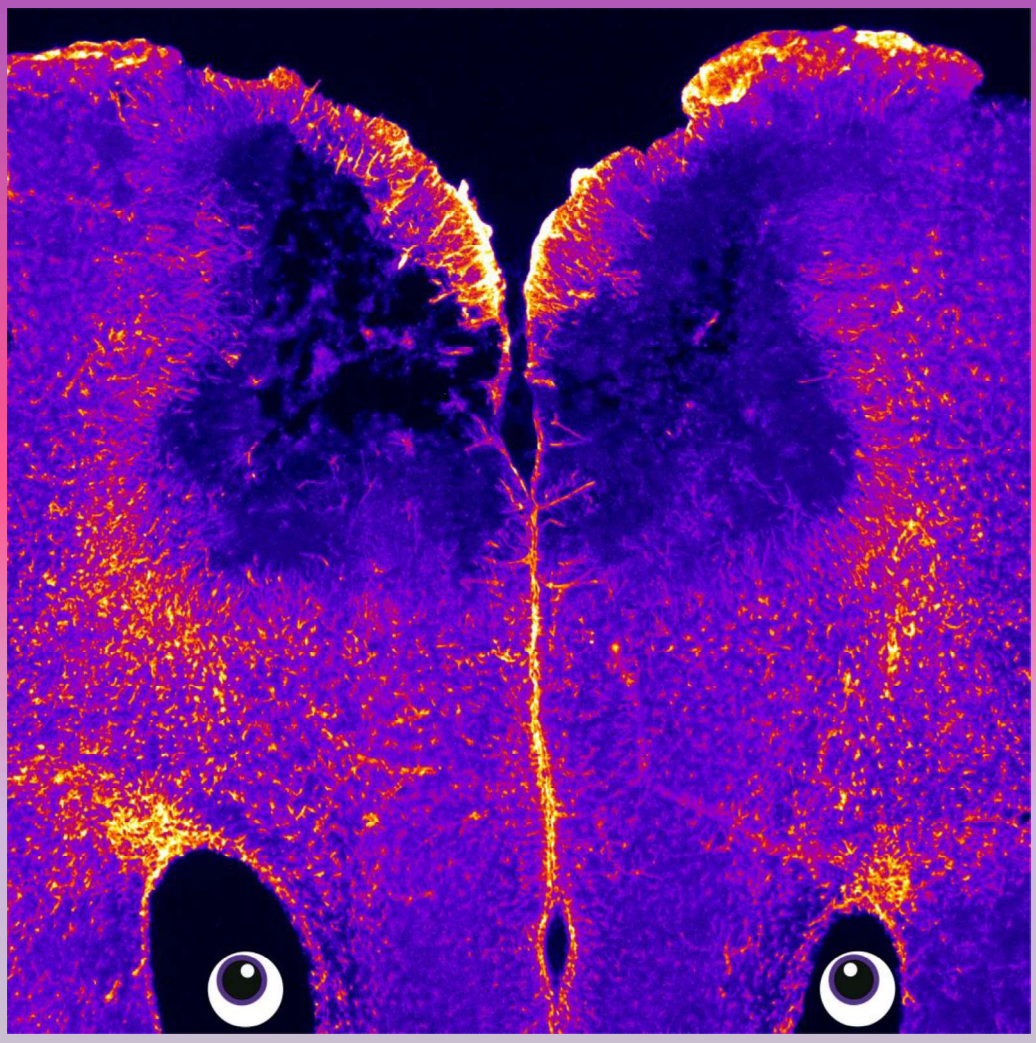
XIV Jornada del Departament de Biologia Cel·lular, Fisiologia i Immunologia

15 de juny del 2023



Something wrong up there_ Elisenda Urpi

Millor fotografia científica de la XIII Jornada del Departament BCFI 2022

XIV Jornades
Departament de Biología Cel·lular, Fisiologia i Immunologia
Universitat Autònoma de Barcelona
15 de Juny de 2023

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TALLER

Pressions en cadena: desmuntar l'estrès per superar-lo
Jordi Fernández-Castro - Stress and Health Research Group

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Dr. Jordi Camps - Unitat de Biologia Cel·lular i Genètica Mèdica

XERRADA

Meeting the Synchrotron: applications of the Synchrotron-Fourier Transformed Infrared Microspectroscopy technique to neuroscience studies
Dra. Beatriz Almolda - Unitat d'Histologia Mèdica

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Caracterización de una nueva familia de péptidos bioactivos y estudio de sus aplicaciones potenciales en salud humana. **Rocío Piñera** (FABC)

Development of a chimeric mouse model to study the effects of human microglia to amyotrophic lateral sclerosis. **Joana García** (FM)

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COMUNICACIONS ORALS

I. PARTICIPANT

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II. COMUNICACIÓ ORAL

Títol: BREAKAGE OF CHROMOSOME BRIDGES DURING MITOSIS: RELEVANCE OF MECHANICAL STRESS

Autors: Marina Rodriguez-Muñoz, Martina Serrat, Teresa Anglada i Anna Genescà

III. RESUM

Chromosomal instability may be caused by different mechanisms that share a common intermediary: the chromatin bridge. Despite the importance of the chromosome bridges in tumor initiation and progression, the mechanisms that determine how and when they are resolved are not well understood. Recent studies argue that chromosome bridges cannot be resolved during mitosis but lead to nucleoplasmic bridges that persist in daughter cells and can be finally destabilized due to mechanical stress or enzymatic digestion. As controversial hypotheses exist on whether the bridges break during mitosis or in interphase, we investigated chromosome bridge breakage in mitotic cells.

Our results show that during anaphase a significant number of bridges break as shown by γH2AX signaling on the bridging DNA. As chromosomes are stretched towards opposite poles during anaphase, we have analyzed if pulling forces exerted by the mitotic spindle could contribute to the bridge breakage. So far, here we employed a model based on CRISPR/Cas9 system to induce dicentric chromosomes with defined distances between centromeres. We found an inverse correlation between the distance between bridge kinetochores in base pairs and the probability of breakage of the chromosome bridges during mitosis. Altogether, we conclude that the discontinuities observed in bridges during mitosis frequently reflect a real breakage of the chromatin and that the mechanisms responsible for chromosome bridge breakage during mitosis may depend on the separation between the bridge kinetochores.

Considering that previous studies identified mechanical stress or biochemical digestion as possible causes of bridge breakage in interphase cells, a multifactorial model emerges for the breakage of chromosome bridges that, consistent with our results, can occur at different stages of the cell cycle and can obey different mechanisms.

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II. COMUNICACIÓ ORAL

Títol: Transmissible cancers: Unravelling the evilness of the Tasmanian Facial Tumor Disease

Autors: Lucía Álvarez-González, Laia Marín-Gual, Hardip Patel, Irene Alfonso, Albert Gubern, Janine Deakin, Marta Farré, Paul Waters, Aurora Ruiz-Herrera

III. RESUM

The cancer paradigm is challenged by transmissible tumours. They can infect individuals through direct transfer of a single or a group of tumorigenic cells without the involvement of an infectious agent. The Tasmanian devil facial tumour disease (DFTD) is one of the extremely rare cases of naturally occurring transmissible tumours, and perhaps the deadliest since it has brought the wild Tasmanian devil populations, an endemic marsupial mammal of Australia, to the verge of extinction. This disease is comprised of two independently evolved transmissible cancers with distinct chromosomal configurations, DFT1 (first reported in 1996 and with a genome highly rearranged) and DFT2 (first identified in 2014 and characterized by a more stable karyotype). Regardless of their genomic difference, both strains show similar prognosis in infected individuals: rapidly progression of locally destructive masses in the oral cavity that prevent animals from feeding, causing death 6 months after infection. Despite their lethal consequences, little is known about the genes and functional pathways altered in this type of infectious cancer, and to what extend genome organization can be related with the tumorigenic process. Here, using the recently described three-dimensional architecture of the Tasmanian Devil genome in combination with epigenetic and functional data we explore the genomic signatures of DFT1 and DFT2 providing new insights into how hallmarks of tumour genomes origin and maintain.

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II. COMUNICACIÓ ORAL

Títol: Caracterización de una nueva familia de péptidos bioactivos y estudio de sus aplicaciones potenciales en salud humana.

Autors: Piñera-Moreno R, Corral M, Verdaguer J, Tort L & Barquinero J.

III. RESUM

A partir del descubrimiento de un péptido derivado del filamento intermedio (FI) de la periferina denominado DIF-P, se observó que inducía una potente secreción de citoquinas proinflamatorias y mostraba una potente actividad citolítica a 55 µM. Tras este hallazgo, se han estudiado péptidos homólogos a éste pero derivados de otros FI, que mantienen una secuencia conservada de 9 aminoácidos en casi todas las especies y se probaron en diversos tipos celulares mediante la adición de colas de 8 argininas en su extremo carboxilo terminal para facilitar su entrada a las células.

Los resultados han permitido identificar uno de estos péptidos, denominado K18 (porque deriva del FI queratina 18), que es el que mayor actividad hemolítica, antimicrobiana, citolítica e inmunoestimulatoria presenta, por lo que nuestros ensayos se han realizado con dicho péptido. Además, se han realizado estudios para dilucidar su mecanismo de acción y saber cómo realmente actúa cuando entra en contacto con las membranas celulares.

Por último, se ha descubierto una nueva aplicación de dicho péptido como agente potencial que mejora la transducción *in vitro,* favoreciendo la entrada de vectores lentivirales a las células, lo que podría mejorar los ensayos de terapia génica *ex vivo*.

Por el momento, se ha probado en líneas celulares murinas y humanas, en progenitores hematopoyéticos humanos, es decir, células CD34 positivas y en células CAR-T, con resultados altamente prometedores.

Actualmente, los ensayos se están centrando en explorar la posible aplicabilidad biomédica de estos péptidos como potenciales optimizadores de la transducción en la terapia con células Natural Killer o NKs, debido al gran desafio y complejidad que presentan dichas células en el tratamiento de diversas enfermedades como el càncer o infecciones virales entre otras.

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II. COMUNICACIÓ ORAL

Títol: Development of a chimeric mouse model to study the effects of human microglia to amyotrophic lateral sclerosis

Autors: Joana Garcia-Garcia and Rubèn López-Vales

III. RESUM

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by motor neuron degeneration. Among others, mutations in SOD1 gene have been described to cause ALS. Microglia has been described to play a key role in ALS pathogenesis. However, mouse microglial transcriptome exhibits altered expression of neurodegenerative disease-associated risk genes compared to the human and the heterogeneity of human microglial states are not recapitulated in mouse microglia. Recently, chimeric mouse models with human microglia have become a promising tool to better understand the role of ALS-associated risk genes in microglial cells, since engrafted human microglia in the mouse CNS mimic more closely the primary human microglia rather than in vitro microglial cells.

In this study, we aim at developing a chimeric mouse model with human microglia to study the effects of microglia to ALS. First, we generated a human embryonic stem cell (ESC) line carrier for the SOD1G93A mutation by CRISPR/Cas9 technology. Then, we characterized in vitro SOD1G93A ESC-derived microglia phenotypically and functionally compared to the control. SOD1G93A microglia exhibited altered phagocytosis and metabolism, as well as, disrupted cytokine expression profile in basal conditions and after LPS stimulation. Our results suggest a cell-autonomous microglial dysfunction driven by mutant SOD1 in human ESC-derived microglia. On the other hand, we evaluated the integration of human microglia in the mouse spinal cord to generate our chimeric mouse model. Microglial precursors were injected in the spinal cord of Rag2−/− IL2rγ−/− hCSF1KI neonates at P4 day. Human CD45+ cells were observed in the mouse spinal cord 2 weeks post-transplantation. Future studies will address whether human SOD1G93A microglia lead to motor neuron death in the mouse spinal cord.

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II. COMUNICACIÓ ORAL

Títol: L'Efectivitat de la teràpia local CoverGel®-anti-integrina en un model experimental de colitis en rata s'associa a canvis en la microbiota del còlon

Autors: Barbosa S.; Bon Romero N.; Vergara P.; Manyé J.; Bartolí R.

III. RESUM

A la Malaltia Inflamatòria Intestinal (MII) les teràpies biològiques com anti-TNF o anti-integrina han millorat el maneig de la MII. Tot i això, se sap poc sobre el seu efecte sobre la microbiota comensal. El desenvolupament d'un nou hidrogel endoscòpic que permet l'elució de fàrmacs (CoverGel®) permet un tractament local per a la inflamació al còlon. Aquesta aproximació comporta una menor necessitat de dosificació i una disminució dels efectes secundaris sistèmics. El nostre objectiu era provar l'eficàcia del tractament local amb vedolizumab utilitzant CoverGel® i avaluar els canvis a la microbiota intestinal. Es va induir una colitis amb en 12 rates mascle SD. Tres dies després de la inducció es va administrar CoverGel®-Vedolizumab (n=5) o solució salina (n=7) per via transrectal. El punt final de l'estudi i la recol·lecció de mostres es va establir 4 dies després del tractament. També s'hi va incloure un grup de colitis simulada (n =7). Es va avaluar el pes corporal, l'àrea de lesió ulcerada al còlon i la ràtio de pes: longitud del còlon. La microbiota luminal cecal es va analitzar per seqüenciació massiva.
Els animals tractats amb CoverGel®-Vedolizumab van perdre significativament menys pes corporal que els animals no tractats. A més, les zones ulcerades al còlon presentaven un àrea significativament més petita. La colitis va conduir a una disminució de la ratio Firmicutes/Bacteroidetes al grup de rates inflamades no tractades. Per contra, aquest canvi microbià no es va produir amb tanta intensitat al grup CoverGel®-Vedolizumab, essent aquesta diferència de ràtio estadísticament significativa. Els resultats del present estudi ens animen a concloure que la combinació de Covergel®-Vedolizumab per via local transrectal en aquest model millora els paràmetres inflamatoris i reverteix parcialment els canvis microbians presents durant la inflamació intestinal.

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II. COMUNICACIÓ ORAL

Títol: Crinosomes act as a major source of beta-cell-derived proteins for peptide presentation by T1D-associated HLA alleles

Autors: M. García Ayala, J. Maggi, S. Auzmendi Aguirresarobe, X. Viñas Margalef, Y. A. Arribas, M. Carrascal, D. Jaraquemada, C. Roura Mir

III. RESUM

T-cell targeting of b-cells in type 1 diabetes (T1D) requires autoantigen processing and presentation by antigen presenting cells (APCs). Various proteins have been described as targets of this autoimmune response. Proteolytic vesicles, which may reflect b-cell status and have their content altered by cell stress, might be the major source of such proteins. Fractions enriched in insulin secretory granules (ISGs) or crinosomes (CBs), the fusion of ISGs and lysosomes, were obtained from human b-cell lines cultured with basal or stressing glucose concentrations. Samples were 1) further trypsin-digested for proteome study and 2) used, together with a complete b-cell lysate, for pulsing moDCs expressing T1D HLA-DR risk alleles to characterise the derived immunopeptidome. All samples were analysed by mass spectrometry. On one hand, more than 800 proteins were identified in each granule fraction, including chromogranin A and secretogranins. About 80% of the proteins were common to both CBs and ISGs, indicating their biological connection. On the other hand, analysis of the pulsed moDC's immunopeptidomes revealed that 47% of the peptide parental proteins were unique to the vesicle-pulsed samples, with most being exclusive to the CB pulse. Interestingly, peptides derived from various ISG proteins could be detected in CB-pulsed samples. Finally, differences were detected in the vesicle proteomes between basal and stressing glucose concentrations, with more than 20% of the proteins being unique to the latter. Differences were also found in the b-cell-derived immunopeptidomes, with only around 30% of the identified peptides being common to both glucose concentrations.
These results point at CBs as a source of ISG proteins and at high glucose as a cause of alterations in the b-cell proteome and derived immunopeptidome, possible triggers the autoimmune recognition of b-cells.

PÒSTERS

Àrea de Biologia Cel·lular

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II. PÒSTER

Títol: Meiotic 3D chromatin dynamics in the marsupial germ line

Autors: Laia Marín-Gual, Laura Gonzalez-Rodelas, Lucía Álvarez-González, Jesús Page, Marilyn Renfree, Paul D Waters, Aurora Ruiz-Herrera

III. RESUM

During mammalian spermatogenesis, homologous chromosome pairing and recombination events are accompanied by the reshuffling of the three-dimensional (3D) chromatin architecture. Exploring the similarities and differences of chromatin folding across evolutionary lineages is central to developing an appreciation of both the dynamics of genome function and, ultimately, the effects on speciation. Due to their key basal position in the mammalian evolutionary tree, marsupials offer a unique opportunity to explore previously uncharacterized meiotic features, from sex chromosome pairing strategies to chromosome occupancy within the nucleus. Here, we combine cytological analysis, fluorescence activated cell sorting and in situ chromosome conformation capture sequencing (Hi-C) to study the meiotic 3D chromatin dynamics in the Australian marsupial tammar wallaby. Ours results show that sex chromosomes pair forming the so-called dense plate following different sex chromosome pairing strategies, which correlates with differential sex chromosomes architecture and specific transcriptional patterns. Moreover, the spatial folding of chromosomes in meiotic (primary spermatocytes) cells showed different patterns of compartmentalisation in the tammar wallaby when compared to eutherian mammals (i.e., mouse). Overall, our results provide new insights into the regulation of chromatin in the germ line.

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II. PÒSTER

Títol: Uncovering the effect of temperature on recombination

Autors: Laura González-Rodelas, Sara Cuesta-Rodriguez, Lukas Kratochvil, Aurora Ruiz-Herrera

III. RESUM

The ability to adapt to environmental changes is key for species' survival. Of the environmental stimuli that can influence chromatin regulation, temperature is the most common. Although it is known that organisms can respond to temperature by activating a common transcriptional programme, the effect on recombination is less explored. Previous studies in plants have shown that the frequency of recombination during meiotic prophase I can be affected when temperatures are extreme, however whether this effect is also conserved in vertebrates is not known at this stage. In this context, the study of the exposure to different temperatures in different species will allow us to understand the possible effect of environmental factors on recombination during the process of gamete formation, especially during meiotic prophase I when homologous chromosomes pair, synapse and recombine. Here, we studied the effect of temperature on recombination in the Ibity ground gecko (Paroedura ibityensis) from the Sauropods. Individuals were exposed to a range of temperature (from 20ºC to 30ºC) for a period of 7 days. Firstly, we performed a cytological analysis of prophase I dynamics, including chromosome pairing and synapsis. Secondly, we analysed the formation of double stand breaks (DSBs) and the formation of crossovers (CO) by immunolocalization of proteins involved in these processes (MLH1 and RPA). Finally, we analysed the frequency and chromosomal location of CO and the levels of DSB formation comparing these individuals. Our preliminary results show hyper-crossover spermatocytes in individuals treated at extremely temperatures (high and low temperatures). Moreover, this significantly increase of COs was related to an increased level of meiotic DSB formation. Overall, our results provide new insights into the effects of environment temperature fluctuations on meiotic recombination.

I. PARTICIPANT **BC.3**

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II. PÒSTER

Títol: COVID-19 compromises spermatogenesis in men

Autors: Maria López-Panadés, Ana Martínez-Marchal, Cristina Madrid-Sandín, Andros Maldonado-Linares, Lluís Bassas, Miguel Brieño-Enríquez, Ignasi Roig

III. RESUM

The SARS-CoV-2 virus uses the TMPRSS2 protease and ACE2 receptor to infect host cells. Even though it is mainly a respiratory disease, both proteins are expressed in many tissues, including several testicular cell types. Furthermore, severe damage caused by inflammation has been detected in the testis of infected men. Thus, our objective was to explore the potential impact of COVID-19 on the male reproductive system. First, we analyzed the morphology of testis sections from patients deceased by COVID-19 and compared them to control samples of similar ages. Overall, COVID-19 samples displayed various anomalies commonly associated with compromised spermatogenesis, such as vacuolization of Sertoli cells, detachment of the germinal epithelium, or thickening of the basal lamina. Next, we studied the presence of different relevant biomarkers. A higher fraction of T lymphocytes and macrophages were detected in the peritubular spaces of COVID-19 samples, confirming the infiltration of immune cells in the peritubular tissue of the testis. In addition, the seminiferous tubules of COVID-19 samples showed fewer UTF1-positive spermatogonia, which represent the spermatogonial stem cell population from which all sperm cells derive, also presenting more DNA damage than control cells, suggesting that COVID-19 could compromise spermatogenesis even after recovery. So, we conducted a study to examine the impact of SARS-CoV-2 infection on the testes of a small group of male individuals who had recovered from the virus infection. These patients also showed a decrease in the number of UTF1-positive spermatogonia, which presented more DNA damage, compared to controls. Finally, viral RNA was found in a fraction of COVID-19 necropsies. Nonetheless, more studies are needed to understand the impact of COVID-19 in spermatogenesis, especially in patients that have recovered from the infection.

I. PARTICIPANT **BC.4**

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II. PÒSTER

Títol: STUDY OF NANOPLASTIC TOXIC EFFECTS ON MAMMALIAN SPERM FUNCTIONALITY

Autors: Clara Feliu, Joan Blanco, Zaida Sarrate, Ester Anton

III. RESUM

Over time, plastic waste scattered around the planet breaks down into polluting particles named nanoplastics (NPs). NPs have been found in a wide spectrum of animal species and it has been proved they can enter the body via ingestion or inhalation.
The involuntary exposure to these particles represents a health and reproductive threat to living organisms. In rodent models, studies suggest that the exposition to plastics induces a decrease in ovarian reserve capacity, oocyte quality and sperm quality and quantity. However, the data are still scarce and preliminary, especially in humans.
To shed more light on the potential adverse effects of NPs in the male reproductive function, we have designed a study to determine the harmful effects of an in vitro exposure to NPs on ejaculated human spermatozoa and epididymal mouse spermatozoa.
Sperm cells have been incubated with polystyrene nanospheres (100 nm diameter). A fraction of the sperm was obtained every 24h and the following sperm functional parameters were analysed: vitality (Trypan Blue stain), membrane integrity (HOS test), and apoptosis (Annexin V stain). Additionally, spermatozoa were incubated with dyed polystyrene nanospheres (Dragon Green) and the plasma membrane stain CellMaskTM (Deep Red). Subsequently, slides were visualized in a confocal microscope to assess whether these nanospheres were adhered or internalized into the cells.
Preliminary results suggest that polystyrene nanospheres adhere to spermatozoa, following a regular pattern specially in mice. In human sperm, a decreased viability, vitality and membrane integrity were observed over time, in a significantly more accused way in NP exposed sperm. This last parameter was also lower in NP exposed mice sperm.
We are currently increasing the number of samples studied and conducting additional functional tests to validate these initial findings.

I. PARTICIPANT **BC.5**

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II. PÒSTER

Títol: SkQ1 Treatment Preserves Ovarian Reserve in Mice

Autors: Nikoleta Nikou, Maria López Panadés, and Ignasi Roig

III. RESUM

In mammals, oocyte development and maturation are critical processes for female fertility. Nevertheless, the genetic mechanisms regulating the ovarian reserve and the female meiotic cells surveillance are just beginning to be described. Natural aging and exogenous factors, such as alcohol consumption, have been linked to a diminished ovarian reserve in humans. Specifically, since ethanol causes oxidative stress, which has been associated with a decline in the quality of aging oocytes, we wondered if moderate alcohol consumption could damage the ovarian reserve in mice and if treatment with a mitochondrial-targeted antioxidant could revert it. To address this hypothesis, we administered, through the drinking water, the antioxidant SkQ1, to young C57BL/6 female mice for 14 weeks. To evaluate SKQ1 efficacy, we administered ethanol and, also, co-administered SkQ1 and ethanol. Our preliminary findings reveal a significant reduction in primordial follicles in mice exposed to ethanol, suggesting that ethanol consumption diminishes the ovarian reserve. In addition, the SkQ1 treatment could revert these effects on the ovarian reserve and even increase the number of primordial follicles to control levels. To test the effects of the SkQ1 treatment in natural aging, we administered SkQ1 dissolved in DMSO into the drinking water of young female mice for 14 weeks. Untreated mice lost around 45% of their primordial follicles, contrary to the SkQ1-treated animals, where the loss was just 22%. So, SkQ1 consumption rescued approximately 40% of the primordial follicles loss during 14 weeks. Based on these findings, we propose that the daily consumption of ethanol could significantly affect the fertility status of mammalian females. However, an SkQ1 treatment could counterbalance the ethanol effects, revert the natural aging effects and highly preserve the mammalian ovarian reserve.

I. PARTICIPANT

BC.6

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II. PÒSTER

Títol: Disentangling the effect of Robertsonian fusions and prdm9 allelic background on meiotic recombination

Autors: Cristina Marín-García, Lucía Álvarez-González, Keren Yam, María Magdalena Garcías-Ramis, Laia Marín-Gual, Covadonga Vara, Jacint Ventura, Aurora Ruiz-Herrera

III. RESUM

Meiotic recombination is a tightly regulated process that generates genetic variability and ensures the proper segregation of homologous chromosomes. Its landscape can be affected by several factors including genomic structural variants (i.e., Robertsonian (Rb) fusions - balanced fusions between acrocentric chromosomes), genetic (the Prdm9 allele), and mechanistic factors (i.e., chromatin state or crossover -CO- interference). Here, we take advantage of wild mice populations from the Barcelona Robertsonian System to evaluate the effect of the high variability of Prdm9 in mice populations and the presence of Rb fusions on meiotic recombination. To this aim, we combined the cytological analysis of COs in primary spermatocytes with inferred recombination rates analysis based on linkage disequilibrium using single nucleotide polymorphisms. Cytological results show that recombination decreases in spermatocytes of Rb mice, but also in standard populations that are homozygous for Prdm9. This reduction was accompanied by the displacement of COs towards telomeres in metacentric chromosomes and increased CO interference in homozygous mice for Prdm9. Furthermore, the estimated historical recombination per population confirmed the reduced recombination in situ in Rb mice. However, the homozygosity of the Prdm9 gene is a major determinant of the recombination at the population level, whereas Rb fusions have limited effects. Next, we analysed the distribution of recombination hotspots and the overlapping between them. When doing so, we observe that the common hotspots between the St and Rb populations which share the same allele of Prdm9 are located at telomeric regions, illustrating the redistribution of recombination due to chromosomal fusions. Altogether, our results provide new insights into the effect of Rb fusions and Prdm9 homozygosis on meiotic recombination.

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II. PÒSTER

Títol: ANALYSIS OF HOMOLOGOUS CHROMOSOMES PAIRING IN MICE PREMEIOTIC SPERMATOGENIC CELLS

Autors: Álvaro Pascual, Joan Blanco, Núria Garcia-Coll, Ester Anton, Zaida Sarrate* and Mireia Solé*.

III. RESUM

Homologous chromosome pairing precedes synapsis and recombination in most model organisms. While synapsis and recombination are well studied and conserved processes, pairing timing and its underlying mechanisms can vary significantly, particularly in the mechanisms of pairing occurring before DSBs formation.
This study was aimed to characterize the timing of homologous pairing in mice premeiotic spermatogenic cells using the following experimental design: 1) Testis disaggregation; 2) Fluorescence-Activated Cell Sorting (FACS) of immunolabeled undifferentiated spermatogonia (GFRα1) and differentiated spermatogonia (cKIT); 3) Fluorescence In-situ hybridization (FISH) for homologous pairing analysis; 4) Confocal image capture; 5) ImageJ/Fiji image analysis; and 6) Statistical analysis.
FACS allowed the selection of a pure GFRα1+ spermatogonia population, representing a 1.12% from the testicular cell suspension. All selected cells exhibited the corresponding membrane stain and a conserved DAPI pattern. On the other hand, cKIT+ population represented a 9.75% of the cell suspension. Its pureness assessment showed that 96% of the cells were negative for γH2AX and SYCP3 meaning that these cells have not entered meiosis. Besides, all cells showed the same DAPI pattern as GFRα1+ cells.
FISH analysis of chromosomes 5 and 16 using painting DNA probes demonstrated that homologous chromosomes occupy a single territory in 33.05% of GFRα1+ spermatogonia, increasing up to 36.60% in cKIT+ spermatogonia. Although both results were higher than those obtained in control somatic cells (24,85%), no statistical significance were found (Chi-squared test; $p>0.05$).
Our preliminary results indicated that the pairing process of chromosomes 5 and 16 does not begin in GFRα1+ and cKIT+ spermatogonia.

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II. PÒSTER

Títol: EFFECTS OF IN VITRO EXPOSURE TO NANOPLASTICS DURING MOUSE OOCYTE MATURATION

Autors: Claudia Fontanet, Andreu Blanquer, Elena Ibáñez

III. RESUM

The global increase in plastic production together with its non-biodegradability and poor waste management have led to massive accumulation of plastics in terrestrial and aquatic environments. Plastic fragments smaller than 5 mm (microplastics, MPLs) and 1 um (nanoplastics, NPLs) have been recently found in all environmental compartments, and in several human tissues and fluids. They have been classified as emergent pollutants due to their potential risks for human health. Among these risks, studies in laboratory rodents have reported adverse effects on the reproductive system of both females and males. However, the impact of MPLs and NPLs exposure on gametes, particularly on female gametes, remains largely unknown.

The present study aims to investigate the effects of NPLs on mouse oocyte maturation in vitro and on the quality of the matured oocytes obtained. Ovaries were collected from CD-1 female mice and follicles were punctured to release the immature oocytes. Cumulus-enclosed oocytes and non-enclosed oocytes were separately cultured in in vitro maturation medium. In the exposed groups, plain or fluorescent polystyrene microspheres (100 nm) were added. After 18 h of culture, cumulus cells were removed and oocyte maturation rates were determined. Oocytes exposed to fluorescent NPLs were fixed, stained with Texas Red-phalloidin, and examined under a confocal microscope to assess NPLs internalization. Oocytes exposed to plain NPLs were stained either with CellROX, to measure reactive oxygen species levels, with TMRE, to label active mitochondria, or fixed and processed for immunofluorescence detection of tubulin. Results of in vitro maturation rates, NPLs internalization, oxidative stress, mitochondrial activity, spindle organization and chromosome alignment in control and exposed oocytes will be presented.

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II. PÒSTER

Títol: Impact of nanoplastics on the zebrafish germ line

Autors: Gala Pujol, Laia Marín-Gual, Laura González-Rodelas, Alexander Goikoetxea, Anna Esquerrà, François Chauvigné, Egle Kelpsiene, Joan Cerdà, Nerea Roher, Mariana Teles, Aurora Ruiz-Herrera

III. RESUM

Pollution from nanoplastics (NPs) is a raising environmental concern whose impacts on biodiversity and human health are far from being understood. In the aquatic environment most species base their reproduction on external fertilization. Yet, the effect of NPs on reproduction is barely known. Gametogenesis is a tightly regulated process by which gametes (oocytes and spermatozoa) are produced by the consecution of two meiotic divisions. In the present study, we explore the consequences of NPs exposure in both female (oogenesis) and male (spermatogenesis) zebrafish germ line. To do so, we evaluated the effects of a short-term (96 h) exposure of zebrafish to differently surfaced charged engineered polystyrene NPs and mechanically broken-down high-density polyethylene (HDPE) NPs. We show that, in males, NPs induced an unusual histological distribution and clustering of germ cells within the testis. The histological effect of NPs resulted in an increased sperm clustering within the seminiferous tubules when compared to the control group, resulting in viable spermatozoa but with reduced motility. Moreover, in females we observed an alteration in oocyte stages frequencies during oogenesis, possibly reflecting alteration in oocyte growth. Overall, our results show that acute exposure to NPs had an effect at the histological levels, in both males and females, compromising their reproductive fitness.

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II. PÒSTER

Títol: Study of TRIP13 function in DNA repair

Autors: Cristina Madrid-Sandín, Andros Maldonado-Linares, Soonjoung Kim, Scott Keeney and Ignasi Roig

III. RESUM

TRIP13 protein is a member of the highly conserved family of proteins AAA+ ATPase whose overexpression is associated with tumor progression. TRIP13 is involved in numerous cellular processes, including regulation of the spindle assembly checkpoint (SAC), DNA damage repair, organization of the meiotic chromosome axis, and the meiotic silencing of the unsynapsed axis (MSUC). TRIP13 is the ortholog of non-vertebrate species Pch2, initially discovered based on its roles in checkpoint responses to meiotic recombination and chromosome pairing defects.
In general, AAA+ proteins use the energy of ATP hydrolysis to change their conformation, resulting in mechanical forces transduced to their substrates. TRIP13 monomers assemble in a hexameric ring which switches between “open” and “close” conformation to remodel their HORMA domain-containing substrates, such as SAC protein MAD2 or the Shieldin complex subunit REV7, which explains TRIP13 roles in the SAC and DNA repair.
We previously reported that Trip13 mutant spermatocytes displayed fewer RAD51 and RPA foci at the onset of meiosis. We have inactivated TRIP13 ATPase function in spermatocytes using genetic and pharmacologic approaches. Interestingly, Trip13 ATPase dead spermatocytes showed no defect in early RAD51 or RPA loading, suggesting that the TRIP13 ATPase function is dispensable to promote RAD51 loading. Moreover, these data indicate that TRIP13 could function as a scaffold protein bringing together proteins required to facilitate RAD51 loading into the chromatin. To elucidate any interactor protein that could help us uncover this unexpected TRIP13 role, we performed tandem mass-spectrometry (MS/MS) analysis of the Co-IP’ed proteins with TRIP13 from mouse testis. In our poster, we will discuss our findings and the candidate proteins we think to cooperate with TRIP13 to promote the loading of RAD51 into resected DSBs.

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II. PÒSTER

Títol: The role of SIRT7 in male meiosis and reproductive aging

Autors: Anna Guitart-Solanes, Mayra Romero, Cristina Madrid, Ignasi Roig, Karen Schindler, Alejandro Vaquero*, Berta N Vazquez*

III. RESUM

Sirtuins are NAD+-dependent deacetylases that play major roles in genome integrity maintenance, cell metabolism, and aging. Sirtuins are paramount regulators of fertility in males and females, being key in different stages of gametogenesis. In this line, we previously described that SIRT7 deficiency in mouse females results in homologous chromosomes synaptic defects, a decreased ovarian reserve, and an overall age-dependent decline in fertility. Interestingly, our most recent data also point to a relevant role for SIRT7 in male gametogenesis during aging, as SIRT7 levels in mouse testes dramatically decrease in old animals. Sirt7-/- males develop premature subfertility, evidenced by fewer offspring, histological defects in the testis, and a reduced testicular volume, as they age. Despite sperm counts being normal, comet assays in H2O2-challenged sperm revealed higher levels of DNA damage in the absence of SIRT7, suggestive of a diminished gamete quality. Analysis of epigenetic marks related to sirtuin activity and/or meiotic progression revealed an age-dependent downregulation of H3K9me3 in Sirt7-/- testes, that correlates with an aberrant distribution of the mark in pachytene spermatocytes. H3K9me3 is a chromatin mark with important implications in homologous chromosome synapsis in prophase I spermatocytes. Interestingly, we observed increased levels of H2AX in Sirt7-/- pachytene spermatocytes, indicative of impaired synapsis, which may impact meiotic progression. Altogether, our findings point to SIRT7 as an essential factor for the preservation of reproductive potential throughout the aging process. Future experiments will provide deeper insights into the precise molecular mechanisms underlying SIRT7 control of gametogenesis and reproductive aging.

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II. PÒSTER

Títol: Desenvolupament d'una metodologia per a l'aillament i cultiu de cèl·lules mare espermatogèniques de ratolí

Autors: Núria Garcia-Coll, Joan Blanco, Álvaro Pascual, Ester Anton, Zaida Sarrate* i Mireia Solé*

III. RESUM

Les cèl·lules mare espermatogèniques (SSC) són un tipus cel·lular indiferenciat localitzat a prop de la membrana basal dels túbuls seminífers amb capacitat d'autorenovació i diferenciació, podent iniciar el procés d'espermatogènesi.
La identificació, aïllament i cultiu d'aquestes cèl·lules és el primer pas cap a la seva aplicació en medicina reproductiva. Tot i així, els avenços en les tècniques d'aïllament de les SSC han estat limitats, principalment per la seva baixa presència en teixit testicular.
L'objectiu d'aquest estudi és optimitzar una metodologia d'aïllament de SSC mitjançant selecció cel·lular activada per fluorescència (FACS) i establir les condicions òptimes per al seu cultiu cel·lular. La metodologia consta de: (1) disgregació enzimàtica del teixit testicular; (2) immunodetecció d'espermatogonis diferenciats (cKIT) i indiferenciats (GFRa-1); (3) FACS; (4) comprovació de la puresa i viabilitat de la fracció seleccionada; (5) cultiu cel·lular.
S'han seleccionat fraccions pures d'espermatogonis cKIT+ i GFRa1+ que representen el 4,67% i 0,9% de la suspensió cel·lular obtinguda. Les cèl·lules aïllades mostraven una viabilitat superior al 70%. Es van establir cultius de 2.000-5.000 cèl·lules en un volum de 500µL de DMEM+KSR i s'han mantingut durant 4 setmanes. Les comprovacions de viabilitat cel·lular amb blau de tripà mostren que les cèl·lules que s'adhereixen a la superfície de la placa de cultiu són viables.
Aquests resultats indiquen que els espermatogonis seleccionats mantenen la seva viabilitat en cultiu in vitro, la qual cosa suggereix que tenen el potencial de créixer i formar colònies. El següent pas consistirà en obtenir una fracció d'espermatogonis GFRa-1+ i sembrar-la sobre cèl·lules nodridores (FSTO) en presència del factor GDNF, amb la finalitat de comprovar la capacitat de proliferació.
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II. PÒSTER

Títol: Multiwalled Carbon Nanotubes, a physical barrier to cell division.

Autors: Núria Pulido Artola, Marina Rodríguez Muñoz, Teresa Anglada Pons, Anna Genescà Garrigosa.

III. RESUM

Fibrous particles are extensively used in a wide variety of industries such as construction, textile or medical industries, nevertheless, at the nanoscale, fibers have been associated with acute toxicological effects. Asbestos is the best-known carcinogenic fiber, since it has been demonstrated that exposure to asbestos increases the risk of development of mesotheliomas and lung carcinomas. Fiber pathogenicity paradigm highlights thinness, length and biopersistence as the principal factors determining pathogenicity and recognizes fibers' geometry as their most important toxicological characteristic. In the last decades, fiber toxicology research has increased, and several types of synthetic and natural fibers have been classified as carcinogenic or probably carcinogenic for humans according to IARC.

Multiwalled carbon nanotubes (MWCNTs) are one of the most exploited nanomaterials nowadays, however, their fiber-like structure and their biopersistence in lung tissues raise potential concerns about possible adverse health effects. Various epidemiological and animal studies suggest that MWCNTs induce mesothelioma in a similar way to crocidolite asbestos. We here focused on the molecular mechanisms through which MWCNTs promote cell transformation features in vitro. Flow cytometry analysis of DNA content demonstrated an accumulation of polyploid cells upon division in cells treated with MWCNTs. We also observed that cell exposure to these fibers increases aberrant morphologies in mitotic spindles and leads to errors in chromosome segregation. Finally, we observed an increase in the frequency of micronuclei in the MWCNT samples when compared with non-treated. Thus, our preliminary results indicate that MWCNTs function as physical barriers for the mitotic machinery components disrupting cell division and promoting genome instability, a key feature of cancer progression.

I. PARTICIPANT **BC.14**

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II. PÒSTER

Títol: Identification of genetic markers associated to adenoma-adenocarcinoma in colorectal pT1 lesions

Autors: Irene Ruiz, Isabel Quintanilla, Luis Zapata, Elena Asensio, Sandra López-Prades, Antoni Castells, Stephan Ossowoski, Miriam Cuatrecasas and Jordi Camps

III. RESUM

Colorectal cancer is manifested after a gradation from healthy mucosa to a malignant tumor. This evolution is caused by genetic anomalies that accumulate over time. These are responsible for the development of an adenoma (AD), which can progress to adenocarcinoma (ADK). This transition occurs within the adenoma, which in its initial stages is called a pT1 polyp. In this study, we intend to determine which numerical chromosomal alterations are present in each region of the polyp. In addition, we would like to define the ploidy of these cells and evaluate their implication in carcinogenesis. Considering this, low-pass whole genome sequencing of both AD and ADK was performed to determine the CNA patterns associated with each state in paraffined pT1 colorectal polyps. We also carried out single-cell FISH analysis with probes that target candidate regions to be lost/gained and that may play a driver role in the transition from AD to ADK. Although overall genomic profiles were similar, some CNAs were identified to be specific of each state. For example, in one of the samples obtained from normal tissue, interphase nuclei presented 2 copies of cMYC (8q) and 2 copies of SMAD4 (18q). In contrast, in carcinoma, FISH probes revealed an increased number of copies of cMYC (3 copies/ nucleus) and a decrease in SMAD4 (1 copy/nucleus). As for the adenoma, while some nuclei showed the same ploidy as the healthy tissue, several cells were identified with the similar counts as to the carcinoma. Our results suggest that variations in CNAs could be driver events for the AD-ADK transition. In prospective experiments more samples will be analyzed with more probes for regions that, if altered, could be key in the development of colorectal cancer.

I. PARTICIPANT

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II. PÒSTER

Títol: Cryptic rearrangement in an apparently balanced translocation: the role of molecular cytogenetic techniques

Autors: Mar Xunclà, Natàlia Rey, Neus Castells, Alberto Plaja, Irene Valenzuela, Anna Maria Cueto, Pedro Antonio Martínez, María Serrano, Lourdes Trobo, Maria Àngels Rigola, Elena García-Arumí, Eduardo Tizzano

III. RESUM

Background: De novo chromosomal translocations are occasionally associated with chromosomal breakage-and-fusion processes. These rearrangements may be cryptic and more complex than initially suspected and may involve several chromosomal breaks that can lead to DNA loss or gain.

Methods: Classical and molecular cytogenetics (G band, subtelomeric FISH, chromosome painting, CGH-microarray, OMG).

Results: We present a 6-year-old girl with ASD and developmental delay. The CGH-array described a deletion at 12q21.33 and another at 13q33.3 that included the ATP2B1 gene. This deletion may explain the neurodevelopmental disorder. Patient's karyotype showed a translocation between chromosomes 12 and 13 with breakpoints difficult to determine by conventional cytogenetics, and did not match the CNV regions described in the array study.
FISH analysis with chromosomal painting for chromosomes 12 and 13 confirmed that only these two chromosomes were involved in the rearrangement, but the sizes of the translocated fragments indicated different breakpoints than those shown by the deletions. Subtelomeric FISH study confirmed the presence of the telomere from chromosome 13 translocated to chromosome 12.

Conclusions: The presence of several CNVs in the same patient may be due to complex processes of chromosomal breaks and reunions that may lead to duplications and deletions, as well as disruption of genes or regulatory regions. This case demonstrates the need to combine different cytogenomic techniques for the correct characterization of highly complex anomalies. The emerging technique of optical genome mapping can be useful in these highly complex rearrangements.

Grant References: Study partially financed by Carlos III Institute and the UE (PI20/01767).

PÒSTERS

Àrea de Fisiologia

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II. PÒSTER

Títol: Localization of the spinal cord neuronal networks controlling manual dexterity in rats

Autors: López-Santos D, Flores Á, García-Alías G.

III. RESUM

Manual dexterity, such as object “reaching and grasping” (R&G), is essential to conduct daily tasks. Stereotypic features of these movements suggest that some level of control may be exerted from the spinal cord (SC). In this project, we aimed to unveil the presence of these networks and their rostrocaudal location within the SC. The approach consisted of inflicting excitotoxic injuries to rats at different SC levels covering from C3 to T3 using kainic acid, for exclusively affecting spinal networks while preserving descending commands. Motor deficits were evaluated by comparing the performance, before vs after the intervention, in multiple behavioural tests of forelimb muscles’ function that likely require distinct neuronal networks. One-week post-intervention, C3-injured animals showed a significant impairment in R&G, staircase and horizontal ladder tests, but not on other tasks. The performance of rats receiving more caudal injuries remained grossly unaffected. Histological analysis revealed a grey matter loss that correlated with the segments of injection, and which presumably is accompanied by the loss of spinal premotor circuits. To dismiss the possibility of the concomitant loss of motoneurons and/or sensory feedback being the cause of the behavioural deficits observed, a subsequent experiment was conducted in which the C3 dorsal and ventral roots were sectioned, therefore eliminating all its direct afferences and efferences but preserving spinal networks’ integrity. Those animals did not suffer manual dexterity impairments. Our results suggest the presence, at spinal segment C3, of a neuronal network necessary for properly executing forelimb skilled movements.

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II. PÒSTER

Títol: The impact of prior severe stressors on contextual fear conditioning is markedly dependent on the type of stressors and the rat strain

Autors: Sara Serrano, Humberto Gagliano, Xavier Belda, Antonio Armario

III. RESUM

Exposure to severe stressors is considered a putative animal model of post-traumatic stress disorder (PTSD), with the potentiation of fear conditioning and/or impaired fear extinction as one of the main behavioural consequences. However, the results in the literature are highly variable, suggesting that the type of stimulus and the strain used might be relevant. Therefore, a context fear conditioning test with rats previously exposed to two severe stressful stimuli, immobilization on boards (IMO) and inescapable foot-shocks (IS), was performed. This impact was studied in Sprague-Dawley (SD) and Long Evans (LE) rats, where in the latter it has been observed that a pre-exposure to IS induces a lasting potentiation of fear conditioning.

Animals were exposed to IS, IMO, or to nothing in the case of the control group, and one week later, all groups performed a context fear conditioning test, consisting of an acquisition, test/extinction and extinction recall phases, and a generalization test after the acquisition phase. After analyzing in the previous phases the freezing time, the distance travelled and the rearings performed, it was observed that the effect of pre-exposure to a severe stimulus was basically absent in SD rats exposed to IMO or IS. However, a moderate effect of IMO and a very marked effect of IS were observed in LE rats, in terms of longer freezing time, and shorter distance travelled and rearings performed throughout all the studied phases. Also, in the analysis of the generalization, it was seen that the animals previously exposed to IS showed a longer freezing time, especially the LE rats.

Thus, the choice of strain and the stressful stimulus is critical to observe this phenomenon, LE rats being particularly sensitive. In addition, these results show a cognitive generalization of fear especially in rats previously exposed to IS.

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II. PÒSTER

Títol: IL-38 characterization in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis

Autors: Néstor López González, Andrea Vera Barrón, Jose Martínez Rodríguez, Rubèn López Vales

III. RESUM

Multiple Sclerosis (MS) is chronic inflammatory disease of the central nervous system with an increasing prevalence last years. Interleukin 38 (IL-38) is the most recently discovered cytokine of the IL-1 family with reported anti-inflammatory effects. However, little is known about the changes in IL-38 expression in MS.

Aims
In this work we aimed to investigate the changes in IL-38 levels in serum samples from MS patients, as well as, in the spinal cord of experimental autoimmune encephalomyelitis mice, a murine model of MS

Methods
Human Serum samples of patients with MS and healthy individuals were collected and IL-38 levels were by quantified by ELISA. Spinal cords from EAE mice were harvested at different stages of the disease and IL-38 levels were assessed by QPCR ELISA

Results
Despite IL-38 has potent anti-inflammatory actions, the protein levels of this cytokine were not increased in MS patients. Similarly, IL-38 transcripts were not detected in the spinal cord of EAE mice at the onset and peak of the disease. However, we observed that IL-38 mRNA levels were induced in the spinal cord of EAE mice at the remission phase of the disease, correlating with increased protein levels, coinciding with the attenuation of the neuroinflammatory response

Conclusions
These results suggest that IL-38 could be involved in attenuating inflammation in EAE mice, and that the induction of this cytokine is defective in MS patients.

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II. PÒSTER

Títol: Assessment of the therapeutic potential of MaR1 for the treatment of acute Spinal Cord Injury and characterization of its receptors in the lesioned spinal cord

Autors: Marc Caro-Cantón, Rubèn López-Vales

III. RESUM

Spinal cord injury (SCI) is a pathology of traumatic etiology that elicits an initial inflammatory response that remains unresolved. Although little is known about the factors that impedes the resolution of inflammation in SCI, we previously demonstrated that this is due, at least in part, to deficiency in the synthesis pathways that lead to the production of specialized pro-resolving lipid mediators (SPMs). These molecules interact with specific G protein coupled receptors (GPCR) to activate different programs that leads to the resolution of inflammation. We previously showed that Maresin 1 (MaR1), a SPM that harbors potent anti-inflammatory and pro-resolutive actions, enhanced inflammatory resolution and conferred protections against functional deficits and demyelination after acute SCI in mice when it was administered exogenously. Here, we aimed to optimize the MaR1 treatment protocol for the acute SCI by performing dose-ranging and therapeutic window studies, as well as in vivo toxicological assays. Furthermore, we also studied the dynamic changes of RNA expression of the two known MaR1 receptors, LGR6 and RORα, in the injured spinal cord in mice and characterized their cell source. This study will therefore contribute to enhance our knowledge of MaR1 for the treatment of neurotrauma.

I. PARTICIPANT **FIS.5**

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II. PÒSTER

Títol: Assessment of regenerative capacities of different peripheral neuronal populations after a nerve injury during the postnatal stage

Autors: Beatriu Molina, Aina Tudela, Sara Bolivar, Natalia Lago, Esther Udina

III. RESUM

Adult peripheral neurons retain regenerative ability after injury, in contrast to most neurons of the central nervous system, but unfortunately, functional outcome after severe nerve injuries is disappointing, in part due to the lack of specific reinnervation of target organs. Therefore, strategies aimed to improve and guide axonal regeneration are needed. Interestingly, the literature points that juvenile neurons possess a stronger regenerative capability and a more relevant preferential regeneration towards the correct target than mature ones. However, studies regarding regeneration at younger stages are scarce, probably due to the massive death of motoneurons induced by nerve injuries at the postnatal period.

The purpose of the current study was to fully characterize the response of three different types of paradigmatic peripheral neurons (motor, proprioceptive and one subtype of nociceptive neurons) to nerve injuries at the postnatal stages, by taking advantage of Cre-Drive TdTomato mice. Neuronal death and axonal regeneration of these three populations of neurons was evaluated after sciatic nerve crush at midthigh level in mice at postnatal stages and compared with juvenile animals.

In contrast to motoneuron, nerve injuries at p10 did not significantly induce loss of Trpv1-nociceptive and parvalbumin-proprioceptive neurons. 2 weeks after the injury, about 50% of nociceptive and 40% of proprioceptive neurons and its axons had regenerated 10 mm distal to the injury site, whereas the percentage was significantly lower in motoneurons. These results are probably due to the massive death of motoneurons in the postnatal stages. Further studies to fully characterize the regenerative response in the postnatal stages are ongoing.

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II. PÒSTER

Títol: Effects of a chronic exposure to the lipid regulator gemfibrozil in goldfish (Carassius auratus)

Autors: Manuel Blonç, Nuria Ruiz, Marta. Llorca, Marinel.la Farré, Asta Tvarijonaviciute Lluis Tort and Mariana Teles

III. RESUM

Lipid regulators, such as fibrates, are pharmaceuticals manufactured to treat dyslipidemias in humans. Their constant use and discard combined to their environmental persistence and poor removal rates from wastewater makes of these emergent contaminants ubiquitous in aquatic systems, with gemfibrozil being the most commonly detected fibrate in water. For this reason, the present study aimed to assess the effects of a 28-day waterborne exposure to both an environmentally relevant concentration (1.5µg/L) and a spiked concentration (15mg/L) of gemfibrozil in adult individuals of the model organism Carassius auratus. To this end, bioaccumulation of this compound in liver and muscle, as well as possible variations on haematological parameters, blood plasma biochemistry, and liver gene expression were investigated. The results indicated that, following exposure to the highest concentration of gemfibrozil, this compound accumulated in both liver and muscle. Similarly, significant differences were observed in haemoglobin levels, and mean corpuscular haemoglobin concentrations in individuals exposed to 15mg/L of gemfibrozil. The biochemical profiling of blood plasma revealed significant decreases of glucose and cortisol levels with exposure to gemfibrozil, as well as a significant increase in the ferric reducing ability of plasma (FRAP). Lastly, real-time qPCR analyses indicated significant upregulation of genes related to antioxidant defences (i.e. gpx, gst) and lipid metabolism (i.e. apoa1). Other genetic markers of lipid metabolism (i.e. pparβ and pparϒ) displayed an increasing trend with increasing gemfibrozil concentrations, although below the threshold for statistical significance. Overall, the results from the present study suggest that bioaccumulation of gemfibrozil in C. auratus alters, to some extent, the metabolism of lipids and triggers antioxidant mechanisms.

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II. PÒSTER

Títol: The sheep as an experimental model for peripheral nerve injury and regeneration studies

Autors: Estefanía Contreras, Sara Traserra, Félix García, Eduardo José-Cunilleras, Ignacio Delgado, Joaquim Forés, Esther Udina, Xavier Navarro, Patri Vergara

III. RESUM

Nerve injuries occur frequently affecting both humans and animals. Despite advances in microsurgery, an effective therapy that promotes regeneration of the injured peripheral nerve and allows full functional recovery has not been established, so the development of animal models remains necessary. The sheep model is considered a good translational option since it presents peripheral nerves with anatomical and physiological characteristics, similar to humans. However, there are no well-established protocols for the study of peripheral nerve injury and its regeneration in this species.

The aim was to establish a surgical protocol to perform lesions of varying severity on the peroneal nerve in sheep, to optimize the methodology of supervision, functional, ultrasound and electrophysiology tests and to perform histological and immunohistochemical techniques to evaluate nerve regeneration.

Peroneal nerve resection was performed under anaesthesia in 20 *ripollesa* sheep and it was repaired by an autograft. Monthly functional tests were performed to evaluate locomotion, proprioception, withdrawal reflex and muscle loss. Electrophysiological and ultrasound test were performed at 6.5 months and at the end of the follow-up, set at 9 months. Samples of the peroneal nerve, tibialis anterior muscle and skin were obtained for histological and immunohistochemical analyses.

Results showed the sheep is a good model for long-gap nerve injuries. No significant clinical signs were observed and animals were able to stand and walk and have good mobility. The functional tests allowed the evaluation of recovery and regeneration. Electrophysiological test allowed the evaluation of reinnervation and ultrasound allowed to measure muscle atrophy.

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II. PÒSTER

Títol: A Novel Gene Therapy Approach for ALS by Overexpressing the Pleiotropic Chronokine α-Klotho

Autors: Sergi Verdés, Mireia Herrando-Grabulosa, Rubén Guerrero-Yagüe, Núria Gaja-Capdevila, Joan Roig-Soriano, Judith Sauleda, Marc Leal-Julià, Andrea Onieva, Laura Rodriguez-Estevez, Javier del Rey, Neus Hernández, Miguel Chillón, Xavier Navarro, Assumpció Bosch

III. RESUM

In Amyotrophic lateral sclerosis (ALS), muscle denervation and degeneration of motoneurons (MNs) result in progressive muscle weakness and atrophy. Preventing axonal detachment from muscles, protecting MNs and promoting reinnervation are key to improve the functional outcome of ALS. α-Klotho is a pleiotropic chronokine with an excellent profile as neuroprotective and myoregenerative agent by means of anti-oxidative and anti-inflammatory properties, promoting myelination, protecting from excitotoxicity, and maintaining mitochondrial ultrastructure and function.

Given the pleiotropic beneficial properties of α-Klotho, we hypothesized that boosting the secretion of α-Klotho in skeletal muscles through a one-time gene therapy treatment would protect muscles from atrophy and prevent axonal retraction and neuronal loss in SOD1G93A mice. Secretion of α-Klotho by muscles, mediated by a myotropic AAV vector, enhances motor function and the strength of the animals and delays the onset of the disease. Neuromuscular functional improvement was reflected as increased compound muscle action potential (CMAP) amplitudes and by larger size and number of functional motor units of hindlimb muscles compared to mock-treated controls. α-Klotho-treated SOD1G93A mice show more surviving MNs and a significant reduction in neuroglial reactivity in the ventral horn of the spinal cord. Increased amplitude of the motor evoked potentials (MEPs) also indicates the preservation of central connectivity between upper and lower MNs. All this correlates with a higher number of innervated motor endplates and a preserved mass of the muscles.

Overall, our results provide evidence that the secretion of α-Klotho in muscles can promote functional improvement in ALS and may open a new avenue for the treatment of this devastating disorder.

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II. PÒSTER

Títol: Viral Nervous Necrosis Virus (VNNV) Inclusion Bodies: A Promising Vaccine Approach for Fish Populations

Autors: Garcia-Ordoñez, M; Aceituno, P; Rojas-Peña, M; Garcia-Martin, JC; N.Roher

III. RESUM

Nowadays, antiviral prophylactic tools against viruses have become essential in the aquaculture industry. The viral nervous necrosis virus (VNNV) is a major viral pathogen that infects a wide range of fish species such as seabass, seabream, and turbot. In this context, vaccination is one of the main methods for controlling and preventing viral diseases in aquaculture, and the development of novel approaches to vaccination is a major focus of fish vaccinology. We designed a new modular oral antiviral vaccine platform based nanostructured viral protein antigens prepared as inclusion bodies (IBs) presented as nanopellets (NPs). The use of NPs to develop vaccines provides a cost-effective and safe way to stimulate an immune response in fish. It has several advantages, such as low cost, high yield, and increased stability. Additionally, NPs are stable under different environmental conditions, making them easy to store and transport. Here, we present one versions of NPs formed by antigenic proteins from relevant virus affecting farmed fish as a proof-of-concept for oral administration: viral nervous necrosis virus (VNNV) coat protein combined with an effector molecule, the interferon gamma (IFNy). In the present study, we performed in vitro assays to characterize the morphology and functionality of modular VNNV-IFN and to explore its role in immune responses. As a proof of concept for oral delivery, we have verified that the NPs are successfully internalized by both sea bream Sab-1 and zebrafish ZFL cells. Encouragingly, analysis of gene expression suggests this NPs evoke an antiviral innate immune response in Sab-1 and ZFL cells. Summarizing, NPs has become a promising platform for the development of prophylactic aquafeed protection in seabream against VNN virus.

PÒSTERS

Àrea d'Immunologia

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II. PÒSTER

Títol: Influence of the proinflammatory environment on cell-based tolerogenic therapies in Multiple sclerosis

Autors: Federico Fondelli, Gerard Godoy, Jana Willemyns, Maria José Mansilla, Silvia Presas Rodriguez, Cristima Ramo Tello, Esteban Ballestar, Eva Martinez Caceres

III. RESUM

Multiple Sclerosis (MS) is an autoimmune disease affecting the central nervous system. Whilst different immunomodulatory treatments are available, a cure for MS doesn't exist. In this context, antigen-specific immunotherapies represent an approach to re-educate immunity toward homeostasis without immunosuppression. We developed an autologous tolerogenic dendritic cell product (VitD3-TolDCs) differentiated from blood monocytes, which showed safety in a Phase I clinical trial in patients with RRMS. However, given the effect MS-intrinsic inflammation, monocytes that we use as starting material could present an inflammatory phenotype in comparison to Healthy Donors (HD), leading to functionally less-than-ideal VitD3-TolDCs. Thus, we compared at a multiomic level monocytes and VitD3-TolDCs from MS patients and HD to identify pathways that could be modulated to produce stronger VitD3-TolDCs.
Methods: 18vs18 DNA monocyte and 7vs7 VitD3-TolDCs samples from naïve active MS patients and HD have been profiled via RNAseq and methylation arrays. Next, targeted gene expression was evaluated through qPCR. Finally, we evaluated the capability of VitD3-TolDCs to induce allogeneic PBMCs proliferation in Mixed Lymphocyte Reactions in comparison to HD.

Results: In MS monocytes we identified methylation changes and enrichment of specific transcription factors binding motifs in genes involved in immune activation. Moreover, RNAseq identified differences among monocytes and tolDCs from patients and controls.

Conclusions: MS monocytes present a more inflammatory phenotype in comparison to HD and when used to produce VitD3-tolDCs generate less powerful tolerogenic cells. Given the involvement of specific TFs in the gene signature of MS patients´ monocytes, we are exploring if these proteins could be targeted to increase the potency of tolDCs derived from MS patients.

I. PARTICIPANT

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II. PÒSTER

Títol: Platelets as possible biomarker candidates to differentiate between neurodegenerative disorders.

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III. RESUM

Background:
Alzheimer's disease (AD), Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are complex diseases that usually overlap in their neuropathological manifestations, which makes a challenge for clinicians to establish a correct clinical diagnosis and management of the disease. Nowadays the study of platelets in the context of neurodegenerative diseases is intensifying with increasing evidence of their role in the pathogenesis of neurodegenerative disorders.
The aim of this pilot study is to explore the interaction of platelets with other immune cells in neurodegenerative disorders.
Methods:
Twenty-three individuals, seven controls (CTRL), six AD, five PD, and five DLB patients, were included in this pilot study. The percentage of platelets attached to T lymphocytes (CD3+CD4+, CD3+CD8+), B lymphocytes (CD19+), monocytes (CD14+), and their activation grade were measured in peripheral blood by Flow cytometry.
Results:
Higher percentage of platelets attached to CD4+ and CD4+ CD25+ T lymphocytes were observed in PD compared to CTRL (p=0,030; 0,048) and DLB (p=0,031; 0,031). A similar result was obtained with CD19+ and CD19+ CD25+ B lymphocytes attached to platelets in PD compared to CTRL (p=0,030; 0,048) and DLB (p=0,015; 0,015). Moreover, the percentage of platelets attached to CD14+ monocytes was higher in PD compared to DLB (p=0,031) as well. The study of CD8+ and CD8+ CD25+ T lymphocyte subpopulation revealed a similar tendency but with no significant results.
Conclusions:
The percentage of platelets attached to B lymphocytes, CD4+T lymphocytes and monocytes could be a promising biomarker to discriminate between PD and DLB. Further studies with higher number of participants are needed to validate these results.

I. PARTICIPANT

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II. PÒSTER

Títol: Somatic revertant mosaicism correlating with clinical improvement in a patient with TNFRSF9 (CD137) deficiency.

Autors: Laura Batlle-Masó, Marina Garcia-Prat, Alba Parra-Martínez, Clara Franco-Jarava, Aina Aguilo-Cucurull, Jacques Riviere, Andrea Martin-Nalda, Pere Soler-Palacin, Ferran Casals, Montserrat Torrent, Laia Alsina, Roger Colobran

III. RESUM

Reversion mosaicism is a naturally occurring event involving a spontaneous correction of a pathogenic mutation in somatic cells. TNFRSF9 (CD137/4-1BB) deficiency is a recently described IEI characterized by lymphocytic defects with early-onset EBV-associated lymphoma.

We report a patient who at 12yo developed severe EBV-related hemophagocytic lymphohistiocytosis. No genetic defects were found at that time and she underwent HSCT from an HLA identical brother with good engraftment. In the following 8y, she presented recurrent EBV reactivations, lymphoproliferation and EBV-associated smooth muscle tumour despite full chimerism. At 21yo, she experienced a spontaneous decrease in EBV viral load and we started an in-depth genetic study of the case in total blood and specific cell populations.

Using whole exome sequencing we identified the homozygous stop-gain variant p.R244Ter in TNFRSF9. Strikingly, the HSCT donor was also homozygous for this variant but without overt clinical symptoms. Sanger sequencing results of the region pointed out a possible somatic reversion event. Using deep-amplicon sequencing we confirmed the presence of two independent somatic reversion events in the patient: a second-site mutation in the same codon (STOP to missense) and a “back mutation” (STOP to wild-type). The revertants were specifically located in CD8-T cells in which single-cell RNAseq experiments are ongoing.

We report the first case of reversion mosaicism in CD137 deficiency. This reversion is probably linked to the recent control of EBV viremia and is proof of principle that sets the ground for future gene therapy strategies in this IEI.

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II. PÒSTER

Títol: Detection and evolutionary dynamics of somatic FAS variants in autoimmune lymphoproliferative syndrome: diagnostic implications.

Autors: Laura Batlle-Masó, Marina Garcia-Prat, Alba Parra-Martínez, Clara Franco-Jarava, Aina Aguilo-Cucurull, Pablo Velasco, Maria Antolin, Jacques Riviere, Andrea Martin-Nalda, Pere Soler-Palacin, Monica Martinez-Gallo, Roger Colobran

III. RESUM

Somatic pathogenic variants at the FAS gene underly up to 20% of ALPS cases. These variants are restricted to double-negative alpha-beta T cells (DNT) which are elevated in ALPS patients but normalized under immunosuppressive treatment. Therefore, the identification of these somatic variants is a major challenge in patients under treatment and can delay the molecular diagnosis.

Here, we present a patient with early-onset ALPS in whom we identified a somatic pathogenic insertion (FAS:c.718_719insGTCG). For that, we used Sanger and deep amplicon sequencing (DAS) in CD3+ cells and peripheral blood. Moreover, we studied samples before and during the treatment with Sirolimus (across five years) to explore the detection limits of the technique and study the evolutionary dynamics of the somatic event.

The variant was first discovered in CD3+ enriched samples (RosetteSep™) by Sanger sequencing but was not detected in peripheral blood (7.4% DNT cells). However, using DAS, it was found in blood and CD3+ cells, even in low DNT counts (0.89%). In that scenario, we demonstrated that the variant allele frequency was doubled in CD3+ enriched samples (1.6% CD3+, 0.68% blood) and that there was an excellent correlation between DNT counts and the frequency of the variant (Pearson's R: 0.98).

Our results evidence that somatic variation is more likely to be detected on CD3+ enriched cells and pre-treatment samples but it can also be discovered in peripheral blood of patients under treatment. This highlights the success of sorting-free sequencing experiments and the importance of somatic studies in previously unsolved ALPS cases.

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II. PÒSTER

Títol: Characterization of HLA-DR immunopeptidome presented by dendritic cells pulsed with the breast cancer tumoral cell line MCF-7 lysates

Autors: Lazaro G, Aran A, Molina E, Carrascal M, Cedano J, Cortes J, Martí M

III. RESUM

The characterization of tumor antigens that drive the immune response has great potential as a tool to develop new treatments, especially antigen-derived therapies. Despite the importance of cytotoxic T cells, the CD4+ T cell response has gained interest due to its major contribution in the maintenance and orchestration of the anti-tumor immune response. Moreover, due to the epithelial etiology of carcinomas, the presentation of peptides by HLA-II is mediated by antigen presenting cells, mainly dendritic cells (DCs). Therefore, we have performed the characterization of the peptides presented by different HLA-DR molecules of monocyte-derived DCs (moDCs) from eight healthy donors. Six moDCs samples were pulsed with protein extracts from the breast cancer cell line MCF-7 and two samples of non-pulsed moDCs were used as controls.
The objectives of this study were: (i) to determine whether there are differences between the peptidome presented by pulsed and non-pulsed moDCs, both at peptide and protein levels; (ii) to determine the percentage of peptide and protein overlap between moDCs with different HLA-DR alleles; (iii) to evaluate the influence of the allele combination on antigenic presentation.
The results showed that: (i) the pulse displaces part of the proteins of the moDCs ligandome, since the non-pulsed samples share most of the proteins with the rest of the pulsed moDCs, but not the other way around; (ii) there is a positive correlation between the abundance of an allele in the samples studied and the level of peptide and protein overlap presented by them but not with the sample size; (iii) the immunopeptidome of the pulsed moDCs is modified by different HLA-DR alleles.
In conclusion, the HLA-DR allele combinatorics has some influence on the immunopeptidome presented by moDCs, which should be considered to better design personalized antigen-directed therapies.