological tumors. This mortality rate is due to the advanced stage of both diseases at diagnosis and its resistance to the standard therapy based on platinum-derived drugs. This makes it essential to identify predictive biomarkers of response to these compounds, improving the use of available treatments for each patient. Exosomes are small double-membrane vesicles released from most types of cells that are involved in intercellular communication through transferring proteins, mRNAs and miRNAs. In the recent decades it has been described that tumor-derived exosomes are involved in tumorigenesis and hence its potential as a source of cancer biomarkers. However their role in the response to anti-tumor drugs is still poorly understood.

Objective: This project is focused on the study of the role of exosomes in response to anticancer treatment and the epigenetic characterization of their content. That could allow identifying cisplatin-resistance biomarkers in NSCLC and ovarian cancer exosomes to be used for liquid biopsies.

Material y Methods: Exosomes from cell culture medium, were isolated by ultracentrifugation. Cell viability curves to cisplatin were developed incubating sensitive cells with resistant exosomes. Transcriptomic and proteomic characterization of exosome content of three paired cell lines, sensitive and resistant, of lung and ovarian cancer were carried out by small-RNAseq and LC-MS/MS respectively. Functional validation of the miRNA candidates was performed overexpressing the specific mimics in sensitive cells followed by the treatment with increasing CDDP doses and analysis of cell viability. Exosomal RNA from 60 NSCLC plasma samples was isolated using ExoRNeasy serum/plasma kit and the analysis of the expression with TaqMan Advanced Assays by qRT-PCR is undergone.

Results: Firstly, our results showed a time of incubation-dependent acquisition of resistance to cisplatin of the sensitive cells seeded with resistant exosomes. Secondly, the global comparison of protein and microRNA content of “resistant vs sensitive” exosomes allowed us to identify 8 miRNA and 20 peptides highly represented in resistant exosomes compared with sensitive ones. Four of those miRNAs were validated by qRT-PCR and currently we are testing their functional role in the acquisition of resistance to cisplatin.

Conclusions: Exosomal miRNAs are involved, at least in part, in the acquisition of resistance to cisplatin in cancer and therefore may be used as potential biomarker of response in liquid biopsy.

Keywords: Exosomes, Chemoresistance, Non-Small Cell Lung Cancer (NSCLC).
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FADD phosphorylation is altered in human T-cell lymphoblastic lymphoma.

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Introduction: T-cell lymphoblastic lymphoma (T-LBL) is an aggressive tumour type derived from immature thymocytes in various differentiation stages. Although FADD canonical function is as a principal adaptor in apoptotic signalling, it has become evident that FADD has a non-apoptotic role related to proliferation and cell cycle control which seems to depend on its phosphorylation status. Moreover, aberrant phosphorylation of FADD in T-LBL samples inversely correlates with the proliferation capacity and tumour aggressiveness.

Material and methods: FADD and S194-FADD protein levels were detected by immunohistochemistry in human T-LBL biopsies which were provided by Biobanks integrated in the Spanish Hospital Biobanks Network. We generated four stable cell lines by lentiviral transduction of the FADD-deficient cell line JURKAT I 2.1: a cell line expressing an empty vector, thus FADD-deficient (NEG), a cell line expressing wildtype FADD (FADD), a cell line expressing a phosphomimetic FADD mutant (S194D) and a cell line expressing a non-phosphorylatable FADD mutant (S194A). Cell proliferation were analyzed using XTT assay. Cell cycle were performed by flow cytometry with propidium iodide. For protein stability assays, the stable cell lines were treated with cycloheximide or anisomycin.

Results: 1) FADD presence rendered the cells significantly more proliferative, in comparison with FADD absence. 2) The FADD-deficient and non-phosphorylatable mutant FADD cell lines are less sensitive to the G2/M-arrest induced by mitotic inhibitors, strongly suggesting that both the presence and the status of FADD phosphorylation are involved in the progression along the cell cycle. 3) The phosphomimetic S194D mutant FADD protein is remarkably more stable than wild-type FADD or the non-phosphorylatable S194A mutant FADD, suggesting that phosphorylation at Ser194 positively affects FADD protein stability.

Conclusions: FADD reduction would impair apoptosis in tumour cells, but we have demonstrated that the phosphorylation status of FADD did not affect such apoptotic role. However, proliferation and cell cycle seem to be affected both by level and phosphorylation of FADD protein. Thus, FADD phosphorylation can be used as a biomarker with prognostic value in human T-LBL, to improve the clinical management of this rare disease and, in turn, to improve cancer survival.

Keywords: T-cell lymphoblastic lymphomas (T-LBL), FADD, phosphorylation, prognostic market.
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Role of cell cycle checkpoint proteins in gastric cancer stem cells.

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Gastric cancer (GC) is the fourth type of tumor more common and one of the first causes of death related with cancer worldwide. The cancer stem cells (CSCs) hypothesis believes that few cells in cancer tissues fuel tumor growth. The characteristics of these cells (self-regenerate, proliferation and multiple differentiation potentials) are responsible for tumor maintenance, recurrence and resistance to therapy. In GC tissues, CSC populations (GCSCs) have been identified. In addition to the known stem cell markers in CSCs, some cell cycle checkpoint proteins have been implicated in the generation and maintenance of GCSCs, such as, Chk1, Mad2 and BubR1. In this study, we aimed to analyze the role of these proteins in GCSCs and their relationship with tumorigenesis and therapy response. First, we isolated GCSCs from different stablished GC cell lines (MKN45, ST2957 and SNU638), by sphere formation assay. RT-qPCR and Western Blot analysis showed that Nanog is increased in all GCSCs, but OCT4 and SOX2 depend on the cell line. Furthermore, flow cytometry demonstrated the increased of CXCR4 in GCSCs. Our data indicated that the expression levels of Mad2 are increased in GCSCs and this situation exacerbates in differentiated cells. The interference of Mad2 decreased the capacity of migration and invasion of GCSCs, indicating the involvement of Mad2 in metastasis. Surprisingly, downregulation of Mad2 increased the generation of tumourospheres. Finally, GCSCs were more resistance to CDDP, BLM and PXL compared with their parental cells; but downregulation of Mad2 do not clearly contributed to therapy response. As a conclusion, our data demonstrated the high inter- and intra-tumor heterogeneity and that Mad2 could be a good prognostic marker. More studies are needed to clarify the behavior of GCSCs, in order to identify easily those populations of cells and use them in programs of discovery of new therapeutic agents.

Keywords: Gastric cancer stem cells; tumorigenesis; Mad2.
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Competing Interests: None declared.

Determination of IGFBP-3 methylation levels at ctDNA could be a prognostic biomarker in advanced stages of NSCLC.

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Introduction: Non-small cell lung cancer (NSCLC) is the 80-85% of the lung cancer. Cisplatin-based chemotherapy is the paradigm of NSCLC treatment; however, it also induces de novo DNA hyper methylation that is associated with gene expression regulation. We have previously reported that the loss of *IGFBP-3* expression by promoter hypermethylation results in reduced tumor cell sensitivity to cisplatin in NSCLC. Liquid biopsies have gained increasing attention in recent years, as they are a convenient and minimally invasive means of interrogating tumor DNA (ctDNA). Many studies have shown concordant genetic and epigenetic alterations between ctDNA and corresponding tumor tissue DNA.

Objective: To study the correlation between the *IGFBP-3* gene promoter methylation levels in both ctDNA and paired tumor tissues from NSCLC patients with advanced stages.

Methodology: 52 FFPE/plasma paired samples from stages III-IV NSCLC patients have been collected in a prospective study at Hospital La Paz together with the associated clinical history. ctDNA and tumor tissue DNA was isolated and bisulfite modified. We then measured the methylation of the *IGFBP3*-promoter by qMSP. To obtain the percentage of methylation for each sample we use the following equation: $Cmeth = 100/ [1+2^{(CT_{CG} - CT_{TG})}]$

Results: When comparing DNA methylation levels from tumor and ctDNA, 69% (33 out of 48) of the patients followed the same methylation pattern. To determine whether *IGFBP-3* methylation data obtained from ctDNA correlates with overall survival (OS) we analyzed 34 NSCLC from our prospective cohort from which we had already collected clinical data associated with progression or decease. We also included a small group of 4 control samples, to set the percentage of baseline methylation levels at circulating DNA, that was 10%. The NSCLC samples were separated into 2 groups based on their *IGFBP-3* methylation levels compared with the control group. The survival functions were plotted using the Kaplan-Meier estimator. We found a clear tendency for a better survival in those patients with lower *IGFBP-3* methylation levels at ctDNA in advanced stages NSCLC patients. Twenty three out of 34 patients (67%), harbored an unmethylated promoter and 11 out of 34 a methylated promoter, these last showed an evident less survival.

Conclusion: DNA methylation status was determined in 48 out of 52 paired samples showing a 92% sensitivity and a positive correlation between tumor/ctDNA from the same patient.
These results indicate that the determination of *IGFBP-3* methylation levels at ctDNA could be an independent prognostic marker, in terms of Overall survival, in advanced stages NSCLC patients that could potentially be used in liquid biopsy.

Keywords: Non-Small Cell Lung Cancer (NSCLC), Liquid Biopsy, Methylation, *IGFBP3*
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Competing Interests: The authors declare not conflict of interest.

Identification of a novel epigenetic biomarker of early lung cancer detection in COPD patients.

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Introduction: Chronic obstructive pulmonary disease (COPD) is a health problem with high prevalence. Currently, it is the fourth leading cause of death in the world, with an increased incidence, which placed it in the coming years in third place globally. Among the leading causes of death in these patients, lung cancer represents the 14-26,5% of all deaths. These data, together with the high prevalence of COPD in lung cancer patients, have shown that COPD is an independent risk for lung cancer factor, increasing in 2-5 times its incidence rates with respect to smoker subjects without COPD. Changes at the DNA methylation profile from non-neoplastic tissues from lung cancer patients could be used to identify potential individuals with higher risk of cancer development. Previous reported studies from our group showed that the methylation of miR-X is an event related to tumor progression in ovarian and lung cancer cells.

Objective: The status of the epigenetic mark involving DNA-methylation of miR-X regulatory region could play a role as new biomarker for clinical use to identify patients at high risk of developing lung cancer among the smoking population with chronic respiratory diseases.

Methodology: We collected the DNA from buccal epithelial samples from 124 stable COPD patients by using buccal swaps at the clinical consultation. DNA was isolated and bisulfite modified. We evaluated the methylation status of the mir-X by qMSP by using double probes. To obtain the percentage of methylation for each sample we use the following equation: $Cmeth = 100/ [1+2^{(CT_{CG} - CT_{TG})}]$

Results: The miR-X levels of buccal epithelial samples between the several phenotypes of COPD were: 19.4±9.9 in exacerbator phenotype, 27.1±10.2 in emphysema, 17.3±9.0 in chronic bronchitis and 16.0±7.2 in asthma-COPD overlap. miR-X levels were significantly higher in emphysema phenotype ($p<0.05$ vs ACO and $p<0.01$ vs exacerbator and chronic bronchitis). In addition, univariate linear regression models of predictors of miR-X methylation in COPD patients showed a significant Pearson's correlation with static hyperinflation (functional residual capacity (FRC)/ total lung capacity (TLC)) ($p<0.001$) and diffusion capacity for carbon monoxide (DLCO) ($p<0.001$). Both variables are related to emphysema, which is the phenotype associated with higher risk of lung cancer development.

Conclusion: The presence of high rates of miR-X methylation levels allows identifying a subgroup of patients with chronic airflow limitation that need to be closely followed with image techniques in order to identify early stages of lung cancer tumor. The early diagnosis in lung cancer patients is associated with a better outcome in terms of overall survival and therapeutic response.

Keywords: COPD, lung cancer, miR-X, Methylation
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VMP1 controls lipid trafficking at ER contact sites.

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Introduction: VMP1 is an endoplasmic reticulum (ER) protein of unknown function which is highly conserved among metazoans but it is absent in yeast. VMP1 has been considered an autophagic protein since around 60% of autophagosomes originates from VMP-enriched ER. However, only a little proportion of VMP1 puncta (5%) are engaged with autophagy suggesting that VMP1 could have additional functions beside autophagosome biogenesis.

Material and methods: Detailed confocal miscroscopy was used to study VMP1 dynamics in mammalian cell culture.

Results: VMP1 is in close proximity to various organelles besides autophagosome at ER-organelles contact sites. Moreover, VMP1 is enriched at ER subdomains where phospholipid synthesizing enzymes are accumulated. Thus, VMP1-enriched ER might function as a platform for phospholipid synthesis and lipid trafficking between organelles. Interestingly, VMP1 regulates the length of Membrane Contact Sites (MCS) between ER and endosomes and is required for proper lipid trafficking between both organelles.

Conclusion: Our results demonstrate for the first time a function for VMP1 in endosome maturation in mammalian cells and suggest that its role could be in maintaining lipid homeostasis in the interface between ER and other organelles.

Keywords: VMP1, Endoplasmic Reticulum, Membrane Contact Sites.
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Synaptic T cell contacts prime DC against pathogen infection.

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Diverse quality control mechanisms ensure mitochondrial functioning during stress conditions. Mitochondrial dysfunction enhances exosome secretion dependent on reactive oxygen species (ROS) signaling alleviating its consequences. We demonstrate that mitochondrial components are transferred through exosomes between immune cells during cognate immune interactions. It has been deeply studied how T cell contacts with antigen-bearing dendritic cells (DCs) result in T cell activation. However, whether this interaction has physiological consequences on DC function is largely unexplored.

Using proteomics, deep RNA sequencing, flow cytometry and high resolution microscopy techniques we describe a new mechanism of intercellular communication between immune cells.

In this study we show that antigen-dependent T cell contacts with DCs trigger anti-pathogenic programs in the latter, priming them against pathogen infection. Information is transmitted through exosomes from the T cell to the DC. T cell transfer of exosomes to DCs triggers transcellular antiviral responses. These events confer resistance to DCs to subsequent viral infections. Together, our results reveal that T cells prime DCs, supporting a specific role for antigen-dependent contacts in conferring protection to DCs against pathogen infection.

Keywords: immune intercellular contacts, extracellular vesicles, exosomes,
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Competing Interests: No Competing interest.

Computational metabolism modeling predicts drug sensitivity in breast cancer cells.

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Not available at this moment

The role of Gasdermin B in ulcerative colitis and inflammation.

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"Insulin receptor substrate 2 (IRS2) deficiency reduces inflammatory and fibrogenic responses of the liver to cholestatic injury".

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Introduction: Hepatocellular injury is the major triggering event of the wound healing response that leads to liver fibrosis. As insulin receptor substrate 2 (IRS2) is one of the key downstream mediators of insulin signaling pathway, which play major roles in liver disease, we investigated whether IRS2 influences the hepatocellular stress response in the liver.

Methods: For that goal, cholestatic liver injury was induced by bile duct ligation (BDL) in wild-type (WT) and IRS2-deficient (IRS2KO) mice. Histological analysis, inflammatory and fibrogenic responses were evaluated in livers from these mice 3, 7 and 28 days following BDL.

Results: Although no differences between genotypes were found at the end of the experiment (28 days), IRS2KO mice displayed less BDL-induced liver histological alterations, including hepatocyte damage and excess deposition of extracellular matrix components compared to WT mice after 3 and 7 days. Moreover, hepatic expression levels of collagen 1 alpha, transforming growth factor 1 and smooth muscle actin were all lower in IRS2KO mice than in WT animals after 3 and 7 days. In parallel, mRNA expression of pro-inflammatory cytokines such us tumor necrosis factor alpha and interleukin 6 was also reduced in livers from IRS2KO mice at these time points. Interestingly, hemeoxygenase 1 expression, used as a maker of oxidative stress, was also decreased in livers lacking IRS2.

Conclusions: Taken together, our results indicate that IRS2 contributes to the progression of cholestatic liver injury since its deficiency reduced inflammatory and fibrogenic responses induced by BDL. Modulation of this protein represents a potential therapeutic strategy for cholestatic liver diseases.

Keywords: Liver, Cholestasis, IRS2
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Functional analysis of Mastl mutations in cancer.

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Introduction: Mastl, also known as Greatwall, is a protein kinase essential for proper chromosome condensation and progression through mitosis and meiosis. It belongs to the AGC kinase family and, particularly, presents a non-conserved insertion of 550 aa at the corresponding T-activation loop site in the C-lobe (usually 20-30 aa). This non-conserved middle region (NCMR) is not considered to have an essential role for Mastl activity but its function in unknown. Mastl is involved in the inhibition of protein phosphatase 2A (PP2A)-B55 complexes to maintain the mitotic state. By using a conditional knockout in mouse generated in our lab, it was shown that mammalian Mastl is essential for mouse embryonic development and cell cycle progression. Mastl was initially found in humans as a gene mutated in thrombocytopenia and preliminary data suggests its overexpression in tumors. However, very little is known about this protein in human disease.

Material and methods: Genomic data from repositories of cancer somatic mutations include MASTL NCMR indel mutations, leading to the generation of a truncated protein. Exome sequencing studies in Mastl in gastric cancers show that mutant tumors present microsatellite instability (MSI). We have studied the prevalence of these mutations by sequencing MASTL in a subset of samples from colon and stomach patients.

To evaluate the therapeutic relevance of this kinase, functional assays have been performed, as complementation studies and kinase assays.

To mimic the cancer mutations we have generated a new mouse model using CRISPR/Cas9 technology. We are currently performing several assays such as focus assays, scratch assays, soft agar and colony formation on cells derived from this model. In addition, we are using a chemical-induced colorectal carcinoma model to study the role of these mutant kinases in cancer.

Results: A heterozygous exonic indel mutation has been found in an MSI+ CRC from the 21 patient samples sequenced.

Functional assays with the mutant enzyme resulted in a partial rescue in DNA segregation observed in Mastl-null cells.

Mastl mutant forms resulted in embryonic lethality in homozygosis. Therefore, our carcinogenesis models are performed in heterozygous mice, thus mimicking cancer mutations.

Conclusions: Mastl indel mutations in the NCMR region lead to the expression of truncated shorter forms. These Mastl mutated forms are not able to fully accomplish the role of Mastl in mitosis. Mutant heterozygous mice, mimicking MASTL cancer mutations, are viable and fertile.

Keywords: Mastl, frameshift mutation, MSI, CRC.
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Deciphering the RNA-dependent DNA synthesis fidelity of retroviral reverse transcriptases: impact of transcriptional inaccuracy threshold.

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In retrovirus, the reverse transcriptase (RT) is the enzyme responsible for the replication of the viral genome. RTs are widely used in biotechnology for their ability to synthesize complementary DNA using RNA templates. Despite its importance, the fidelity of RNA-dependent DNA polymerization catalyzed by RTs has not been properly determined, although there are many studies reporting on the accuracy of the enzyme using DNA templates. In M13mp2 *lacZ*α forward mutation assays measuring intrinsic fidelity of DNA-dependent DNA synthesis, wild-type human immunodeficiency virus type 1 (HIV-1) RTs of group M/subtype B previously showed >10-fold higher error rates than murine leukaemia virus (MLV) and avian myeloblastosis virus (AMV) RTs.

Here, we provide error rates and mutant frequencies in RNA-dependent DNA synthesis reactions for several RTs, including wild-type HIV-1_{BH10}, HIV-1_{ESP49}, AMV and MLV RTs, and the high-fidelity mutants of HIV-1_{ESP49} RT K65R and K65R/V75I, by using an adapted version of the forward mutation assay. In order to analyze the transcriptional inaccuracy threshold of the RNA template, kinetics of T7 RNA polymerase were also studied by using single-nucleotide incorporation assays with correct and incorrect nucleotides using different transcription conditions (pH and Mg^{2+} concentration).

Our results showed that there were less than two-fold differences in fidelity between the studied RTs, with error rates ranging from $2.5x10^{-5}$ to $3.5x10^{-5}$. These results were consistent with the existence of a transcriptional inaccuracy threshold, generated by the RNA polymerase while synthesizing the RNA template used in the assay. A modest but consistent reduction of the inaccuracy threshold was achieved by lowering the pH and Mg^{2+} concentration of the transcription reaction.

Despite assay limitations, we conclude that HIV-1_{BH10} and HIV-1_{ESP49} RTs are less accurate when copying DNA templates than RNA templates. Analysis of the RNA-dependent mutational spectra revealed a higher tendency to introduce large deletions at the initiation of reverse transcription by all HIV-1 RTs except the double-mutant K65R/V75I. With RNA templates (as well as with DNA), the HIV-1_{BH10} RT remained as the least accurate enzyme, while the K65R/V75I mutant was one of the most faithful RTs. A better assessment of reverse transcription fidelity is expected to be helpful to improve next-generation sequence platforms that require retroviral RTs for RNA sequencing.

Keywords: reverse transcriptase, fidelity, retrovirus, RNA polymerase, HIV, transcription
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Abstracts

PhD Programme in Neuroscience

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Noradrenaline innervation in the primate thalamus: similarities and differences in macaques and humans.

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Introduction: Noradrenaline (NA) in the thalamus is interesting because of its role in modulating the transmission of information, and because of the abnormalities described in some conditions. For instance, NA is depleted in many thalamic nuclei of Parkinson's disease patients. Also, noradrenergic drugs are used for diverse purposes, including anesthesia, posttraumatic stress disorder or attention deficit hyperactivity disorder. Despite this interest, the distribution of NA axons has not been fully explored in the healthy primate thalamus.

Materials & Methods: We performed immunohistochemistry against dopamine-β-hydroxylase (monkey) and the NA transporter (monkey and human) on coronal sections containing the thalamus. Afterwards, we built high-resolution maps of immunoreactive (-ir) axons based on 20x magnification pictures using Neurolucida® software.

Results: In the human and monkey thalamus, the distribution patterns of the NA innervation are overall similar: NA-ir axons are present in all thalamic nuclei, albeit with notable density differences. Midline and intralaminar nuclei (central medial, paraventricular, paracentral) receive the densest NA innervation. Most of the remaining nuclei receive a moderate innervation. The lateral geniculate nucleus holds the lowest innervation. The reticular nucleus is sparsely innervated by NA-ir axons.

Differences between the macaque and human thalamus are also present. In macaques, the medial sector of the mediodorsal nucleus (MD) is densely innervated, whereas in humans MD receives a moderate innervation, only denser in the most ventral-medial region. The NA innervation in the centromedian-parafascicular (CnMd-Pf) intralaminar complex is also different in monkeys and humans: In the macaque thalamus, Pf is heavily innervated by NA axons and CnMd holds an average density; whereas in human thalamus, CnMd, in particular at caudal levels of the CnMd-Pf complex, is notably innervated while Pf holds a weaker density of NA axons. Also, in the human thalamus, the ventral posteromedial (VPM) and ventral posterolateral (VPL) nuclei appear more densely innervated by NA than in the monkey.

Conclusions: In primates, NA may play a prominent role in thalamo-striatal circuits acting through the intralaminar and midline nuclei. Because NA axons are located as well in nuclei supporting cortico-thalamo-cortical and subcortico-thalamo-cortical transmission, NA likely modulates transmission also in those pathways. The role of NA in somatosensory transmission through the thalamus might be more relevant in humans than in macaques, since the relative innervation of the first order nuclei in this system (VPM and VPL) is denser in the human than in the macaque thalamus.

Keywords: Noradrenaline, Thalamus, Primate, Macaque, Human.
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Medial prefrontal cortex modulation of thalamic whisker responses in urethane-anesthetized rats.

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Introduction: The medial prefrontal cortex (mPFC) has been implicated in novelty detection and attentional processes so it could modulate sensory responses in the cortex and/or in the thalamus. The effect of mPFC stimulation on whisker responses recorded in the ventroposterior medial thalamic nucleus (VPM) or the posterior thalamic nucleus (POm) has been studied in urethane-anesthetized rats.

Material and Methods: Local field potentials and unit recordings were performed in the VPM or POm nuclei and in the Zona Incerta (ZI). Somatosensory evoked potentials were elicited by whisker deflections. Current pulses were delivered by bipolar stimulating electrodes aimed at the prelimbic (PL) and infralimbic (IL) areas of mPFC or lateral Superior Colliculus (lSC).

Results: Current train stimulation (50 Hz, 500 ms) on PL induced a long-lasting facilitation of whisker responses in the VPM nucleus, but only a short-lasting inhibition in the POm nucleus. Similar train stimulation on IL induced a short-lasting facilitation of whisker responses in both VPM and POm nuclei. This facilitatory effect was underlied by corticofugal projections since it was reduced after S1BC cortical inactivation with lidocaine, and by activation of the NMDA glutamatergic receptors which were blocked by D-APV (50 µM). Paired stimulation of the mPFC and whisker deflections revealed an inhibitory effect at short intervals (50 - 100 ms), which may be due to TRN and ZI activation. Likewise, PL-lSC paired stimulation provided a facilitatory effect on the majority of recorded incertal neurons even at long intervals (50 – 300 ms) while inhibition could be seen on the 29% of these neurons only up to 100 ms. IL-lSC paired stimulation showed a facilitatory effect on the 25% of the incertal neurons in a very short interval (50 ms) and a inhibitory effect on the 33% of these neurons which remained up to 100 ms.

Conclusion: Multi-synaptic prefrontal modulation of somatosensory drives in the thalamus may require the recruitment of different GABAergic populations as those found in the TRN and ZI nuclei.

Keywords: medial prefrontal cortex; zona incerta; posterior thalamic nucleus; ventroposterior medial thalamic nucleus; whisker sensory system; electrophysiology.
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Competing Interests: none.

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Gait evaluation of patients with mild cognitive decline and mild alzheimer´s disease.

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Mild Cognitive Decline (MCD) is a clinical stage characterized by a memory and/or cognitive function loss which does not interfere with daily routines. That condition is part of a process that in a noticeable percentage of patients progresses towards Mild Alzheimer´s Disease (MAD), which is characterized by a cognitive loss that affects the patient´s daily life activities. Early detection of which MCD patients will convert into MAD remains a challenge that would represent a key to design appropriate therapeutic approaches. Patients with MCD and MAD suffer gait alterations that deteriorate progressively and can be described observationally within a wide spectrum of impairment even preceding the cognitive alterations. We reckoned that Instrumental Gait Analysis (IGA) should allow us to describe accurately the gait kinematic changes that coexist with abnormal cognitive function, with the hypothesis that we should find a gait biomarker to differentiate both conditions.

We recruited 25 patients with MCD (56%women; median age 71yrs, IQR 9.0yrs; median MMSE score 25pts, IQR 6pts) and 26 patients with MAD (53%women; median age 69yrs, IQR 7.3yrs; median MMSE 20pts, IQR 5.3pts) diagnosed in a specialized unit at the H.U. Infanta Sofía. 79 voluntary subjects were also recruited as a control group (CG, 56% women; median age 68yrs, IQR 10yrs; median MMSE 30pts, IQR 1pt). 43 gait left and right lower limb spatiotemporal and kinematic parameters were extracted in a single session from 4-5 cycles from each subject. Those had been acquired by a 3D-optoelectronic system (CODAmotion) and processed with homemade software for validation. We assessed differences CG vs. MCD, CG vs. MAD and MCD vs. MAD by means of Cohen's d (an effect size statistic that is calculated as the difference of groups means normalized by a pooled standard deviation) and their confidence intervals (95%CI).

Walking speed and cadence are similar in MCD and MAD patients but lower than CG being the variability higher in patients. Double support time is significantly increased in patients. Compare to CG, there is a trend by which the range of hip flexion, knee flexion, ankle flexion and foot progression angle is decreased in MCD patients and this is statistically significance for MAD patients. However, there are no significant differences between MCD and MAD except in the ranges of ankle dorsiflexion in swing and stance.

These results suggest that conversion MCD-MAD occurs within a spectrum of changes in the lower limb joint control by which gait is progressively slower, more variable at expense of lower push off. This conditions a shorter advance of the oscillating leg and therefore lower cadence and lower walking speed and longer support time

Keywords: Mild Cognitive Decline, Alzheimer´s Disease, Gait, Kinematic Parameters
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Competing Interests: Authors declare no conflict of interests.

Dopamine innervation of the mediodorsal and reticular thalamic nuclei in MPTP parkinsonian monkeys.

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Introduction: Dopamine loss in parkinsonism affects most intensely the mesostriatal system. Understanding how dopamine loss affects brain structures beyond the striatum is, nonetheless, important to understand barely explained pathogenic mechanisms and clinical manifestations. Because the primate thalamus is densely innervated by dopaminergic axons, we hypothesised that a dopamine deficit might be present in the thalamus of the parkinsonian brain. Also, given the heterogeneity of the thalamus, differences might be present among the different nuclei. We have explored the mediodorsal and reticular nuclei because of their relevant role in attention, cognition and brain synchronization.

Materials and Methods: We used immunohistochemistry against the dopamine transporter (DAT) in brain sections of control and parkinsonian monkeys. The toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was administered to adult macaque monkeys via a slow intoxication protocol. The intoxicated monkeys were distributed in four groups by motor tests: asymptomatic, recovered, mild parkinsonian and severe parkinsonian. After immunoreaction (ir) of the brain sections, we estimated the total length of DAT-ir axons in the mediodorsal and reticular thalamic nuclei using a 3D fractionator. We also generated maps of the distribution of the DAT-ir axons.

Results: Compared to control animals, parkinsonian monkeys exhibited less DAT-ir axonal length in the MD nucleus. The dopamine denervation was already present in monkeys that were asymptomatic at the time of sacrifice and showed moderate substantia nigra cell loss (40-60%). The dopamine denervation was most pronounced in the severe parkinsonian animals. By contrast, parkinsonian monkeys had an increase of DAT-ir axonal length in the reticular nucleus relative to control monkeys. The maps of DAT-ir axons support the quantitative findings.

Conclusions: The above results show a heterogeneous reaction of thalamic dopamine to MPTP intoxication. Changes in the dopaminergic innervation of the thalamus may result in dysfunction of thalamocortical and intrathalamic transmission and may contribute to motor and non-motor manifestations of PD, especially attention defects and sleep disturbances.

Keywords: Dopamine, Thalamus, Parkinson's Disease.
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Myelination-promoting drugs acting on oligodendrocyte precursor cells generated by direct lineage conversion from adult rat mesenchymal cells.

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Introduction: Multiple Sclerosis is characterized by a disruption in the CNS myelin, while failures in migration, proliferation and/or differentiation of oligodendrocyte precursor cells (OPCs) lead to its progression. Our group has recently generated functional OPCs (iOPCs) through direct cell reprogramming by overexpressing Sox10 + Olig2 + Zfp536 in adult rat adipose tissue-derived stromal cells (rADSC). In the last years, high-throughput drug screenings have identified compounds that promote oligodendrogenesis but had been indicated for other uses. Some of them belong to various pharmacological categories, such as muscarinic receptor antagonists (benztropine), glutamatergic agonists (kainate), antifungals (miconazole), fluorinated glucocorticoids (clobetasol) or GABA-agonists (baclofen). We aimed to test if these drugs could promote proliferation and/or differentiation of oligodendrocytes (OLs) derived from iOPCs.

Material and Methods: We studied the effects of these five drugs on 2-4 months cultured iOPCs. These iOPCs were generated through direct lineage conversion of rADSC, by lentiviral transduction with Tet-O-FUW-Sox10 + Tet-O-FUW-Olig2 + Tet-O-FUW-Zfp53 + FUW-M2rtTA. Five-week old cultures of rat spinal cord neural stem cells (rNSC) were also tested for comparison. The iOPCs were molecularly characterized by end-point PCR for the expression of different markers, such as CNPase, GFAP, MBP, MOG, MAG or PLP1. To test myelination, iOPCs or rNSC were co-cultured onto dorsal root ganglion neurons (DRGn) from E15 rat embryos. Immunopositivity to O4 monoclonal antibody (O4+) was used as marker of iOPCs, and myelin basic protein (MBP+) as marker of mature-myelinating OLs. The effects of the drugs in iOPCs complexity was analyzed by fractal dimension on randomly-chosen cells.

Results: All five drugs increased percentage and complexity of O4+ cells as it follows: kainate and benztropine stimulated the proliferation of O4+ cells; miconazole, clobetasol and baclofen stimulated the conversion of O4− cells into O4+ cells; clobetasol produced the highest percentage of these cells, whereas baclofen-treated iOPCs exhibited the most complex morphology (size and arborization). Up to the date, both kainate and benztropine appear to promote myelination in culture and additional experiments are ongoing with the remaining drugs.

Conclusions: The tested drugs can stimulate myelination by acting not only on differentiation but also on proliferation of iOPCs population. This might further support the potential of using both these iOPCs and drugs for therapy of demyelinating lesions and provide an in vitro model to test other treatments that promote either proliferation or differentiation of the oligodendroglial lineage.

Keywords: cell reprogramming, mesenchymal cells, multiple sclerosis, oligodendrocytes, remyelination, repurposed drugs.
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Endocannabinoid mediated NMDAR independent ltd of glutamatergic synaptic transmission at layer v pyramidal neurons of rat infralimbic cortex.

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Introduction: Some forms of spike timing-dependent LTD at cortical glutamatergic synapses require the coactivation of presynaptic NMDA and CB1 receptors, being crucial the release of endocannabinoids by postsynaptic calcium spikes.

Methods:

•Electrophysiology: In vitro. Patch-Clamp recordings at layer V pyramidal neurons in whole-cell voltage-clamp and current-clamp configurations.
•Calcium Imaging. Cytosolic calcium elevations analysis with Fluo-4 indicator.

Results: Here we have analyzed the role of calcium spikes on the induction of LTD at glutamatergic synapses of layer V pyramidal neurons of rat infralimnic cortex. After recording the control postsynaptic currents (PSCs) evoked by electrical stimulation in the basal dendrites, we increased the stimulation intensity to generate a postsynaptic potential (PSP) followed by an action potential (AP) and a calcium spike (PSP-AP-Ca2+ spike responses). Repeating the stimulation 60 times at a frequency of 0.2 Hz induced a robust long-term depression (> 40 minutes) on the PSC peak amplitude (48.9 ± 4.15% compared to control). The LTD was blocked under Nifedipine (20µM) plus D-AP5 (50 µM) when APs were blocked by intracelullar QX-314 (5mM). However LTD was unaffected by intracelullar QX-314 (5mM) or Nifedipine (20µM) plus D-AP5 (50 µM) applied alone. A similar LTD was obtained when EPSCs were isolated under PiTX (50 microM). This LTD was mediated presynaptically because the decrease in the amplitude of the EPSCs was associated with a change in the coefficient of variation, being unaffected the postsynaptic currents evoked by local puff application of glutamate. The LTD of the PSCs was prevented by intracellular BAPTA or by AM251 bath superfussion. In addition, AM251 was not able to prevent the LTD of the EPSCs recorded under PiTX.

Conclusion: Taken together our results suggest that the cytosolic calcium increase mediated by the PSP-AP-Ca2+ spike responses release endocannabinoids from layer V pyramidal neuron that reduce the GABAAR mediated inhibition allowing the induction of a NMDAR independent presynaptic LTD of the EPSCs.

Keywords: Infralimbic cotex, calcium spike, LTD.
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Abstracts

PhD Programme in Pharmacology and Physiology

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Visfatin/Nampt: A new therapeutic target in the vascular dysfunction associated with ageing and metabolic diseases?

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Introduction: Vascular ageing, obesity, and type 2 diabetes mellitus are associated with high circulating levels of pro-inflammatory adipokines, as IL-1β or visfatin/nicotinamide phosphoribosyltranferase (Nampt). We investigated whether the *ex vivo* treatment and the *in vivo* infusion of these adipokines in mice can produce vascular alterations, analyzing the signaling mechanisms involved.

Material and methods: *Vascular reactivity*. Mesenteric arteries from control mice were pre-incubated *ex vivo* with visfatin/Nampt (50 ng/mL) and/or its specific inhibitor FK866 (10 µmol/L), IL-1β (5 ng/mL) and/or IL-1-receptor antagonist anakinra (100 µg/mL), as well as with apocynin (NADPH oxidase inhibitor, 10 µmol/L), SQ-29,548 (thromboxane A2 receptor antagonist 10 µmol/L) or anakinra (100 µg / mL). Osmotic mini-pumps with visfatin/Nampt (100 ng/kg/day) and/or FK866 (2.4 mg/kg/day), as well as IL-1β (12 mg/kg/day) were implanted in mice for 7 days. Some mice received also anakinra (100 mg/kg/day, 3 doses i.p.). Microvessels from mice with visfatin/Nampt infusion were pre-incubated with apocynin, SQ-29,548 or anakinra. Vascular reactivity was studied by a small vessel myograph, inducing vasoconstriction with noradrenaline (NA; 3µM), and endothelium-dependent relaxations with cumulative concentrations of acetylcholine (ACh; 10nM – 10µM). On the other hand, aortic tissues from these animals were homogenized to detect and analyze NF-κB phosphorylated (p-p65), NF-κB (p65) and β-actin protein expression.

Results. *Ex vivo* treatment with visfatin/Nampt impaired endothelial relaxations in isolated microvessels from control mice, which was blocked by FK866, apocynin and SQ-29,548. *In vivo* infusion of visfatin/Nampt induced a similar effect, which was prevented by FK866, apocynin, SQ-29,548, or anakinra. *In vivo* IL-1β produced endothelial dysfunction antagonized by anakinra. NF-kB (p-p65) signaling increased in aortic tissue from mice visfatin/Nampt infusion, which was reduced in the presence of FK866.

Discussion. In vivo infusion of visfatin/Nampt and IL-1β produce endothelial dysfunction, similar to the produced by the same adipokines *ex vivo*, which was mediated by a FK866-sensitive enzymatic activity, involving NADPH oxidase-derived superoxide anions and thromboxane A2 receptors. The blockade of IL-1 receptors with anakinra abolished the *in vivo* effects of visfatin/Nampt but not the *ex vivo* actions. Visfatin/Nampt produces a FK866-sensitive activation of NF-κB in the aortic tissue. We conclude visfatin/Nampt is not only a biomarker but also a mediator of the cardiovascular damage and premature ageing associated to metabolic alterations and, therefore, can become a pharmacological target for the prevention of those diseases.

Keywords: visfatin, IL-1β, endothelial dysfunction.
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The noncanonical notch ligand dlk1 regulates renal inflammation.

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INTRODUCTION AND AIMS: The main goal of this work is to study the role of the notch signaling pathway non-canonical ligand DLK1 in experimental renal damage. Notch signaling pathway is highly activated during embryonic development, but it is inhibited in adult tissues. DLK1 is suggested to be a Notch pathway endogenous inhibitor *in vitro*; however, there are no studies about its role *in vivo*.

METHODS: Progressive renal damage model of unilateral ureteral obstruction was done in wild-type and dlk1-null mice of SvJ-129 genetic background. These animals were sacrificed after 2, 5, 10 and 14 days of obstruction. Left kidney was obstructed, whilst right kidney was used as control (contralateral).

RESULTS: Non-canonical ligands DLK1 and DLK2 (DLK1 homolog) were analysed. From 5 days of obstruction, both genes increased their expression in WT vs contralateral (*dlk1*) and in obstructed kidneys of dlk1-null mice vs WT (*dlk2)*. Obstructed kidneys of dlk1-null mice presented an increase in NICD, fragment of Notch receptor which is translocated to the nucleus and activates the effector genes, *hes* and *hey.* Moreover, a significant increase of *hes-1* gene expression levels was observed in obstructed kidneys of dlk1-null mice when compared to obstructed WT kidneys at 14 days, as well as *hey- 1* at 5 days. The evaluation of renal damage through PAS tinction revealed a significant increase in inflammatory infiltrate as focal aggregates in obstructed kidneys of transgenic mice at 14 days. These aggregates were associated to a significative increase of CD3+, CD4+, F4/80+ infiltrating cells and neutrophils, as well as Th17 lymphocytes. Studying the possible inflammatory mechanism, it was observed that p-IκBα was increased in damaged kidneys of dlk1-null mice when compared to their littermates at 14 days. In addition, a significant increase in *ccl-2* gene expression was observed in obstructed kidneys of transgenic mice from 10 days, following 14 days. It is important to remark the increase of the Th17 response in these damaged kidneys of dlk1-null mice when compared to the WT ones, as we demonstrated here by an augment of IL17A renal production and the transcription factors that are implicated in this immune response: RORγt and STAT3.

CONCLUSIONS: The deletion of the non-canonical ligand DLK1 from Notch pathway involves the overactivation of this pathway in a renal experimental damage. This confirms that DLK1 acts as an endogenous antagonist on Notch receptor in pathological processes in the kidney. Notch activation in dlk1 absence is associated in an increase of renal inflammatory infiltrate and in an activation of Th17 immune response, demonstrating the importance of Notch pathway in renal inflammatory processes.

Keywords: DLK, Jagged, Notch, inflammation, renal damage.
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Competing Interests: No conflicts of interest.

Angiotensin-(1-7)/Mas axis attenuates endothelial cell senescence by Nrf2 activation.

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Introduction: Endothelial senescence is one of the major mechanisms contributing to vascular ageing, a complex process associated with vascular inflammation, endothelial dysfunction and atherosclerosis. Angiotensin (Ang)-(1-7) is a heptapeptide of the renin-angiotensin system (RAS), considered as physiological antagonist of Ang II. Little is known on the capacity of Ang-(1-7) to protect against vascular ageing. In this study, we tested whether Ang-(1-7) could mitigate the senescence of cultured human umbilical vein endothelial cells (HUVEC) induced by Ang II or interleukin (IL)-1. We further aimed to identify protective cellular pathways activated by Ang-(1-7), with particular focus on the antioxidant and anti-inflammatory nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2)/heme oxygenase (HO)-1 axis.

Methods: Cultured HUVEC were stimulated with Ang II (100 nM) or IL-1β (2,5 ng/ml) for 18 h. Cell senescence was quantified by positive senescence-associated β-galactosidase (SA-β-gal+) staining. Vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were quantified by flow cytometry, while leukocyte-endothelium adhesion was determined using a flow chamber assay. Nrf2 and HO-1 were determined by Western blot.

Results: Both Ang II and IL-1β enhanced the fraction of SA-β-gal+ cells, together with increased expression of ICAM-1 and VCAM-1, resulting in a higher leukocyte adhesion to HUVEC monolayers. Ang-(1-7) (100 nM) attenuated all these actions through a mechanism that was prevented by the Mas receptor antagonist A779 (1 µM). Ang-(1-7) enhanced Nrf2 and HO-1 levels. Indeed, the Nrf2 activator sulforaphane (1 µM) mimicked the effects of Ang-(1-7) on the SA-β-gal+ cells fraction. Interestingly, the HO-1 inhibitor Sn protoporphyrin (1 µM) dampened the anti-senescence action of both Ang- (1-7).

Conclusions: Ang-(1-7) counteracts endothelial cell senescence triggered by both RAS-dependent and -independent stimuli. Nrf2/HO-1 pathway seems to be on the basis of these protective properties of Ang-(1-7). Overall, the Ang-(1-7)/Mas axis arises as a novel pharmacological tool to attenuate endothelial senescence and vascular aging.

Keywords: Angiotensin-(1-7), endothelium, senescence.
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The biological drug anakinra prevents endothelial senescence and vascular smooth muscle cell inflammation elicited by interleukin-1β.

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Introduction: Vascular complications are the main cause of mortality in patients suffering from type 2 diabetes mellitus (T2DM). In these patients, vascular damage is associated with enhanced inflammation and premature vascular ageing, among other. The cytokine interleukin (IL)-1β is also an adipokine overexpressed by the adipose tissue in the context of T2DM. Our goal was to determine the direct impact of IL-1β on endothelial cell senescence and vascular smooth muscle cell inflammation and to pharmacological interfere with the action of the cytokine.

Materials and Methods: In this study, we used cell cultures of human aortic smooth muscle cells (HASMC) and human umbilical vein endothelial cells (HUVEC). The Griess method and a lucigenin-derived chemiluminescence assay were used to determine the activation of NF-κB and NADPH oxidase in HASMCs, respectively, while the levels of iNOS were determined by western blot. Regarding HUVECs, endothelial senescence was determined using a β-galactosidase staining kit, while a double indirect immunofluorescence was used to determine total and telomeric DNA damage.

Results: IL-1β (2.5 ng/ml) promoted cell senescence in human umbilical vein endothelial cells (HUVEC), as determined by the number of cells exhibiting positive senescence-associated β-galactosidase staining (SA-β-gal+). This was accompanied by increased total and telomeric DNA damage. The biological drug anakinra (0.01 to 1 µg/ml) was able to prevent the increase in SA-β-gal+ elicited by IL-1β in a concentration-dependent manner.

Moreover, IL-1β (10 ng/ml) promoted the activation of human vascular smooth muscle cell (HASMC) in terms of NADPH oxidase and NF-κB activity and inducible nitric oxide synthase expression. In a high glucose environment (22 mM vs 5.5 mM) the pro-inflammatory cascade activated by IL-1β was significantly exaggerated. Anakinra 1 µg/ml) prevented not only the pro-inflammatory action of IL-1β but also the exacerbation observed under high glucose conditions.

Conclusion: IL-1β may be a direct player in promoting vascular damage associated to T2DM by favoring vascular ageing and inflammation, which is turn potentiated by high glucose concentrations. Biological IL-1β blockers, such as anakinra, may be useful as pharmacological tools to treat or delay vascular complications.

Keywords: IL-1β, Endothelial senescence, Inflammation
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Pharmacogenetic algorithm for acenocoumarol dosing improve anticoagulation control: a multicenter randomized clinical trial.

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Background: It has been demonstrated that there is a strong association between genetic polymorphisms and acenocoumarol dose requirements. Our group designed and validated a pharmacogenetic dosing algorithm for acenocoumarol, indicated in patients with thromboembolic disease who are going to start treatment with this drug.

Material and methods: The design of this multicenter, single blind, randomized clinical trial has been published (Trials, 2012;13:239; PMID: 23237631). Patients were randomized to one of the two arms: common clinical practice or following an individualized pharmacogenetic algorithm. The main endpoint was: percentage of patients with INR within the therapeutic range on day 7 after initiation of acenocoumarol treatment. The variables included in the algorithm were: demographic, clinical and pharmacogenetic variables (VKORC1, CYP2C9, CYP4F2 and ApoE). The follow-up was three months since the beginning of acenocoumarol treatment.

Results: 149 patients were recruited, (144 patients conformed the ITT population). Mean age was 59.42 (±18.53) years old and 77 (55.5%) were males. In the experimental group, 34 of 72 patients (47.22%) and 14 of 64 patients (21.8%) in the control group had INR value within therapeutic range (p=0.023). No statistically significant differences were found in the time to achieve therapeutic INR values (p=0.286) or in the INR mean during the last two months of study (p=0.1796). When classified by phenotype (low, intermediate and high stable dose requirement) the INR at day 3 and 7 in both groups were:

Phenotype	Day 7 (visit 3)		p
	Experimental	Control	
Low	2.72 (2.19;3.26)	3.16 (2.53;3.80)	0.288
Intermediate	2.63 (2.29;2.96)	2.14 (1.81;2.46)	0.039
High	1.74 (1.55;1.92)	1.47 (1.29;1.65)	0.044

Conclusions: Better anticoagulant control was obtained at day 7 of the beginning of the drug with the pharmacogenetic algorithm. Difference is observed in the value of INR between both groups, being more evident in the groups of extreme dose requirement.

Keywords: acenocoumarol; pharmacogenetics, clinical trial
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New chiral melatonin-derivatives as multitarget directed ligands to treat Alzheimer's disease.

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Introduction: Alzheimer's disease (AD) is the most prevalent neurodegenerative disease (NDD) with around 35 million people currently affected worldwide, according to World Health Organization statistics [1]. As there is not an effective therapy for AD and NDDs, considered as multifactorial diseases [2], the new paradigm of multitarget directed ligands, seems promising. In this context, we designed our compounds towards control neuro-inflammation and oxidative stress, by combining the induction of the Nrf2 transcription factor, restoration of cholinergic function and free radical scavenging effect, which would promote the neuroprotection.

Materials and methods: A seven step convergent-lineal enantioselective synthesis was performed to obtain the new molecules. The pharmacological studies included evaluation as free radical scavengers using the ORAC test; as Nrf2 inducers employing a luminescent method in the AREc32 cell line; as acetylcholinesterase inhibitors with Ellman's method, and as neuroprotective agents against okadaic acid in cell line SH-SY5Y, an *in vitro* model of Tau hyperphosphorylation.

Results: A library of fifteen compounds has been obtained with very good yields and enantiomeric excesses. All of them are excellent free radical scavengers and inhibitors of acetylcholinesterase around the 10 µM range. Furthermore, some of them are Nrf2 inducers and neuroprotectants.

Conclusion: We have obtained a new family of melatonin chiral derivatives, Six out of fifteen compounds showed all the desired properties to a promising degree. Therefore, we plan to continue and complete their pharmacological profile to choose our lead compound.

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[2] A. Cavalli et al. J. Med. Chem. 2008, 51, 347- 372

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Generation and potential applications of an X-linked dyskeratosis congenita model in human hematopoietic stem cells.

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Introduction: X-linked dyskeratosis congenita (X-DC) is a low prevalent inherited bone marrow failure syndrome caused by mutations in the *DKC1* gene. This gene encodes for the dyskerin nucleolar protein, which is part of the telomerase complex. These mutations impair telomerase activity leading to premature telomere length attrition. For this reason X-DC is also classified as a telomere biology disorder. The major cause of premature death in 80% of the DC patients is bone marrow failure (BMF). To date, the only curative treatment for BMF is hematopoietic stem cell (HSC) transplantation. However, the difficulties to find compatible donors, risks derived from conditioning regimes and graft versus host disease postulate that gene therapy may constitute a promising alternative in treating DC patients.

Materials and methods: Due to the complications related to the use of primary HSCs from DC patients for experimental studies, this work is focused on the generation of X-DC-like CD34$^+$ cells by lentiviral delivery of *DKC1* short hairpin RNAs (shRNAs) to knock down the expression of dyskerin of umbilical cord blood derived HSCs.

Results: Three shRNAs were selected, among a library of seven shRNAs, based on the efficacy to inhibit *DKC1* gene expression. Interfered CD34$^+$ cells showed a downregulated *TERC* expression, a reduced telomerase activity, a decreased cell expansion, as well as an impaired clonogenic and hematopoietic reconstitution potential in NSG mice. Moreover, an upregulation in p21 expression was observed in *DKC1*-interfered CD34$^+$ cells, consistent with an increased rate of cell senescence induction and DNA damage.

Conclusions: Development of X-DC-like CD34$^+$ cells will facilitate the understanding of the HSC defects characteristic of X-DC and contribute to the development of new therapeutic strategies for the treatment of the BMF in X-DC patients.

Keywords: Dyskeratosis congenita, bone marrow failure syndromes, hematopoietic stem cells, short hairpin RNA and *DKC1* gene.
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Competing Interests: The authors declare no competing financial interests.

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Effect of antidepressants of clinical use on neuronal nicotinic acetylcholine receptors.

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Introduction: Depression is the most common affective disorder and the main cause of disability. The current treatment of this pathology is based on the monoaminergic theory, which attributes its origin to a deficit in monoamine neurotransmitters, mainly serotonin and noradrenaline. Despite current antidepressants are effective, a 30% of patients do not respond to this therapeutic strategy. Besides, although monoamine levels are increased after a few days of treatment, the onset of the therapeutic effect takes at least two weeks to appear. To shed light on these questions, we based our work in the alternative "cholinergic theory" of depression, that considers the overactivity of the cholinergic system observed in depression as one of its main causes. Thus, we wanted to exploit this field by studying the effect of several antidepressants on cholinergic neurotransmission, to determine whether they possess an additional mechanism of action.

Materials and methods: For this study, five antidepressants, one from each group of current clinical use was selected. Their effect on nAChRs was studied on bovine chromaffin cells (BCCs), that secrete catecholamines upon physiological nAChR activation by ACh. In this experimental model we assessed the effect of these compounds on: the secretion of catecholamines measured by amperometry in cell populations, the intracellular calcium levels measured by fluorescence in cell populations, and the nicotinic and calcium currents in single cell measured by patch-clamp techniques.

Results: Some of the antidepressants blocked the catecholamine release in BCCs populations when they were physiologically stimulated with ACh, but not with a depolarizing solution of high potassium. Besides, the calcium entry in BBCs upon ACh stimulation was blocked in their presence in a concentration dependent manner, an effect that was not observed when they were stimulated with high potassium. Finally, some of the studied compounds were able to concentration-dependently block the nicotinic inward current elicited by ACh in single BCCs, while their effect on calcium currents, that would be related to an activity on voltage-dependent calcium channels (VDCCs), cannot be related to an interaction with subtypes of these channels involved in catecholamine secretion.

Conclusions: These results indicate that some of the studied antidepressants are able to specifically block the nAChRs in BCCs, with no effect on VDCCs involved in catecholamine secretion, and thus conferring them an additional mechanism of action. Our study might help to a better understanding of the therapeutic effect of these antidepressants, and eventually contribute to the development of more effective drugs for depression.

Keywords: depression, antidepressant, cholinergic theory, neuronal nicotinic acetylcholine receptor, chromaffin cell.
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Multitarget compounds for the treatment of neurodegenerative diseases: Nrf2-EpRE pathway as key target.

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Introduction: Neurodegenerative diseases (NDDs) share several pathophysiological mechanisms such as presence of aberrant protein aggregates, mitochondrial dysfunction, oxidative stress and neuroinflammation. Given the role of these factors in the development of NDDs, compounds able to regulate them simultaneously are of great interest.At physiological conditions, the main route to combat oxidative stress and neuroinflammation is the Nrf2/EpRE pathway. Besides, it has been observed, that the Nrf2 factor is deregulated in neurodegenerative disorders[1]. Based on this hypothesis we carried out the design, synthesis and pharmacological evaluation of a new family of Nrf2 inducers with complementary activities for the treatment of NDDs[2].

Materials and methods: The biological evaluation of the compounds was carried out by the use of bioluminescence and fluorescence techniques, to determine the Nrf2 inducing and antioxidant capacity of the compounds, respectively. Also, its capacity for neuroprotection in cell lines and immunomodulatory capacity in primary glia cultures was studied. Besides, inmunofluorescence techniques were used to study the pharmacological properties.

Results: Newly developed compounds were able to induce the Nrf2-EpRE pathway, and also demonstrated a scavenger and anti-inflammatory effect. Besides the compounds showed an interesting neuroprotective effect against in vitro and ex vivo model models of oxidative stress and toxicity induced by protein aggregation.

Conclusions: Compounds targeting the Nrf2-EpRE pathway present interesting properties for the treatment of neurodegenerative diseases. Altogether, results obtained indicate these compounds should be further evaluated in in vivo models of NDDs.

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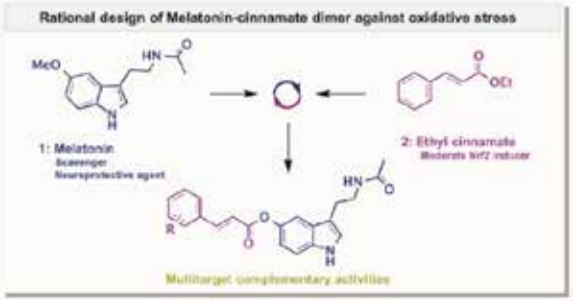

Keywords: Nrf2-EpRE, oxidative stress, neuroinflammation, multitarget compounds.
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Competing Interests: None declared

Oxidative stress is linked to lifetime cardiovascular risk stratification in young/middle age individuals.

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Loss of NLRP6 expression increases the severity of kidney injury.

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Nlrp6 is a nucleotide-binding oligomerization domain-like receptor (NLR) that forms atypical inflammasomes. Nlrp6 protects the gut epithelium, regulating the interaction with microbiota. However, the expression and function of Nlrp6 in the kidney, a sterile environment, have not been characterized.

In a transcriptomics array of murine acute kidney injury (AKI) induced by a folic acid overdose, Nlrp6 and Naip3 were the only significantly downregulated NLR genes (fold-change 0.55 and 0.80, respectively). The functional implications of Nlrp6 downregulation were explored in mice and in cultured murine tubular cells. Nlrp6 was expressed by the healthy murine and human kidney tubular epithelium, and expression was reduced in mice during AKI induced by a folic acid overdose and in chronic kidney injury induced by unilateral ureteral obstruction. Low kidney Nlrp6 was observed in human kidney injury. Genetic Nlrp6 deficiency resulted in upregulation of kidney ERK1/2 and p38 MAP kinase phosphorylation, more severe AKI and more severe kidney fibrosis in mice. Nlrp6 downregulation induced by specific siRNA in cultured tubular cells resulted in upregulation of ERK1/2 and p38 phosphorylation and chemokine mRNA expression and downregulation of the nephroprotective gene Klotho. MAP kinase inhibition prevented the inflammatory response in Nlrp6-deficient cells.

In conclusion, Nlrp6 has a role in suppressing sterile inflammation during kidney injury.

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PD-L1 is overexpressed on endotoxin tolerant septic patients via HIF1α imparing the adaptive immune response.

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Background: Endotoxin tolerance (ET) is an important feature of sepsis-associated immunosuppresion, however, a clear underlaying mechanism is still lacking and its implication in the outcome of patients is controversial, as some authors suggest it to be protective and others to be detrimental.

Materials and methods: We performed a prospective study classifying at admission 48 patients with sepsis into 3 subgroups according to their ex vivo response to lipopolysaccharide. We evaluated the differences between groups in both clinical parameters and immune response assays. To check the endotoxin tolerance implication in these observations, we studied these immune features in a well stablished in vitro model.

Results: We observed a worse APACHE II, qSOFA and clinical outcome in the non-responder/tolerant subgroup. Futhermore, the monocyte PD-L1 expression was upregulated in these patients corresponding with reduced T lymphocyte proliferation. PD-L1 knockdown on monocytes and PD-1 blocking assays reverted the impaired T cell response. HIF1α, which was showed to be overexpressed under ET, controlled PD-L1 expression on septic patients' and in the in vitro model.

Conclusions. These findings give a new role of HIF1α explanation controlling PD-L1 overexpression observed in sepsis patients. Moreover, classifying patients according to the ex vivo lipopolysaccharide response, could be considered an interesting field of study directed toward a precision medicine and more personalized therapies in sepsis.

Keywords: Monocytes, sepsis, PD-L1,HIF α, endotoxin tolerance.
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CompetingInterests: All authors: No reported conflicts of interests.

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Alterations in the stimulus-secretion coupling related to aging in the murine model of accelerated senescence SAMP8.

Andrés M. Baraibar[1], Carmen Nanclares[1], Inés Colmena[1], Isabel Gameiro-Ros[1], Iris Álvarez-Merz[1], Alicia Muñoz-Montero[1], Jesús M. Hernández-Guijo[1], Luis Gandía[1].

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Introduction: The nervous system is especially vulnerable to aging. Its vulnerability is manifested by the existence of neurodegenerative pathologies like Alzheimer's disease (AD), Parkinson's disease (PD) or Amyotrophic Lateral Sclerosis (ALS). During these diseases, alterations of neurotransmitter systems has been reported. Although, numerous changes can also be observed in many individuals during non-pathological aging. Our working hypothesis suggests that, with the progression of age, alterations in the stimulus-secretion coupling can occur, compromising the release of neurotransmitters and causing cognitive deficits.

Materials and Methods: In this work, mice of the senescence-prone strain 8 (SAMP8) have been used which show age-related behavioural deterioration such as deficits in learning and memory, emotional disorders and altered circadian rhythm, being therefore used as a model of spontaneously occurring Alzheimer's disease. In parallel, their resistant senescence (SAMR1) brothers have been used as a control. We used the chromaffin cell as a model of neurosecretion in SAMP8 and SAMR1 mice at 2, 6 and 12 months of age. By means of the patch-clamp technique, we have studied the ionic currents involved in the secretory process of catecholamines and in the transmission of the nerve impulse (nicotinic, Na^+, Ca^{2+} and K^+ currents). We have also assessed cell excitability by measuring membrane potential and spontaneous and triggered action potentials. Moreover, we have studied the release of the neurotransmitters by the amperometric technique using K^+ as stimuli. Finally, we have used the Y-maze to evaluate the cognitive behaviour of the mice.

Results: We have observed that there is an increase in all ionic currents with the age in both, SAMP8 and SAMR1 mice, but this increase occurs before and more remarkable in SAMP8 mice. Regarding membrane potential and spontaneous and triggered action potentials we have observed that there is a hyperpolarization of membrane potential in both types of mice during the aging, moreover, the depolarization that ACh produces is lower throughout the age and again, this phenomenon occurs before and is more remarkable in SAMP8 mice. Furthermore, the number of action potentials generated at rest and those produced by depolarizing pulses decreases during the aging, being higher the amplitude and posthyperpolarization area. Although, the number of action potentials evoked by ACh increases with aging due the less depolarization being more remarkable in SAMP8 mice.

Regarding the release of the neurotransmitters we have seen that when we stimulate with K^+ there is an increase in the catecholamine secretion with the aging, happening before in SAMP8 mice. Moreover, this increase in the catecholamine release is accompanied by changes of secretory spikes.

Finally, with Y-maze, we have seen that these alterations in the release of neurotransmitters are associated with a cognitive deficit, since the SAMP8 mice explore all the arms equally, whereas the R1 always explore more the new arm.

Conclusion: We have found that during the aging the mechanisms of exocytosis of neurotransmitters is altered. These alterations could be correlated with the changes that occurs in some neurodegenerative diseases and also causing cognitive deficits.

Keywords: exocytosis, chromaffin cell, aging, Alzheimer.
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NLRP3 inflammasome inhibition improves motor and behavioral outcome in a mouse model of traumatic brain injury.

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Introduction: traumatic brain injury (TBI) is one of the first causes of death and disability in young adult population in our society. Recently, TBI has been called "the silent epidemic", since it is a frequent serious problem and the consequences of the pathology are unknown. In this context, neuroinflammation that takes place after TBI plays a key role in the development of secondary lesions, which can become chronic. Thus, the aim of this study is to analyze the effect of different compounds that can modulate the inflammatory response in *in vitro* models of inflammation, as well as in an *in vivo* model of TBI.

Material and methods: we have evaluated the effect of MCC950 (inhibitor of inflammasome and IL-1β release) on the release of two proinflammatory cytokines (IL-1β and TNF-α) in primary glial cultures after stimulation with LPS and ATP. In order to evaluate the potential beneficial effects of the compounds, we used the mice TBI model called "Closed Head Injury". After the induction of the lesion and subsequent resuscitation, we assessed the neurological functions of the animal 1 hour and 24 hour after TBI using Neurological Severity Score (NSS) scale. It consists of the evaluation of different motor and behavioral parameters with a maximum score of 10 points, considering a severe trauma the value of 7-8 points. The treated groups were injected intraperitoneally with MCC950 (3 mg/kg or 10 mg/kg) immediately following the 1-hour test.

Results: in primary glial cultures, MCC950 reduces IL-1 β release but not TNF-α. In the TBI model, we obtained a score of 7 after 1 hour, while after 24 hours it decreased to 5 in non-treated animals. Both dosages decreased the value obtained at 24 hours, even though it was only significant at 3 mg/kg (value of 3.8). Furthermore, we analyzed the edema measuring the content of water in the brain, as well as the blood brain barrier (BBB) impairment using Evans Blue dye. We obtained a reduction of water content and a decrease of BBB permeability at 3 mg/kg and 10 mg/kg.

Conclusions: although an extensive evaluation of molecular parameters and biomarkers related with inflammation is needed, treatment with MCC950 improves motor and behavioral conditions that correlate with a reduction of brain edema and a decrease in BBB permeability. Therefore, inflammation could be a potential target to treat detrimental effects of traumatic brain injury.

Keywords: traumatic brain injury, inflammation, inflammasome, interleukin-1 beta, MCC950.
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Competing Interests: The author declares no conflict of interest.

Control of inflammation by microglial heme-oxygenase- 1 is differentially regulated with aging.

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Introduction: Neurodegenerative diseases (NDDs) share pathological mechanisms such as oxidative stress, protein aggregation or chronic inflammation, processes in which microglial cells have a pivotal role. Microglia are the cells of the innate immune system of the central nervous system. It has been proven that microglial heme-oxygenase 1 (HO-1) enzyme has anti-inflammatory, antioxidant and neuroprotective effects. However, in patients with Alzheimer's disease and during aging, the expression and activity of HO-1 is increased compared to adult and healthy subjects. Also, there are ferrous deposits derived from the metabolism of HO-1, which can be cytotoxic. Therefore, our aim was to understand the role of HO-1 under inflammatory conditions, both in adult and aged wild type (WT) mice and LysMCreHmox1ΔΔ (KO HO-1) mice, which lack the HO-1 enzyme in microglial cells.

Method and Results: Initially, 3-month-old mice were treated i.p. with 0.5 mg/Kg of LPS. The results of behavioral parameters (locomotion and social interaction) showed that the LysMCreHmox1ΔΔ animals treated with LPS presented a worse behavioral profile than the WT mice at 4 and 8 hours after the injection of the inflammatory stimulus. In addition, the absence of microglial HO-1 was related with a higher release of pro-inflammatory cytokines (TNF-α and IL-1β) and with an increase in pro-oxidant enzymes. In addition, WT mice treated 2 hours before the injection of LPS with 20 mg/Kg of zinc protoporphyrin (ZnPP), as a pharmacological tool to inhibit the activity of total HO-1, provided similar results to the ones observed in LysMCreHmox1ΔΔ animals. To study the implications of the absence of HO-1 in the inflammatory response in aged animals, 15-months-old mice treated with 20 mg/Kg of ZnPP or LysMCreHmox1ΔΔ were used. The results of behavioral parameters and biochemical analysis showed that WT animals treated with ZnPP and LysMCreHmox1ΔΔ mice subjected to LPS presented a better anti-inflammatory response compared to their respective controls treated with LPS.

Conclusion: Taken all together, these results highlight the importance of microglial HO-1 in resolving inflammation with age; its absence in young animals is related to a higher inflammatory profile, while its absence in aged mice seems to be beneficial. Therefore, regulation of HO-1 to resolve the inflammatory state that underlies many NDDs is tightly dependent on age.

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Keywords: HO-1; inflammation; aging
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Neuroprotective effects of new compounds directed to PP2A, a promising therapeutic target for Alzheimer's disease.

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Introduction: Alzheimer's disease (AD) is a progressive neurological disease that causes a progressive memory loss. Main histopathological hallmarks of AD are senile plaques and neurofibrillary tangles, generated by aggregation of the microtubule associated protein tau. In addition to these pathological characteristics, there are other alterations, such as oxidative stress or loss of cholinergic transmission, among many others. One of the most promising approaches in AD treatment is to inhibit neurofibrillary degeneration produced by an abnormal tau hyperphosphorylation. In this sense, serine/threonine phosphoprotein phosphatase 2A (PP2A) is the major phosphatase in brain that accounts for over 70% of tau dephosphorylation. It has been shown that PP2A activity is significantly decreased in post-mortem AD brains, partly due to the increase of endogenous inhibitors that bind the PP2A catalytic subunit C.

Hypothesis: Okadaic acid (OA) is a natural toxin capable of inhibiting PP2A, leading to tau hyperphosphorylation. Our working hypothesis is based on antagonizing the inhibitory effects of OA on PP2A by designing analogues of OA, capable to bind to PP2A but without exerting inhibition, and thus preventing the attachment of endogenous inhibitors.

Material y Methods: The compounds synthesized, analogues to C19-C27 OA fragment, have been pharmacologically studied, evaluating them in several in vitro models of AD, such as: tau hyperphosphorylation induced by OA, oxidative stress caused by the toxic cocktail rotenone and oligomycin A or the glutamate induced excitotoxicity, all of them by the MTT method. Furthermore, we have measured the cellular phosphatase activity by the pNPP method. In order to carry out these objectives, we have used SH-SY5Y neuroblastoma cells and rat embryonic cortical neurons. Finally, we confirm our theory by docking studies.

Results and conclusions: Our molecules are not toxic in SH-SY5Y cells or in cortical neurons, and they are capable of reducing the neurotoxicity induced by OA. Some of them also showed good profile in the cell stress model induced by R/O A in SH-SY5Y cells, and in the glutamate-induced excitotoxicity in cortical neurons. The new compounds maintained the serine/threonine phosphatase activity, depressed by the action of two PP2A inhibitors: OA and citostatin. Molecular docking studies indicated that compound ITH12680 is capable of binding to PP2A similarly to OA, but it does not interact with the catalytic site, thus confirming our starting hypothesis. Taking into account these results, we conclude that our compounds could have potential indication for the treatment of neurodegenerative diseases based on the maintenance of PP2A activity.

Keywords: tau hyperphosphorylation, PP2A, okadaic acid, neuroprotection
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Galectin-3 and Monocyte Chemoattractant Protein-1, as new biomarkers for patients with diabetes and high risk of cardiovascular diseases.

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Introduction: Coronary artery disease (CAD) is a leading cause of death in developed countries. Also, type-II diabetes mellitus (DM2) has been recognized as an independent cardiovascular risk factor. In this sense, new specific biomarkers may allow clinicians to early diagnose CAD to classify high-risk patients and to initiate appropriated treatments.

Material and Methods: Plasma samples from 989 patients with stable coronary artery disease (SCAD) were analysed 6 months after an Acute Coronary Syndrome [non-ST elevation acute coronary syndrome (NSTEACS) or ST elevation myocardial infarction (STEMI)] by enzyme-linked immunosorbent assays (ELISA). Galectin-3, pro-protein convertase subtilisin/kexin type 9 (PCSK-9), lipoprotein-A (LpA) and monocyte chemoattractant protein-1 (MCP-1) plasma levels were quantified in parallel to reference biomarkers such as N-terminal fragment of brain natriuretic peptide (NT-proBNP) and high sensitivity C-reactive protein (hsCRP), as well as the lipid profile (LDL-c, HDL-c, total cholesterol, triglycerides). Values were compared in patients with DM2 (fasting glucose ≥ 126 mg/dl or anti-diabetic treatment) (n=237) and without DM2 (n=752).

Results: DM2 patients with SCAD showed higher levels of galectin-3 (8.30 [6.44-10.48] vs 7.76 [5.94-9.77]; p=0.042) and MCP-1 (144.53 [112.13-194.94] vs 132.83 [105.33-173.30]; p=0.009) as well as CRP (1.40 [0.56-4.42] vs 1.13 [0.304-2.985]; p=0.005). Total cholesterol (140.0 [121.0-157.0] vs 145.0 [126.0-166.0] mg/dl; p<0.001), LDL (73.0 [61.0-88.0] vs 79.0 [65.0-94.0] mg/dl; p=0.003), HDL (38.0 [33.0-44.0] vs 42.0 [36.0-48.8] mg/dl; p<0.001) were lower in patients with DM2 than in those without this disorder. Opposite to this, triglycerides were higher in DM2 patients (113.0 [81.0-162.5] vs 97.0 [75.0-135.0] mg/dl; p<0.001). NT-proBNP, high sensitivity troponin I, PCSK-9 and Lp(a) levels were unchanged.

Conclusion: Galectin-3, a β-galactoside-binding lectin involved in cardiovascular remodelling, and MCP-1, a chemoattractant factor implicated in vascular inflammation and atherothrombosis, may serve as biomarkers to detect inflammation in DM2 patients with high risk of cardiovascular disease as ACS.

Keywords: diabetes mellitus, myocardial infarction, serum biomarkers, galectin-3
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Competing Interests: Authors declare that there are no conflicts of interest.

Huntingtin overexpression is associated to altered excitability and exocytosis in chromaffin cells from the R6/1 mouse model of Huntington's disease.

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Introduction: Adrenal medullary chromaffin cells (CCs) from transgenic mouse models of Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) have been reported to undergo functional changes of cell excitability, ion currents, cytosolic calcium transients ($[Ca^{2+}]_c$), the quantal release of catecholamines and the kinetics of the exocytotic fusion pore, with respect their wild type (WT) counterparts. In this frame, we planned this investigation trying to answer these questions in the slow-developing Huntington's disease (HD) R6/1 mouse model at 2-months of age (absence of motor symptoms, pre-disease stage) and at 7-months of age (motor deficits present, disease stage).

Material and methods: We here present a thorough investigation on the functional changes undergone by CCs from 2-months and 7-months aged WT and R6/1 mouse model of Huntington's disease (HD), stimulated with acetylcholine (ACh) or K^+. To study this, we used different approaches such as immunohistochemistry assay, patch clamp and amperometry techniques.

Results: With respect WT cells, some of the changes were already observed in HD mice at 2 months; however, they were more pronounced at 7 months, when motor deficits were clearly established. They were as follows: (i) nuclear huntingtin overexpression as nuclear aggregates; (ii) smaller CC size with decreased dopamine β-hydroxylase expression, indicating lesser number of chromaffin secretory vesicles; (iii) reduced adrenal tissue catecholamine content; (iv) membrane hyperpolarisation with reduced ACh-evoked action potentials; (v) reduced Na^+ and Ca^{2+} currents; (vi) reduced $[Ca^{2+}]_c$ transients with faster clearance; (vii) diminished quantal secretion with smaller vesicle quantal size; (viii) slower kinetics of the exocytotic fusion pore, pore expansion, and faster closure.

Conclusion: These profound changes demonstrate that the altered neurotransmitter release occurring in the brain of HD patients may also occur in peripheral sympathetic-like adrenal CCs. As in central neurons, these peripheral functional changes may be attributed to the pathological accumulation of mutated huntingtin in CCs. The drastic reduction of the quantal release of neurotransmitter indicates that in human HD patients, their response to stress may be hampered by the drastic decrease of catecholamine release from adrenal medullary CCs.

Keywords: Huntington's disease, R6/1 mice, chromaffin cells, exocytosis
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Competing Interests: The authors declare no competing financial interest

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Implication of Interleukin-17A in renal dysfunction progression due to arterial pressure changes.

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Objectives: Hypertension is a vascular disease that damages several organs. In the kidneys, the disorder related to this damage is called hypertensive nephropathy. This disease involves an interstitial and periglomerular fibrosis, tubular atrophy and inflammation. There are several cytokines, chemokines and immune system cells engaged in repairing tissues. Th17 lymphocytes and their main effector cytokine, Interleukin-17A (IL-17A), take part in the response against extracellular pathogens, autoimmune and chronic inflammatory diseases. Our aim was to study the participation of IL-17A in renal damage and its relation with arterial pressure elevation.

Methods. The presence of IL-17A positive cells was evaluated in renal biopsies of hypertensive nephropathy patients and in experimental models of hypertension: systemic infusion of Angiotensin II (AngII) in Wistar rats and in C57BL/6 mice (dose of 100 or 1000 ng/kg/min, respectively, during 15 days). The effect of IL-17A in the kidney was studied using two approaches: 1) neutralizing antibody against IL-17A (100 µg/mouse intraperitoneally every 4 days) in the AngII-infusion model in mice and 2) a model of systemic administration of IL-17A in mice at a dose of 1.5 ng/g mouse.

Results. IL-17A positive cells were found in renal biopsies of hypertensive patients, as well as in the kidneys of hypertensive models. Accordingly, IL-17A renal levels were also elevated in hypertensive animals compared to controls (evaluated by ELISA). IL-17A systemic administration in mice increased systolic pressure (117 ± 4 mm Hg vs 88 ± 3 mm Hg; n=7 $p \leq 0.05$, at 15 days compared to controls). These mice also had elevated renal kallikrein-1 levels, observed by immunohistochemistry and quantitative PCR, compared to controls. Interestingly, renal gene expression levels of markers Kim-1 and N-gal were upregulated in IL-17A-treated compared to control mice. The evaluation of renal lesions showed the presence of inflammatory cells in the kidneys of IL-17A-treated mice, including CD3, CD4 lymphocytes and neutrophils. Moreover, upregulation of proinflammatory factors was also found. In the model of AngII-induced hypertension, IL-17A blockade decreased renal inflammatory cell infiltration and proinflammatory factors overexpression.

Conclusions. The presence of IL-17A positive cells in the kidney of hypertensive patients suggests that these cells could be involved in the pathogenesis of hypertension. The experimental studies presented here show that elevated IL-17A circulating levels induce an elevation of blood pressure and contributes to the inflammatory process in the kidney and, therefore, this cytokine participates in renal dysfunction associated to hypertension.

Keywords: Interleukin-17A, hypertension, inflammation
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NLRP3 inflammasome inhibition reduces infarct volume, Blood-Brain-Barrier breakdown and inflammation in cerebral ischemia.

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Introduction: Cerebral ischemia is the third cause of death and the main cause of adult disability worldwide. Currently, intravenous tissue plasminogen activator (tPA) is the only pharmacological treatment for acute ischemic stroke. However, only 3% of patients benefit from tPA administration, due to its limited therapeutic window and the risk of intracerebral hemorrhage. NLRP3 inflammasome, a key component of the innate immune system, has a critical role in inflammation damage in ischemic injury. NLRP3 inflammasome activation leads to caspase-1-dependent cleavage of pro-IL-1β to active IL-1β and pyroptosis.

Material and Methods: In this study, we wanted to investigate the role of NLRP3 in post-ischemic inflammation, using MCC950, a potent inhibitor of NLRP3 inflammasome. For that purpose, we used transient middle cerebral artery occlusion (tMCAO) during 1 hour in mice as a model of cerebral ischemia. Furthermore, we analyzed the blood brain barrier (BBB) permeability using Evans Blue dye 24 hours after reperfusion. Finally, we wanted to know whether MCC950 could be acting on tight junction of endothelial cells.

Results: Administration of MCC950 1h after reperfusion reduced infarct volume in a dose-dependent manner (1, 3, 10 mg/kg; 53,23% ,50,57%, 107,87%, respectively). As a clinical outcome parameter, MCC950 at 3 mg/kg improved neuro-motor function and reduced expression of different pro-inflammatory cytokines (IL-1β and TNF-α) and NLRP3 inflammasome component. We observed that tMCAO produced BBB disruption that was improved in animals treated with MCC950 3 mg/kg. In MCC950-treated animals, we observed a functional recovery of endothelial proteins that forms the tight junctions of BBB (VE-cadherina, Cd31, ZO-1).

Conclusion: From these results we can conclude that i) inhibition of NLRP3 inflammasome with MCC950 significantly reduces infarct volume and improve neuro-motor function, and ii) MCC950 preserves BBB integrity through stabilization of the tight junctions. Hence, the inhibition of NLRP3 may be a promising target in cerebral ischemia.

Keywords: cerebral ischemia, NLRP3 inflammasome, blood-brain barrier.
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Bisphenol A induces autophagy and oxidative stress in experimental chronic kidney injury and in tubular cells.

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Introduction and Aims: Uremic toxins accumulated as a result of chronic kidney disease (CKD) contribute to the complications of the disease and its progression. . Bisphenol A (BPA) is a ubiquitous environmental toxin accumulated in CKD. Autophagy is a degradative lysosomal process that eliminates misfolded and protein aggregates or damaged organelles to maintain intracellular homeostasis and cellular integrity. Recent evidences suggest that autophagy is implicated in tubular and glomerular cell damage in renal diseases. Nevertheless, the role of autophagy on pathophysiology of renal disease is still unknown. The aim of this study is to explore the role of autophagy as mechanism of BPA toxicity on experimental chronic kidney injury.

Methods: The role of BPA in chronic kidney injury was studied in a experimental model of subtotal nephrectomy in mouse c57bl/6. Some animals were injected with BPA (120mg/kg/day) intraperitoneally during 5 weeks. Furthermore, in vitro studies were developed in human proximal tubular epithelial cells (HK-2) stimulated with BPA at different concentrations (50 µM, 100 µM and 200 µM). Gene Expression were measured by qRT-PCR and proteins levels were analyzed by several techniques such as western blot and immunohistochemistry.

Results: We observed that in subtotal nephrectomized mice, the BPA exposure during 5 weeks led to oxidative stress, autophagy and inflammation by increasing the gene expression of specific autophagy markers such as Atg5, Atg7, LC3B and Beclin. On the other hand, BPA exposure in mice increased the expression of oxidative stress and Nrf2 target genes as Hemo-oxygenase 1 (HO-1) and NAD(P)H dehydrogenase [quinone] 1 (NQO-1). Additionally, in cell culture the stimulation with BPA increased in a dose dependent manner the gene expression of proinflamatory factors as CCL-2, CCL-5 and IL-6 and the autophagy markers LC3B and Beclin.

Conclusions: These data suggests that BPA causes oxidative stress, inflammation, and autophagy in experimental chronic kidney injury developping a fundamental role into CKD progression.

Keywords: Chronic Kidney Disease, Autophagy, Bisphenol A.
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Clopidogrel response is defined by CYP2C19 metabolizer status in patients undergoing percutaneous neurointervention procedure.

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Introduction: Clopidogrel is a widely prescribed thienopyridine prodrug which inhibits platelet aggregation. It is prescribed to prevent atherothrombotic and thromboembolic events in patients who are given a stent implant in carotid, vertebral or cranial arteries. CYP2C19 is the most studied enzyme involved in clopidogrel metabolism. The most common *CYP2C19* no function polymorphisms (*2 and *3) have been associated with hyporesponse to clopidogrel, showing lower levels of the active metabolite. On the contrary, the presence of the increased function allele (*17) has demonstrated enhanced platelet inhibition and clopidogrel hyperresponse.

Methods: This observational retrospective study assessed antiplatelet response and clinical events after clopidogrel treatment in patients who underwent percutaneous neurointervention, related to *CYP2C19* metabolizer status (normal (NM), intermediate/poor (IM-PM) and ultra-rapid (UM); inferred from *2, *3 and *17 allele determination by real-time PCR).

Results: One hundred twenty-three patients were analysed (59 men and 64 women, mean age 64 years), of which 83% had cardiovascular risk factors. The most common type of intervention was angioplasty with stent (60.2%). According to the aggregation value, 58.7% of the patients were responders to clopidogrel; moreover, 4.1% required dose reduction and 12.2% change of treatment. Related to their genotype, 32 (26%) patients were classified as IM-PM; 53 patients (43.1%) as NM and 38 patients (30.9%) as UM. CYP2C19 IM-PM had higher aggregation value (201.1 vs 137.6 NM, 149.4 UM, $p<0.05$) and lower response rate (37.5% vs. 69.8% NM, 61.1% UM), along with higher treatment change rate (25% vs. 5.7% NM, 10.5% UM). Moreover, 20% of the patients suffered from a subsequent clinical event. The highest ischemic events incidence occurred in NM (11.3% vs. 6.3% IM, 10.5% UM; $p=0.358$) and haemorrhagic events in UM (13.2% vs. 0% IM and 3.8% NM; $p=0.041$). No differences found regarding ischemic events' onset time, while haemorrhagic events' frequency in UM was higher with shorter onset time ($p=0.047$). Additionally, 53% of the patients were receiving concomitant treatment with proton-pump inhibitors (PPIs), which showed significantly higher aggregation value when compared to those not receiving PPI concomitant treatment (178.1 vs. 134.4; $p=0.009$).

Conclusion: CYP2C19 no function and increased function alleles defined clopidogrel response. CYP2C19 genotyping and platelet reactivity quantification help to determine whether a patient could be at risk of ischemic or haemorrhagic event. CYP2C19 UM patients have increased bleeding risk after percutaneous neurointervention. This is the first study to associate CYP2C19 with clinical outcomes in this cohort of patients. Therapeutic recommendations should include an alternative therapeutic option in IM-PM or UM patients.

Keywords: CYP2C19; phenotype, antiplatelet; clopidogrel; neurointervention, haemorrhage, ischemia
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Role of the mitochondrial Na+/Ca2+ exchanger in NLRP3 inflammasome activation.

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Introduction: The pathophysiology of multiple neuroinflammatory diseases involve the activation of NLRP3 inflammasome. The inflammatory response triggered by the inflammasome can be activated by different danger stimuli in the cell. Several studies propose mitochondria as key elements in the activation of this inflammatory signalling pathway, through the production of reactive oxygen species (ROS) and mitochondrial dysfunction. However, the exact mechanisms operating in this process are poorly understood. Previous results have shown that inhibition of the mitochondrial Na^+/Ca^{2+} exchanger (NCLX) by the benzothiazepine CGP37157 exerts a protective effect in several *in vitro* models of neurodegeneration. Moreover, NCLX inhibition reduces ROS induced by hypoxia. Since mitochondrial ROS participate in the activation of NLRP3 inflammasome, we proposed to study the possible participation of NCLX in this process.

Materials and Methods: To this end, we have used the compound ITH12575, a synthetic derivative of CGP37157, and we have studied its effect in glial primary cultures of mouse and in the murine macrophage cell line J774 A.1 exposed to NLRP3-activation conditions.

Results: Stimulation of glial cultures with lipopolysaccharide (LPS) 1 µg/ml during 3'5 hours, followed by ATP 5 mM 30 min induced NLRP3 inflammasome activation and IL-1β release. Inhibition of NCLX by ITH12575 reduced the release of this pro-inflammatory cytokine in a concentration-dependent manner (1, 3 and 10 µM). Oxidative stress parameters (ROS and RNS) induced by LPS treatment of glial cultures were also reduced by ITH12575. LPS treatment of macrophages during 24 hours induced the stabilization of the hypoxia-inducible factor 1-alpha (HIF-1α) and pro-IL-1β expression, an effect that was notably potentiated under hypoxic conditions (1% O_2). In these conditions, pharmacological inhibition of NCLX by ITH12575 reduced both HIF-1α stabilization and pro-IL-1β protein levels, which suggests a possible mechanism by which mitochondria can be participating in the activation of the inflammasome.

Conclusion: From these results we can conclude that (i) inhibition of mitochondrial NCLX by ITH12575 significantly reduces IL-1β release in glial cultures stimulated with LPS+ATP and (ii) NCLX inhibition by ITH12575 reduces oxidative stress parameters induced by LPS in glial cultures.

Keywords: NLRP3 inflammasome, mitochondrial Na^+/Ca^{2+} exchanger (NCLX), mitochondria, inflammation, Reactive oxygen species (ROS).
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Competing Interests: The authors declare no conflict of interests.

Mitochondria function and morphology alterations precede neurosecretion impairment in chromaffin cells of the SOD1G93A mouse model of amyotrophic lateral sclerosis.

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Introduction: Amyotrophic lateral sclerosis (ALS) is characterized with a selective loss of motor neurons that cause paralysis and respiratory failure. Hyperexcitability and Ca2+-dependent glutamate excitotoxicity has been hypothesized to be involved in ALS pathogenesis. Exploring the chromaffin cell (CC) of SOD1G93A mouse model of familiar ALS, we found that the fusion pore kinetics of exocytosis is slowed but with higher catecholamine quantal size when the disease is already established (Calvo-Gallardo et al., Am J Physiol Cell Physiol 2015;308:C1-C19). To go further in the study of these neurosecretion alterations, we investigate the exocytosis before the disease onset (30 days postnatal), and we focus in the study of mitochondrial ultrastructure and function as a crucial organelle involved in the process.

Material and Methods: Mitochondrial ultrastructure was explored by transmission electron microscopy (TEM) and analyzed with ImageJ software. Luminometer was used to measure ATP levels in CC cultures with the commercial CellTiter-Glo® kit. Reactive oxygen species (ROS) production was monitored 30 minutes with the fluorescent dye H2DCFDA. Fusion pore kinetic was studied by amperometry, eliciting exocytosis by 1 minute acetylcholine stimulus.

Results: TEM showed that mitochondria from SOD1G93A CCs have the following alterations with respect to wildtype CCs: i) more number and small sized; ii) increased mitochondrial intermembrane space; iii) lower number and swollen cristae. These ultrastructural changes suggesting mitochondrial fission and ultrastructure damage were accompanied by lower ATP production and a higher rate of ROS generation. However, we fail in observe such significant differences in the fusion pore kinetics.

Conclusion: The described mitochondrial alterations shown an interesting non-motor neuron degeneration in this ALS model at presymptomatic stages. However, the kinetic of the exocytotic fusion pore have not been affected, contrary to the slowed secretion observed once the paralysis is already established. These results evidence that this mitochondrial alterations precede the functional changes linked to neurotransmitter release. In spite of having lower clinical relevance than in later stages, it could generate some clues about the initiation and progression of the disease. Our data consolidate the mitochondria as a potential target and the sympathetic-adrenal system affectation as an interesting new approach for ALS diagnosis.

Keywords: ALS, $SOD1^{G93A}$, chromaffin cell, mitochondrial ultrastructure, fusion pore, exocytosis.
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Competing Interests: Authors have no conflict of interest to the presented work.

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Vascular damage in obesity associated to pge2 derived mpges-1 through aldosterone/mineralocorticoid-receptor route.

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Adipocytes are more than fat storage cells since they release a number of factors contributing to energy homeostasis and vascular tone and structure. We demonstrated that adipocytes are a source of aldosterone in response to Ang II and that this is facilitated by mPGES-1-derived ProstaglandinE_2 (PGE_2) (unpublished). However, whether this pathway is activated in obesity is unknown. Mineralocorticoid-Receptor (MR), is involved in the stiffness and the endothelial dysfunction observed in obesity. We determined if mPGES-1 participates in aldosterone production from adipocytes in obesity and whether this is involved in endothelial dysfunction and vascular stiffness observed in this pathology.

Epididimal fat from DBA mPGES-$1^{+/+}$ and mPGES-$1^{-/-}$ mice fed with normal or high fat diet (HFD) was analyzed. CYP11B2 and MR expression were studied by qRT-PCR. Changes in vascular function and stiffness were studied using wire and pressure myographs. 3T3-L1 adipocytes were stimulated with 16,16-DimethylProstaglandinE_2 ($DPGE_2$). Furthermore, visceral fat was obtained from patients and gene data were correlated with parameters of vascular stiffness.

We found that CYP11B2 mRNA expression is augmented in the adipose tissue of the mPGES-$1^{+/+}$ HFD mice, but not in mPGES-$1^{-/-}$. However, we did not find differences in the MR. Moreover, $DPGE_2$ increases CYP11B2 mRNA expression in 3T3-L1 adipocytes. HFD provokes endothelial dysfunction in both genotypes, which is prevented by eplerenone. HFD induces vascular stiffness in mPGES-$1^{+/+}$ but not in mPGES-$1^{-/-}$mice. Preliminary data in patients show positive correlations between mPGES-1, CYP11B2 and MR gene expression. Moreover, there is a positive correlation between pulse wave velocity and CYP11B2 gene expression.

Our study suggest that mPGES-1-derived PGE_2 is involved in the excessive aldosterone synthase expression observed in adipose tissue in obesity and this might have a role in the vascular damage observed in this pathology.

Keywords: vascular damage, obesity, mPGES-1.
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Competing Interests: No competing interests.

Abstracts

Other PhD Programmes

PPRV effects on the differentiation of sheep monocyte-derived dendritic cells.

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Introduction: Peste des Petits Ruminants virus (PPRV) is the causative agent of an economically important disease, which affects small ruminants, limiting the productivity of the livestock. PPRV produces immunosuppression which can derive in opportunistic infections that increase mortality in infected animals. PPRV is a Morbillivirus, *Paramyxoviridae* family, closely related to measles and rinderpest virus. PPRV infects immune cells through the signalling lymphocyte activating molecule (SLAM) and this tropism could contribute to its immunosuppressive properties. Since dendritic cells (DC) are professional antigen presentation cells essential to the development of an effective adaptive immune response, we evaluated PPRV effects on DC differentiation and activity.

Material and Methods: $CD14^+$ cells (monocytes) were isolated from ovine Peripheral Blood Mononuclear Cells (PBMC) using anti-CD14 antibody magnetic sorting, and differentiated into immature monocyte-derived DC (iMoDC) with GM-CSF and IL-4 for 72h. To determine PPRV effects on DC differentiation, $CD14^+$ cells were infected with the virulent ICV-89 PPRV strain prior to differentiation.

Results: After 72h with GM-CSF and IL-4, ovine monocytes presented classic DC characteristics such as changed morphology (increased size, multiple dendrites), increased DC cell markers expression levels (CD11b, CD11c, DC-SIGN, MHC-II, MHC-I, CD1, CD1w2, DC80 and CD86) and increased phagocytic activity. Thus $CD14^+$ monocytes were successfully differentiated into iMoDC after 72h. Viral production was detected in iMoDC culture supernatants differentiated from PPRV-infected monocytes. iMoDC infection was further confirmed by anti-PPRV-N antibody staining. A slight increase in cell death was observed in PPRV-infected iMoDC. At 72h, PPRV-infected iMoDC decreased CD11b, CD11c, MHC-II, CD1 and CD1w2 expression, while CD80 and CD86 co-stimulation markers levels remain unchanged. PPRV infection reduced the phagocytic activity of iMoDC as detected by flow cytometry and confocal microscopy. Furthermore, in allogeneic MLR assays, the antigen-presenting capacity of infected iMoDC was reduced.

Conclusions: We were able to successfully differentiate functional iMoDC from sheep $CD14^+$ monocytes. PPRV infection in monocytes disrupted their differentiation to iMoDC by downregulating a number of iMoDC surface markers and reducing their phagocytic and antigen-presenting ability. PPRV disruption of professional antigen presenting cell function could therefore contribute to the immunosuppressive effects of the virus.

Keywords: PPRV, sheep, monocyte-derived dendritic cells, differentiation, cell markers, phagocytosis
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Competing Interests: The authors declare that they have no competing interests.

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Palbociclib radiosensitizes colon and lung cancer cell lines in a p53-dependent manner.

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Introduction: Cell cycle regulatory proteins are one of the most used targets in cancer-targeted therapy. One example is palbociclib, a CDK4/6 inhibitor that suppresses their enzymatic activity by the inhibition of their phosphorylating capacity making them unable to phosphorylate their substrate: the retinoblastoma protein (pRB), which results in G1-phase cell cycle arrest.

On the other hand, radiotherapy is one of the main tools in cancer therapy, in fact, it has been estimated that approximately 50% of patients need their use at some point during the course of the disease. In this sense, the use of radiosensitizing drugs becomes very important, because allows greater treatment effectivity.

Recent studies points to the potential of palbociclib as a radiosensitizer agent, nevertheless, it has not been established its molecular mechanism of action.

Material and methods: The aim of this study was to check the radiosensitizing effect of palbociclib, as well as establishing its molecular mechanism of action in colorectal (HCT116 y HT-29) and lung (A549 y H1299) cancer cell lines. We evaluated the palbociclib toxicity by MTT. We measured the functionality of palbociclib in two ways, by Western Blot (measuring pRB phosphorylation) and by flow cytometry (analyzing cell-cycle arrest). Furthermore, to analyze the needed of p53 for the radiosensitizing effect, we use the HCT116 isogenic model p53+/+ and p53 -/-; and we knockdown p53 by shRNA in A549 cells.

Results: Palbociclib shows the same toxicity in all the employed cell lines, and moreover induces the same G1-phase cell cycle arrest and inhibits the phosphorylation in pRB. Palbociclib induces radiosensitivity if the cell line presents a fully functional p53 protein, on the contrary, in those lines lacking p53 or expressing a non-functional p53 protein we do not observe this radiosensitizing effect.

Conclusion: Our results demonstrate the radiosensitizing effect of palbociclib and the needed of a functional p53 protein for this effect, providing a new clinical use for this inhibitor. These results could allow doctors to predict the effectiveness of the treatment in a patient using the p53 protein as a biomarker.

Keywords: Palbociclib, radiosentitizing, p53, radiation.
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Competing Interests: The authors declare that they have no conflicting interests.

Erk5 pathway is a new indirect target of sorafenib.

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Introduction: Sorafenib is a tyrosine kinase inhibitor that was developed as a B-RAF inhibitor. Afterwards it was discovered that Sorafenib could also inhibit other kinases like VEGF-R and PDEGF-R. The fundamental therapeutic mechanisms of Sorafenib are anti-angiogenesis and anti-cell proliferation. Its use has been approved in Renal Cell Carcinoma (RCC), Gastrointestinal Stromal Tumor (GIST), Thyroid Cancer and Hepatocellular Carcinoma.

Material and Methods: An experimental model of human epithelioid cervix cancer cells (Hela) was used to analyse how Sorafenib impacts on MEK5 function assessing the inhibition of the phosphorylation of its immediate downstream MAPK, ERK5. Moreover, cell viability by MTT assay**,** and migration of cancer cells by wound healing assay were also studied.

Results and Conclusion: In this study, we demonstrated that MEK5/ERK5 signaling pathway is a new target of Sorafenib. Thus, we showed that Sorafenib provokes a partial inhibition of MEK5 activity upon treatment with EGF or by expression of a constitutively active MEK5 mutant form. Furthermore, we have confirmed our observations by using specific shRNA against ERK5.

Therefore, our data indicate that part of the therapeutic effect of Sorafenib could be mediated through the inhibitory effect exerted onto ERK5 signalling pathway, discarding other MAPK as ERK1/2 or p38MAPK pathways. In sum, our data demonstrate that ERK5 pathway is an indirect target of Sorafenib, providing new therapeutic opportunities in the use of this novel tyrosine kinase inhibitor.

Keywords: Sorafenib, ERK5, MAPK.
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Competing Interests: All the authors declare that there is no competing financial interest.

Mitochondrial activity plays a critical role in multiple myeloma resistance.

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Introduction: Mitochondria control crucial biological pathways such as proliferation, apoptosis and cell growth. The implication of its activity in the pathogenesis of Multiple Myeloma (MM) stills remains unknown.

We have studied the impact of mitochondrial genes, protein expression, and activity in MM progression and treatment resistance. Furthermore, we have studied the potential exploitation of mitochondrial activity as a functional target in the MM therapy.

Methods: We have performed gene expression studies by RT-PCR of known factors that regulate and are involved in the mitochondrial function (c-Myc, TFAM, EF-Tu, NRF1 and hnRNPK) in a total of 34 sample patients at different MM stages. To validate gene expression results, we developed an immunohistochemistry (IHC) assay of COX II, representative protein of mitochondrial burden in a total sample of 49 patients. We analyzed the mitochondrial activity with the study of COX2 histoenzymatic reaction in 15 patients. Finally, we have tested the effect in plasma cells of Metformin and Tigecycline, in monotherapy and in combination with Bortezomib over four MM cell lines (JJN3, L363, NCI-H929 and NCI-H929 R20). Besides, we analyze the mitochondrial DNA (mtDNA) burden in these cell lines. Our further studies include *in vivo* validation in NSG mice models of results obtained *in vitro*.

Results: We have observed a significant overexpression of genes C-Myc, TFAM, EF-Tu, and a higher expression trend of hnRNPK in MM relapsed patients compared with MGUS and newly diagnose MM groups (p-value *< 0.05; p-value ** < 0.001) (Fig. 1A). Moreover, IHC reveals overexpression of mitochondrial COXII protein in newly diagnose MM and relapsed groups compared with MGUS (Fig. 1B). By a functional assay we have demonstrated that gene and protein overexpression drives to an increase of activity, comparing MGUS and MM at diagnosis versus MM at relapse (p-value ***< 0.0001) (Fig. 1C). We confirmed the correlation between higher mitochondrial burden and resistance to bortezomib in JJN3 and L363, and NCI-H929 and its resistant, NCI-H929 R20 (p-value*<0.05) (Fig. D). *In vitro* drug assays showed a synergistic effect of tigecycline with bortezomib, suggesting that could be used as a potential therapy in combination for MM patients.

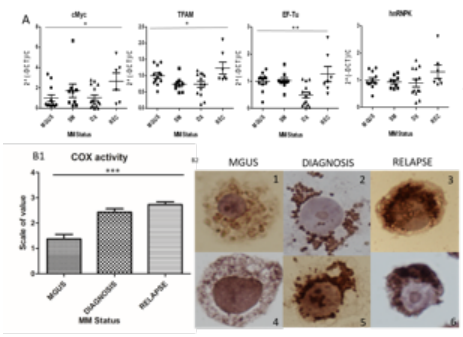

Figure 1: A. Primary MM samples of different stages showed an incrased of cDNA level of the genes involved in the mitochondrial function. B. Mitochondrial activity study on MM group patients. B1. Histoenzimatic staining of COX in different MM status (1: low intensity; 2: mid intensity; 3: mid-high intensity; 4: high intensity). B2. Image examples of each MM status with histoenzimatic staining of COX.

Conclusions: Mitochondrial machinery plays a critical role in the development, progression and resistance of MM patients. Mitochondrial protein components that generates the activity could be prospective targets for MM treatment. Tigecycline demonstrates synergistic effect with Bortezomib suggesting potential use as novel drug combination therapy in MM patients.

Keywords: multiple myeloma, resistance, mitochondria.
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From stem cells to unique neurons: Specification of the Drosophila melanogaster Orcokinin A neurons.

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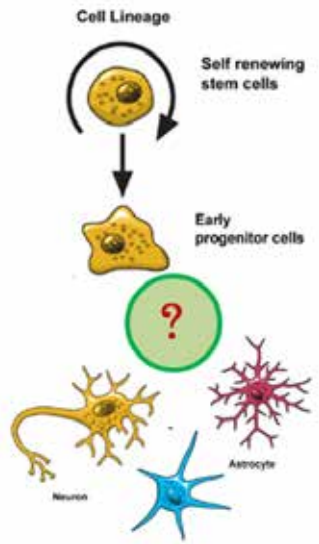
One of the major challenges in the field of Developmental Neurobiology and Regenerative Medicine based on Stem Cells (SCs) is to understand the basic mechanisms of cell specification. Discovering how SCs produce a progeny that differentiates into diverse cell fates is essential to understand the normal development of an organism, but also to understand some pathological situations and take advantage of the therapeutic potential of this system. This is particularly interesting in tissue repair and regeneration after any damage or pathology.

Regarding the Central Nervous System (CNS), it is critical to understand how each neural subtype is generated under physiological conditions. Thus, we could acquire the capacity to produce specific neurons from neural SCs to replace those that have been lost or damaged.



Nowadays, improving *in vivo* studies of neural SCs manipulation becomes essential. However, the high conservation of the basic processes that regulate the neural progenitor differentiation supports the study of simpler model organisms.

Therefore, the main objective of this project is to advance in a logical and efficient way in our understanding about generation and maintenance of neuronal diversity. For that purpose, we study the specification of the abdominal Orcokinin A neuropeptidergic neurons in *Drosophila melanogaster*.

First, we have found that these cells are generated at late stage of lineage progression in the Neuroblast 5-3 (NB 5-3) during the *castor-grainyhead* temporal window. Second, we have found that their correct specification depends on Hox genes input. In particular, *Ubx* and *abd-A* appear to be involved in the establishment of the Orcokinin A terminal fate, while *Antp* and *Abd-B* seems to trigger a different fate in the segments they govern.

We have found that although the Dpp pathway is active in these neurons, the pathway does not seem to determine the Orcokinin A fate. Additionally, the Notch pathway is inactive in these neurons, which suggest that they are the Notch-OFF counterpart in the case they were sibling neurons born from a GMC cell.

Finally, we have identified the cis-regulatory modules (CRM) that direct the expression of Orcokinin A to address the molecular mechanisms involved in the specification of these neurons. We have begun dissecting their organization by extensive *in vivo* studies. These involve mutant and misexpression analysis and transcription factor binding site mutagenesis. In addition, the CRISPR/Cas9 system will be use to delete this specific CRM to validate its *in vivo* necessity. With this set of experiments, we attempt to resolve key questions that are hampering progress in the knowledge of Developmental Neurobiology and Stem Cells Biology.

Keywords: neuron, stem cells, development, cell fate specification.
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A comprehensive study of Trichomonas vaginalis infection: a step forward to understand the pathobiology of isolates from Madrid, Spain.

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Introduction: The protozoan parasite *Trichomonas vaginalis* (TV) is the causative agent of the most common non-viral sexually transmitted infection (STI) in the world. Trichomonosis is a very complex disease characterized by a broad range of symptoms, as TV isolates vary in their virulence, pathogenicity and drug resistance, and a remarkable ability to evade the host immune system using a wide variety of mechanisms. The last WHO report estimates an incidence of 276 million new cases every year.TV infection has been associated with an increased risk of several diseases as cervix and prostate cancer, adverse pregnancy outcomes, infections caused by HIV, *Chlamydia trachomatis*, *Neisseria gonorroheae*, *Treponema pallidum*, and human papilloma virus (HPV), especially the high-risk HPV type 16. Despite the fact that trichomonosis exceeds the incidence of chlamydia, gonorrhea and syphilis together, nowadays this parasitic infection is not a reportable disease.

Procedure: Our study is phased in three stages: (1**) Epidemiological study** in a STI clinic and a hospital of Madrid (Spain) to study the trichomonosis in this region, (2) **biological characterization** of TV isolates to identify relevant phenotypic characteristics as metronidazole resistance, presence of endosymbionts in the parasite (TVV and *Mycoplasma*), for the correct parasite's classification between type 1 or 2, and (3) **molecular characterization** of TV isolates to identify length polymorphism microsatellite as genetic markers which support biologic characterization for intraespecific differentiation.

Results: As to date, 35 TV isolates have been received, 16 of which have been completely characterized, and 19 are being analyzed. The epidemiological study showed that 8 of each 1000 vaginal swabs analyzed in the Hospital, was positive to *T. vaginalis* in 2017, and comparing with the previous year, the incidence of trichomonosis in women attending this hospital during 2017 increased a 118 percent. In addition, 2017 was the first year which broke the falling trend in the incidence of trichomonosis in the hospital since 2013.

Discussion: Our findings demonstrated a high prevalence of *T. vaginalis* isolates infected with TVVs or *M. hominis*. The MIC to metronidazole in *M. hominis*-infected *T. vaginalis* isolates tend to be higher than the respective non-infected strains. The opposite result was found in TVV- infected *T. vaginalis* isolates, whose MIC tend to be lower than the respective non-infected ones. This is the first study evaluating incident *T. vaginalis* in women attending a Hospital of Madrid since the 90's. Further investigation should assess the benefits of routinely screening women in STD clinics and hospitals for *T. vaginalis.*

Keywords Trichomonosis, resistance, epidemiology, sexually transmitted infection, *Trichomonas vaginalis* virus, *Mycoplasma,* genotype, microsatellite.
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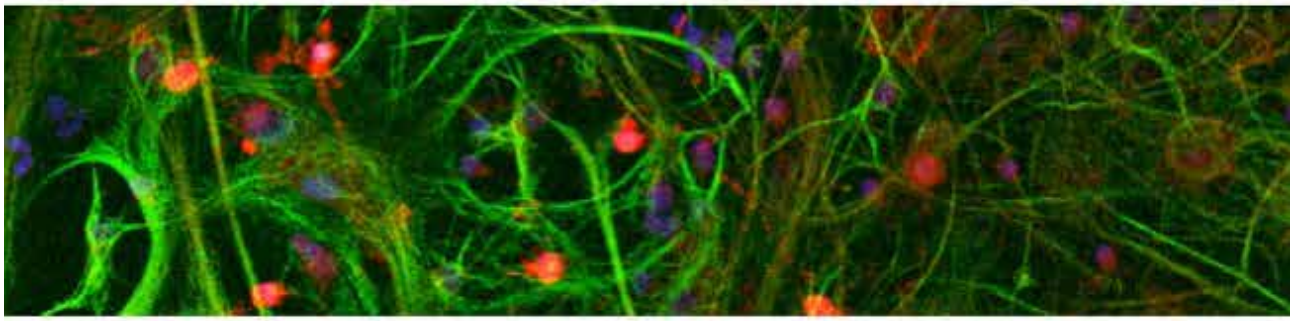

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