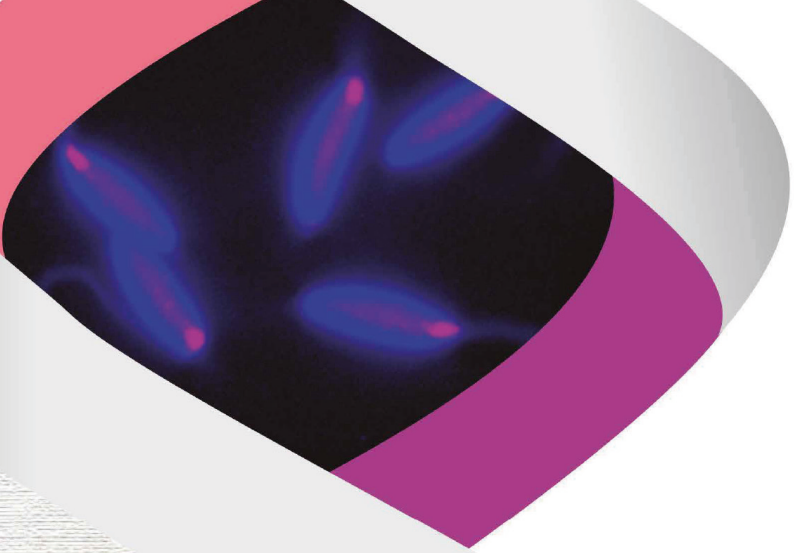

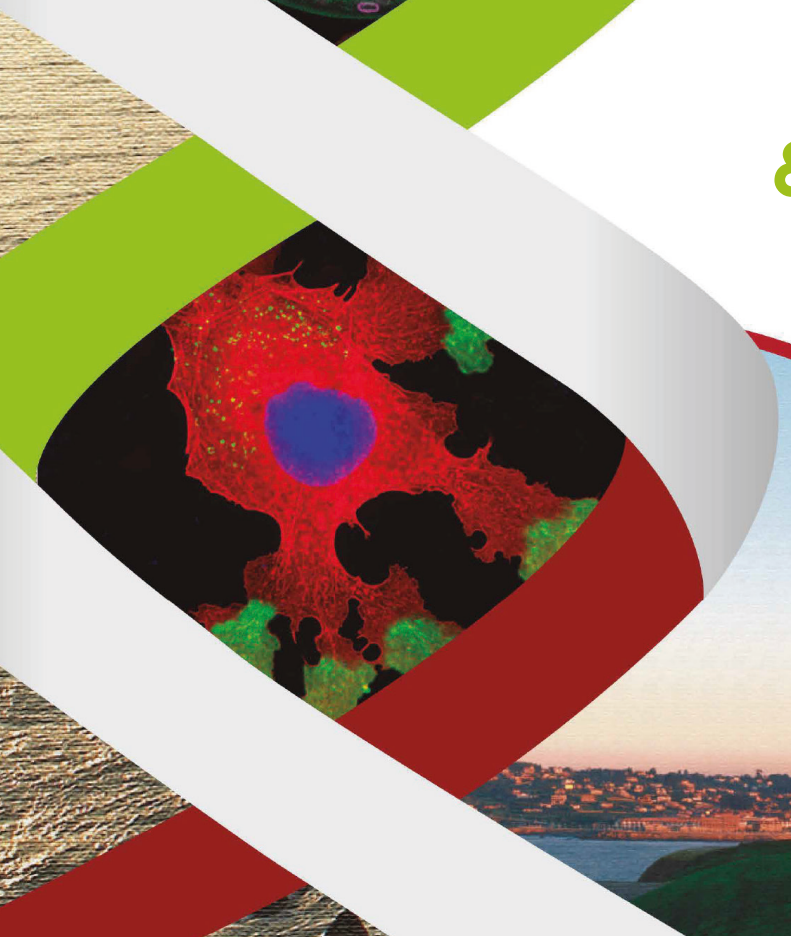

Joint Congress 2017

OCTOBER 24-27 th

GIJÓN
PALACIO DE CONGRESOS

PROGRAM & ABSTRACT BOOK

SOCIEDAD ESPAÑOLA DE BIOLOGÍA CELULAR
SPANISH SOCIETY FOR CELL BIOLOGY
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SEBD
SPANISH SOCIETY FOR DEVELOPMENTAL BIOLOGY

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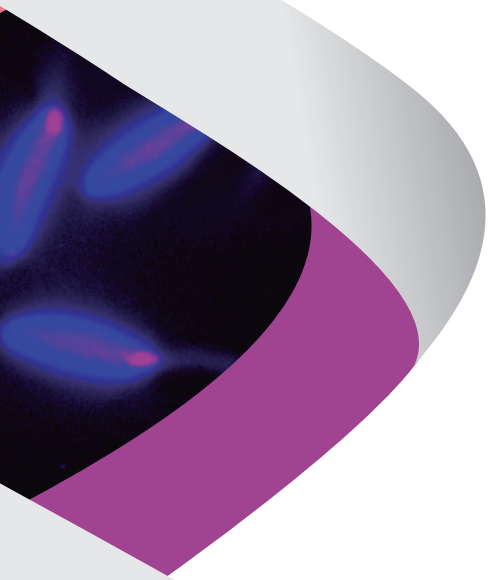
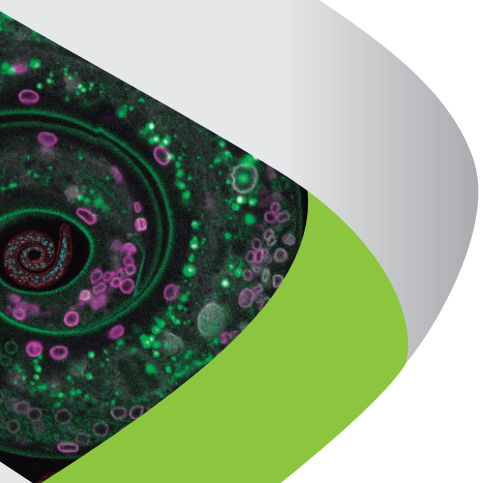
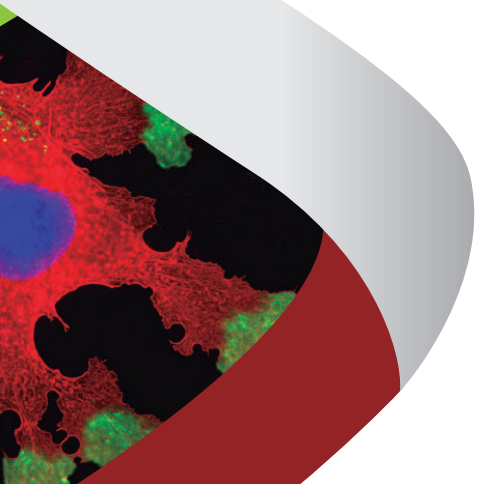
INDEX







Dear colleagues,

On behalf of the Spanish Societies of Genetics, Cell Biology and Developmental Biology, it is a great honour to open the call for this special meeting. This edition of the Congress is special because we join three societies with common and synergic interests to offer a unique scientific event that aims to foster the exchange of ideas between closely related fields of Biology. Creative science flourishes at the crossroads and young students need to have the opportunity to see and experience by themselves the benefits of looking beyond their daily realm of activities. As organizers of this joint congress, we are confident that new ideas and collaborative projects will emerge from this seed. In this spirit, we have composed an internationally open program with excellent speakers and topics to stimulate your attendance.

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VENUE

The congress will take place in the
PALACIO DE CONGRESOS DE GIJÓN
located at C/ Doctor Fleming, 481, 33203, Gijón, Spain.

Salón de Actos: 1st floor

Sala Anfiteatro: 1st floor

Sala Asturias: 1st floor

Sala Columnas: Ground floor

SCIENTIFIC PROGRAMME

Joint Congress 2017 | OCTOBER 24–27th | GIJÓN PALACIO DE CONGRESOS

	TUESDAY 24		WEDNESDAY 25	THURSDAY 26	FRIDAY 27
8,30 - 9,30	Registration				
9,30 - 11,00	ST I*	ST III***	S1 Autophagy, Stress and Cell Death	S3 Development by design	S5 Plant Biology
11,00 - 11,30	COFFEE BREAK				
11,30 - 13,00	ST II**		S2 Evolution	S4 Chromosome Mechanics Mónica Bettencourt - Dias (Instituto Gulbenkian, Oeiras, Portugal) "Centrosome regulation in development" "The EMBO Keynote Lecture" SPONSORED BY: EMBO excellence in life sciences	Closing Lecture Ginés Morata (Center for Molecular Biology "S. Ochoa"). CSIC-UAM. Madrid. "Cell competition" SPONSORED BY: fundación pryconsa
13,00 - 15,30			Posters + Lunch (odd number)	Posters + Lunch (even number)	Award Session
15,30 - 17,00	Junta SEG		SS1 Genomics of adaptation SS2 Inter-and intra-cellular Trafficking SS3 Pattern formation and differentiation	SS7 Regulation of gene expression SS8 Cytoskeleton and cell architecture SS9 ON GROWTH AND FORM Upcoming special edition of the journal "On Growth and Form – 100 years on" SPONSORED BY: Development	
17,00 - 18,30	Registration		SS4 Genetic basis of pathogenicity in fungi SS5 Cellular basis of pathology and aging SS6 Developmental genomics	SS10 Genome instability SS11 Cell signaling SS12 Tissue homeostasis and regeneration	Salón de Actos Sala Anfiteatro Sala Columnas Sala Asturias
18,30 - 19,00	Opening		Coffee or soft drinks	Coffee or soft drinks	
19,00 - 20,00	Opening Lecture Elly Tanaka Institute of Molecular Pathology "A molecular genetic approach to axolotl limb regeneration"		ISDB-MOD PLENARY LECTURE Arturo Álvarez – Buylla (University of California, San Francisco, USA) "Adult Neural Stem Cell Relay" SPONSORED BY: ISDB International Society of Developmental Biologists; Mechanisms of Development	Business SEG Business SEBC Business SEBD	*Satélite STI (SEG). Cambio climático. Estrategias genéticas para retos del futuro: alimentación, medio ambiente y cambio climático. **Satélite STII (SEG). Estrategias genéticas frente a las enfermedades raras. ***Satélite STIII (SEBC). Cell adhesion, migration and invasion.
20,00	Reception				
21,00				GALA DINNER EL LLAGAR DE CASTIELLO Camino de San Miguel, 807. Gijón, Asturias	

THURSDAY, OCTOBER 24th, 2017

OPENING LECTURE

Elly Tanaka (Institute of Molecular Pathology, Vienna, Austria)
"A molecular genetic approach to axolotl limb regeneration"

WEDNESDAY, OCTOBER 25th, 2017

S1 (SEBC) AUTOPHAGY, CELL STRESS AND CELL DEATH

Patricia Boya (Centro de Investigaciones Biológicas, CSIC, Madrid)
"Autophagy, metabolism and cell fate"

José Antonio Enríquez (Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain)
"Mitochondria define organismal metabolism"

The OXPHOS system is the only process in animal cells with components encoded by two genomes, mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). MtDNA is polyploid, maternally inherited, suffers marginal recombination and has a rate of mutation one order of magnitude higher than nDNA, thus allowing high variability of healthy mtDNA haplotypes. Nuclear OXPHOS gene diversity is achieved with a lower mutation rate and recombination and has alternative options due to allele variants and tissue-specific variants, which confront identical nDNA with diverse mtDNA within the same individual. This asymmetry leads to the physical match constraint: the fact that mtDNA-encoded proteins have to physically assemble with the nuclear-encoded ones to build the respiratory complexes. We found that diverse wild type mtDNA variants promote the generation of mitochondrial electron transport chains (mtETC) with differences in organismal metabolism affecting healthy ageing. We intend to understand the long range consequences of this observation. In particular, whether modifications in key nuclear genes involved in mitochondrial function modulate the impact of alternative mtDNAs, whether the co-existence of two wild-type mtDNAs in the same cytoplasm impact in metabolism, and what are the signals involved in this regulation. For this, we use conplastic mice (identical nucleus but interchanged mtDNA C57 vs. NZB), heteroplasmic mice (both mtDNAs in the same cell). Our recent analysis supports: (1) mtDNA variants induce functional OXPHOS differences under/in the same nuclear contexts. (2) Cells adapt their OXPHOS performance to generate healthy animals, regardless the nDNA/mtDNA match. (3) Different cell types show specific sensitivity to the nDNA/mtDNA match. (4) nDNA/mtDNA match induces significant metabolic differences that are overtly manifested in adulthood and dramatically impact on ageing and longevity.

Seamus J. Martin (Trinity College, Dublin, Ireland)
"Inflammatory outcomes of 'death receptor' activation"

Receptors for TRAIL and FasL have been dubbed 'death receptors' as these can receptors can act as potent initiators of apoptosis in many settings. As a consequence, 'death receptor' signaling has been studied almost exclusively in the context of cell death outcomes. However, TRAIL and FasL can also induce NFκB CSPα-dependent expression of multiple pro-inflammatory cytokines and chemokines, which may be exploited by certain cancers to recruit macrophages, neutrophils and myeloid-derived suppressor cells. We have recently found that TRAIL and Fas receptor

engagement leads to the assembly of an NFκB CSPα-activating 'FADDosome' complex where, rather surprisingly, caspase-8 plays a critical scaffold role in the process leading to inflammatory cytokine production. Thus, in addition to their well-known roles as a 'death ligands', TRAIL and FasL can promote inflammation in a variety of settings. Here I will discuss the contexts in which 'death receptors' promote inflammation rather than cell death.

S2 (SEG/SEBD) EVOLUTION

Abderraham Khila (Institut de Génomique Fonctionnelle, Lyon, France)
"Development, selection, and species diversification"

Patrick S. Fitze (Museo Nacional de Ciencias Naturales, Madrid, Spain)
"Drivers of sexual selection and consequences for the maintenance of genetic polymorphisms"

It is generally accepted that sexual selection importantly influences evolution and that it can produce rapid sympatric speciation. Theory suggests that the intensity of sexual selection and sexual conflict may affect population dynamics and thereby the persistence of species. In this talk I will focus on the determinants of sexual selection and sexual conflict, their consequences, and the role of sexual selection in maintaining genetic variation over time. To this end, I will introduce the basic theories and present results from population experiments on lizards allowing to disentangle among them.

David Posada (Universidad de Vigo, Pontevedra, Spain)
"Genomic evolution of intratumoral heterogeneity"

Cancer is an evolutionary process that results in ample intratumoral genomic heterogeneity (ITH), where multiple tumor regions display distinct genetic configurations. Multiple somatic evolutionary processes like mutation, drift, selection and/or migration are responsible for ITH to a different extent in distinct tumors. In this talk I will present our current progress in understanding how ITH is generated using population genomics and phylogenetic approaches on NGS data from multiple regions of the same tumor.

SS1. (SEG) GENOMICS OF ADAPTATION

Dra. Juliette de Meaux (University of Cologne, Germany)
"Genomics of adaptation in the Arabidopsis genus"

Dr. Antonio Barbadilla (Universidad Autónoma de Barcelona, Spain)
"Genome adaptation during the life cycle of Drosophila melanogaster"

From 30 to 50% of fixed non-synonymous mutations in D. melanogaster are estimated to be adaptive. Is this amount of molecular adaptation randomly distributed with respect to different phenotypic traits such as organs or morphological body parts? To address this question we have made a survey of selection acting on coding genes across Drosophila melanogaster embryonic anatomy. Our approach integrates genomic variation, spatial gene expression patterns and development to map adaptation over the entire embryo's anatomy. We have traced an adaptation map analyzing gene expression spatial information for 5,969 genes from text-based annotations

of in situ hybridization data directly from the BDGP database and polymorphism and divergence in these genes (from the project DGRP).
The proportion of non-synonymous substitutions that are adaptive, neutral or slightly deleterious were estimated for the set of genes expressed in each embryonic anatomical structure using a robust derivative of the McDonald and Kreitman test. We also explore whether different anatomical structures differ in the phylogenetic age, codon usage or expression bias of the genes they express and whether genes expressed in many anatomical structures show more adaptive substitutions than other genes.
We found that: (i) most of the digestive system and ectoderm-derived structures are under selective constraint (ii) the germ line and some specific mesoderm-derived structures show high rates of adaptive substitution (iii) the genes that are expressed in a small number of anatomical structures show higher expression bias, lower phylogenetic ages and less constraint.

GENOME-WIDE COMPARATIVE ANALYSIS OF ADAPTIVE AND PURIFYING SELECTION IN TWO HEPATITIS C VIRUS SUBTYPES

GONZÁLEZ-CANDELAS, FERNANDO*; PATIÑO-GALINDO JUAN ANGEL. *UNIVERSITY OF VALENCIA

RNA viruses are among the most important emerging pathogens and represent a very serious problem for public health. Lack of effective vaccines and a tremendous capacity to evade the immune system response and antiviral treatments reflect their high mutation and evolutionary rates. These responses lead to very fast adaptation to rapidly changing environments. Hepatitis C virus (HCV) has a ssRNA, positive-sense genome of almost 10 Kb and can cause chronic infection ultimately leading to hepatocarcinoma. About 170 million persons are estimated to be infected with HCV world-wide. Genotype 1 of HCV is the most prevalent variant of this virus. Its two main subtypes, HCV-1a and HCV-1b, are associated to differences in epidemic features and risk groups, despite sharing similar features in most biological properties. We have analyzed the impact of positive selection on the evolution of these variants using complete genome coding regions, and compared the levels of genetic variability and the distribution of positively selected sites. We have also compared the distributions of positively selected and conserved sites considering different factors such as RNA secondary structure, the presence of different epitopes (antibody, CD4 and CD8), and secondary protein structure. About 10% of the genome was found to be under positive selection, and purifying selection was the main evolutionary process acting in both subtypes. We found differences in the number of positively selected sites between subtypes in several genes (Core, HVR2 in E2, P7, helicase in NS3 and NS4a). Heterozygosity values in positively selected sites and the rate of non-synonymous substitutions were significantly larger in subtype HCV-1b. Logistic regression analyses revealed that similar selective forces act in both subtypes at the genome level: RNA secondary structure and CD4 T-cell epitopes are associated with conserved sites, while CD8 T-cell epitopes are associated with positive selection in both subtypes. These results indicate that similar selective constraints are acting along HCV-1a and HCV-1 b genomes, despite some differences in the distribution of positively selected sites at independent genes. Notably, most sites (around 2200, about 75% of the analyzed codons) in the HCV-1 genome are under strong purifying selection, with only a small fraction of codons evolving neutrally. This is one of the first comprehensive analyses of selection acting on complete viral genomes.

GENOMIC DETERMINANTS OF THE ADAPTIVE RADIATION OF THE GENUS DYSDERA IN CANARY ISLANDS

HINOJOSA, SILVIA*; ESCUER PAULA; FRÍAS-LÓPEZ CRISTINA; SÁNCHEZ-HERRERO JOSE; VIZUETA JOEL; ARNEDO MIQUEL A.; SÁNCHEZ-GRACIA ALEJANDRO; ROZAS JULIO. *DEPARTAMENT DE GENÈTICA, MICROBIOLOGIA I ESTADÍSTICA

Spiders compose the most diverse group of chelicerates (Arthropoda), including more than 45,000 described species that are the dominant predators in most terrestrial ecosystems. Using the adaptive radiation of the genus Dysdera in the Canary Islands as a case study, we investigate the genomic signatures associated with species diversification, including the specific ecological (dietary specialization) shift processes occurred in some of its members after speciation. In particular, we focused the study in five island endemic Dysdera species, two pairs of closely related generalist-specialist members of this genus, plus a generalist species as outgroup. For the study, we have sequenced the complete transcriptome (RNAseq) of these species, and have identified (in each species pair), several genomic changes associated with speciation, both at the gene expression, copy number and nucleotide (in coding sequences) levels. We propose a set of candidate genes that could be involved in dietary shifts, including genes associated to metabolism and heavy metal tolerance. Furthermore, and to better characterize the candidate gene regions from the transcriptome analysis and to identify new putative genomic signatures of the adaptive radiation, we have sequenced and assembled the complete genome of these five above-mentioned Dysdera species using different sequencing platforms and libraries.

THE GENETIC BASIS OF THE REPEATED EVOLUTION OF AN ADAPTIVE CHARACTER: HYPERTROPHIED LIPS OF CICHLIDS

MACHADO-SCHIAFFINO, GONZALO*; HENNING FREDERICO; KAUTT ANDREAS; TORRES-DOWDALL JULIAN; MEYER AXEL. *UNIVERSITY OF KONSTANZ, GERMANY

Despite the great and long-standing interest in the genomics of adaptation, little is known yet about the genetic mechanisms underlying parallel evolution. With the advent of genomics, it is becoming more feasible to determine the genetic basis of trait variation with certainty in non-model organisms.
The extraordinary convergence of colouration, body shape, and trophic morphologies, such as those found in cichlid fishes has led to the question of whether there are biases in the generation of phenotypic variation that direct adaptive evolution towards certain trajectories. One of the most notable cases of convergence among cichlid lineages is the evolution of hypertrophied lips. In a multidisciplinary approach, using QTL mapping, fine mapping, population genomics and feeding performance experiments we disentangle the genetic basis and the adaptive significance of hypertrophied lips in both African and Central American cichlid fish species.
Interestingly, we found that the genetic architecture of hypertrophied lips differs significantly between African and Neotropical cichlids, being much more polygenic in the former. Moreover, we assessed the role of disruptive selection during the early stages of divergence and found a functional trade-off in feeding behavior between thick- and thin-lipped ecotypes suggesting that this trait is a target of disruptive selection and could thus facilitate ecological speciation.

SS2. (SEBC) INTER – AND INTRA – CELLULAR TRAFFICKING

Andrés Alcover (Institut Pasteur, Paris, France)
"Interplay between intracellular traffic and cytoskeleton to control T cell activation"

María Mittelbrunn (Fundación de Investigación Hospital 12 de Octubre and Centro de Biología Molecular "Severo Ochoa", Madrid, Spain). Coauthors: DESDÍN-MICÓ GABRIELA; BAIXAULI FRANCESC.
"Mitochondria – Lysosomal Crosstalk in the Immune System"

To analyze how modulation of T cell metabolism and adaptive immune response contributes to the progression of age-associated human diseases, we have recently generated a mouse model in which mitochondrial function is compromised by genetic ablation of the mitochondrial transcription factor A (Tfam) specifically in T cells. Lack of mitochondrial transcription factor A (Tfam) in T lymphocytes induces a severe decrease in mtDNA content, and a failure to express the key components of the electron transport chain, promoting severe mitochondrial dysfunction, impaired oxidative phosphorylation (OXPHOS), and mitochondrial ATP production. Despite these mitochondrial abnormalities, Tfam-deficient T cells are viable, proliferate and rely on a metabolic program characterized by a glycolysis. By using this model, we have demonstrated a critical role for mitochondria in the regulation of T cell inflammatory responses by controlling lysosome function (Baixauli et al. Cell Metabolism 2015). Abnormal lysosomal function and sphingomyelin accumulation in Tfam-deficient cells linked for the first time a primary mitochondrial dysfunction to lysosomal storage disorders and exacerbated inflammatory responses. Our recent results extend these previous investigations and support a role for T cell metabolism in the regulation of overall organism homeostasis. Thus, CD4Tfam-/- mice show reduced lifespan, exhibit signs of premature aging, such as hyperkyphosis, reduced body weight and altered glucose homeostasis, and present an accelerated development of age-associate diseases.

MECHANISMS FOR EGFR SIGNALING REGULATION BY INTRACELLULAR TRAFFICKING IN GLIOBLASTOMA

PORTELA, MARTA*; SEGURA BERTA; JARABO-BLÁZQUEZ PATRICIA; HERNÁNDEZ-SANMIGUEL ESTHER; ARGUDO IRENE; SÁIZ ALMUDENA; GARGINI RICARDO; SÁNCHEZ-GÓMEZ PILAR; CASAS-TINTÓ SERGIO.
*CAJAL INSTITUTE CSIC, MADRID, SPAIN

The most frequent genetic lesions in glioblastoma (GBM) include EGFR which show constitutive kinase activity and Ras signaling to drive cellular proliferation and migration. We use a GMB model in Drosophila melanogaster based on the expression of constitutively active EGFR and PI3K in glial cells, this model reproduces the highly proliferative and invasive neoplastic cells that create transplantable tumor-like growths, mimicking human GBM. An in vivo genetic screen to identify new modulators of GBM progression was performed. As a result a member of the secretory pathway was identified: Kish. Downregulation of kish rescues glioma formation, proliferation, neurodegeneration, alterations of the circadian rhythms and viability of the animals. There is a kish human orthologue TMEM167A which is conserved. We found that TMEM167A is upregulated in human GBM samples and TMEM167A interference inhibits human glioma cells (U87) growth. Our aim is to understand how Kish/TMEM167A modulate EGFR signaling during its intracellular trafficking. The majority of EGFR signaling occurs at the plasma membrane, but it is known that activated EGFR-mediated signals continue from endosomes. With this goal we have measured EGFR accumulation in specific endosomes comparing GBM and GBM brain samples upon kish

downregulation. The results show that when the secretory pathway is altered by kish inhibition in a GBM, EGFR accumulates in early endosomes and lysosomes where they are targeted to degradation. Moreover, kish inhibition in GBM restored total EGFR protein to control levels. Finally, we performed a biased drug screen using a collection of compounds that affect vesicular transport, looking for a drug that is able to rescue the lethality induced by the GBM. We found an interesting candidate that we are currently analyzing. These and future results will provide basic information on the glioma mechanisms and may be key for the design of future therapeutic strategies against specific targets involving EGFR signaling.

SS3. (SEBD) PATTERN FORMATION AND DIFFERENTATION

Wieland Huttner (MPI for Molecular, Cell Biology and Genetics, Dresden, Germany)
"Neural stem and progenitor cells and neocortex expansion in development and evolution"

Our group studies neural stem and progenitor cells in the context of the expansion of the neocortex in development and evolution. Two major classes of cortical stem and progenitor cells (CSPCs) can be distinguished. First, CSPCs that reside in the ventricular zone (VZ), i.e. neuroepithelial cells, apical radial glia (aRG) and apical intermediate progenitors, collectively referred to as apical progenitors (APs). Second, CSPCs that reside in the subventricular zone (SVZ), i.e. basal radial glia (bRG) and basal intermediate progenitors, collectively referred to as basal progenitors (BPs). Neocortex expansion is thought to be linked to an increased abundance and proliferative capacity of BPs. To gain insight into the genomic changes that underlie neocortex expansion, notably in humans, we have analyzed the transcriptomes of human vs. mouse VZ and SVZ, and of human vs. mouse aRG and bRG. This led to the identification of the human-specific gene ARHGAP11B as a major player. Specifically, ARHGAP11B promotes the generation of BPs from aRG and the subsequent BP proliferation, thereby increasing BP abundance. Moreover, ARHGAP11B is able to induce folding of the embryonic mouse neocortex, which normally is smooth. The ability of ARHGAP11B to amplify BPs is based on a single C-to-G base substitution which creates a novel splice donor site; this leads to the removal of 55 nucleotides upon mRNA splicing, resulting in a reading frame shift and generating a human-specific 47-amino acid sequence that is thought to be key for BP amplification. Given that the mouse is secondarily lissencephalic, we searched for relicts of features of the gyrencephalic ancestror and found these in the mouse E18.5 medial neocortex. Specifically, the mouse medial neocortex exhibits an outer SVZ and a high abundance of bRG which express the human bRG-enriched marker Hopx. Disruption of Hopx expression in mouse medial neocortex reduces bRG abundance. Conversely, forced expression of Hopx in mouse lateral neocortex increases bRG abundance. Thus, Hopx is necessary and sufficient to achieve a gyrencephalic-like bRG abundance. To compare neural stem cell division between human and great ape developing neocortex, we have performed live imaging using iPSC-derived 3D cerebral organoids. This revealed a specific lengthening of metaphase during AP mitosis in human as compared to chimpanzee and orangutan.

Carlos Estella (Centro de Biología Molecular "Severo Ochoa", Madrid, Spain). Coauthors: REQUENA DAVID; ÁLVAREZ JOSÉ ANDRÉS; GABILONDO HUGO; LOKER RYAN; MANN RICHARD S.
"Molecular Logic of Appendage Formation"

The insect wing is a key evolutionary innovation that was essential for insect diversification. Yet despite their importance, there is still debate about their evolutionary origins. Two main hypotheses have been proposed: the paranotal hypothesis suggests that wings evolved as an extension of the dorsal thorax, while the gill-exite hypothesis proposes that wings were derived from a modification of a pre-existing branch at the dorsal base (subcoxa) of the leg. Here we address this question by studying how wing fates are initially specified during Drosophila embryogenesis, by characterizing a cis-regulatory module (CRM) from the snail (sna) gene, sna-DP (for dorsal primordia). sna-DP specifically marks the early primordia for both the wing and haltere, collectively referred to as the dorsal primordia. We found that the inputs that activate sna-DP are distinct from those that activate Distalless, an early marker for leg fates. Further, in genetic backgrounds in which the leg primordia are absent or ablated, the dorsal primordia are still partially specified. However, lineage-tracing experiments demonstrate that cells from the early leg primordia contribute to both leg and dorsal appendage fates. Together, these results suggest that the wings of Drosophila have a dual developmental origin: cells from both dorsal and ventral positions of the embryo, initially specified by distinct inputs, give rise to the mature wing. These developmental observations are consistent with a dual evolutionary origin of the wing, in which cells of the subcoxa of the leg coalesced with dorsal outgrowths to evolve a dorsal appendage with motor control.

PROPER DIFFERENTIATION OF INNER EAR NEURONS REQUIRES ADJACENT VASCULAR SIGNALS

ALSINA, BERTA*; TABERNER LAURA; BAÑÓN AITOR. *UNIVERSITAT POMPEU FABRA

The Statoacoustic ganglion (SAG) of the inner ear is composed by bipolar afferent neurons that transmit acoustic and vestibular information to their corresponding nuclei in the hindbrain. Until now, efforts have focused on the signals produced by the inner ear and the SAG itself to regulate SAG´s development. However, the influence of the vascular system surrounding the inner ear in neuronal development has poorly been studied. Since in the adult and developing brain, it has been shown that vascular signals regulate either the quiescence, proliferation and/or migration of neuronal progenitors, we investigated whether the head vasculature has an influence in cranial ganglia debvelopment. In particular we focused on the maturation and growth of the SAG. First, we have used different transgenic lines reporting the expression of markers for neurons (Tg[neurog1:dsRed] and Tg[neurod:GFP]) and vessels (Tg[Kdrl:ras-mCherry] and Tg[Kdrl:GFP]) to generate a precise 3D anatomical map of the neurovascular system of the inner ear at various stages (Taberner et al., submitted). Secondly, we have compared the size of progenitors and differentiated pools in wild-type and avascular (cloche mutant) embryos and found a strong reduction on the number of post-mitotic differentiated neurons (islet1-positive cells). Together, with a role of vasculature in SAG maturation, defects in axonal patterning to the sensory patches are also observed. Altogether, our results highlight for the first time the role of the vascular system in sensory neural differentiation and axon guidance in the otic system.

SIGNALING CROSSTALK BETWEEN EPITHELIAL CELLS AND THE STROMA IN TUBE FORMATION

MARTIN BELMONTE, FERNANDO*. *CBMSO

Epithelial organs require an exquisite control of cell proliferation and differentiation in order to achieve their final form and function during development, which depends on the regulation of polarity proteins and signaling receptors such as Frizzled and Notch. The general aim of our research is to characterize new genes involved in epithelial morphogenesis, patterning and regeneration, and to further understand their function and molecular mechanism using 3D cultures, and in vivo models. I will discuss our more recent unpublished work focused in the role of Frzb, a regulator of Wnt signaling and matrix metalloproteinases, in the normal formation of the branched epithelia ducts of mouse mammary glands. Frzb is expressed by the stromal cells of the fat pad and negatively regulates proliferation and the branched development of the epithelial ducts.

SS4. (SEG) GENETIC BASIS OF PATHOGENICITY IN FUNGI

Eduardo Espeso (Centro Investigaciones Biológicas, CSIC, Madrid, Spain)
"Role of alkaline pH responsive Slt pathway in fungal virulence"

Filamentous fungi populate a wide range of environments, counting soil, plants, animals and human hosts. They are also capable of growing in extreme environmental niches and fungi survive to various forms of stresses including osmotic stress, oxidative stress, nutrient deprivation, changes in pH and heat shock. Fungi have developed sophisticated mechanisms to alleviate the extracellular stress associated with these harsh conditions.
We have determined that three regulatory systems coexist in the fungal model Aspergillus nidulans and other filamentous fungi to provide with adequate response to ambient alkaline pH and excess of cations. The calcineurin-dependent transcription factor CrzA is required for growth at alkaline pH and in conditions of high extracellular calcium concentrations. PacC is the well known regulator of ambient pH signaling and SltA is required for tolerance to high alkali-cation concentrations and to alkalinity. CrzA and PacC homologues are present in almost all known fungal genomes, however SltA homologues can only be found in species of Pezizomycotina subphylum. All these transcription factors are subjected to posttranslational modifications for modulating their activities. Particularly PacC and SltA are subjected an irreversible PTM modification, the regulated proteolysis. Here we present our analysis of the Slt regulatory system in a phytopathogenic fungi. Colletotrichum gloeosporioides SltA homologue was identified and conservation of signaling pathway element SltB reveal functional conservation of PTMs. We have determined the role of CgSltA in tolerance to cations, development of appressorium and virulence was studied. Our results indicate a major role of CgSltA in the formation of lesions in avocado and mango fruit.

Michael Thon (University of Salamanca, Spain). Coauthors: ARMIJOS JARAMILLO VINICIO; SANZ MARTÍN JOSE; SUKNO SERENELLA.
"Horizontal gene transfer contributes to virulence in a plant pathogenic fungus"

Horizontal gene transfer (HGT) is the stable transmission of genetic material between organisms by means other than vertical inheritance. HGT has an important role in the evolution of prokaryotes but is relatively rare in eukaryotes. We studied the importance of HGT in plant pathogenic fungi by identifying horizontally transferred genes in the genomes of members of

the genus Colletotrichum. We identified eleven HGT events from bacteria and one from plants to Colletotrichum spp. or their ancestors. The horizontally transferred genes from bacteria encode proteins involved in amino acid, lipid and sugar metabolism as well as lytic enzymes. Four of the eleven genes have homology to known virulence factors, suggesting that HGT may be important for niche adaptation and virulence. The putative minimal dates of the HGT events were calculated using a time calibrated phylogenetic tree, revealing a constant flux of genes from bacteria to fungi throughout the evolution of subphylum Pezizomycotina. HGT appears to be a constant, albeit rare phenomenon in the Pezizomycotina, occurring at a steady rate during their evolution. The gene acquired from plants encodes a protease which we call CPLS (Colletotrichum plant-like serine protease). Transcriptional profiling and pathogenicity assays of CPLS null mutants show that CPLS is expressed at the early stages of pathogenesis and has a role in virulence. Together, our results suggest that HGT is an important evolutionary process in fungi that contributes to the evolution of plant pathogens.

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"Investigating appressorium-mediated plant infection by the rice blast fungus Magnaporthe oryzae"

To cause rice blast disease, the fungal pathogen Magnaporthe oryzae develops a specialised infection structure called an appressorium. The appressorium is a single dome-shaped cell that accumulates internal turgor pressure that is translated into mechanical force to breach the cuticle of the leaf by the reorientation of a septin-dependent F-actin cytoskeleton at the base of the infection cell. The appressorium produces a rigid and narrow penetration peg that ruptures the tough and waxy leaf cuticle to allow colonization of the plant tissue. In this work, we show that appressorium mediated plant infection by M. oryzae is tightly linked with cell cycle control and more specifically, requires two independent S-phase cell cycle checkpoints. The first checkpoint regulates the formation of appressoria on the rice leaf surface and acts through the DNA damage response (DDR) pathway, involving the Cds1 kinase. Appressorium repolarization and plant infection however, involves a novel, DDR-independent S-phase checkpoint, triggered by appressorium turgor generation and melanisation. This second S-phase checkpoint regulates septin-dependent, NADPH oxidase-regulated F-actin dynamics to organise the appressorium pore and facilitate the entry of the fungus into the plant cell. We show that a minimum turgor threshold in the appressorium depends on melanin production and it is necessary to trigger this unusual S-phase cell cycle checkpoint necessary for the appressorium to function properly.

EXPERIMENTAL EVOLUTION IN THE FUNGAL PATHOGEN FUSARIUM OXYSPORUM TO STUDY MECHANISMS OF GENOME PLASTICITY AND HOST ADAPTATION.

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Filamentous plant pathogens undergo rapid evolution, leading to shifts or expansions in host range. The Fusarium oxysporum species complex collectively causes vascular wilt disease in more than a hundred different crops, provoking devastating losses in global agriculture. The evolutionary mechanisms underlying host adaptation and host range dynamics in this pathogen remain poorly understood. Here we followed an experimental evolution approach, involving serial passages of the tomato pathogenic isolate Fol 42-87, either through plants or artificial media

plates. Independently evolved populations obtained after ten consecutive passages displayed notable phenotypic differences with respect to the initial clonal isolate, including alterations in growth, sporulation and virulence. Strikingly, four of the five plate-passaged populations had significantly reduced virulence on tomato plants. Resequencing of the evolved populations revealed the presence of segmental duplications and deletions, particularly in the transposon-rich lineage-specific regions of the genome. In addition, single nucleotide changes and small Indels were detected in the evolved lines, some of which affected genes with known functions in fungal development and virulence. Collectively, our findings suggest that chromosome plasticity acts as a major evolutionary driver in F. oxysporum, and provide new insights into the genetic mechanisms underlying host adaptation in this important fungal pathogen.
This research was financially supported by the Project BES-2014-070450 (Ministerio de Economía y Competitividad, MINECO, Spain) and Burroughs Welcome Investigator Award.

AN RNAI-BASED FUNCTIONAL GENOMICS PLATFORM TO IDENTIFY NEW VIRULENCE DETERMINANTS IN MUCORMYCOSIS

NICOLÁS MOLINA, FRANCISCO ESTEBAN*; NAVARRO-MENDOZA MARÍA I.; PÉREZ-ARQUES CARLOS; MURCIA-FLORES LAURA; MARTÍNEZ-GARCIA PABLO; LAX CARLOS; LÓPEZ-GARCIA SERGIO; PÉREZ-RUIZ JOSE A.; NAVARRO EUSEBIO; GARRE VICTORIANO. *UVERSIDAD DE MURCIA

Mucorales are an emerging group of human pathogens that are responsible for the lethal disease mucormycosis. Unfortunately, functional studies on the genetic factors behind the virulence of these organisms are hampered by their limited genetic tractability, since they are reluctant to classical genetic tools like transposable elements or gene mapping. In this work, we describe an RNAi-based functional genomic platform that allows the identification of new virulence factors through a forward genetic approach firstly described in Mucorales. This platform contains a whole-genome collection of Mucor circinelloides silenced transformants that presented a broad assortment of phenotypes related to the main physiological processes in fungi, including virulence, hyphae morphology, mycelial and yeast growth, carotenogenesis and asexual sporulation. Selection of transformants with reduced virulence allowed the identification of mcplD, which encodes a Phospholipase D, and mcmyo5, encoding a probably essential cargo transporter of the Myosin V family, as required for a fully virulent phenotype of M. circinelloides. Knock-out mutants for those genes showed reduced virulence in both Galleria mellonella and Mus musculus models, probably due to a delayed germination and polarized growth within macrophages. This study provides a robust approach to study virulence in Mucorales and as a proof of concept identified new virulence determinants in M. circinelloides that could represent promising targets for future antifungal therapies.

SS5. (SEBC) CELLULAR BASIS OF PATHOLOGY AND AGING

Lola Ledesma (Centro de Biología Molecular "Severo Ochoa", Madrid, Spain). Coauthors: PÉREZ-CAÑAMÁS AZUCENA; GABANDÉ-RODRÍGUEZ ENRIQUE; SOTO-HUELIN BEATRIZ.
"Sphingomyelin induced neuronal and microglia pathology in the lysosomal storage disorder Niemann Pick type A"

BACKGROUND: Niemann Pick disease type A (NPA) is an inherited lysosomal storage disorder caused by loss of function mutations in the gene encoding for the acid sphingomyelinase (ASM). Sphingomyelin (SM) accumulates in the cells of NPA patients and ASMko mice, which mimic the disease, leading to motor and cognitive impairment, neurodegeneration and early death.

RESULTS: We have characterized brain cell anomalies in ASMko mice and NPA patients. Besides previously reported alterations in synaptic function and autophagy we will discuss recently observed anomalies in neuronal calcium homeostasis and microglia activation. We show that high SM levels impair the activity of the plasma membrane calcium ATPase (PMCA) leading to increased intracellular calcium in neurons. SM accumulation also corrupts the protective function of M2 microglia leading to lysosomal exocytosis, Cathepsin B release and neurotoxicity. We will also present pharmacological and genetic strategies to counteract these anomalies.
CONCLUSIONS: Our results unveil the relevant contribution of SM to neuron and microglia physiology by influencing synaptic function, autophagy, calcium homeostasis and inflammation. These findings open therapeutic perspectives for a currently untreatable disease such as NPA.

Mario Fraga (CINN – CSIC, IUOPA, Oviedo, Spain). Coauthors: FERNÁNDEZ RAÚL; TEJEDOR JUAN RAMÓN; BAYÓN GUSTAVO F; FERNANDEZ AGUSTÍN F.
"DNA methylation changes during aging and cancer: similar but not identical"

Cancer is an aging-associated pathology, but the underlying molecular link is still poorly understood. Recent genome wide studies have identified gene promoters that share a common chromatin signature in ES cells which become frequently hypermethylated in both aging and cancer. In addition, there is also a global DNA hypomethylation in aging and cancer. However, it is unknown whether the loss of DNA methylation occurs at similar DNA regions in both aging and cancer, or whether these regions share a common chromatin signature. To address this issue, we obtained DNA methylation data from control and cancer samples and identified several thousands of CpG sites differentially methylated in aging and cancer. A systematic analysis of the principal histone modifications from the ENCODE project confirmed that, in general, hypermethylated genes in aging and cancer share a similar repressive chromatin signature. In contrast, hypomethylated DNA sequences in cancer and aging occurred in very different chromatin contexts: in aging, hypomethylated sequences were enriched at DNA regions marked with the activating histone posttranslational modification H3K4me1, whilst in cancer, they were primarily associated with H3K9me3. Our results suggest that the role of DNA methylation as a molecular link between aging and cancer is more complex than previously thought.

DECIPHERING THE ROLE OF TMPRSS4 IN LUNG CANCER REVEALS BIOLOGICAL CHANGES IN REPLICATION AND MIGRATION LEADING TO TUMOR GROWTH AND METASTASIS.

EXPÓSITO, FRANCISCO*; VILLALBA MARÍA ; REDRADO MIRIAM; DE ANDREA CARLOS; VICENT SILVESTRE; L DE ABERASTURI ARRATE; PIO RUBÉN; CALVO ALFONSO. *UNIVERSIDAD DE NAVARRA

Proteases have been involved in promotion of cancer cell growth and metastasis. We have previously identified the serine protease TMPRSS4 as a protein overexpressed in NSCLC that promotes tumor development. In order to investigate molecular mechanisms elicited by TMPRSS4 in lung cancer we have developed tetracyclin-inducible shRNA and overexpression vectors to modify endogenous levels of this protein in lung cancer cell lines. Overexpression in LKR13 cells led to increased clonogenicity, migration and subcutaneous tumor growth. In a model of multiorganic metastasis, TMPRSS4 induced liver, bone and suprarenal colonization. In knock-down experiments, abrogation of TMPRSS4 in H358 and H2170 cell lines impaired migration and caused a very strong reduction in proliferation, clonocenicity and subcutaneous tumor growth. Tumor engraftment was abruptly reduced in mice injected with cells lacking TMPRSS4. Cell cycle

analysis showed reduction in S and G2/M phases and increased in cell death (as evaluated by annexin-V/sytox analysis). Microarray expression analysis in knock-down cell clones revealed changes in 136 genes (64 up-regulated and 72 down-regulated). Gene Ontology, STRING and Ingenuity pathways showed that most genes belonged to cell replication and migration/motility categories. A set of down-regulated genes was associated with the replisome and nucleotide availability, including MCM3, MCM6, primase, E2F1 and cyclin E, suggesting and impairment in DNA replication. E2F1 was identified bioinformatically as an upstream regulation of 12 target down-regulated genes, one of which was thymidilate synthase, a gene involved in sensitivity to fluoropirimidine-based and antifolate chemotherapy. Our results show that TMPRSS4 plays an important role in lung cancer growth and metastasis and suggest that its blockade may enhance sensitivity to chemotherapy.

RELEVANCE OF THE INSULIN-DEPENDENT GLUT4 TRANSPORTER IN PROSTATE CANCER PROGRESSION

GONZALEZ-MENENDEZ, PEDRO*; HEVIA DAVID; CERNUDA-CERNUDA RAFAEL; ALVAREZ-MUJICA MIGUEL; SAINZ ROSA Mª. *INSTITUO DE UNIVERSITARIO DE ONCOLOGIA DEL PRINCIPADO DE ASTURIAS/DEPARTAMENTO DE MORFOLOGIA Y BIOLOGIA CELULAR

The overexpression and membrane translocation of GLUT transporters are associated with an increase in glucose uptake in cancer cells, which is considered a hallmark in their pluripotency. Among the 12 members, GLUT1 is the main responsible for most of the tumors but others can be involved. Recently, our group described for the first time the production of the insulin-dependent GLUT4 in prostate cancer cells, which might be connected with the important role of insulin in this carcinoma. Thus, this study aimed to find evidence about the participation of GLUT4 in PCa progression. For that, androgen-sensitive LNCaP and androgen-insensitive PC-3 cells and TRAMP (Transgenic Adenocarcinoma of Mouse Prostate) mice were employed. First, we found that insulin stimulation increased glucose uptake significantly in androgen-insensitive PC-3 cells. In addition, the overexpression of GLUT4 in androgen-sensitive LNCaP cells reduced nuclear levels of androgen receptor (AR) and showed a higher PSA release, both markers of the castration-resistant phenotype. In vivo, there was a direct association between GLUT4 levels and prostate cancer progression in TRAMP model along with androgen-insensitivity. After castration, GLUT4 protein levels decreased, and treatment with testosterone restored GLUT4 activity. Finally, these results were confirmed in human samples. Patients treated with insulin showed an increase of GLUT4 production and an increment of AR translocation in prostate cancer. Moreover, the treatment with the antidiabetic drug metformin decreased GLUT4 and AR production. In conclusion, our work confirms that GLUT4 is androgen-independent regulated in prostate cancer and might be related to the inverse relation with diabetes.

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A NEW GENETIC MODEL OF DYSTROGLYCANOPATHIES IN THE RETINA: THE CRX-CRE+/POMT1 CONDITIONAL KO MOUSE

MARTÍN NIETO, JOSÉ*; URIBE MARY LUZ; RUBIO FERNÁNDEZ MARCOS; VICENTE TEJEDOR JAVIER; GERMAIN FRANCISCO; SUSÍN LARA CRISTINA; MONTOLIU LLUIS; DE LA VILLA PEDRO; CRUCES JESÚS. *DEPARTAMENTO DE FISIOLOGÍA, GENÉTICA Y MICROBIOLOGÍA, FACULTAD DE CIENCIAS, UNIVERSIDAD DE ALICANTE.

Dystroglycanopathies (DGP) constitute a heterogeneous group of recessive congenital neuromuscular diseases coursing with different grades of severity of muscular impairment, brain malformation and ocular abnormalities. These include retinal malformations, vitreoretinal dysgenesis, optic nerve hypoplasia and blindness. The main DGP-underlying cause is a deficiency in the process of glycosylation (mainly O-mannosylation) of dystroglycan. This is a key component of the dystrophin-glycoprotein complex in muscular and CNS tissues, acting as a nexus between the extracellular matrix (ECM) and the cytoskeleton and composed of two subunits: a-DG (extracellular) and ß-DG (transmembrane). a-DG is a heavily glycosylated protein with the potential to bind, by virtue of its O-mannosyl glycans, to a large number of ECM proteins, including laminin and pikachurin, the latter exclusive of the retina. Protein O-mannosyltransferase 1 (POMT1) is the first enzyme to act in the O-mannosylation of a-DG. However, the molecular basis of the retinal involvement of POMT1-associated DGPs has remained elusive. In order to address this issue, we have generated a conditional knockout (cKO) mouse experiencing an internal Pomt1 deletion during retinal development, mediated by the Cre recombinase expressed from the photoreceptor-specific Crx gene promoter. In this mouse, retinal a-DG was found to be unglycosylated and incapable of binding laminin. Immunohistochemical analyses revealed the absence of ß-DG and pikachurin in the outer plexiform layer (OPL) of the retina. This was accompanied by a misalignment of presynaptic terminals and shortening of photoreceptor axons, together with sprouting of postsynaptic dendrites into the outer nuclear layer. These deficits correlated with significant alterations observed at the ultrastructural level in mitochondrial morphology and ribbon synapses established between photoreceptors and their postsynaptic retinal neurons. At the functional level, retinal POMT1 deficiency caused a remarkable impairment of both electroretinographic recordings (b-wave reduced amplitude and increased implicit time) and optokinetic reflex in Pomt1 cKO mice. Our results allow to conclude that O-mannosylation of a-DG in the retina carried out by POMT1 is crucial for the establishment of proper synapses at the OPL and the transmission of visual information from cones and rods to their postsynaptic neurons. Its mutational loss thereby mimics a good part of the retinal pathology found in DGP-suffering patients Funding: Instituto de Salud Carlos III grants PI15/00073 and PI13/02098, RETICS RD12/0034/0006, and Comunidad de Madrid ‘VISIONANIMAL’ Biomedicine project S2010/BMD2439, all of them cofinanced by the European Regional Development Fund (ERDF/FEDER).

SS6. (SEBD) DEVELOPMENTAL GENOMICS

Eileen Furlong (EMBL, Heidelberg, Germany)
"Generating robustness and precision in development programs"

Jorge Ferrer (Imperial College London, UK)
"Noncoding genome function in pancreas development and diabetes"

EXPLORING THE ROLE OF NUCLEAR ORGANIZATION IN ZEBRAFISH DEVELOPMENT WITH HICHIP USING HISTONE MARK ANTIBODIES

DOMÍNGUEZ ACEMEL, RAFAEL*; SANTOS-PEREIRA JOSÉ MARÍA; NARANJO SILVIA; TENA JUAN JESÚS; GÓMEZ-SKARMETA JOSÉ LUIS. *CENTRO ANDALUZ DE BIOLOGÍA DEL DESARROLLO CSIC/UPO, SEVILLA, SPAIN

Animal development requires the precise transciptional regulation of thousands of genes in every cell of the embryo. Interestingly, transcriptional regulation in metazoans seems to be heavily influenced by the 3D organization of the genome in several ways. On the one side, active and inactive chromatin regions tend to cluster together in the nucleus forming the so called A and B compartments. Some developmental genes are known to switch from one compartment to another while getting activated or repressed. On the other side, developmental genes are often controlled by large sets of enhancers that activate them in a spatio-temporal specific manner during embryogenesis. In order to regulate the transcription from a promoter, enhancers need to be close to them. Specific 3D structures named chromatin loops can facilitate the interaction in space between enhancers and their target promoters, even though they might lay far apart in the linear sequence. In the same way, undesired interactions between enhancers and promoters can also be prevented at the structural level. Chromatin conformation capture techniques, such as HiC, have been key to understand these roles of the 3D configuration of the genome in gene regulation. In short, HiC experiments provide the frequency of interactions between every two pair of loci in the genome. It has been shown recently that by coupling the regular HiC protocol with a step of chromatin selection with a given antibody it is possible to sample only those interactions involving a protein of interest, such as CTCF or cohesin. In this work, we perform HiChIP experiments in 24hpf zebrafish embryos using antibodies against three histone marks: H3K4me3 (marking active promoters), H3K27ac (marking active promoters and enhancers), and H3K27me3 (marking polycomb repression) reaching 5kb resolution with a moderate sequencing effort. We provide evidence that the HiChIP experiments using H3K4me3 and H3K27ac antibodies are sufficient to predict active promoters and enhancers at 24 hpf stage and assign these predicted enhancers to their targets promoters, all at once. In addition, HiChIP using the H3K27me3 antibody confirmed that the presence of polycomb mediated repressive interactions, such as those taking place between the HoxD and Dlx1-2 gene clusters in mouse, are conserved in zebrafish. Compartments A and B are also readily identified using this approach.

COMPOSITION AND DOSAGE OF A MULTIPARTITE ENHANCER CLUSTER CONTROL DEVELOPMENTAL EXPRESSION OF IHH (INDIAN HEDGEHOG)

GARCÍA LUPIÁÑEZ, DARÍO*; WILL ANJA J.; COVA GIULIA; OSTERWALDER MARCO; CHAN WING-LEE; WITTLER LARS; HEINRICH VERENA; VINGRON MARTIN; VISEL AXEL; MUNDLOS STEFAN. *BERLIN INSTITUTE FOR MEDICAL SYSTEMS BIOLOGY (BIMSB), MAX DELBRUECK CENTER FOR MOLECULAR MEDICINE (MDC), BERLIN, GERMANY

Copy number variations (CNVs) often include noncoding sequences and putative enhancers, but how these rearrangements induce disease is poorly understood. Here we investigate CNVs involving the regulatory landscape of IHH (encoding Indian hedgehog), which cause multiple, highly localized phenotypes including craniosynostosis and synpolydactyly. We show through transgenic reporter and genome-editing studies in mice that Ihh is regulated by a constellation of at least nine enhancers with individual tissue specificities in the digit anlagen, growth plates, skull sutures and fingertips. Consecutive deletions, resulting in growth defects of the skull and lo chieved by complex multipartite enhancer ensembles. Alterations in the composition of such clusters can result in gene misexpression and disease.

ISDB-MOD PLENARY LECTURE

Arturo Álvarez – Buylla (University of California, San Francisco, USA)
"Adult Neural Stem Cell Relay"

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S3 (SEBD) DEVELOPMENT BY DESIGN

Matthias Lutolf (École Polytechnique Federal, Laussane, Swiss)
"Engineering stem cell self – organization"

Ron Weiss (Massachusetts Institute of Technology, Cambridge, USA)
"Mammalian synthetic biology and programmable organoids"

Eduard Batlle (Institute for Research in Biomedicine, Barcelona, Spain)
"Organoid-based approaches to study colorectal cancer"

S4 (SEG) CHROMOSOME MECHANICS

Ana Losada (CNIO, Madrid, Spain)
"Distinct contribution of cohesion variants to 3D chromosome organization"

In addition to its function in sister chromatid cohesion, the Cohesin complex plays a central role in the spatial organization of the genome together with CTCF. Two versions of Cohesin containing SMC1, SMC3, RAD21 and either STAG/SA1 or SA2 are present in all cell types of vertebrate organisms. While specialized functions for Cohesin-SA1 and Cohesin-SA2 have been reported in cohesion and DNA repair, none have been described regarding genome architecture and gene regulation. To address this issue, we have examined the genome-wide distribution of the two complexes and the consequences of their downregulation in human mammary epithelial cells. We found that Cohesin-SA1 drives stacking of cohesin rings at CTCF-bound sites and contributes to the stabilization and preservation of topologically associated domain (TAD) boundaries. In contrast, a more dynamic Cohesin-SA2 promotes cell type-specific contacts between enhancers and promoters within TADs independently of CTCF. Loss of SA2 results in increased intra-TAD interactions, most likely aberrant, and alters gene expression. These findings provide insights on how cohesin mediates chromosome folding and establish a novel framework to address the consequences of STAG2 mutations in human cancer.

Antonio J Giráldez (Yale University, New Haven, USA)
"Deciphering the gene regulatory code during the maternal to zygotic transition"

Mónica Bettencourt – Dias (Instituto Gulbenkian, Oeiras, Portugal)
"Centrosome regulation in development"
"The EMBO Keynote Lecture"

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SS7. (SEG) REGULATION OF GENE EXPRESSION

Juan Carlos del Pozo (Centro de Biotecnología y Genómica de Plantas, UPM – INIA, Madrid, Spain).
Coauthors: SILVA.-NAVAS JAVIER; MORENO-RISUEÑO MIGUEL ÁNGEL; MANZANO CONCEPCIÓN; TÉLLEZ-ROBLEDO BÁRBARA; NAVARRO-NEILA SARA; POLLMANN STEPHAN; GALLEGO F. JAVIER.

"Hormones and flavonols: coordination of cell division and differentiation in the root meristem"

Roots normally grow in darkness, but they may be exposed to light. However, plants cultivated in vitro are growing with the root system in presence of light. We have demonstrated that root illumination is a stress that affects root growth and responses to environmental clues. In roots, light stimuli alters the levels of a large number of genes and plant metabolites. Among them, flavonols accumulated to high levels along the root but to low levels in the apical meristem. Dark-grown roots, after perceiving light, bend to escape from illumination (root light avoidance). In this response, flavonols rapidly accumulate at the side closer to light in the transition zone. This accumulation promotes asymmetrical cell elongation and causes a differential growth between both sides, leading to root bending. If the illumination persists, flavonol content increased to high levels and root growth is retarded. We found that high level of flavonols diminishes root growth and cell division by reducing auxin signaling, PLETHORA gradient and superoxide radical

content in the root meristem. In other hand, cytokinin and hydrogen peroxide, which promote root differentiation, induce flavonol accumulation in the root transition zone, decreasing root meristem size. We propose that flavonols function as positional signals, integrating hormonal and ROS pathways to regulate root growth direction and rate in response to light.

Raúl Méndez (Institute of Research in Biomedicine, Barcelona, Spain)
"The CPEB-family of RNA-binding proteins, mechanisms of action and new functions in cell cycle and cancer"

The Cytoplasmic Polyadenylation Element Binding (CPEB)-family of RNA-binding proteins regulates pre-mRNA processing and translation of CPE-containing mRNAs in early embryonic development and synaptic activity. However, the specific functions of each CPEB in the adult organism are poorly understood. We show that CPEB is required to suppress high fat diet- and aging-induced endoplasmic reticulum (ER) stress, and its subsequent hepatic steatosis. Stress-activated expression of CPEB in the liver is controlled through a double layer of regulation. First, Cpeb is transcriptionally regulated by the circadian clock and then, its mRNA translation is regulated by the Unfolded Protein Response (UPR) through the upstream Open Reading Frames (uORFs) present in its 5' UTR. Thus, CPEB is synthesized only upon ER-stress but the amplitude of the induction is circadian. In turn, CPEB activates a second wave of UPR-translation required to maintain ER and mitochondrial homeostasis. Our results suggest that combined transcriptional and translational regulation of CPEB generates a "circadian sensor", which coordinates the UPR sensitivity with periods of high liver ER folding activity preventing Non-alcoholic fatty liver disease (NAFLD). The impact of thes CPEB4 stress-mediated response in tumor development will be discussed.

FORMATION OF BACTERIAL LINEAGES IN SALMONELLA ENTERICA BY A PLEIOTROPIC EPIGENETIC SWITCH

GARCIA PASTOR, LUCIA*; PUERTA-FERNÁNDEZ ELENA; GUTIÉRREZ GABRIEL; SÁNCHEZ-ROMERO MARIA ANTONIA; CASADESÚS JOSEP. *DPTO. DE GENÉTICA. UNIVERSIDAD DE SEVILLA

The std locus of Salmonella enterica, an operon acquired by horizontal transfer, encodes fimbriae that permit adhesion to epithelial cells. The operon includes two downstream genes whose products are unrelated to the synthesis of fimbriae. The products of these genes, StdE and StdF, are cytoplasmic proteins that possess DNA-binding ability and control expression of nearly 200 genes. The list includes loci downregulated by StdEF such as the Salmonella pathogenicity island 1, genes involved in flagella and chemotaxis, and loci involved in biofilm formation. In turn, StdE and StdF upregulate expression of the conjugal transfer operon in the virulence plasmid. StdEF thus control multiple phenotypic traits: invasion of epithelial cells, motility, biofilm formation, and conjugal transfer of the virulence plasmid. Transcription of std is bistable, and generates a major subpopulation of StdOFF cells (97-99%) and a minor subpopulation of StdON cells (1-3%). Formation of StdOFF and StdON subpopulations differing in multiple phenotypic traits may thus be viewed as an example of prokaryotic cell differentiation. Because StdOFF cells can cause acute infection and StdON cells can cause chronic infection, std bistability may be tentatively interpreted as a bet hedging strategy.

ROLE OF AHR-REGULATED ALU TRANSPOSON IN CHROMATIN STRUCTURE MODIFICATIONS AND INSULATION OF PLURIPOTENCY GENES OCT4 AND NANOG

GONZÁLEZ RICO, FRANCISCO JAVIER*; ROMÁN ÁNGEL CARLOS; MONTOLIÚ LLUIS; GÓMEZ SKARMETA JOSÉ LUIS; FERNÁNDEZ SALGUERO PEDRO M. *UNIVERSIDAD DE EXTREMADURA

Local chromatin accessibility (CA) defines spatial and temporal gene expression patterns during cell differentiation. This chromatin conformation is modulated by transposable elements (TE), likely because of their ability to bind specific transcription factors (TF) and other associated proteins, becoming insulators and boundaries elements. How these three elements, CA, TEs and TFs are interacting at the molecular level in differentiation processes is largely unknown. By using enhancer blocking assays (EBAs) we report that, in the human genome, three Alu(s) retrotransposon located in the flanking regions of pluripotency genes NANOG and OCT4 have potent insulation activity conferred by binding the transcription factor dioxin receptor (AhR) to consensus elements present in the transposon sequence. Insulators can exert their regulatory activity by modifying chromatin compaction. The analyses of histone marks revealed different methylation patterns of me3H3K4, me3H3K9 and me3H3K27 upon AhR expression. Notably, these epigenetic profiles changed during differentiation with retinoic acid (RA). At the genomic level, insulation can also induce long-range chromatin reorganization. We have used chromosome conformation capture (3C) technique to address long-range physical interactions between the Alu(s) flanking NANOG. The results obtained showed that the interaction frequency changes drastically with RA differentiation, suggesting the formation of a new chromatin loop. Interestingly, such loop dissapears by AhR knock-down, implying the involvement of AhR in the process. In order to unveil the role of nucleosomes in regulation of gene expression, accessibility of control regions was addressed via Micrococcal Nuclease digestion, obtaining a different cleavage pattern after RA treatment. Purified DNA was sequenced, revealing target genes whose expression patterns also changed with the different experimental conditions. In addition, to extract the protein complex contained in the NANOG loop without contamination from other regions, we used a modification of CRISPR-Cas9 protocol known as enChIP-Cas9, where specific genomic regions are immunoprecipitated with antibody against a tag (FLAG) fused to a catalytically inactive form of Cas9 (dCas9), which is co-expressed with a guide RNA (gRNA) and recognizes endogenous DNA sequence in the genomic region. Then, enChIP-mass spectrometry (enChIP-MS) targeting endogenous loci identified associated proteins. We propose that AhR-regulated Alu(s) elements represent evolutionary conserved genome-wide insulators that control developmental, oncogenic or toxicological-dependent processes via physical heterochromatin modifications.

A NEW FACET OF VITAMIN B12: GENE REGULATION BY A NEW AND WIDESPREAD FAMILY OF ADENOSYLCOBALAMIN-DEPENDENT PHOTORECEPTORS IN BACTERIA

PÉREZ-CASTAÑO, RICARDO*; JESÚS FERNÁNDEZ-ZAPATA; MARCO JOST; MARÍA DEL CARMEN POLANCO; CATHERINE L. DRENNAN; PADMANABHAN S.; MONTSERRAT ELÍAS-ARNANZ. *DEPARTAMENTO DE GENÉTICA Y MICROBIOLOGÍA (UNIDAD ASOCIADA AL IQFR-CSIC), UNIVERSIDAD DE MURCIA, 30100 MURCIA.

Photoreceptor proteins enable living organisms to sense and respond to light. They are ubiquitous in all domains of life and rely on bound chromophores such as retinal, flavins or linear tetrapyrroles for light sensing. We have discovered a new, widespread family of bacterial photoreceptors that sense light using 5'-deoxyadenosylcobalamin (AdoCbl), a form of vitamin B12 best known as an

enzyme cofactor (1,2). The prototype, CarH, is a transcriptional repressor that mediates light-dependent gene regulation, whose molecular mechanism of action is based on AdoCbl and light modulating its oligomeric state (2,3). Our high-resolution structures of CarH in three relevant states: free or bound to operator DNA in the dark, or after exposure to light, together with corroborative structure-based mutational analysis, provided visualizations at high resolution of how AdoCbl mediates CarH tetramer formation in the dark, how its unusual mode of DNA-binding represses transcription, and how light activates transcription (3). These studies reveal a remarkable functional repurposing of AdoCbl, from an enzyme cofactor to a light sensor, and of known protein modules in assembling a B12-dependent photoregulator (3). Besides a striking architecture and DNA-binding mode, CarH also appears to alter the photochemistry of AdoCbl from that established for this cofactor when it is free in solution or bound to enzymes (4,5). We will present our current knowledge about these B12-dependent photoreceptors, their distribution and mode of action, and the structural and photochemical basis of how they orchestrate signal transduction and control gene expression. 1. Padmanabhan et al Annu Rev Biochem 86, 485-514 (2017) 2. Ortiz-Guerrero et al PNAS 108, 7565-7570 (2011) 3. Jost et al Nature 526, 536-541 (2015) 4. Kutta et al Nat Commun 6, article no. 7907 (2015) 5. Jost et al Biochemistry 54, 3231-3234 (2015) FUNDING: Ministerio de Economía y Competitividad (Spain) grants to MEA (BFU2015-67968-C2-1-P, co-financed by FEDER-UE) and to SP (BFU2015-67968-C2-2-P), and Fundación Séneca (Spain) grant 19429/PI/14 to MEA.

SS8. (SEBC). CYTOSKELETON AND CELL ARCHITECTURE.

Nathalie Spassky (Institute de Biologie de L'Ecole Normale Supérieure de Paris (IBENS CNRS), Paris, France)
"Mechanisms of multiciliated ependymal cell development"

Alexis Gautreau (Université de Paris – Saclay, Palaiseau Cedex, France)
"A mechanosensitive G1 checkpoint monitors cortical branched actin"

The actin cytoskeleton of a cell generates and senses forces. Signals that regulate cell proliferation are associated with actin rich structures: membrane protrusions triggered by growth factors, cell adhesions to the extracellular matrix and to neighboring cells. Here we show that the pathway that generates cortical branched actin is critical for cell cycle progression. The lamellipodial branched actin, nucleated by the WAVE complex and antagonized by Arpin, is monitored by a sensing mechanism that controls the G1-S transition in a p21WAF1/CIP1 dependent manner. This signalling pathway integrates soluble stimuli and mechanotransduced signals from cell adhesions, such as substrate rigidity or epithelial cell stretching. Most cancer cells, which become independent from these biochemical and biophysical signals, lose the requirement for cortical branched actin, but not tumor cells transformed by the RAC1 oncogene, which are sensitive to Arp2/3 inhibition. This novel cytoskeletal checkpoint provides novel diagnostic and therapeutic options.

SHAPING DROSOPHILA LEG: MORPHOGENETIC MECHANISMS THAT SCULPT TARSAL JOINTS

CÓRDOBA CASADO, SERGIO*; ESTELLA CARLOS. *CENTRO DE BIOLOGÍA MOLECULAR SEVERO OCHOA - UAM

The legs of arthropods are characterized by the presence of joints, a structure that subdivides them and makes them movable. During prepupal development, tarsal joints are prefigured by the formation of folds in the epithelium of the leg disc that will later maturate to shape an adult joint.

The lack of such folds correlates with the absence of joints in the adult. Therefore, joint formation is an excellent model to study the complex cellular dynamics that control organ morphogenesis. In Drosophila melanogaster, Notch signaling is completely required for joint formation. We identified the transcription factor dysfusion (dys) as a direct target of Notch that globally coordinates the formation of tarsal joints. The lack of dys cause the loss of tarsal joints, while its misexpression generate folds in the epithelium that resemble joint formation. We found that Dys regulates two key morphogenetic events during joint formation, cell death and the regulation of cytoskeleton dynamics. We observed that the pattern of Rho1 activity is severely altered in dys mutants, and that blocking the activity of Rho1 impairs formation of epithelial folds and adult joints. Interestingly, inhibition of cell death does not cause alterations on Rho1 activity. Furthermore, downregulation of Rho1 downstream effectors, rok, MyoII and Diaphanous cause partial defects in joint formation, while ectopic activation of Rho1, MyoII or Dia cause autonomous folding of the epithelium. We propose that dys finely regulates Rho1 activity locally at the presumptive joint domain, thus generating the apical constriction necessary to form the folds.

HIERARCHICAL REGULATION OF MICROTUBULE NUCLEATION IN MAMMALIAN CELLS

GANDOLFO DOMÍNGUEZ, PABLO*; GAVILAN MARIA P.; BALESTRA FERNANDO R.; ARIAS FRANCISCO; BORNENS MICHEL; RIOS ROSA Mª. *CELL DYNAMICS AND SIGNALLING DEPARTMENT, CABIMER-CSIC, 41092-SEVILLE, SPAIN

In most interphase mammalian cells, microtubules (MTs) are nucleated at the centrosome and the Golgi Apparatus (GA). Several components of the MT nucleation machinery are common to both organelles, suggesting a co-regulation of MT assembly. By combining genetic ablation of these components and the PLK4 inhibitor centrinone, we show that centrosomal MT nucleation is independent of the GA whereas GA-nucleation activity is dictated by the number of centrosomes. Inhibition of GA-associated MT nucleation in centrosome-less cells lead to MT growth from scattered cytoplasmic foci. In the absence of centrosomes, either the GA or cytoplasmic foci are able to grow a cell-wide although disorganized MT network. Our results show that the centrosome controls the number of MTs, not only by nucleating them, but also by acting as a negative regulator of their nucleation elsewhere in the cell and reveal a hierarchical regulation of the MT nucleation process.

P73, AN ARCHITECT OF EPITHELIAL TISSUES

MARÍN, MARÍA DEL CARMEN *; FUERTES ÁLVAREZ SANDRA; MAESO ALONSO LAURA; MARTÍN LÓPEZ MARTA; VILLENA CORTÉS ALBERTO; LIZÉ MURIEL; MARQUÉS MARGARITA M. * IBIOMED. UNIVERSIDAD DE LEÓN

p73 transcription factor belongs to one of the most important gene families in vertebrate biology, the p53 family. Although p73 shares many biological functions with p53, it also plays distinct roles during development. Trp73 knockout mice, which lack all p73 isoforms, show multiple and apparently unrelated phenotypes, as gastrointestinal and cranial hemorrhages, rhinitis or hippocampus dysgenesis and enlarged ventricles. Recent studies have revisited the Trp73 deficient mice phenotype. In this regard, our work demonstrated, for the first time, that p73 is essential for ependymal ciliogenesis and for the cytoarchitecture of the subventricular zone brain stem cell niche. Lack of p73 resulted in loss of ventricular wall integrity and severe defects in cilia formation. Moreover, this work revealed a p73 function in the establishment of Planar Cell Polarity. Ependymal cells are polarized within a plane orthogonal to the apico-basal axis. This polarization,

known as Planar Cell Polarity (PCP), is observed not only at single-cell level, where cilia basal bodies adopt a common orientation (rotational PCP), but is also displayed at tissue scale, with all the cilia clusters polarized to the rostral side of the ependymal cells (translational PCP). In ependymal cells, PCP signaling has been reported to have a role upstream of cilia formation; however, the causal relationship between the cilium and PCP signaling remains controversial. In this regard, our results demonstrate that p73 deficiency not only affects multiciliogenesis, but also severely impairs the establishment of PCP on ependymal cells. Here we describe the mechanisms by which p73 could be regulating PCP signaling independently of ciliogenesis. Altogether, our data supports the role of p73 as an architect of epithelial tissues since the cell shape, the tissue structure and the coordinated behavior across epithelial sheets is established by the action of the planar cell polarity pathway.

SS9. (SEBD). ON GROWTH AND FORM

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Development

UPCOMMING SPECIAL EDITION OF THE JOURNAL "ON GROWTH AND FORM – 100 YEARS ON"

Arkhat Abzhanov (Imperial College / Natural History Museum, London, UK)
"Evolution of the Animal Face: from morphology to development mechanisms"

Dagmar Iber (Computational Biology, ETH, Basel, Swiss)
"How to shape an organ? – Computational Models of Organogenesis"

IDENTIFICATION OF A NOVEL POPULATION OF WT1 POSITIVE CARDIOMYOCYTES DURING HEART DEVELOPMENT

GARCÍA MELERO, ANA*; VELECELA VICTOR; CASELLAS-DÍAZ SERGI; REINA MANUEL; HASTIE NICK; MARTÍNEZ-ESTRADA OFELIA. *CELLTEC UB, DEPARTMENT OF CELL BIOLOGY, PHYSIOLOGY AND IMMUNOLOGY, UNIVERSITY OF BARCELONA AND INSTITUTE OF BIOMEDICINE, UNIVERSITY OF BARCELONA

During heart development Wt1 expression has mainly been described in the epicardium and epicardial derived cells. Wt1 is essential for epicardial development and furthermore it is essential for myocardial growth through paracrine signalling from the epicardium. Given the enormous interest in this topic we decided to use a different approach than the traditional immunohistochemistry and isolate and characterize the global expression profiles of Wt1 positive cells during embryonic heart development using as a mouse model the Wt1GFPKI mice. Detailed FACS analyses revealed two distinct types of Wt1GFP positive cells in the embryonic heart on the basis of their GFP expression levels a Wt1GFPbright (GFP++) and a Wt1GFPdim (GFP+) population of cells. Consistent with the GFP expression real time-PCR and Western Blot analyses demonstrate the expression of endogenous Wt1 in these cells. In order to characterize the signature of these two population of cells microarray analyses were performed on RNA isolated from freshly isolated Wt1GFP sorted cells at different stages of heart development from

E11.5 to E16.5. Interestingly, the examination of the transcripts abundantly expressed in the Wt1GFP+ population of cells demonstrate its cardiomyocyte identity. Here we show that the role of Wt1 in myocardial growth and development also extends to a previously unidentified expression of Wt1 in a population of cardiomyocytes that is not derived from the epicardium and is present from the early heart tube, which is formed before the epicardium. The deletion of Wt1 in a cardiomyocyte specific manner leads to wall thinning revealing a new role for Wt1 in heart development. During a heart attack millions of cardiomyocytes are lost, the results presented here offer new insights into the cellular and molecular framework underlying cardiac development, and could be used in therapeutic strategies for heart disease.

CONFOCAL MICROSCOPY FOR IN TOTO IMAGING OF THE BONE GROWTH PLATE

FERNÁNDEZ-IGLESIAS ANGELA*; FUENTE ROCÍO; GIL-PEÑA HELENA; SANTOS FERNANDO; LÓPEZ JOSÉ MANUEL. * RESEARCH GROUP OVIPED. DEPARTMENT OF MEDICINE. UNIVERSITY OF OVIEDO

The growth plate comprises a relatively low number of chondrocytes that are highly organized in columns and are responsible for longitudinal bone growth. During its maturation cycle in the growth plate, a chondrocyte increases 10-15 times in volume and changes its shape. These two coupled processes are of utmost importance for final length or height. The increase in cell volume occurs by both increase of cytoplasm components (hypertrophy) and also by increase of water content (swelling). Osmotic active and inactive fractions are present in chondrocytes and their relative changes are of critical importance for cell volume increase. Osmolarity is dramatically influenced by the extracellular matrix surrounding chondrocytes in the tridimensional space. Histological techniques, including sectioning, alter drastically the cell environment resulting in modification of osmolarity and, in turn, of the osmotic active and inactive fractions. To avoid this artefact, we performed an “in toto” analyses on growth plate chondrocytes followed by confocal microscopy. Bone samples were removed using a handheld circular saw and subsequently dehydrated using ascending concentrations of ethanol and embedded in low-viscosity epoxy resin Durkupan-ACM (Sigma). The embedded samples were ground down to a thickness of 100 μm and analyzed using a white light laser confocal microscope Leica TCS SP8. The method, without any physical slicing, lets chondrocytes to preserve their structural three-dimensional interaction with the surrounding extracellular matrix. The use of the method allowed us to obtain three-dimensional images of intact chondrocytes and to perform quantitative analysis of position, volume and relative density of the cytoplasm. The results obtained are of high interest in order to understand the cellular processes associated to the modulation of longitudinal growth. The study presented here was designed to find out cellular changes underlying growth retardation induced by pathologies like chronic renal failure and also to evaluate the growth-promoting effect of growth hormone treatment.

SS10. GENOME INSTABILITY

Óscar Fernández – Capetillo (Centro Nacional de Investigación Oncológicas, Madrid, Spain)
“Exploring the role of replication stress in cancer and ageing”

Andrés Joaquín López – Contreras (Panum Institute, University of Copenhagen, Denmark)
“Defining novel therapeutic targets in the DNA damage response pathway”

Ignacio Pérez de Castro (Instituto de Salud Carlos III, Madrid, Spain)
“Search for chromosome instability biomarker with prognostic value in cancer”

Chromosome Instability (CIN) is defined as the condition in which the cells are unable to properly

segregate whole chromosomes or are prone to their structural rearrangements. The immediate consequence of chromosomal instability is aneuploidy. Although CIN and aneuploidy have been associated with cancer development and progression and resistance to antitumoral therapies, no CIN associated marker is currently used in clinical practice. Our goal is to obtain an optimized CIN signature with high diagnostic / prognostic value. We have carried out a bio-computational analysis of 60 human cancer cell lines (NCI60 collection) that has allowed us to find a mRNA/miRNA/protein profile associated with high CIN. Preliminary characterization and validation of some of the members of this signature will be presented.

A FUNCTIONAL SCREEN IDENTIFIES NOVEL PHOSPHATASES REQUIRED FOR CHECKPOINT RECOVERY IN G1

GARCÍA SANTISTEBAN, IRAIA*; LLOPIS ALBA; VAN DEN BROEK BRAM; MEDEMA RENÉ H.; ZUBIAGA ANA MARÍA. *DPT. OF GENETICS, PHYSICAL ANTHROPOLOGY AND ANIMAL PHYSIOLOGY, UNIVERSITY OF THE BASQUE COUNTRY UPV/EHU, LEIOA, SPAIN

DNA damage checkpoints are of crucial importance for maintaining genomic stability. Following DNA damage, cells must stop cell cycle progression, maintain the arrest until the repair has been completed, and finally restart the cell cycle. Although the basic machinery that detects the damage is common throughout the cell cycle, subsequent checkpoint establishment and maintenance are different in G1 and G2. In sharp contrast to G2 cells, which lose their ability to recover within a few hours after irradiation-induced DNA damage, G1 cells maintain the recovery competence for several days. After DNA damage in G1, ATM kinase phosphorylates and activates Chk2, triggering a downstream signaling cascade that prevents cell cycle progression. An initial ATM-dependent response is essential to install a checkpoint arrest. By contrast, ATM is dispensable to perpetuate it, even after one hour following damage. Instead, checkpoint maintenance mainly depends on the downstream target Chk2, which keeps its own activity and sustains the arrest independently of ATM, even when most of the DNA lesions are already resolved. These data suggest that Chk2 might be activated in a DNA damage template-independent manner. An important question that remains unanswered is what inactivates the signaling cascade that allows the progressive recovery of G1 cells after DNA damage. We hypothesize that an active phosphatase inactivates Chk2 in those cells that recover from damage in G1. To test this hypothesis, we set up a screen with a siRNA library spanning all phosphatases and regulatory proteins. Silencing the phosphatase that dephosphorylates Chk2 could potentially prevent the cell cycle recovery of G1 cells following DNA damage. Results from this screen, which are being validated using deconvoluted siRNA and CRISPR/Cas9 approaches, are enabling us the identification of novel phosphatases required for checkpoint recovery after DNA damage in G1.

EWSR1 DEFICIENCY COMPROMISES NUCLEOLAR FUNCTION AND LEADS TO THYMOMA DEVELOPMENT IN MICE

LAFARGA, VANESA*; NIETO-SOLER MARIA; MORGADO ISABEL; LECONA EMILIO; MURGA MATILDE; LAFARGA MIGUEL; CHEDIN FREDERIC; AGUILERA ANDRES; LOPEZ-CONTRERAS ANDRES; FERNANDEZ-CAPETILLO OSCAR. *GENOMIC INSTABILITY GROUP, SPANISH NATIONAL CANCER RESEARCH CENTRE (CNIO), MADRID, SPAIN.

Ewing Sarcomas are the most frequent pediatric bone tumor, and are initiated by translocations between EWSR1 and a member of the ETS family of transcription factors, most frequently FLI.

While most of the focus has been placed on understanding how the translocation affects the transcriptional landscape of FLI1, it is becoming clear that EWSR1 also plays important cellular functions, including genome maintenance and RNA metabolism. Still, what are the true roles of EWSR1, and whether a compromised EWSR1 activity can also contribute to tumorigenesis is not known. Here we have developed a conditional mouse knockout model to study the physiological impact of EWSR1 deficiency in mammals. In agreement with its role in genome maintenance, EWSR1 nullyzygosity is embryonic lethal, associated with increased levels of embryonic DNA damage. In addition, we found evidences indicating that EWSR1-deficient MEF have specific defects in nucleolar integrity, which include a deficient nucleo/cytoplasmic export of the rDNA, an increase in R-loops and DNA breaks at the rDNA, and an overall alteration of the nucleolar structure when analysed by EM. Remarkably, when EWSR1 is ubiquitously deleted in adult mice, it leads to a fully penetrant early onset of thymomas, which also present aberrant nucleoli, and from which animals die within the first six months of life. Our data and ideas about this project will be discussed.

SS11. (SEBC) CELL SIGNALING

Roger Gomis (Institute for Research in Biomedicine of Barcelona, Barcelona, Spain)
"Mechanism of tissue specific metastasis"

Ana Cuenda (Centro Nacional de Biotecnología, Madrid, Spain)
"Novel signaling pathways in inflammation and cancer"

CONTROLLING THE PARTIAL EMT PROGRAM IN MODELS OF ORGAN FIBROSIS

KASS YOUSSEF, KHALIL*; FAZILATY HASSAN; ABAD DIANA; VEGA SONIA; NIETO ANGELA. *INSTITUTO DE NEUROCIENCIAS CSIC-UMH

Epithelial homeostasis is essential to preserve tissue organization and function and, therefore, is robustly regulated in adulthood. In pathological conditions, the reactivation of developmental Epithelial-to-Mesenchymal (EMT) programs in cancer and fibrosis1 includes profound deregulation and impairment of epithelial homeostasis. EMT is triggered by different EMT transcription factors (EMT-TFs). While different EMT-TFs can promote cellular switches from epithelial to mesenchymal like programs is still unclear how the different EMT-TFs are coordinated to orchestrate the phenotypic changes. Thus, it is now crucial to understand how different EMT-TFs support different functional programs both during morphogenesis and in disease. In kidney fibrosis, de novo expression of several EMT transcription factors (EMT-TFs) induces a partial EMT program in epithelial tubular cells2. This reactivation fosters the progression of the disease essentially through (i) the dedifferentiation of the renal epithelial cells, and (ii) the release of different pro-inflammatory and fibrogenic signals to the interstitium. We have now studied the temporal course of EMT-TFs reactivation in epithelial cells during the development of renal fibrosis induced by unilateral ureteral obstruction (UUO) in mice. We followed this EMT-TFs stereotypic expression signature together with the dynamic changes of epithelial and mesenchymal markers as well as with the alterations in cell architecture and function of tubular kidney units. We are currently developing in vitro and in vivo tools to specifically control the expression of single EMT-TFs to assess their specific contribution to the EMT program. Together, we hope to (i) better characterize the modality of EMT-TFs program driving the pathological EMT in kidney fibrosis (ii) extend our findings to the programs activated during the progression of carcinomas to the metastatic disease. References 1.- Nieto et al. Cell (2016) 2.- Grande et al. Nat Med (2015).

CANCER ACTOMYOSIN CONTRACTILITY SUPPORTS IMMUNOSUPPRESSIVE TUMOUR MICROENVIRONMENTS

MIRELLA GEORGOULI*; CECILIA HERRAIZ; JOSE L. ORGAZ; BRUCE FANSHAWE; IRENE RODRIGUEZ-HERNANDEZ; OSCAR MAIQUES; GILBERT O. FRUHWIRTH; SOPHIA N. KARAGIANNIS; VICTORIA SANZ-MORENO.* TUMOUR PLASTICITY LABORATORY, RANDALL DIVISION OF CELL AND MOLECULAR BIOPHYSICS, NEW HUNT'S HOUSE, GUY'S CAMPUS, KING'S COLLEGE LONDON, LONDON, SE1 1UL, UK.

Malignant melanoma is the most serious type of skin cancer and one of the few cancers which incidence is continuously increasing. Being very plastic, melanoma cells can switch between different modes of migration to efficiently metastasise. Previous work has shown that high levels of ROCK-driven actomyosin contractility are crucial for rounded-amoeboid fast melanoma cell migration. However, it is less understood whether actomyosin contractility in cancer cells has an impact on tumour microenvironment. By evaluating human melanoma biopsies, we find that melanoma cell roundness and actomyosin contractility levels are both regionally increased in the invasive fronts of primary melanoma tumours, where communication with the stroma occurs. In the same tumour regions, we detect an increase in tumour-associated macrophages (TAMs), blood vessel density and T regulatory cells (Tregs). Importantly, tumour-stroma organization in metastatic melanoma lesions mirrors the structure observed in the invasive fronts of primary tumours. Using proteins arrays, we show that contractile melanoma cells are highly secretory and secrete pro-inflammatory and immunomodulatory factors. Contractile melanoma cells recruit human primary monocytes and induce tumour-promoting macrophage polarisation in vitro and in vivo. Mechanistically, actomyosin contractility in cancer cells can be self-perpetuated through signal amplification via protein secretion coupled to NF-κB driven transcription. In such a way, highly contractile and rounded-amoeboid melanoma cells establish a crosstalk with NF-κB to reprogram the immune microenvironment. Overall, our data suggests that actomyosin contractility in melanoma cells can direct the formation of an invasive tumour-promoting immune microenvironment. Importantly, we can switch a tumour promoting- to a tumour suppressive- microenvironment by decreasing actomyosin contractility in melanoma cells. (Answering reviewers' comments in Cell. CELL-D-17-00632).

SS12. (SEBD) TISSUE HOMEOSTASIS AND REGENERATION

Irene Miguel – Aliaga (Imperial College London, UK)
"Sex differences in adult organ size and plasticity"

Internal organs are constantly exchanging signals, and can respond with profound anatomical and functional transformations, even in fully developed organisms. Such organ plasticity results from a need to integrate and respond to both environmental information and internal state, and is key to maintaining homeostasis and driving adaptive changes. We are interested in understanding the mechanisms by which organs sense change and respond to it: the molecules, cellular events and physiological adaptations involved. The intestine and its neurons are a fantastic system with which to tackle these questions. Our investigations in Drosophila have uncovered functional similarities between the invertebrate and vertebrate enteric nervous systems (Cognigni 2011 Cell Metab). They have also characterized an adaptive mechanism, reminiscent of neurovascular interactions in mammals, which points to a key role for the intestinal vasculature in adaptations to malnutrition (Linneweber 2015 Cell). More recently, we have begun to explore the physiological plasticity of the intestinal epithelium: an obvious cellular target of the enteric neurons. I will

present some of this work, which has revealed unexpected sexual dimorphisms in intestinal stem cells, as well as intestinal contributions to reproductive success (Hudry 2016 Nature, Reiff 2015 eLife). I will also discuss some of our ongoing work, aimed at exploring how widespread cell-intrinsic sex differences are beyond fly stem cells using mouse organoids and human embryonic stem cells.

Cedric Blanpain (Université Libre de Bruxelles, Belgium)
"Epithelial stem cells during homeostasis and repair"

MIR-106B FACILITIES SKELETAL MUSCLE REGENERATION IN DYSTROPHIC MUSCLE BY BALANCING SATELLITE CELL STATUS

RODRÍGUEZ-OUTEIRIÑO LARA*; ARANEGA AMELIA; HÉRNANDEZ-TORRES FRANCISCO; VALLEJO DANIEL; RAMÍREZ FELICITAS. * UNIVERSITY OF JAEN.

Following injury, skeletal muscles can regenerate from muscle specific stem cells, called satellite cells. Quiescent in uninjured muscles, satellite cells become activated, proliferate and differentiate into myotubes. Several recent evidences indicate that miRNAs are previously unrecognized regulators of satellite cell proliferation, fate specification, and differentiation during adult myogenesis. Previous data reported by our laboratory demonstrated that miR-106b modulates satellite cell proliferation and myogenic commitment. Here, we show that miR-106 is able to arrest myogenic differentiation of satellite cells, being its down regulation needed for a proper myogenenesis in vitro and in vivo. Importantly, miR-106b expression is increased in dystrophic muscles of DMD/mdx mice and inhibition of miR-106b by intramuscular injection leads to improve muscle regeneration with a significant functional recovery in DMD/mdx mice. In addition, genome-wide RNA profiling data support the notion that inhibition of miR-106b in dystrophic muscles leads to an enrichment on muscle regeneration transcriptome profile while repress inflammatory pathways. These findings support the notion that miR-106b plays a key role during the process of muscle regeneration and may open new therapeutic perspectives by identifying new molecular tools to improve the regenerative capacity in muscular dystrophies.

MYC-DRIVEN ENDOGENOUS CELL COMPETITION PROTECTS PLURIPOTENT STEM CELL POOLS BY ELIMINATING DIFFERENTIATION-PRIMED CELLS

DIAZ, COVADONGA*; FERNÁNDEZ DE MANUEL LAURA; JIMÉNEZ-CARRETERO DANIEL; MONTOYA MARÍA C.; CLAVERÍA CRISTINA; TORRES MIGUEL. *NATIONAL CENTER OF CARDIOVASCULAR RESEARCH (CNIC)

In the early mouse embryo and in embryonic stem cell (ESC) cultures the transcription factor Myc exhibits a cell-to-cell heterogeneous pattern. Cells expressing low levels of Myc are eliminated from the population by cell competition. Myc has been reported to promote cell reprograming to pluripotency and to regulate cell anabolism and proliferation in ESCs; however, the biological role of Myc-dependent endogenous cell competition and the dynamics and regulation of Myc during this process remain unknown. Here we develop a new image analysis tool that allows us to track the temporal evolution of endogenous Myc levels, perform neighbourhood analysis in ESC cultures and generate 3D+t computerized data. We show that despite Myc degradation and resynthesis during mitosis, Myc levels are mostly heritable in ESC lineages. Cell competition results from random interactions between cells with high discrepancies in Myc levels. Myc-low cells ("losers") temporally integrate contacts with Myc-high cells ("winners"), which leads to a progressive decrease in their own Myc levels until dying. Interestingly, endogenous Myc levels correlate with the pluripotency status; differentiation-primed cells express low Myc levels and are outcompeted by Myc-high naive ESCs. Indeed, cell competition inhibition results in an accumulation of primed cells. These observations in ESCs correlate with findings in the mouse epiblast. Moreover, we show

that Myc levels directly determine the competitive ability of ESCs irrespective of the pluripotency status. Our results identify Myc as a mediator between differentiation status and competitive ability of pluripotent cells. Myc-driven endogenous cell competition thus acts as a mechanism to protect pluripotent stem cell pools from differentiation.

FRIDAY, 27th OCTOBER, 2017

S5 (SEG/SEBD/SEBC) PLANT BIOLOGY

Antonio di Pietro (Universidad de Córdoba, Spain)
"Understanding pathogen adaptation to the plant host"

Filamentous plant pathogens pose a severe threat to global food security. These organisms often show exquisite host adaptation, but also undergo rapid evolution leading to shifts or expansions in the host range. The genetic mechanisms of pathogen-host adaptation remain poorly understood. In the soil-inhabiting vascular wilt fungus Fusarium oxysporum, individual isolates tend to exhibit high specificity towards a given plant host, while the species complex collectively attacks more than a hundred different crops. In addition, F. oxysporum is also an emerging human pathogen that provokes lethal systemic infections in immunocompromised individuals. Remarkably, a single field isolate of this fungus can kill tomato plants, immunodepressed mice and insects. By following a combination of reverse genetics and experimental evolution approaches, we found that F. oxysporum uses multiple strategies to adapt to different host environments. These include recruitment of conserved fungal signaling pathways or hijacking of host regulatory mechanisms for new virulence mechanisms. Strikingly, fungal populations evolved after serial passaging through different environments displayed large-scale chromosomal reorganizations in transposon-rich accessory regions of the genome, suggesting that chromosome plasticity could act as a major evolutionary driver in F. oxysporum. Understanding the genetic mechanisms that govern virulence evolution and host adaptation may reveal new ways to control diseases caused by filamentous pathogens and improve plant health.

Enrico Coen (John Innes Centre, Norwich, UK)
"Resolving conflicts: Genetic control of tissue morphogenesis"

Salomé Prat (Centro Nacional de Biotecnología, Madrid, Spain). Coauthors: MARTÍNEZ CRISTINA; ESPINOSA ANA.
"Br levels plays a pivotal role in thermomorphogenic growth"

Brassinosteroids (BRs) promote plant growth by inactivating the GSK3-like kinase BRASSINOSTEROID INSENSITIVE2 (BIN2), a critical repressor of BR signaling shown to phosphorylate the BRASSINAZOLE RESISTANT1 (BZR1) and BRI1 EMS SUPPRESSOR (BES1) factors, in addition to the PHYTOCHROME INTERACTING FACTORs 4 and 3 (PIF4 and PIF3) with a central role in light signaling. BRs in fact play an antagonistic role to light, mutants defective in BR biosynthesis shown to exhibit a de-etiolated response and display pleiotropic dwarf phenotypes. BES1 and BZR1 negatively regulate BR levels by recognizing a conserved BRRE element in the promoters of BR biosynthetic genes. These BR-related factors were also reported to function as PIF4 co-activators by binding in a complex with the PIF4 factor the promoters of many of its direct regulated targets. PIF4 transcription is regulated by the circadian clock, its transcript levels being shown to increase during late night to be suppressed at dusk. Expression of PIF4 preceding phyB light activation allows stabilization of the protein and leads to maximal hypocotyl growth at dawn. However, functional significance of feed-back regulation of BR levels and PIF4 co-activation function of the BES1 and BZR1 factors in diurnal plant growth remains little understood.
Here, we show that BES1 and PIF4 recognize different promoter elements upon formation of homodimeric or heterodimeric complexes. While the BES1 homodimer binds both BRRE and G-box motifs enriched in the BR biosynthesis promoters and in BES1/BZR1-downregulated genes, the BES1-

PIF4 heterodimer shows a strong binding affinity for the CATGTG PBE (PIF binding element) motif, found to be over-represented in the PIF4 and BES1/BZR1 up-regulated targets and in multiple auxin-related genes peaking at dawn. Notably, in time course studies of BR biosynthetic gene expression, we observed the DWF4, CPD and BR6ox genes to display also a dawn phased peak of expression, regardless not to be direct regulatory targets of PIF4. This suggests that PIF4 stabilization up-regulates BR levels at dawn, by competing for formation of the repressive BES1-BES1 homodimeric complex, hence contributing to increase nuclear bioactive PIF4 and BES1 levels, by inactivating BIN2. Consistent with this model of regulation we show that BR levels are strongly increased in PIF4ox lines. We also show that this mechanism of control plays a pivotal role in thermomorphogenesis, where PIF4 up-regulation during the night correlates with an earlier peak of BR biosynthetic gene expression, that is not observed in pifq mutants. Moreover, increased elongation of the hypocotyl and petioles of plants grown at elevated ambient temperatures is suppressed by the inhibitor brassinazole. Together, our results underscore a pivotal role of the PIF4 factor in BR biosynthesis and as such in modulating BIN2 kinase activity.

CLOSING LECTURE

Ginés Morata (Center for Molecular Biology "S. Ochoa"). CSIC-UAM. Madrid.
"Cell competition"

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P1 - LOSER CELL DEATH IN MAMMALIAN CELL COMPETITION

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Cell competition has emerged as an important cell quality control system, essential for the homeostasis of the organism and during embryo development. According to this phenomenon, cells interact with their neighbours and their intrinsic cellular properties are compared. As a result, suboptimal cells or those misplaced or not well adapted are non-autonomously eliminated, contributing to the general fitness of the tissues. Recent works have described Cell Competition in multiple models and tissues, relating it with relevant topics as homeostasis, tissue regeneration and cancer. (reviewed in Amoyel & Bach, 2014; Clavería & Torres, 2015; Gogna, Shee, & Moreno, 2015). In our lab, it was described that during the early mammalian embryo, cells with low level of transcription factor c-Myc are eliminated by neighbouring cells with higher Myc levels, a process termed Myc-mediated endogenous Cell Competition (Clavería et al. 2013). By using mouse embryonic stem cells (mESCs), we are exploring several cellular stresses and cell death pathways regulating the death of the less fit cells during Cell Competition. Up to now, different stresses as DNA damage, oxidative stress or metabolic imbalance have been tested without significant results. Multiple RNAseq data analysis and previous results generated in the lab, suggest some important candidates for the mechanisms controlling Cell Competition death, for example: p53, Htra1 serine-protease, pro-apoptotic proteins Puma (Bbc3) and Noxa (Pmaip1) and caspases 7 and 9. These components are being validated at this moment. p53 activation has been associated with a loser phenotype and it has been described as necessary for loser cell death in other types of CC (Bondar and Medzhitov 2010; Wagstaff et al. 2016; Zhang et al. 2016). Recent data generated in the lab indicate an activation of p53 in low-Myc cells. Therefore, we are interested

in study this pathway in our model and its relation with loser cell death. For that, we are using chemical inhibitors as pifithrin-α and a genetic construction for the inducible inhibition of p53.

P2 - ULTRASTRUCTURAL MITOCHONDRIAL FEATURES AND COENZYME Q REGULATION THROUGH FATTY ACIDS

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Biological membranes adapt their phospholipid composition according to the major lipid source present in the diet. Different dietary sources could modify the lipid pattern producing biochemical alterations in cells, especially in mitochondrial membranes. Concretely, polyunsaturated sources, such as soybean or fish oil, will generate membranes more susceptible to oxidative stress than saturated or monounsaturated ones, like animal fat and olive oil, respectively. Previous studies have revealed that different dietary fats also influenced the mitochondrial levels of coenzyme Q (Q), which is a lipid present in all the organisms that, apart from participating as electron carrier in the mitochondrial electron transport chain, plays an important role in numerous cellular functions such as metabolism, antioxidant protection and the regulation of signal transduction. Using a hepatocellular model of Hepa1-6 cells treated with different lipid emulsions, we focused on the regulation of the Q system and the ultrastructure of the mitochondria. Our results have shown that unsaturated fatty acids were able to increase Q levels, which was directly related with an increase in Q biosynthetic rate in the case of polyunsaturated fatty acids (PUFA) but not in the case of monounsaturated fatty acids (MUFA). Moreover, our results indicate that PUFA regulate the different Q isoforms, promoting the biosynthesis of Q10 over Q9 thus decreasing Q9/Q10 ratio. Since most of the cellular Q is located in mitochondria, the structure, number, size and distribution of this organelle greatly influences the overall content of Q in cells. We studied the ultrastructure and the abundance of this organelle in order to evaluate whether the regulation of Q levels by fatty acids involved an alteration of mitochondrial ultrastructure and/or mitochondrial abundance. Electron microscopy micrographs of cells showed that n-3 PUFA significantly increased mitochondrial volume and the number of mitochondria per cell, explaining partially the increase of Q levels previously described. However, these alterations in the mitochondrial ultrastructure could not explain the alteration of the Q9/Q10 ratio. Further experiments focused on the mevalonate pathway showed that the alteration of the Q9/Q10 ratio can be explained by the role that PUFA have as inhibitors of the farnesyl diphosphate synthase, a key enzyme in this pathway. However, the observed increase of Q biosynthesis might imply additional target(s) in the Q biosynthetic branch, still to be identified. Further studies will be needed to fully understand the exact regulation that fatty acids exert on Q metabolism and mitochondrial ultrastructure.

P3 - ORIGINS AND REGULATION OF AN EUTHERIAN NOVELTY: THE BGW CLUSTER

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Two related gene subfamilies known as BEX and TCEAL (also known as WEX) map to a genomic region specific to Eutheria (placental mammals), located on the X chromosome. These families are part of a gene cluster, named “BGW cluster”, together with the ARMCX family and Hnrnph2. Some of the BEX/TCEAL genes have been related to control the balance between proliferation and differentiation, while others promote apoptosis in a p75-dependent manner, but most of them remain poorly studied. The ARMCX family and Hnrnph2 are derived from retrocopies of the

Armc10 and Hnrnph1 genes respectively –conserved across bilateria, and located in autosomal chromosomes–, whereas no orthologs have been found for the BEX/TCEAL family outside of Eutheria. However, all these genes share an intriguing feature: a sequence motif in their proximal promoter region that appears to be crucial for their expression, the BGW motif. To further understand the evolution of this gene cluster, we investigated the origin of the BEX/TCEAL genes and traced it to an atypical formation in the ancestor of eutherians. Furthermore, novel features associated with BEX/TCEAL suggest a more complete scenario for the origin of the cluster: the BGW motif was already present at the Hnrnph2 locus in the ancestor of therian mammals, being subsequently duplicated and coopted in the eutherian lineage by the BEX/TCEAL ancestor and, posteriorly, by the ARMCX ancestral gene. Finally, we also studied the expression of the BEX/TCEAL genes during mouse development using in situ hybridization. We found that they are highly expressed in the brain and placenta, which are structures that require a well-tuned control of cell cycle during their development in eutherian mammals. Here we propose a scenario for the origin of the BEX/TCEAL family and for the formation of the BGW cluster where they belong. Their uncommon origin, their pattern of expression, and their putative biological function during development makes these genes an interesting subject of study to understand how lineage-specific genes could contribute to mammalian evolution.

P4 - OH MY, WHAT BIG (AND NEW) EYES YOU HAVE!

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Evolutionary innovations are biological revolutions: new organs are critically associated with the emergence of new species and their exploitation of new niches. Despite their importance in the history of life, how morphological novelty arises and evolves is a long-standing question in Evolutionary Biology. How the genetic network associated to the new structure appears? How this new structure is functionally and anatomically integrated into the pre-existing body plan? One of the most striking examples of a sexually dimorphic novel structure occurs in males of the mayfly species Cloeon dipterum. Cloeon males develop, in addition to the compound eyes (shared by males and females), an extra pair of extremely large dorsal, turban-shaped eyes. Thus, by comparing males versus females, this mayfly species provides a privileged system to understand the origin and integration of new structures. To answer these questions, first, we have successfully established a C. dipterum culture in the lab. Next, we describe the development of the eye and its integration with the optic lobes of male and female Cloeon nymphs using confocal and electronic microscopy. Furthermore, we compare sex-specific gene expression in nymphal heads, with a special focus on genes of the highly conserved Retinal Determination Network (RDN), to show how RDN elements could have evolved to play a role in the origin of this novel sexually dimorphic visual organ. Finally, the use of Cloeon to study evolutionary novelties goes well beyond the sexually dimorphic turbanate eyes. Insect wings are perhaps the most iconic morphological innovation in animals and their origin led to the conquest of the sky and the adaptation to a huge diversity of new ecological niches. This key structure first appeared among the ancestral mayflies, thus, the establishment of the first mayfly model system in the lab is an unprecedented opportunity to test the different proposed hypotheses about the origin of wings in their extant relatives.

P5 - TO BRANCH OR NOT TO BRANCH, A CONSERVED CARBON STARVATION RESPONSE IS INDUCED DORMANT BUDS

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Plant shoot branching patterns determine leaf, flower and fruit production, and thus reproductive success and yield. Branch primordia or axillary buds arise in the axils of leaves and their decision to either grow or enter dormancy is coordinated at the whole plant level. Comparisons of transcriptional profiles of axillary buds entering dormancy have identified a shared set of responses that closely resemble a Low Energy Syndrome. This syndrome is aimed at saving carbon use to support essential maintenance functions, rather than additional growth, and involves growth arrest (thus dormancy), metabolic reprogramming and hormone signalling. This response is widely conserved in distantly related woody and herbaceous species, and not only underlies but also precedes the growth-to-dormancy transition induced in buds by different stimuli that lead to eco para and endodormancy.

P6 - CONE SNAIL PHYLOGENOMICS AND CONOTOXIN TRANSCRIPTOMICS

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Cones are predatory venomous marine snails, which produce small peptides termed conotoxins that paralyze their preys once injected using hollow harpoon-like radular teeth. To accomplish their paralyzing effect, conopeptides target with great specificity voltage-gated and ligand-gated ion channels. The transcriptomes from the venom gland of several cone species are being assembled in the search for conopeptide precursors. In addition, these transcriptomes could be used to extract orthologous nuclear as well as mitochondrial gene for phylogenetic inference purposes. Here, we present a phylogeny of main genera within the family Conidae based on compete mitochondrial genomes and selected nuclear genes. We use this phylogeny to gain insights on the evolution of conotoxin superfamilies and disentangle the processes underlying their great diversity.

P7 - PORCINE Y-CHROMOSOME VARIATION IS CONSISTENT WITH THE OCCURRENCE OF PATERNAL GENE FLOW FROM NON-ASIAN TO ASIAN POPULATIONS

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Pigs (Sus scrofa) originated in Southeast Asia and expanded to Europe and North Africa approximately 1 MYA. Analyses of porcine Y-chromosome variation have shown the existence of two main haplogroups that are highly divergent, a result that is consistent with previous mitochondrial and autosomal data showing that the Asian and non-Asian pig populations remained geographically isolated until recently. Paradoxically, one of these Y-chromosome haplogroups is extensively shared by pigs and wild boars from Asia and Europe, an observation that is difficult to reconcile with a scenario of prolonged geographic isolation. To shed light on this issue, we genotyped Y-linked SNPs and one indel in a worldwide sample of 236 pigs and wild boars

and partially resequenced seven loci distributed along the Y-chromosome. We performed an approximate Bayesian computation analysis focused on the patterns of Y-chromosome variation of wild boars and local pig breeds in which we compared three demographic models: two isolation models (I models) differing in the time of isolation and a model of isolation with recent unidirectional migration (IM model). Our results suggest that the most likely explanation for the extensive sharing of one Y-chromosome haplogroup between non-Asian and Asian populations is a recent and unidirectional (non-Asian > Asian) paternal gene flow.

P8 - MOLECULAR IDENTIFICATION OF ECONOMICALLY IMPORTANT SHRIMP SPECIES BY SPECIES-SPECIFIC PCR ASSAY

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During the last few years prawn demand has increased among consumers, trend partly promoted by the worldwide popularity of the seafood products like sushi, sashimi or the wide variety of frozen processed foods. According to the European Union regulations, developed to ensure the control and traceability of seafood, the commercial (trade) name, the scientific name, the production method (catching or harvesting) and the catch area have to be declared on the label of any seafood product. However, despite the legal framework, the absence and inaccuracy of labelling have been documented. In this context, species identification is particularly relevant for authorities and the seafood industry in order to avoid species substitution, economic fraud and potential human health risks as well as to protect the sustainability of fisheries. Some species may be easily identified by external traits; however, in seafood products visual identification is difficult and even impossible when external parts of the animal anatomy are absent as a result of the processing. Therefore, molecular tools are required as unequivocal identification system to seafood traceability. In the present study, we developed a molecular identification method of six prawn species: Palaemon serratus, Palaemon elegans, Aristeus antennatus, Penaeus monodon, Pleoticus muelleri and Fenneropenaeus indicus. It concerns a reliable, fast and cheap method based on species-specific PCR assays targeting a fragment of the mitochondrial cyt b. Moreover, the analysis can be carried out in both fresh and frozen tissue. Accordingly, we provide a potential molecular tool for the administration and seafood industry to verify labelling compliance.

P9 - PHYLOGEOGRAPHY OF THE EUROPEAN LITTORAL PRAWN PALAEMON ELEGANS REVEALS COMPLEX POPULATION GENETIC PATTERNS AND CRYPTIC SPECIES

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Marine species with high dispersal ability frequently show substantial genetic differentiation owing to biological specific traits, environmental factors and historical processes. Hence, marine populations can present unexpected complex phylogeographic patterns when they are genetically analysed. Moreover, morphological indistinguishable lineages within one single supposed species can exhibit dramatic genetic differences. This is the case of cryptic species, a widespread

phenomenon across the oceans. The common European littoral prawn Palaemon elegans is a decapod distributed throughout the Atlantic Ocean and Mediterranean, Black, Caspian and Baltic Seas. This species is characterised by its capability to adapt to highly variable environmental conditions. Due to its broad ecological niche and recent geographic expansion this prawn is considered an important species within European coastal fauna. A range-wide phylogeographic study was carried to go into detail about the population genetics of this species. Mitochondrial marker cytochrome c oxidase subunit I (COI) was analysed in 521 individuals collected from 41 localities. Statistical parsimony haplotype network, analysis of molecular variance (AMOVA), spatial analysis of molecular variance (SAMOVA), FST pairwise indices, principal components analysis (PCoA) and Mantel test were performed with this dataset to delve into P. elegans population structure. Overall, the results obtained from all these analyses strongly support the existence of three different COI haplogroups (type I, type II and type III) previously described. Here, we have increased the sampling effort generating 249 sequences from 10 new localities and we further extended the analyses. Haplogroup type I was found in Atlantic Ocean whereas type II and type III were found in Mediterranean Sea, mostly in sympatry. A phylogeographic break was reported between Atlantic (type I) and Mediterranean localities (type II) corresponding to the Almería-Orán Front, an oceanographic barrier which restricts gene flow. Haplogroup type III showed a high genetic divergence from type I and type II, which indicate that type III is a cryptic species within P. elegans. In order to clarify the phylogenetic relationships between this three types and the origin of type III, Maximum-likelihood (ML) tree and Bayesian (BI) tree were inferred for the first time within P. elegans complex. Two clusters were recovered clearly separating type III from the other two types. Both ML and BI trees suggest that type III was the ancestral species and both type I and type II derived from type III ancestors. Subsequently, this work provides new insights into current population structure and evolutionary history of this prawn species.

P10 - MORPHOTYPE DIFFERENTIATION IN GOOSENECK BARNACLE POLLICIPES POLLICIPES (GMELIN, 1789) BASED ON GENE EXPRESSION PATTERNS

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Gooseneck barnacle Pollicipes pollicipes is a marine crustacean, whose life cycle shows two phases: free-life larvae and sessile adults. It inhabits coasts exposed to strong waves and tides from North of France to Senegal. In Spanish and Portuguese markets, its economic value is currently quite high due to its demand in fisheries, reaching prices higher than 250 €/kg in certain seasons of the year. Two different phenotypes were described according to certain morphological characters, barnacles of sun and barnacles of shade. These morphological traits different between both morphotypes include the size, thickness and weight of the peduncle and the colouring of the oral aperture. Barnacles of sun have high commercial values whereas barnacles of shade barely have them. In order to explain these morphological differences, genetic and morphometric analyses were performed. Specimens of both morphotypes were obtained from Roncudo (Galicia, NW Spain) and measures of different morphological traits were carried out. Statistical analysis to detect significant differences of each trait between both morphotypes were calculated by T-Student tests. DNA was extracted from five different tissues belonging to both morphotypes and cDNA was synthesised. Microsatellite markers were genotyped in 15 individuals of each morphotype following the instructions described in Seoane-Miraz et al., (2015). Six reference genes were developed and assessed using four different algorithms to use them as normalisers. Furthermore, five specific genes related to integrity and turgidity of peduncle were used as genes

of interest. Statistical comparisons of morphological traits showed significant differences between morphotypes for ratios of rostrum-carinal length/total length and capitulum humid weight/total humid weight (p-values<0.0007). Microsatellite analyses showed no genetic differences between barnacles of sun and barnacles of shade. Reference genes analysed to select the reference gene that showed more stable expression levels, detected HSP70 as the most stable one. After gene expression standardisation using HSP70 as reference gene, slight differences in expression levels were detected between genes of interest in both morphotypes. These differences in expression levels of muscularity and integrity genes in sun morphotype regarding shade morphotype could explain the morphological differences reported in gooseneck barnacle P. pollicipes related to water uptake and peduncle's integrity and turgidity. The implementation of these findings in management programmes of this natural resource would let a more sustainable exploitation due to sun morphotype would be mainly harvested, whilst, shade morphotype (less economically important) could act as larvae deliver, maintaining the population sizes.

P11 - BIOINFORMATIC AND ANALYTICAL TOOLS FOR THE ANALYSIS OF WHOLE-GENOME SEQUENCE POLYMORPHISM DATA

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Most of current NGS tools for sequence analysis were designed to minimize the false-positive rate in the genotyping process (i. e., the detection of SNPs across genome). Consequently, the estimates of variability obtained by these methods may be biased when low read depth data is studied. Also, the analysis of variability using pooled data is still very simplistic and in many cases, the raw frequency of variants is directly used as a proxy of the diversity levels. We have developed statistics and tests of neutrality for estimating the patterns of variability using NGS data containing large number of missing data. We have also developed new algorithms that allow an accurate estimation of the levels and patterns of variability in diploids but also in pooled data. Finally, we have designed bioinformatic tools that allow the user to use these algorithms and statistics in an efficient way.

P12 - DEVELOPMENT OF A COLLECTION OF INTROGRESSION LINES OF SOLANUM INCANUM, A DROUGHT TOLERANT WILD RELATIVE, IN THE GENETIC BACKGROUND OF EGGPLANT

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The development of sets of introgression lines is a powerful tool for the genetic dissection of traits of interest, as well as for crop breeding. However, the development of such collections requires several generations of backcrossing and genotyping in these segregating generations. Here we present the development of a collection of introgression lines of Solanum incanum in the genetic background of eggplant (S. melongena). Solanum incanum is a wild species related to eggplant that grows in desert and semi-desert areas in North Africa, the Middle East and the Middle East. Therefore, Solanum incanum represents a potential source of variation for breeding for resilience in cultivated eggplant. Throughout a process begun in 2008, successive generations

of backcrossing have been obtained using one accession of S. incanum as donor and one accession of S. melongena as recipient. Different types of markers, as they were available, were used for the genotyping of the materials of segregating generations: COSII and SSRs in the early stages and SNPs (including mass genotyping by sequencing) in the latter. As a result, advanced introgression materials covering 99% of the S. incanum genome have been obtained. In addition, a set of 45 fixed (immortal) introgression lines covering 71.7% of the S. incanum genome are already available. Each of these lines contains between 0.1% and 10.9% of the S. incanum genome, with an average value of 3.4%. Through in silico analysis, within this collection of introgression lines, we have been detected 68 candidate genes potentially involved in drought tolerance. The introgression lines obtained will be of great use in the genetic dissection of characters of interest for the improvement of eggplant, in particular for drought tolerance, as well as for studies related to the domestication of this crop. On the other hand, since most of the genome of these materials correspond to cultivated eggplant, these are elite materials that can be incorporated directly into commercial improvement programs. The preliminary results of agronomic characterization obtained so far indicate that these introgression materials can be very useful in the development of a new generation of varieties with greater tolerance to drought.

P13 - GPI ANCHOR REMODELING ACTS AS A QUALITY CONTROL SYSTEM LICENSING ER EXPORT OF MATURED GPI-ANCHORED PROTEINS.

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Quality control in the endoplasmic reticulum (ER) prevents packaging of immature secretory proteins into COPII vesicles, but the precise mechanisms involved in this process have not been defined for most cargos. Glycosylphosphatidylinositol-anchored proteins (GPI-APs) are luminal secretory proteins anchored to the membrane by the glycolipid GPI. After protein attachment in the ER lumen, lipid and glycan parts of the GPI anchor are structurally remodeled. In yeast, GPI-lipid remodeling concentrates GPI-APs into specific ER exit sites (ERESs) via a lipid-dependent sorting mechanism and GPI-glycan remodeling induces subsequent recruitment of the specialized ER export machinery that enables vesicle formation from these specific ERESs. Here, we present evidence suggesting that GPI anchor remodeling operates as a sophisticated quality control system preventing the premature ER export of free GPI molecules and increasing the efficiency of the GPI-anchoring process.

P14 - CHARACTERIZATION OF EARLY OCULAR PHENOTYPES OF THE ZEBRAFISH BUGEYE LRP2A KNOCK-OUT LINE

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LRP2 is a transmembrane protein that belongs to the LDL receptor family (May et al., 2007) and is involved in the transport and internalization of multiple molecules, including SHH, BMP4, vitamin and hormone binding proteins and apolipoproteins, among others. According to previous studies, LRP2 is expressed in cells of the proximal renal tubule, choroid plexus, developing neural tube, intestine, thyroid, and inner ear. Inside the eye, LRP2 is expressed in pigment epithelial cells of the retina, as well as in ciliary epithelial cells (Fisher & Howie, 2006). LRP2 was identified by

our group as a candidate gene in congenital glaucoma patients by exome analysis. Mutations in LRP2 have been previously associated with Donnai-Barrow syndrome (Kantarci et al., 2008), that includes ophthalmological alterations, among other congenital malformations. In the last years the zebrafish (Danio Rerio) has become an attractive animal model for the study of genetic diseases including glaucoma (Bibliowicz et al., 2011). In zebrafish, LRP2 presents two orthologs (lrp2a and lrp2b) and the mutant line, bugeye, which presents lrp2a loss of function, has been described to reproduce several glaucomatous phenotypes including intraocular pressure elevation, retinal ganglion cells loss and increased eyeball size (Veth et al., 2011). As the mechanisms that lead to these phenotypes is unknown, in this work we characterized the ocular histological defects previous to the appearance of the macroscopic phenotype in the bugeye zebrafish line. Fluorescent immunohistochemistry in OCT embedded eye sections of adult bugeye fishes without increased eye size (5 months) showed structural changes in ciliary margin zone (CMZ) and increased corneal thickness. In addition, the use of the reporter line Tg(sox10:GFP) as a marker of neural crest cells also showed that Lrp2a-/- KO larvae had decreased sox10 expression. Hopefully, these analyses will contribute to the understanding the role of LRP2 in congenital glaucoma. This study has been supported by research grants from the Regional Ministry of Science and Technology of the Board of the Communities of “Castilla-La Mancha” (PEII-2014-002-P) and the “Instituto de Salud Carlos III/FEDER” (RD12/0034/0003, RD16/0008/0019 and PI15/01193).

P15 - ANALYSIS OF PCP CORE PROTEIN VANGL2 IN SPROUTING ANGIOGENESIS

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Angiogenesis is the formation of new blood vessels from pre-existing ones and is a complex and coordinated process. Angiogenesis has been involved in several pathologies, including cancer. During tumor development, tumor cells have to acquire the ability to induce the formation of its own vasculature through the process of sprouting angiogenesis. This is called “angiogenic switch” and is a fundamental step in tumor progression. To form new vessels, endothelial cells must get front-back polarization in order to determine the direction of the migration, and apicobasal polarity to form the vessel lumen, but also have to be oriented in the plane of the tissue. While the mechanisms underlying the intercellular communication that allow this coordinated polarization are not fully understood, it is believed that Planar Cell Polarity (PCP) regulatory pathways could play a key role in them. PCP is established trough the asymmetrically distribution of PCP core proteins like Vangl or Celsr in the cells. It has been reported that Vangl2 is expressed in endothelial cells and that inhibition of PCP signaling disrupts endothelial cell growth, polarity, and migration. However not much is known about the regulation of PCP core proteins in vascular morphogenesis and tumor angiogenesis. We hypothesize that that PCP signaling may play an important role in vascular morphogenesis and its deregulation be important during formation of tumor vasculature. In this work, we describe the use of two different models to address the role of PCP during vascular morphogenesis: mouse retina and embryonic stem cells 3D differentiation assay to analyze the localization of different PCP proteins.

P16 - WT1 IS INVOLVED IN PANCREAS DEVELOPMENT AND ADULT PANCREATIC HOMEOSTASIS

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The embryonic mesothelium lining the visceral organs gives rise to mesenchymal cells through a localized epithelial-mesenchymal transition (EMT). This has been extensively studied in some cases, such as the heart, where the epicardium gives rise to epicardial-derived cells that contribute to the cardiac vascular and connective tissues. In other organs, such as the lungs, liver and gut, the developmental fate of the mesothelial-derived mesenchyme and their importance for visceral morphogenesis has also been demonstrated (reviewed in Ariza et al., Dev Dyn, 2016, 245:307-22). Hepatic stellate cells (HSC) are located in the perisinusoidal space of the liver. It has been described that cells derived from the liver mesothelium through an EMT contributes to the HSC population and also to the sinusoidal endothelium during development (IJpenberg et al. Dev Biol, 2007, 312: 157–170; Asahina et al., Hepatology, 2011, 53:983-95). Thus, we checked is a similar developmental origin accounts for pancreatic stellate cells (PSC), a population of pancreatic stromal cells that share many features with HSC. In normal adult pancreas, PSC are quiescent, star-shaped cells with a periacinar distribution. When activated by profibrogenic stimuli such as inflammatory cytokines or oxidative stress, PSC transform into myofibroblast-like cells. Thus, PSC are the major source of extracellular matrix in the adult pancreas, but their embryonic origin remains unknown. The Wilms' tumor suppressor gene (Wt1) is highly expressed in the embryonic mesothelium. For this reason, we have used two lines of transgenic mice for lineage tracing of mesothelial-derived cells, systemic (Wt1Cre; R26REYFP), tamoxifen-inducible (Wt1ERT2; R26REYFP) and we have also used the inducible driver for conditional deletion of Wt1 (Wt1ERT2; Wt1 flox) in adult mice. Our results confirm that WT1 protein is only expressed in the mesothelium of the developing pancreas, allowing for reliable tracing of the mesothelial-derived cells. During the early stages of pancreas morphogenesis, its mesothelium shows the typical features of EMT. Mesothelial-derived cells, identified by constitutive YFP expression, differentiate into a major part of the PSCs and also contribute to other connective and vascular cell type, including endothelium. Thus, mesothelial-derived cells originated by EMT seem to constitute an important subpopulation of mesodermal cells during pancreas development, contributing to its morphogenesis. On the other hand, systemic deletion of Wt1 in adult mice causes a severe atrophy of the pancreas, although this factor is only expressed in the pancreatic mesothelium. In addition, we have observed that adult PSC express Wt1 in the caerulein-induced pancreatitis model. Our results suggest that: 1) normal pancreatic function is maintained by a Wt1-dependent signaling mechanism acting from the mesothelium and 2) Wt1 plays a role in PSC activation in adult mice. These observations point to a relevant function of the Wt1 gene in pancreatic development and function.

P17 - ANALYSIS OF THE ROLE OF P73 IN ADIPOCYTE DEVELOPMENT.

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The study of adipose tissue and its regulation have gained importance because of the high prevalence of obesity and its comorbidities in the developed countries. The main component of adipose tissue are adipocytes, whose generation requires two processes: adipogenesis and lipogenesis. Adipogenesis is a complex differentiation program that gives rise to adipocytes from

mesenchymal stem cells. This program is executed in two phases: commitment of mesenchymal stem cells to the adipocyte lineage and terminal differentiation of the preadipocytes, which acquire mature adipocyte features. All these processes are tightly controlled by more than a hundred of transcription factors. The p53 family of transcription factors is composed by p53, p63 and p73. These genes share similar structure and sequence identity. In addition to their tumor suppressor functions, p53 family members have a relevant role regulating several differentiation processes. Our group has demonstrated that p73 is a positive regulator of endothelial cell differentiation and vasculogenesis, while we and other groups have revealed its role in neurogenesis. It has been reported that p53 negatively regulates white adipose tissue adipogenesis while it is a positive regulator of brown adipose tissue generation. However, a possible role of p73 in adipocyte development has not been addressed. To study the involvement of p73 in adipogenesis we used in vivo and in vitro models. Adipocyte differentiation from induced pluripotent stem cell give us information about the whole process of adipogenesis, even the first stages of differentiation from mesenchymal stem cells. In addition, differentiation into adipocytes from mouse embryonic fibroblasts recapitulates the late phase of adipogenesis. We also utilize in vivo models, analysing adipose depots in wild type and Trp73 deficient mice.

P18 - MEMBRANE TYPE-4 MATRIX METALLOPROTEINASE (MT4-MMP) IS REQUIRED FOR ANGIOGENESIS DURING BRAIN DEVELOPMENT

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During brain angiogenesis, endothelial cells (EC) from blood vessels of the perineuronal vascular plexus (PNVP) on the brain surface invade and migrate into the neuroepithelium. Good postulated candidates for regulating sprouting angiogenesis in the neural tube are matrix metalloproteinases (MMPs). MMPs constitute a large group of endopeptidases that degrade all components of the extracellular matrix. Within this family are the membrane-type MMPs (MT-MMPs) that are anchored to the cell membrane. We have focused on MT4-MMP (MMP17) that is tethered to the cell surface via a glycosylphosphatidylinositol moiety. Information about its function and substrates is very limited to date and little is known about its role during development. Here, we show that Mt4-mmp is initially expressed in the PNVP and by E10.5 is also located in EC of the first blood vessels that migrate and enter the neural tissue. Our results also demonstrate that the presence of MT4-MMP is necessary for VEGF-induced EC migration. Thus, mouse lung EC derived from Mt4-mmp-/- mice showed reduced migration compared to wild type animals in transwell migration assays. Accordingly, MT4-MMP is required for the proper formation of the vascular plexus in the developing brain. Whole-mount immunostaining of the embryonic hindbrain revealed vascular defects characterized by limited number and density of junctions and a reduced vessels area as well as compromised number of macrophages in the mutant embryos. Our preliminary data indicate similar results in the analysis of the angiogenesis in the postnatal retina.

P19 - GENERATION AND PRELIMINARY CHARACTERIZATION OF ZEBRAFISH MYOC TRANSGENIC AND KNOCK-OUT LINES: IMPLICATIONS IN GLAUCOMA PATHOGENESIS

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Myocilin is an extracellular glycoprotein of poorly understood function. Mutations in the MYOC gene are involved in both juvenile and adult-onset glaucoma, optic neuropathies characterized by a progressive and irreversible visual loss and frequently associated with elevated intraocular pressure (IOP). Although certain molecular mechanisms such as homoallelic complementation, haploinsuffiency or negative dominant effect have been proposed to explain the pathogenesis of myocilin glaucoma, most experimental evidences support the gain of function theory. Both Myoc transgenic and knock-out mice models have been developed, but none of them have shown phenotypic defects in eye anterior segment nor glaucoma. Mice models for other glaucoma causing genes such as Foxc1 or Cyp1b1 have equally failed to reproduce the IOP increase observed in humans suggesting that this animal model is limited to recapitulate the human glaucoma phenotype. The zebrafish (Danio Rerio), is receiving attention as a powerfull genetic model to study human ocular diseases such as glaucoma. Moreover, zebrafish knock-out models for glaucoma related genes such as foxc1 and gpatch3 recapitulate ocular phenotypes such as edemas, microphthalmia and colobomas. Myoc is also expressed in the ocular anterior segment of zebrafish larvae although neither spatio-temporal expression pattern nor phenotypes associated to changes in myoc expression have been described. The primary objective of this work was to establish and functionally characterize MYOC transgenic and knock-out zebrafish lines. We used the Gateway/Tol2 technology (http://tol2kit.genetics.utah.edu) to produce the myoc overexpressing fish line Tg(ßactin:myoc)+P2AmCherry. The CRISPR/Cas9 technology was employed to generate a myoc knock-out fish line. Preliminary analysis of these zebrafish lines showed that ubiquitous overexpression of myoc resulted in craniofacial dismorphologies in Tg/+ adult fishes including lower jaw hypoplasia and operculum overgrowth, but no obvious ocular phenotypes. Myoc knock-out zebrafish resulting from a 10 nucleotides insertion in exon 1 of myocilin produced growth retardation as well as periocular edemas in adult fishes. The obtained zebrafish lines will allow the analysis of myoc related histological defects underlying the observed phenotypes and the analysis of genetic interactions with other glaucoma related genes. Hopefully, these animals will also be useful to elucidate the enigmatic biological function of myocilin.

P20 - FUNCTIONAL RELATIONSHIP BETWEEN SNAIL1 AND PRRX1 EMT TRANSCRIPTION FACTORS IN NEURAL CREST DEVELOPMENT

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The epithelial-mesenchymal transition (EMT) endows cells with migratory and invasive properties and it is crucial for the formation of many tissues and organs during embryonic development. This cellular program is triggered by the activation of transcription factors, referred to as EMT-TFs. Prrx1 was recently identified as a novel EMT-TF in our lab (Ocaña et al. 2012). We have observed a complementarity between the expression patterns of Prrx1 and the classical EMT-TF Snail1 during embryonic development. The described phenotype of single mutants indicate that although they differ, they affect similar cell populations, including neural crest derivatives. We are generating mouse models in which the expression of both transcription factors is compromised, with the aim of better understanding putative genetic interactions and/or cooperation during

embryonic development, which can also shed new light into the interpretation of the phenotypes observed in pathological contexts. In particular, we are focusing in the neural crest, which is a cell population that undergoes EMT from the dorsal part of the neural tube and expresses both EMT-TFs. The analysis of the phenotype of the single and double Prrx1/Snail1 heterozygous embryos showed that a copy of both transcription factors is sufficient to allow normal neural crest cell migration. However, preliminary results indicate that the loss of function for both genes causes defects in the migratory neural crest. Due to the early lethality of Snail1 mutants, we are generating new conditional mouse lines that will allow us to better characterize these defects in the neural crest and its derivatives.

P21 - PRRX1 CONTROLS CARDIAC PACEMAKER DEVELOPMENT.

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The cardiac pacemaker plays an essential role in the control of rhythmicity of heart contractions, and the abnormal development of the cardiac conductive system is a major contributor of lethal cardiac arrhythmias. During the early stages of heart development, the fusion of the two precardiac regions in the embryonic midline results in the formation of a linear heart tube that displays an initial slow and peristaltic contraction pattern. The posterior addition of progenitor cells to the venous pole at the time of heart looping, generates the sinoatrial node (SAN), a dominant cardiac pacemaker that eventually leads to a coordinated and sequential atrial-ventricular contraction pattern. We have recently shown that in zebrafish heart looping occurs as a result of a left-right (L/R) asymmetric activation of the Prrx1a EMT inducer,. This asymmetric expression generates L/R differential cell movements towards the midline that lead to a leftward displacement of the cardiac posterior pole and the subsequent dextral looping (Ocaña et al., 2017). We have now found, that in addition to heart laterality, Prrx1a is also involved in heart morphogenesis. As such, prrx1a downregulation by morpholino injection leads to a defective posterior pole with a smaller atrium the lack of a defined sinus venosus. Interestingly, prrx1a morphants exhibit severe defects in rhythmic contraction of the heart, displaying bradycardia and a sinus block phenotype, two problems associated with a defective pacemaker. Prrx1a expression colocalizes with that of Islet1 and Tbx18, two posterior pole markers involved in the development of cardiac pacemaker and both genes are repressed in the Prrx1 morphant embryos. Thus, Prrx1a regulates the morphogenesis of the posterior pole of the heart and controls pacemaker development.

P22 - ABNORMAL AXIAL SKELETON DEVELOPMENT IN THE ABSENCE OF MEIS

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Skeletal defects have been described in a large group of genetic disorders. The skeleton arises from three different origins: the somites (paraxial mesoderm), lateral plate and neural crest cells. The axial skeleton, that comprises vertebrae and ribs, derives from the somites, and each somite differentiates into a ventral and a dorsal part, the sclerotome and dermomyotome, respectively. The sclerotome gives rise to the axial skeleton and the dermomyotome generates the appendicular and the axial musculature, as well as the dorsal epithelium. However, deletion of different somite markers either from the sclerotome or the dermomyotome produces skeleton malformations. On the other hand, Hox genes are key regulators of regional pattern formation along the embryonic antero-posterior axis among others and consequently mutations in these group of genes lead to

homeotic transformations observed in the axial and in the appendicular skeleton. We have been studying Meis mutant skeletal pattern using different mouse Cre lines. The most striking defects in Meis mutants are anterior homeotic transformations in the cervical region and fusions between different vertebrae and ribs. These defects are very similar to those observed in the absence of sclerotomal and myogenic factors such as Pax1, Myf5 and Myf6 and, in Hox mutants such as HoxA4, Hox5 and HoxA6. Meis transcription factors belong to the TALE homeodomain family and are well-known early patterning regulators during embryogenesis. Meis factors bind to DNA and interact with Hox proteins increasing their affinity and selectivity. Accordingly, we are analysing Hox gene expression patterns in Meis mutants in order to detect if altered expression patterns could explain the phenotype observed in our mutants. In addition, we are using the same strategy with sclerotomal and myogenic factors looking for changes in the specification of the somites that could cause the skeletal defects observed. We showed that Meis transcription factors have a role in skeleton development since Meis mutants exhibit important skeletal defects. However, it is not understood how Meis affects skeletogenesis. We suspect that the phenotype observed might result from a general effect of the loss of Meis in different independent processes. Therefore, our objective is to characterize Meis function and determine if Meis acts by regulating Hox genes, by directly affecting somite specification or both.

P23 - UNCOVERING THE CLONAL DYNAMICS OF THE HINDBRAIN PROGENITORS: THE CELLULAR CONTRIBUTION TO HINDBRAIN GROWTH

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During embryonic development, a vast diversity of cellular types is generated within the central nervous system (CNS). These neural cells derive from multipotent progenitors, which follow patterning cues before proneural genes specify the distinct neuronal populations. Proneural genes commit neural progenitors to a given fate and initiate neurogenesis by promoting cell cycle exit and activating a downstream cascade of differentiation genes. Neuronal subtype identity is defined by the expression of neuronal homeodomain proteins, which are critical for determining the neurotransmitter phenotype of the mature neuron, as well as its axonal connectivity and circuit assembly. It has been proposed that proneural genes also play a role in specifying the neuronal subtype that the committed progenitors will acquire during differentiation. Our main goal is to understand how spatiotemporally controlled cell specification and differentiation occur alongside morphogenesis in the construction of the functional hindbrain, whose neuronal populations regulate a wide range of processes vital for the organism. The orchestrated division of neural progenitors and patterning of lineages leads to the assembly of a well organized hindbrain, where progenitors end up located in the ventricular zone, whereas differentiated neurons move to the mantle zone where they will project their axons out of the neural tube. We have characterized the spatiotemporal relationship between the different proneural domains and the putative neuronal subtypes arising from them. Preliminary results on loss-of-function experiments suggest context-dependent mechanisms in which cell specification occurs in different proneural domains: some neuronal populations rely in a single proneural gene for fate acquisition, whereas others seem unaffected by the loss of a single proneural gene -where redundant and regulatory relationships between overlapping and neighboring proneural clusters are expected-. To understand the structural divergence, and how this beautifully organized architecture is maintained over extensive cell proliferation and organ morphogenesis, we combined life-monitoring of specific mosaic and clonal lineage tracing, and compare it with specific neuronal progenitor pools. Thus, our findings

provide information about how morphogenesis impacts cell allocation, by tracing the lineage of a given progenitor population and assess its mode of growth, proliferative potential and neurogenic capacity while cell specification and differentiation is occurring simultaneously.

P24 - GENERATION AND PRELIMINARY CHARACTERIZATION OF A ZEBRAFISH GUCA1C KNOCK-OUT LINE BY CRISPR/CAS9 GENOME EDITING: IMPLICATIONS IN GLAUCOMA PATHOGENESIS

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GUANYLATE CYCLASE ACTIVATOR 1C (GUCA1C) has been identified in our laboratory as a candidate gene in congenital glaucoma by Whole-Exome Sequencing. GUCA1C encodes GCAP3, a member of the Guanylate Cyclase Activating Protein family that belong to the Calmodulin-like neuronal Ca2+-binding proteins (NCBPs) superfamily. GCAP3 is mainly expressed in the retina and is involved in the regulation of photoreceptor guanylate cyclase during phototransduction (Imanishi et al., 2002). Besides, GCAP3 has a significant role in regulating aqueous humor secretion in the ciliary body and in controlling the cellular volume of trabecular meshwork and Schlemm's canal cells (Dorette et al. 2011), physiological processes that are involved in glaucoma development. To generate and functionally characterize a guca1c Knock-Out zebrafish line as a genetic tool to evaluate the role of GUCA1C in congenital glaucoma. CRISPR/Cas9 genome editing was used to generate a zebrafish GUCA1C knock-out line. Guca1c mRNA stability was evaluated using RT-qPCR. Gross ocular phenotypes were assessed by microscopic examination of anesthetized adult fishes (6 months). Gelatin embedded eye sections of adults fishes (6 months) were used for hematoxylin and eosin staining and fluorescent immunohistochemistry. The tissue sections were visualized in an LSM710 Zeiss confocal microscope. We generated a Guca1c Knock-out zebrafish line carrying a p.(Leu41ArgfsTer47) mutation in exon 1 of guca1c that resulted in reduced guca1c mRNA and protein levels. Preliminary characterization of gross phenotypes indicated the existence of retinal and craniofacial alterations in adult fishes. Immunohistochemistry showed guca1c expression in specific tissues involved in glaucoma. We have generated a zebrafish guca1c knock-out line that is being used to evaluate the role of the candidate gene GUCA1C in congenital glaucoma. Acknowledgements This study has been supported by research grants from the Regional Ministry of Science and Technology of the Board of the Communities of "Castilla-La Mancha" (PEII-2014-002-P) and the "Instituto de Salud Carlos III/FEDER" (RD12/0034/0003, RD16/0008/0019 and PI15/01193)

P25 - ROLE OF MEIS TRANSCRIPTION FACTORS IN LIMB DEVELOPMENT

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Meis 1 and 2 are members of the TALE homeobox Transcription factor (TF) family. These TFs are expressed in the lateral plate mesoderm in the early embryo and then, as the limb bud grows, their expression becomes restricted to a proximal domain. Meis genes have been shown to regulate proximo-distal patterning along the limb bud and have been traditionally proposed as proximalizing factors. By studying Meis1 and Meis2 conditional knock-out deleted with different Cre lines, we conclude that Meis genes are not only essential for stylopod specification but also for zeugopod specification and determinant for limb antero-posterior pre-patterning.

P26 - LAMININ LEVELS REGULATE TISSUE MIGRATION AND ANTERIOR-POSTERIOR POLARITY DURING EGG MORPHOGENESIS IN DROSOPHILA

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Basement membranes (BMs) are specialized extra- cellular matrices required for tissue organization and organ formation. We study the role of laminin and its integrin receptor in the regulation of tissue migration during Drosophila oogenesis. Egg produc- tion in Drosophila involves the collective migration of follicle cells (FCs) over the BM to shape the mature egg. We show that laminin content in the BM in- creases with time, whereas integrin amounts in FCs do not vary significantly. Manipulation of integrin and laminin levels reveals that a dynamic balance of integrin-laminin amounts determines the onset and speed of FC migration. Thus, the interplay of ligand-receptor levels regulates tissue migration in vivo. Laminin depletion also affects the ultrastruc- ture and biophysical properties of the BM and results in anterior-posterior misorientation of developing follicles. Laminin emerges as a key player in the regu- lation of collective cell migration, tissue stiffness, and the organization of anterior-posterior polarity in Drosophila.

P28 - OCOXIN ORAL SOLUTION (OOS)® EXERTS ANTITUMOR EFFECT AND IMPROVES CHEMOTHERAPY REDUCING STROMAL CELL-MEDIATED RESISTANCE IN PANCREATIC CANCER CELL LINES IN VITRO

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Pancreatic cancer is one of the most lethal malignancies nowadays. Although big effort has been made to improve the treatments, their efficacy is compromised mainly through stromal cell mediated cancer cell chemoresistance. The current trend is to combine different treatments in order to improve the efficacy of chemotherapeutic agents. OOS® is a nutritional complement with potent anticancer properties described. AIM: We aim to test OOS® as a citotoxic agent in pancreatic cancer cell lines and combine it with two chemothepareutic drugs, Paclitaxel and Gemcitabine. In addition, we want to analyze the ability of OOS to overcome the tumor resistance favored by fibroblast, when treated with both drugs. Methods: Different concentrations of OOS®, Paclitaxel and Gemcitabine were tested in 4 human pancreatic cells lines, BxPC-3, CF-PAC, Capan-2 and SW1990. The cytotoxic potential of these treatments was measured after 48 hours by means of viability assay. Cytotoxic assays were carried out using different combinations of chemotherapeutics and OOS for 48 hours. Moreover, we also evaluated the potential of OOS® to overcome stromal cell mediated chemoresistance in pancreatic cancer cells. Cells were pretreated with human fibroblast-derived medium for 24 hours, and incubated with Paclitaxel, Gemcitabine and the combination of both with or without OOS®, diluted in normal or fibroblast-derived medium for 48 hours. Results: OOS® showed a significant cytotoxicity at 1/50 and 1/100 dilutions with no effect at the other tested concentrations. Paclitaxel reduced the cell viability in concentrations ranging from 50 uM to 1 uM. Gemcitabine had the same effect at 5, 2 and 1 uM in all cell lines except for Capan-2 cell line, which shows a strong resistance to this drug. The addition of OOS® at a dilution of 1/50 increased the cytotoxic effect of either 1 uM Paclitaxel or 1 uM Gemcitabine, included Capan-2. Finally, fibroblast-derived conditioned media partly suppressed the cytotoxicity of the chemotherapeutic agents, which was partially reverted by the addition

of OOS®. Conclusion: OOS® exerts antitumor effects in pancreatic cancer cell lines in vitro, while improving the cytotoxic potential of Paclitaxel and Gemcitabine. Even more, OOS® was able to diminish the resistance of Capan-2 to Gemcitabine. Furthermore, OOS® partially reverted the chemoresistance induced by fibroblast derived soluble factors in all the cell lines. These results point out OOS® as a potential complement in the treatment of patients undergoing chemotherapy for pancreatic cancer by improving the efficacy of antitumor drugs used routinely in the clinics.

P29 - SOD2 OVEREXPRESSION PROMOTES TUMOR PROGRESSION IN THE PROSTATE WITH A NEUROENDOCRINE PROFILE

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Mitochondria are the main source of reactive oxigen species (ROS). Manganese superoxide dismutase (SOD2/MnSOD) is located in the mitochondrial matrix where it is the first antioxidant barrier to fight the overproduction of superoxide (O2.-) produced by the electron transport chain. SOD2 overexpression has been implicated in tumor progression as well as in the resistance to anti-cancer therapies. We have found that overexpression of SOD2 induces neuroendocrine differentiation of androgen dependent prostate cancer cells. Neuroendocrine differentiation is a phenomenon observed along with the progression of prostate tumors and other cancer types. This type of differentiation is characterized because the cells show similar features to neuroendocrine cells, and is usually associated with a poor prognosis because these cells display a high resistance to apoptosis and they are androgen-independent. The TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) is a mouse model with a tumor progression similar to humans. SOD2 expression is increased in 24 and 32 weeks-old TRAMP mice, and is markly reduced in castrated mice. We mated SOD2 knockdown heterozygous mice (Sod2+/-) and SOD2 overexpression mice (Sod2++/+) with TRAMP mice to compare the initiation and the progression of prostate tumors in TRAMP, TRAMPSod2+/- and TRAMPSod2++/+ offspring. These animals were orchiectomized at 12 weeks-old and sacrificed after 24 and 32 weeks of age. After castration TRAMP mice showed an atrophic prostate morphology while TRAMPSod2++/+ mice developed poorly differentiated malignant tumors compared to TRAMP and TRAMPSod2+/- mice. Moreover, after castration TRAMPSod2++/+ animals showed a very high rate of relapses with a neuroendocrine phenotype and developed multiple metastasis that ultimately causes a significantly lower survival rate compared to the parental TRAMP mice.

P30 - DETERMINATION OF EARLY MELANOMA BIOMARKERS BY COMPARATIVE EXOPROTEOMA ANALYSIS OF MELANOCYTES AND MELANOMA CELLS

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The highly metastatic nature of malignant melanoma -even when identified in early (I-II) stages- supports the need of early molecular markers to accurately classified patients. Biomarkers give us information regarding the diagnosis or prognosis of the disease; moreover they may also be directly linked to a deregulated pathway and therefore, provide us with information to determine most suitable treatment. Nevertheless, not just accuracy but also molecular stability is

necessary for any selected biomarker in order to favor good technical standardization methods. In previous studies, our group described serum vitronectin (VTNC) and dermcidin (DCD) levels as two prognosis biomarkers for melanomas identified at early stages. Exosomes, on the other hand, are cell-derived extracellular vesicles with a size ranging from 30 to 100 nm and originated from multivesicular endosomes (MVE). These membrane-surrounded structures contain proteins as well as RNA molecules, including miRNAs and have been named as metastasis messengers due to their capability to modulate other cells to favor metastasis. Regardless of their specific role in metastasis, exosomes represent a stable reservoir for molecules travelling throughout the body and may play an important role in long distance signaling. All in all, characteristics described for exosomes made them optimal for biomarker studies. Previous studies have focused on the characterization of the protein content of exosomes derived from melanoma cells lines. Nevertheless there is no data concerning a comparative analysis of the exoproteoma derived from melanocytes and malignant melanoma cells. In this study, we have isolate exosomes from HeMn-LP and HeMn-MP melanocytes as well as several malignant melanoma cells, including primary melanomas (e.g. A375) as well as largely invasive cells lines (e.g. A2058). Isolation of the exosomal fraction has been characterized by electron microscopy as well as by the presence of well described protein markers (e.g. Tsg101, Hsp90) together by the absence of non exosomal protein calnexin. Preliminary LC-MS/MS data support the exosomal nature of our fractions. More importantly, VTNC and DCD, previously mentioned serum markers, have been detected in the exosomal fraction. These data suggest that exosomes may contribute to observed biomarker differences in patient serum samples.

P31 - COMPARATIVE LIPIDOMIC STUDY OF NORMAL SKIN MELANOCYTES, NEVUS MELANOCYTES AND MELANOMA CELLS

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Cell metabolism is robustly established as a hallmark of cancer. In this regard, tumour cells show an increased metabolic plasticity that supports their proliferative phenotype and confers them the ability to not only survive but also grow in harsh microenvironmental conditions. This metabolic rewiring generates modifications in the conventional metabolic pathways. Concretely, lipid metabolism is commonly altered in cancer what is translated into important modifications in the composition and quantity of some lipids. Actually, these alterations may drive the malignification from melanocytes to melanoma. Hence, the main objective of this work was to identify and compare the lipidome of normal skin melanocytes, melanocytes isolated from nevi biopsies and melanoma cell lines, in order to detect the current lipids that can be used as biomarkers and help in the discrimination of normal skin and melanoma. For this purpose, we studied the lipidome of 19 different cell lines: 3 skin melanocytes, 5 melanocytes isolated from nevi, 5 primary melanomas and 6 metastatic melanomas. Besides, since these cell lines were routinely grown in different culture mediums, we sought to determine the influence of these media in the lipid composition of the cells. Therefore, we cultured the A375 primary and Sk-Mel-28 metastatic melanoma cell lines in different growth mediums (DMEM, EMEM and 254 Medium) and conditions (10% and 1% Fetal Bovine Serum). After culturing the cells, we isolated the lipids of each sample and analysed their lipid composition performing an Ultra High Performance Liquid Chromatography and a tandem mass spectrometry with electrospray ionization. Taking together all the findings, we identified hundreds of lipids of different classes and subclasses. Among these, a set of them

was significantly different when comparing normal melanocytes and melanoma. Besides, we also found that culturing the cells in different growth conditions does not alter their lipidome significantly, being more important the phenotype of each cell line than the acylic composition of the culture mediums to differentiate between the distinct cell lines. Consequently, the variations identified in the lipidome of melanoma and melanocytes were strictly due to the differences between these two cell types. Thus, we found a good methodological process that allowed us to find a panel of lipids that could be used as new lipidomic biomarkers in melanoma.

P32 - COMPARATIVE ANALYSIS OF 4-HPR EFFICIENCY IN 2D AND 3D CULTURES OF COLON CARCINOMA C26 AND BREAST CANCER 4T1 CELLS IN THE PRESENCE OF STROMAL CELLS.

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Introduction: The development of solid cancers, malignancy and consequently drug resistance presents a high dependence on the tumour microenvironment, which is composed by highly complex tumor-stroma cross-interactions. The 4-HPR or fenritinide, an analogue of the transretinoic acid, has shown promising results in bidimensional mono-cellular cultures as anti-tumor compound. However in vivo results have been discouraging. The aim of this study is to analyze the effect of co-culture in either a two dimensions (2D) or a three dimensions (3D) conditions in the cytotoxicity of 4-HPR. Materials and methods: The effect of 4-HPR towards the viability of the murine breast carcinoma 4T1 cell line, and the murine colon carcinoma C26 cell lines cultured alone or in the presence of the fibroblast 3T3 cell line was analyzed. The cultures were establishes by using either a 2D or 3D approximation using a gelatine coating or matrix respectively. Viability was quantified by means of PrestoBlue™ based on resazurin assay, and cell cycle analyzed by means of flow cytometry with the propidium iodide assay. Results and discussion: The 4-HPR effect was determined showing sensibility to increasing concentrations in either mono or co-culture in 2D conditions. However, the anti-tumoral effect of 4-HPR was significantly decreased by culturing the tumor cells in a gelatine scaffold. This protective effect was further increase in the presence of fibroblasts. Interestingly, the viability of 3T3 cells or their co-culture with tumor cells cultured in a gelatine matrix increased after incubation at low 4-HPR concentrations. Conclusion: Hence, these results show that mono- or co-cultures of tumor cells and fibroblasts present higher resistance to 4-HPR cultured under 3D culture, suggesting a role for the tumor microenvironment in the development of de novo drug-resistance during cancer progression. These 3D multicellular model might constitute an efficient method for addressing the efficacy of neoadyuvant therapies and also may unveil new biomarkers and targets directed to the tumor stroma.

P33 - ENDOPLASMIC RETICULUM-MITOCHONDRIA TETHERING MEDIATED BY MFN2 FACILITATES CALCIUM EXCHANGE TO BOOST MITOCHONDRIAL METABOLISM

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Mitochondria and endoplasmic reticulum (ER) are not isolated entities within the cell. They interact with each other, which is necessary for proper function of both organelles. Several studies have shown that calcium is the mediator by which ER and mitochondria communicate with each other. Cyclical calcium exchange between both organelles is crucial for mitochondrial function since calcium stimulates mitochondrial oxidative metabolism by activating three important dehydrogenases from tricarboxylic acid cycle and other enzymes catalyzing oxidative phosphorylation. Mitochondria-ER contact region is known as mitochondria-associated endoplasmic reticulum membrane (MAM). It is in MAM where calciumexchange takes place. Hence, the juxtaposition of the ER with mitochondria is a prerequisite for a proper calcium exchange between ER and mitochondria. MAM dysfunction has been recently proposed to be linked to human pathologies such as neurodegenerative diseases or cancer. In this specialized domain, proteins responsible for calcium handling or tethering between both organelles, such as mitofusin 2 (Mfn2), can be found.

Mfn2 belongs to mitochondrial fusion-fission machinery, formed by a group of dynamin-related large GTPases. Mfn2 and mitofusin 1 (Mfn1) are responsible for outer mitochondrial membrane fusion. These two mitofusins have non-redundant roles since Mfn1 can compensate for some but not all of the functions of Mfn2. Mfn2 but not Mfn1 is enriched in MAM and located in both mitochondria and ER, promoting tethering between both organelles by means of transorganellar homotypic and heterotypic interactions with mitochondrial Mfn2 and Mfn1, respectively. Mfn2 has been demonstrated to intervene both in mitochondrial dynamics and mitochondrial metabolism. Reduction of Mfn2 protein levels causes mitochondrial dysfunction, but the mechanism by which Mfn2 regulates mitochondrial metabolism is not fully understood yet. Therefore, this current study aims to elucidate whether a relationship between Mfn2 tethering role and mitochondrial function exists and how it works.

Here we show that mitochondrial metabolism regulation by Mfn2 could relay in its mitochondria-ER tethering function. We observed that artificial mitochondria-ER re-joining with synthetic protein ChiMERA restores proper mitochondrial function in Mfn2 knockout fibroblasts. Furthermore, we found that blocking cyclical calcium exchange between these organelles, recovery of bioenergetics by ChiMERA or Mfn2 expression was suppressed. Thus indicating that mitochondria-ER tethering mediated by Mfn2 facilitates Ca2+ exchange to maintain mitochondrial bioenergetics. This tethering requires Mfn2 expression at the ER, since we demonstrated that expression of ER-targeted Mfn2 restores mitochondrial membrane potential in Mfn2 KO fibroblasts.

P34 - CHARACTERIZATION OF FIBROBLASTS ISOLATED FROM DIFFERENT BASAL CELL CARCINOMA SUBTYPES

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Tumor microenvironment is a critical factor modulating cancer cells and one of its components, cancer associated fibroblasts (CAFs) has been implicated in tumor progression as well as therapeutic resistance therapy in numerous types of cancer. Basal cell carcinoma (BCC) is the most prevalent skin cancer worldwide that appears in photoexposed areas like face, neck or extremities, and, although its low mortality rate, it is highly mutilating. We have described BCC CAFs through different markers expression, that have been scarcely studied in BCC, and its knowledge would be of great help to the study of the tumor process within the framework of its microenvironment. Thus, 12 BCCs from patients were classified by histological evaluation into superficial, nodular or invasive group, and their surrounding fibroblasts were isolated from biopsies. These samples were compared with fibroblasts from 4 healthy (control) skins. CAFs were morphologically characterized and the expression of the following markers, by immunofluorescence, RT-PCR and/or Western Blot was analyzed: Vinculin, Endoglin, a-SMA, FAP-1, S100A4 and MMP-1. In general, all the isolated fibroblasts from BCCs presented characteristics of typical CAFs, including lower expression of Vinculin and Endoglin, and although variable, higher expression of a-SMA and MMP-1. They did not show differences in S100A4 expression compared to control group, but its distribution in the cell was associated with the aggressiveness. However, all types of BCC CAFs presented lower expression of FAP-1. A better knowledge of the surrounding tumor fibroblasts would help to determine the potential invasiveness features of the BCC as well as to optimize or design actual o novel therapies for BCCs.

P35 - TRANSMISSION OF FLUORESCENTLY-LABELLED EXTRACTS OF BETA AMYLOID PLAQUES OBTAINED FROM ALZHEIMER`S DISEASE HUMAN SAMPLES

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Alzheimer`s Disease (AD) is the major cause of dementia worldwide, characterized by severe cognitive deficits. From a neuropathological point of view, AD is characterized by the deposition of Aß in the brain parenchima, as well as hyperphosphorilation of tau protein, forming plaques and neurofibrillary tangles, respectively. However, apart from these two well-known isolated factors, the composition of these structures is extremely complex. On the other hand, it has been hypothesized that AD is developed in a prion-like manner. Moreover, microvessels-endothelial cells are intimately in contact with soluble Aß peptide and plaques. We focused this study in elucidating if 1) components of solubilized plaques are transmited from endothelial to endothelial cells and 2) if Aß has the main role in this prion behavior. To this end, we have solubilized plaques by optimizing a protocol using human samples from patients with different stages of AD (IDIBAPS, BTCIEN and BIOBANC-MUR as part of the Spanish Biobanks Network). After using different buffers, we quantified the amount of Aß peptide in the Aß enriched fractions by western blot and dot blot. Then, extracts were fluorescently labelled and 10 micrograms loaded into host HUVEC cells. Once loaded, cells were plated with receptor HUVEC cells stained with calcein orange-AM, MitoTracker DEEP Red or ER Tracker-Red and images were taken for 10 h into a Cytation 5 high content imaging

equipment at 37ºC. In parallel, the procedure was repeated using fluorescently labelled synthetic Aß25-35 peptide. Our results indicate that plaque-fluorescence is transmitted between HUVECs from very short times, whereas no transmission of synthetic Aß was found. Moreover, plaques-fluorescence is trafficked to the endoplasmic reticulum as showed co-localization with ER tracker. Though these are preliminary results, the amount of evidences reinforces reliability of the prion hypothesis, but suggest that the responsible factor must be a protein/peptide different to Aß peptide but intimately associated to amyloid plaques. Acknowledgements: Authors are indebted to the IDIBAPS, BTCIEN and BIOBANC-MUR Biobanks for the sample and data procurement. Supported by the Spanish Ministry of Economy and Competitiveness FEDER (grants SAF2016-79311-R and SAF 2016-75768R to MDP and AJMM, respectively).

P36 - NEURODEGENERATION IN A GLIOMA MODEL ALTERS CIRCADIAN RHYTHMS

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Glioblastoma (GBM) is the most common tumor from the central nervous system. GBM is an infiltrative, invasive and highly aggressive tumor with a poor-prognosis. Currently, there is no efficient treatment against it. GMB causes neurological symptoms in patients such as sleep disturbances. Our group has proposed a novel approach to study GMB, considering it as a neurodegenerative disease. We use a GMB model based on the expression of constitutively active EGFR and PI3K in glial cells. Our results indicate that GBM affects neuronal function by inducing synapse loss, one of the first events in neurodegeneration. Specifically, we focus our analysis on the effect of GMB in circadian clock neurons and disturbances in circadian rhythms. Our preliminary results show that the progression of GBM triggers a progressive disruption of circadian rhythmicity. Moreover, in absence of GBM, a decrease in the number of synapses in clock neurons induces changes in circadian rhythms. Our final aim is to protect the neurons from neurodegeneration and prevent phenotypes associated to the neurodegeneration linked to GBM, as a consequence, to improve the life expectancy and quality of life. Furthermore, we want to understand the role of circadian disturbances in GBM disease through the study of clock neurons. To perform these experiments, new genetic tools were created during this study.

P37 - RESMINOSTAT INDUCES PHENOTYPIC CHANGES IN HEPATOCELLULAR CARCINOMA CELLS AND SENSITIZES THEM TO SORAFENIB-INDUCED APOPTOSIS

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Background: Currently, the only approved systemic therapy in advanced hepatocellular carcinoma (HCC) is the multikinase inhibitor sorafenib. However, patients are often primarily resistant, or develop resistance during therapy. Resminostat, a novel inhibitor of class I, IIb, and IV of histone deacetylases, was studied in advanced HCC patients after relapse to sorafenib (SHELTER study). In this phase I/II clinical trial the combination of sorafenib and resminostat in HCC patients was safe and showed early signs of efficacy. However, the molecular mechanisms that could explain this synergism are not explored yet. In this work, we aimed to analyze whether resminostat regulates epithelial-mesenchymal and stemness phenotype as a mechanism of sensitization to sorafenib.
Methods: HCC cell lines with differences in their epithelial/mesenchymal phenotype were treated with resminostat and sorafenib alone, or in combination. Cell viability, cell death, cell cycle, invasive growth and colony formation assays were analyzed in vitro. The expression of

EMT and stemness related genes was analyzed by qRT-PCR. Protein expression was analyzed by immunofluorescence and western blot analysis.
Results: Resminostat prevents growth and induces cell death in all the HCC cell lines tested, in a time and dose dependent manner. Furthermore, a collaborative effect between resminostat and sorafenib to induce apoptosis was detected in the mesenchymal HCC cells, which were insensitive to sorafenib-induced cell death. Expression of mesenchymal-related genes was decreased in resminostat-treated HCC cells, concomitant with an increase in the expression of epithelial-related genes and increased organized tight junctions. This change in the phenotype correlated with decreased invasive growth. Moreover, resminostat down-regulated CD44 expression in HCC cells, coincident with decreased capacity to form colonies at low cell density.
Conclusions: Resminostat shifts mesenchymal cells towards a more epithelial phenotype, lower invasive and stemness properties, which may contribute to the sensitization to sorafenib-induced apoptosis.
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P38 - THE ROLE OF WT1 IN THE CENTRAL NERVOUS SYSTEM

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Wilms' tumor protein 1 (Wt1) is a multifunctional transcriptional factor involved in the correct development of several organs. So far, splice variants of WT1 have been linked to the proliferation capacity of progenitors in the peripheral sensory system. However, questions about its presence and functions in the central nervous system (CNS), and in the cells that populate it, still remain unanswered. Recently, WT1 overexpression has been linked to several neurodegenerative pathologies, such as Alzheimer and Huntington disease. Moreover, it has been found to be repressed by PRC2 in striatal samples, among other neurodegenerative markers; and overexpressed when this repressor is absent.
We have detected the expression of specific isoforms of WT1 in mature neurons populating the rodent brain all throughout adulthood, being expressed from postnatal stages as lineage tracing experiments evidence. Also, we have detected that Wt1 expression is modulated throughout aging, being overexpressed in old mice. As a whole, these data suggest a potential role for Wt1 in neural regulation and neurodegeneration of the CNS.

P39 - CARDIOGENESIS IMPAIRMENT PROMOTED BY EMBRYONIC AND PATERNAL BISPHENOL A EXPOSURE: EPIGENETIC BASIS

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Bisphenol A (BPA) is a chemical compound used for manufacturing of domestic (food and beverage packaging) and industrial (epoxy resins) devices. Humans are daily exposed to this toxic through ingestion, inhalation or even dermal contact. Several studies have reported that exposure to environmental pollutants may be transgenerationally inherited if the germ line results affected. In previous studies in zebrafish, we have demonstrated that adult male exposure to BPA promotes the transmission of defective cardiac development up to the F2 generation. Our hypothesis is that cardiogenesis impairment occurs via epigenotoxic effects of BPA. The risk of disease as well as its transmission depends on the susceptibility window during which the exposure to the toxin takes place. In this study, animals were treated with BPA during two different windows of heritable epigenetic damage: during migration of primordial germ cells to the genital ridge (when genome-wide epigenetic erasure occurs) -the first 24 hours of development in zebrafish- and during spermiogenesis (a period of whole chromatin remodeling). The study of heart development and its related changes in epigenetic profile, was performed in embryos exposed during 24hpf (F0) to 100, 2000 and 4000 μg/L of BPA as well as in non-exposed embryos obtained from males exposed to 100 and 2000 μg/L of BPA (F1). Results showed that both embryonic and paternal exposure to higher doses of BPA led to an impairment of cardiac development. The expression of a set of genes encoding transcription factors implicated in zebrafish cardiogenesis (gata5, hand2 and bmp4) was analyzed, the three of them resulting similarly de-regulated in both kind of embryos. In zebrafish, cardiac progenitors can be firstly tagged at blastula stage and heart gene expression relies on the accessibility of regulatory DNA regions which depends on DNA methylation and histone modifications. Both 5mC and histone acetylation were evaluated by whole mount inmunofluorescence in F0 and F1 blastomeres. Moreover the expression of genes encoding DNA-methyltransferases and histone de-acetylases was analyzed. Although methylation pattern did not change, expression of dnmt3, dnmt5 and dnmt8 was altered in F0 and F1 embryos. Moreover, H3 acetylation was sharply increased after embryonic and paternal BPA exposure at blastula stages, the embryos experiencing an upregulation of histone deacetylases at later stages. Since histone 3 acetylation is closely related to cardiomyocyte differentiation,changes in H3ac promoted by BPA could lie behind the mechanism of its inheritable effects on cardiogenesis.

P40 - AUTOPHAGY IS REQUIRED TO EXERCISE-INDUCED ADAPTIVE RESPONSES IN BRAIN

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Molecular mechanisms underlying exercise-induced adaptations in brain are not well known and even whether the type, intensity or duration of the exercise have different effects over this process. Since exercise is considered as an autophagy inductor, we used the atg4b-/- mouse model, with a systemic reduction of autophagy, to determine these issues. Therefore, we considered two types of exercise, endurance and resistance, and two exercise periods, 2 and 12 weeks, and explored their effects on different brain areas. First, we analyzed adult neurogenesis in hippocampus by means of doublecortin (DCX) cell quantification. Our results indicated that exercise was able to

increase neurogenesis in Wt mice, independently of type and duration. Interestingly, atg4b-/- mice did not show differences in DCX+ cells. Moreover, we were not able to detect DCX+ cells in any of these mice in the 12-week training, when they were 6 months old. We next explored basal autophagy levels and we did not find changes in hippocampus, striatum and cerebellum in our model. However, basal autophagy was increased in cerebral cortex of both Wt and atg4b-/- after exercise. Nonetheless, while Wt mice showed basal autophagy enhancement after 12 weeks of exercise, independently of the type, endurance atg4b-/- mice increased their basal levels after 2 weeks of exercise. Finally, since autophagy is known to be required during synaptogenesis and to participate in synaptic function, we further explored the effects of autophagy impairment on the chaperone proteins CSPα and α-synuclein.. Our results show that both were not affected by exercise in Wt mice, but they were decreased in atg4b-/- cerebellum, independently of the type and duration. In summary, our results reinforce autophagy role in brain health favoring important processes such as adult neurogenesis and synaptic protein recycling at the synapse, and highlight the benefits that exercise can provide to cellular homeostasis.

P41 - EPIGENETIC EFFECTS OF BPA DURING SPERMATOGENESIS: BASIS FOR PATERNAL TRANSGENERATIONAL INHERITANCE

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Bisphenol A (BPA) is a well-known endocrine disruptor that mimics estrogens actions binding to estrogen receptors. In males, BPA has been described impairing fertility, interfering with meiotic processes as well as decreasing methylation in zebrafish gonads and acetylation in rat testis (Xie et al. 2016; Liu et al. 2016; Chen et al. 2017). Moreover we have demonstrated the transmission of the toxic effects of BPA to the non-exposed progeny, pointing to specific effects on the germinal cells which are transmitted upon fertilization (Lombó et al. 2015). The present work is aimed to analyze the potential involvement of BPA in zebrafish spermatogenesis, focusing on their epigenetic effects on germinal cells. Adult males were exposed to 100 and 2000 ppb of BPA during 21 days and testes were extracted. mRNA expression level of different epigenetic-remodeling enzymes, estrogen receptors and sycp3, involved in sinaptonemal complex formation, was assessed by qPCR. DNA methylation and histone acetylation levels were evaluated by immunodetection and flow cytometry, according to the different testicular cells. Apoptosis was determined by TUNEL assay and flow cytometry. Results showed an upregulation of hdac4 and kdm6b genes, corresponding to a H3K14 deacetylase and to H3K27 demethylase, respectively. An increase in the H3 acetylation level was also observed after BPA treatment in premeiotic cells. Besides an increase in the percentage of apoptotic testicular cells similar to that reported in mice (Xie et al. 2016) or rats (Urriola-Muñoz et al. 2014) was observed. Our data showed an upregulation of gper-1, an estrogen receptor related to the maintenance of the meiotic arrest in fish oocytes (Thomas 2017) and with the initiation of apoptosis (Chimento et al. 2013), and a downregulation of sycp3 gene, suggestive of a reduced meiosis rate. The present study demonstrated that paternal BPA exposure contributed to meiotic disruption, affecting spermatogenesis, and modified the epigenetic profile of the transcriptionally and translationally active premeiotic cells. Such an effect may compromise the RNA populations transmitted to the spermatozoa, whose important role in the control of early embryo development upon fertilization is no longer discussed. Supported by the Spanish MINECO (AGL2014-53167-C3-3-R), JCyL (Spain) (EDU/1083/2013) and Fondo Social Europeo.

P42 - CERKL, A RETINITIS PIGMENTOSA GENE, IS ASSOCIATED TO RNA-STRESS GRANULES IN THE RETINA

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CERKL, a retinitis pigmentosa and cone-rod dystrophy gene, shows a high transcriptional complexity, with many isoforms produced by alternative splicing and alternative promoters. The CERKL protein displays lipid kinase and mRNA binding domains, as well as nuclear localization and nuclear export signals. The precise physiological function of CERKL is still being elucidated, but it is related to cell resilience to stress since its overexpression protects cells from the apoptosis triggered by oxidative stress. In vitro studies on cultured cells showed that CERKL binds mRNA and contributes to the formation of stress granule complexes. These in vitro results have been further confirmed in murine isolated retinal neurons (retinal ganglion cells and photoreceptors) as well as in human retinal epithelium cells, where CERKL co-localizes with RNA and RNA-binding proteins and is a component of the stress granules produced under oxidative-stress conditions. Moreover, differential localization of CERKL isoforms in rods and cones has been shown using a panel of in-house antibodies. This differential isoform specifity is highly suggestive of specific functional roles for CERKL in different photoreceptor cell types. Future work will address the impact of CERKL mutations in rods and cones related to human visual pathophysiology.

P43 - MODULATION OF APOLIPOPROTEIN D EXPRESSION IN A CELLULAR MODEL OF MULTIPLE SCLEROSIS INDUCED BY CUPRIZONE

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Apolipoprotein D (Apo D) is a lipocalin to which it has been attributed an important role in aging, as well as in several neurodegenerative diseases, related with lipid metabolism and antioxidant mechanisms. One of the diseases that shows an altered Apo D expression is Multiple Sclerosis (MS), although its role on this pathology is still unknown. Therefore, the aim of this work was to study the Apo D expression, by immunocytochemical techniques, in a cuprizone cellular model of MS, and its possible changes in response to an antipsychotic drug that is able to increase Apo D levels. For this purpose, we treated an oligodendroglial cell line (HOG) with different concentrations of cuprizone (0,05 – 1mM), an antipsychotic drug (0,1 – 5μM) or both, during 24 or 48 hours. Once the treatments were concluded, we analyzed the effects on cellular viability by a MTT viability assay and we quantified Apo D immunosignal in these experimental conditions. The results have shown that treatment with cuprizone or antipsychotic drug alone induced very few changes in the Apo D level in the oligodendroglial cell line. However, when we treated cells with the two compounds we observed that antipsychotic drug was able to restore the loss of cell viability caused by treatment with cuprizone while increasing the expression of this apolipoprotein. According with these results, cuprizone, unlike other cytotoxic molecules like H2O2 or Lipopolysaccharide, does not induce the expression of Apo D probably due to the long term effect demonstrated for this copper chelator. More remarkably is the statistical significant increase in the Apo D expression triggered by the antipsychotic drug that seems behind its neuroprotective effect in the cuprizone cellular model probably due to the antioxidant role of Apo D in the arachidonic acid metabolism. All this highlights that the cuprizone cellular model of MS is a useful approach for the study of Apo D expression and open new avenues for investigate its implication in the pathophysiology of MS. Acknowledgements: This work was supported by FISS Instituto de Salud Carlos III and FEDER (Fondo Europeo de Desarrollo Regional) (PI15/00601) grant.

P44 - NEW RETINAL DYSTROPHY MOUSE MODELS USING CRISPR/CAS9 TO EDIT NR2E3 AND CERKL GENES

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Retinal neurodegeneration, characterized by the dysfunction or apoptosis of photoreceptor cells, is a major cause of genetic blindness. So far, mutations in over 200 genes are associated to inherited monogenic retinal diseases (prevalence 1:3000 worldwide), but we are still far from completely understanding their ethiopathology. Therefore, animal models are an essential tool to complement in vitro and cell culture assays. We aimed to generate two different mouse models by genome editing of Cerkl and Nr2e3, two retinal dystrophy genes in human, to dissect and characterize their precise role in photoreceptor cells. We generated two different mouse models by causing small and large deletions of Nr2e3 and Cerkl genes, using the CRISPR/Cas9 system. For Nr2e3, which encodes a dual transcription factor involved in photoreceptor fate, several modified alleles generated by small/medium deletions are under study. These alleles alter the coding sequence of the last exon of this orphan nuclear receptor gene, thereby affecting the dimerization and repressor domains as well as the mRNA and protein stability. For Cerkl, we have deleted the full locus, approximately 100 kb, with the aim to investigate the phenotypic effect of this knockout mutant, particularly the effect of the lack of CERKL in oxidative stress response. We are currently assessing the effect of these gene deletions in the retinal phenotype of wildtype, heterozygous and homozygous littermates. Retinal morphology and functionality is being assessed and compared to other knockout and knockdown mouse models. Grants: This work has been supported by SAF2013-49069-C2-1-R and SAF2016-80937-R (Ministerio de Economía y Competitividad, Spain), La Marató TV3 (project 201417.30), SGR2014-0932 (Generalitat de Catalunya), CIBERER (Instituto Carlos III, Spain) ACCI-2015 y ACCI-2016.

P45 - EVOLUTION OF THE PROFILE OF COQ GENE EXPRESSION ALONG AGING IN MICE

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Coenzyme Q (CoQ) is a key factor in essential activities in cells, tissues and organs. This lipid with redox activity transfer electrons at the electron transport chain in mitochondria and when its levels are low, as in some patients suffering rare diseases due to CoQ-synthesis deficiency, ataxia, myalgia, hearing loss and kidney diseases occurs. Further, CoQ is also a key component in the prevention of oxidative damage in cell membranes and in plasma lipoproteins playing, then, an essential role in the development of oxidative-damage related diseases. It has been described that during aging the capacity of CoQ synthesis decays affecting in different way different tissues and organs. In order to determine if the expression of genes involved in CoQ synthesis is affected during aging we studied their levels in organs of C57BL/6J mice at different ages: young (8 months); mature (18 months) and old (24 months). RNA was extracted by using trizol and RNA purification columns (Qiagen). We analysed the levels of mRNA from brain, liver, kidney, muscle and heart by using specific probes for qPCR designed and checked for specificity. Levels of proteins in liver were determined by WB by using different commercial available antibodies. Liver was the organ showing higher changes in the levels of COQ genes mRNA. Interestingly the mature group of animals showed a rise in comparison with both, young and old animals. In fact,

young and old animals showed a similar pattern of expression. Kidney showed a similar pattern of expression but showing lower modifications in the levels of mRNA. Brain and skeletal muscle showed the lower changes along aging whereas heart showed only low variations in some of the genes determined. Protein variations along aging and in some cases the evolution of proteins involved in the synthesis of CoQ showed opposite variations to the changes found in mRNA liver. The age and the organ studied are important factors in the study of CoQ synthesis during aging. We found higher variations in mitotic organs such as liver and kidney and low variations in postmitotical organs such as brain and skeletal muscle. When we analysed all the variations of these genes in an organ we found that only some of them showed a similar behaviour whereas other did not show any relationship with others suggesting a non-coordinated regulation of the expression.

P46 - MODERATE REPLICATIVE AGEING CONTRIBUTES TO PROLIFERATION HETEROGENEITY.

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Irene DELGADO-ROMÁN, Lidia DELGADO-RAMOS, Mari Cruz MUÑOZ-CENTENO and Sebastián CHÁVEZ. Departamento de Genética, Universidad de Sevilla, Spain, Instituto de Biomedicina de Sevilla (IBiS), Hospital Virgen del Rocío-CSIC-Universidad de Sevilla, Spain. Microencapsulation allows the isolation of single yeast cell within an alginate particle. When cells from a clonal yeast culture are individually encapsulated, each single cell forms a microcolony inside the capsule. We have already shown that these microcolonies differ in size as a consequence of their heterogeneity in proliferation capacity. In this work we have focused on investigating microencapsulated cells that generate very small microcolonies, in order to understand the molecular basis coupling moderate replicative ageing and cell cycle regulation. Previous transcriptomic results showed that these small microcolonies were enriched in the expression of the genes implicated in respiration metabolism and some others related to cell cycle-control. WHI5 was a representative example of this category and the most overexpressed gene in small microcolonies. WHI5 is an inhibitor of the G1-S transition and the yeast functional homologous of the human Retinoblastoma protein. We have found that small microcolonies are predominately founded by moderately aged cells. Moreover, we also found that the frequency of small microcolonies increases when we encapsulate a cell population enriched in moderately aged cells. We will discuss a set of experimental results that seems to link this phenomenon to the enhanced expression of WHI5.

P47 - DIFFERENTIAL EXPRESSION OF DISCOIDIN DOMAIN RECEPTORS IN THE TUMOR STROMA OF DUCTAL INVASIVE CARCINOMA PATIENTS.

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Introduction: Breast cancer is the most common carcinoma in women worldwide, being the ductal invasive carcinoma (DIC) the one with the highest incidence. Due to the implication of tumor microenvironment in cancer development, the study of stromal features could further characterize the DIC, as prognostic and therapeutic tools. Among receptors expressed in the stromal compartment Discoidin Domain Receptors (DDR-1 and -2), have been linked with numerous

human cancers. However, the differential expression of DDRs in the tumor microenvironment from DIC tissues and its association with clinic and histopathological data remain poorly defined. Thus, the aim of the present study is to characterize DDRs expression in microenvironment compartments of samples from DIC patients according. Material and Methods: To do so, samples collected from patients suffering of DIC and adjacent normal breast tissue were obtained from a total of 25 patients for histological analysis (hematoxylin/eosin staining), analysis of the expression of DDR1/2, a-SMA (fibroblasts) and CD-68 (macrophages) by immunohistochemistry, and matrix deposition analysis by Picric-sirius red staining was performed by immunochemistry. These biomarkers were related to clinical features, including tumor size, grade and stage, lymph nodes invasion and e-cadherin and HER-2 levels. Results: A differential expression of DDR1 and DDR2 was detected not only between healthy and tumor tissue, also this expression showed different patterns of expression in tumor cells and the tumor stroma. Comparing to healthy tissues, carcinoma cells show a variable DDR1/2 expression, while increase significantly in the tumor stroma. Also, the deposition of collagen was higher in tumor stroma in associated with areas of ASMA positive cells, and the amount of non collagenous extracellular matrix was associated with areas of lower DDRs expression in tumor cells. Also, CD68 expression was increase in the tumor stroma comparing with the healthy one. Conclusions: These results show a differential expression of DDR1/2 in the tumor and stromal area correlated with a type of tumor cells and extracellular matrix deposition. Further studies will confirm if DDR1/2 may account as a potential marker of disease subtype, progression, and development of “de novo” therapy resistance and a therapeutic target for patients suffering from DIC.

P48 - DISRUPTION OF PRE-ESTABLISHED MTOCS INHERITANCE PATTERN REDUCES CELL LIFESPAN IN BUDDING YEAST

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The mitotic division of eukaryotic cells usually ends up with an equal distribution of the replicated genome and the cellular components between the resulting cells. However, the asymmetric segregation of cellular material during cell division is an essential mechanism of cell diversification. Asymmetric divisions commonly involve the age-dependent partition of various cellular components, including specific RNAs, post-translationally modified or damaged macromolecules. By means of this asymmetric distribution of cellular components, the mother and the newly generated daughter cell can be endowed with a different potential to self-renew or differentiate into a particular cell type. The segregation of the chromosomes during mitosis is possible by their attachment to the mitotic spindle, a structure formed by microtubules that nucleate from microtubule-organizing centers (MTOCs) located at the spindle poles: the centrosomes in higher eukaryotes and the spindle pole bodies (SPBs) in budding yeast. MTOCs are inherently asymmetric, both in terms of their morphology and their age, and interestingly, they represent a highly conserved example of age-dependent segregation, since many stem cells non-randomly segregate the old and the newly-synthesized MTOC during mitosis. This asymmetric age-dependent segregation of MTOCs has been described in the Drosophila male germ line, the neocortex and radial glia cell progenitor from mice and in human neuroblastoma cell lines. Remarkably, SPB inheritance in budding yeast is also asymmetric. Specifically, the daughter cell, which guarantees the immortal lineage, inherits the old SPB, while the mother cell maintains the new SPB. However, little is known about how pre-established MTOC inheritance patterns

are generated and, more importantly, about the biological function of this asymmetry. We have explored the consequences of disrupting a pre-established MTOC inheritance pattern, and our results show that SPB asymmetric inheritance is necessary to preserve the immortal lineage of new daughter cells by coupling asymmetric partitioning with the inheritance of specific age-determinants during mitosis.

P49 - OPTIMIZATION OF A CELLULAR MODEL FOR THE SCREENING OF NEW CHEMOTHERAPEUTICS AGAINST CANCER STEM CELLS IN HUMAN GLIOBLASTOMA

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Glioblastoma multiforme in one of the most prevalent and lethal tumors of the central nervous system. Cancer Stem Cells (CSC) are thought to be responsible for the glioblastoma recurrence, as they are able to resist usual treatments and to generate new tumors. The CD133 protein, usually detected with the AC133 antibody, has been proposed as a marker for this cell population in glioblastoma. Due to the need of identification of new active drugs against this cell population, our aim was the implementation of a cellular model to test new therapies active against the CD133+ population in glioblastoma. Two different approaches were used: genetic modification strategies using lentiviral reporter systems and cell culture strategies, in order to enrich the Cancer Stem Cell population. The use of lentiviral reporter vectors would allow the monitoring of CD133+ population under different conditions or treatments, generating a great tool to screen for new possible therapeutic compounds active against this cell population. Since classical glioblastoma cell lines have a very small percentage of CD133+ cells, different methods have been reported to enrich for glioma CSC, like the culture of the glioblastoma cell lines in serum-free medium or under hypoxic conditions. The formation of tumor spheres in suspension has also been described as a valuable approach for CSC-enrichment and in vitro modelling of GBM. With this aim, classical glioblastoma cell lines were cultivated as tumor spheres in different conditions, and expression of CD133 was analysed. Stablished Glioma Stem Cell lines seemed the best cellular model to test possible candidate treatments. Different drugs were tested in glioma stem cells and their effects were compared with classical glioblastoma cell lines, in order to identify new therapeutic compounds active against the glioma stem cell population.

P50 - EXPRESSION PATTERN OF DYSTROGLYCANOPATHY-ASSOCIATED PROTEINS IN MAMMALS: THEIR DISTRIBUTION PROFILE IN THE MOUSE RETINA

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Dystroglycanopathies (DGPs) are clinically and genetically heterogeneous neuromuscular dystrophies offering a shortened life expectancy and whose severe symptoms extend to the retina. They include the Walker-Warburg syndrome, muscle-eye-brain disease and Fukuyama congenital muscular dystrophy, among other milder disorders. Their causative genes, 18 identified so far, mostly encode glycosyltransferases involved in the O-glycosylation of a-dystroglycan (a-DG).

This is a glycoprotein responsible for linking muscular and nervous cells to the extracellular matrix and the establishment of synapses in the CNS, including the retina. The role of these genes and their protein products is not well established in the adult nervous system, and knowledge on their expression in the mammalian retina is fairly scarce. In our group we have determined by means of RNA-Seq that all known DGP-associated genes are expressed in the adult human retina, though at low to moderately-low levels (1). Among these, we have validated by RT-PCR the presence of POMT1, POMT2, POMGNT1, FKTN/fukutin and FKRP mRNA transcripts in the neural retina of a number of mammalian species, including primates (human and monkey), cow and rodents (rat and mouse), as well as in the 661W photoreceptor cell line (2). In the present work we have focused in analyzing the expression of proteins encoded by the above genes in the mammalian retina. We have evidenced by western blotting that their five protein products are detected in the retina of all mammals analyzed and in 661W cells. Next, we have used immunohistochemical techniques in order to characterize their expression pattern in mouse retinal sections, by means of colabeling for molecular markers of the endoplasmic reticulum (ER), such as calreticulin or KDEL, or the Golgi complex, such as GM130. Confocal fluorescence microscopy observations revealed that these proteins were located in the ER (POMT1/2 and fukutin) or the Golgi (POMGnT1 and FKRP) of retinal neurons, including photoreceptors (inner segments and axon terminals) and/or 661W cells. By contrast, POMGnT2 (a protein functionally related to POMGnT1) was found in both organelles in mouse retinal neurons. These results are indicative of a relevant role of DGP-associated proteins not only in the brain and muscle, but also in a-DG glycosylation in the neural retina of adult mammals. References (1) Campello, L. and Martín-Nieto, J. (2013) EMBnet.journal 19 (Suppl. A), 40-41. (2) Martín-Nieto, J. et al. (2012) FEBS J. 279 (Suppl. 1), 311. Funding: Instituto de Salud Carlos III grant PI15/00073, cofinanced by the European Regional Development Fund (ERDF/FEDER).

P51 - THE IMPACT OF AGING, CALORIE RESTRICTION AND DIETARY FAT ON MITOCHONDRIAL ULTRASTRUCTURE, DYNAMICS AND AUTOPHAGY MARKERS IN MICE SKELETAL MUSCLE.

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Calorie restriction (CR) without malnutrition improves survival and delays the onset of age-associated diseases. In skeletal muscle, aging induces sarcopenia (i.e. a loss in muscle mass and function), a phenomenon that has been proved to be significantly delayed by CR. Aging also induces changes in subsarcolemmal (SSM) and intermyofibrillar (IMM) mitochondria ultrastructure and content and impairs autophagy. Furthermore, alterations of mitochondrial fission and fusion markers have also been described during aging. Recently it has been reported in mice that dietary fat plays an important role in determining lifespan extension with 40% CR. In these conditions, animals fed with lard as dietary fat, showed an increased longevity compared with mice fed diets containing soybean or fish oil. Most of the mammalian muscles are composed by two different types of fibers with strong differences in physiology and ultrastructure and differential response to aging: the so-called slow-twitch (type I or red) and fast-twitch (type II or white) fibers. From an ultrastructural point of view, red and white fibers can be differentiated by the

higher number of mitochondria in the red ones. In this work, we study the effect of these dietary fats on mitochondrial mass and ultrastructural parameters of autophagy in type I skeletal muscle fibers from gastrocnemius muscle from mice maintained on 40% CR for 6 and 18 months. Besides the structural analysis, we also investigated possible changes in the expression patter of protein related to mitochondrial fusion/fission (Mfn1, Mfn2 and OPA1), autophagy (LC3 and Beclin-1), mitophagy (Pink1 and Parkin) and mitochondrial content (VDAC and mitochondrial complexes) in homogenates from hind limb skeletal muscles. Our structural analysis on red fibers shows that aging differentially affected mitochondrial mass and ultrastructural features of mitophagy in SSM and IMM. However, different results were obtained depending on the dietary fat in CR fed mice. On the other hand, the study performed in hindlimb homogenates showed that aging increased mitochondrial fusion markers, a situation that was partially abrogated by CR. Similarly, aging resulted in increased levels of proteins related to autophagy and mitophagy. However, these changes were accompanied by an increased number of mitochondria showing altered ultrastructure and megamitochondria in red fibers. Nevertheless, CR alleviated the accumulation of altered mitochondria in different extent depending on the dietary fat. These results indicate that dietary fat modulates skeletal muscle structure and function in CR mice and plays an essential role in the determination of health span in rodents.

P52 - NUCLEAR RETENTION OF POLY(A) RNAS IN NUCLEAR SPECKLES AND RNA GRANULES IN MOTOR NEURONS OF THE SMA MICE

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Spinal muscular atrophy (SMA) is caused by the deletion or mutation of the survival motor neuron 1 (SMN1) gene, leading to reduced SMN protein levels and, consequently, resulting in the degeneration of motor neurons (MNs). The most well-known function of SMN is the biogenesis of spliceosomal snRNPs, which leads to widespread splicing defects. We used the SMN?7 mouse model of SMA to investigate in MNs the cellular reorganization of polyadenylated mRNAs associated with the splicing dysfunction. We demonstrate that SMN deficiency induced the abnormal nuclear accumulation of poly(A) RNAs in both nuclear speckles of splicing factors and RNA granules enriched in the splicing regulator Sam68. However, these granules lacked RNA-binding proteins essential for pre-mRNA processing and nuclear mRNA export. Moreover, poly(A) RNA retention was accompanied by the cytoplasmic depletion of polyadenylated mRNAs for their translation. These effects were associated with the retention of the intron-containing pre-mRNAs of Chat, Chodl, Myh9 and Myh14 genes, which are important for MN functions. Nuclear retention of polyadenylated mRNAs appears to be a stress-related neuronal response during the late stages of MN degeneration. This response might represent an essential component of RNA metabolism dysfunction in MNs and, therefore, an important contributor to SMA pathogenesis.

P53 - ADCK2 DEFICIENCY REDUCES WEIGHT GAIN AND INCREASES BODY TEMPERATURE OF MICE IN HIGH FAT DIET

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Primary coenzyme Q (CoQ) deficiency causes a heterogenic group of mitochondrial diseases with high variability in severity and tissues affectation. It has been identified that ADCK2 gene is involved in CoQ biosynthesis and its mutation is responsible of a mitochondrial myopathy and liver dysfunction in humans. ADCK2 knockout mice in heterozygosis developed a skeletal muscle mitochondrial dysfunction and myopathy, liver steatosis and defects in oxidation of fatty acids under standard diet (ST). To understand the role of lipids in its phenotype, we have studied the consequences of a high fat diet (HFD) in ADCK2 knockout mice. Wild type (WT) or ADCK2 knockout were assigned to either ST or HFD for seven months (WT-ST n=5, WT-HFD n=6, +/- ADCK2-ST n=4, +/- ADCK2-HFD n=4) and weights were obtained. Animals were housed individually to control weekly food ingestion. Ratio kilocalories ingested per gram of body mass (BM) was determined. Rectal body temperature was explored. Strength and running performance were investigated. Western blots and RT-PCR were performed. Descriptive statics and one-way ANOVA analyses were used. WT animals presented a higher weight than mutants after 31 weeks of study (WT: 31.16g vs. ADCK2 KO: 27.55g in ST and WT: 43.47g vs. ADCK2 KO: 35.20g in HFD; $p<0.05$). Weight gain significantly differed between the genotype and diets (WT: 2.00g vs. ADCK2: 2.50g in ST and WT: 14.43g vs. ADCK2: 9.55g in HFD; $p<0.05$). There were no significant differences in kilocalorie intake among mice in the same diet independently of their genotype. Mutants in HFD presented a higher ratio kilocalorie ingested per gram of BM ($p<0.05$), they tend to exhibit higher body temperature. This group showed higher levels of mRNA and protein of UCP1 in brown adipose tissue, UCP2 in liver and UCP3 in quadriceps, proteins involved in dissipation of energy as heat. ADCK2 KO showed significantly higher insulin resistance on HFD ($p<0.05$) than control, and also showed decreased strength and running performance. MEFs from ADCK2 KO mice showed dysfunctional respiration by fatty acids as bioenergetics substrate. ADCK2 KO showed a defective fat accumulation under HFD due to decreased weight gain associated to higher rectal temperature, which is apparently due to upregulation of UCPs proteins. These results support the role of ADCK2 encoded protein in mitochondria oxidation of fatty acids and lipid metabolism. As skeletal muscle functions highly depend on fatty acids, our results support the decrease of strength and running performance of ADCK2 KO in HFD.

P54 - 1ALPHA,25-DIHYDROXYVITAMIN D3 INHIBITS THE PROTUMORAL PROPERTIES OF COLORECTAL CANCER-ASSOCIATED FIBROBLASTS AND HIGH EXPRESSION OF VITAMIN D RECEPTOR IN THESE CELLS PREDICTS A BETTER CLINICAL OUTCOME

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Vitamin D deficiency is associated with high colorectal cancer (CRC) incidence and mortality. Accordingly, 1alpha,25-dihydroxyvitamin D3 (1,25(OH)2D3, the most active vitamin D metabolite) inhibits the proliferation and promotes the differentiation of colon carcinoma cells. 1,25(OH)2D3 action is mediated by the vitamin D receptor (VDR), a transcription factor of the superfamily of

nuclear receptors that upon ligand binding regulates the expression of its target genes. Given the strong influence of tumor stroma on cancer progression, we investigated the potential effects of 1,25(OH)2D3 on CRC stroma. First, we found that high VDR protein expression in tumor stromal fibroblasts is associated with better overall and progression-free survival in a cohort of 658 metastatic CRC patients. Then, we established primary cultures of colon normal fibroblasts (NFs) and cancer-associated fibroblasts (CAFs) from fresh surgical specimens resected from CRC patients. We found that both types of fibroblasts express VDR and respond to 1,25(OH)2D3. Moreover, 1,25(OH)2D3 inhibits two protumoral properties of fibroblasts: the ability to reorganize collagen fibers and contract collagen gels, and the capacity to paracrinally promote the migration of colon carcinoma cells. Global transcriptomic analyses showed that 1,25(OH)2D3 regulates the gene expression profile of NFs and CAFs, and imposes in CAFs a gene expression signature that correlates with longer survival of CRC patients. Our results indicate that the antitumor action of 1,25(OH)2D3 on CRC is mediated not only by its direct action on carcinoma cells, but also through the inhibition of the protumoral properties of CAFs, suggesting that treatment of CRC patients with 1,25(OH)2D could be explored even in the absence of VDR expression in carcinoma cells.

P55 - THE NAD+ PRECURSOR NMN IMPROVES RESPIRATION IN MITOCHONDRIAL DISEASES CAUSED BY MTDNA DEFECTS.

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Most of the mitochondrial diseases caused by mtDNA defects have no effective therapies, except mitochondrial replacement techniques and mitochondria-targeted nucleases to eliminate mtDNA mutations, which involve embryos and/or germline. However, patients suffering these diseases needs a treatment once they are diagnosed, which has to reach all cells. We propose here a pharmacological approach based on nicotinamide mononucleotide (NMN) treatment that will raise NAD+ biosynthesis. NMN improves mitochondrial function in age, ataxia telangiectasia and nuclear-encoded mtDNA defects mice models, and complex I deficient fibroblasts. NAD+ improves mitochondrial bioenergetics providing Krebs cycle substrate and regulating SIRT3 and SIRT4, and nDNA through SIRT1. We aim to characterize NMN as a new therapeutic drug for mitochondrial diseases through a multidisciplinary study treating human trans-mitochondrial cybrids with mtDNA defects. Although in recent years there have been considerable progresses in NAD+ metabolism and their precursors, the exact mechanism and targets of NMN are still unknown. NMN supplemented mutant cybrids showed a significant increase of growth and survival fed in galactose for five days, but did not show significant effect in either control or mutant cybrids grown in glucose. NMN treatment induced the increase of maximum respiratory capacity in cybrids mutated in tRNA12s, ND6 (complex I), COX1 (complex IV), and with less effect in ATP6 (complex V) mtDNA encoded genes, and a significant increase of oxygen consume coupled to ATP production in tRNA12s and ND6 mutant cybrids but not in controls. NMN increased glycolysis in control cybrids but it was not observed in mutant cybrids indicating the specific activation of mitochondrial pathway in mtDNA mutant cells. These results on enhanced respiratory capacity in these mutants were associated to a decrease of endogenous mitochondrial oxidative stress, indicating an improvement of the mitochondria efficiency. Preliminary results indicate that NMN supplementation induced the expression of mtDNA genes but not nuclear genes in both mutant and control cybrids. We propose NMN as a pharmacological treatment of mtDNA defects by activating oxidative phosphorylation and the regulation of NAD+ dependent mitochondria functions. Funded by CIBERER ACCI 2015 and FIS PI14-1962.

P56 - ACTIVATION OF AUTOPHAGY AND CATHEPSINS INVOLVED IN CELL DEATH DURING STRESS-INDUCED MICROSPORE EMBRYOGENESIS IN BARLEY

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Microspore embryogenesis is an in vitro system in which the haploid microspore is reprogrammed by the application of external stress treatment and enters into an embryogenesis pathway. Despite the usefulness of stress-induced in vitro embryogenesis in breeding programs, the efficiency of the system in many species of economic interest is still limited since it is greatly affected by many factors, and primarily by the occurrence of cell death induced by the stress applied to trigger embryogenesis. Autophagy is a universal degradation pathway that recycles cell materials upon stress conditions or during specific developmental processes, thereby promoting cell survival. In addition to this survival role, autophagy can also play critical roles as cell death initiator and/or executioner. Cathepsins are Papain-like C1A cysteine-proteases that are well known lysosomal proteases with a role in autophagy and cell death, in animals. In plants, cathepsins are involved in many physiological processes, including programmed cell death and proteolysis induced by stress. In this study, we have analysed autophagy and cathepsin involvement in cell death during stress-induced microspore embryogenesis in Hordeum vulgare, barley. After the inductive stress, cell death levels increased and autophagy was activated, including up-regulation of HvATG5 and HvATG6, and the increase in the levels of ATG5 and ATG8 proteins, and autophagosomes. Concomitantly, cathepsin L/F-, B- and H-like proteolytic activities were induced, cathepsin-like genes HvPap-1 and HvPap-6 were up-regulated, and HvPap-1, HvPap-6 and HvPap-19 proteins increased. Treatments with inhibitors of autophagy (3MA) and cysteine protease activities (E64) reduced cell death levels and enhanced embryogenesis initiation rate. These findings reveal that both autophagy and cathepsins play a role in stress-induced cell death during microspore embryogenesis induction, opening up new possibilities to enhance microspore embryogenesis efficiency with modulators of autophagy and/or cysteine proteases. Authors thank Dr. M. F. Suárez (University of Málaga, Málaga, Spain) for the generous gift of the anti-ATG5 antibody. Work supported by project AGL2014-52028-R funded by the Spanish Ministry of Economy and Competitiveness (MINECO) and the European Regional Development Fund (ERDF/FEDER).

P57 - MEARRAYS TO IDENTIFY MICROENVIRONMENTS THAT PROMOTE MESENCHYMAL TO EPITHELIAL TRANSITION IN TUMOUR CELLS

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steps (Nieto et al. Cell 2016). The main inducers of the EMT are transcription factors of the Snail, Zeb, Twist and Prrx families. Disseminated tumour cells show EMT features (Reviewed in Nieto, 2016) and the reversion to an epithelial phenotype through the mesenchymal to epithelial transition (MET) and acquisition of stem-like properties in the metastatic niche is necessary for the cells to grow and form metastatic deposits. MET requires the downregulation of members of the EMT-TFs family. However, the mechanisms that regulate the repression of the EMT-TFs and the interaction of migratory cancer cells with the metastatic niche are still unknown. Increasing evidences suggest that the key determinants that allow a population of disseminated tumour cells to grow as macrometastases may be dependent on the interactions of the cells with their microenvironment and the state of the host environment in the metastatic niche (reviewed in McAllister and Weinberg, Nat Cell Biol., 2014). We are using a high-throughput unbiased approach in which we grow cancer cells on microarrays composed of extracellular matrix molecules and other extracellular proteins in different combinations called MEArrays. We monitor morphology and phenotypic markers in cells after growing them in the different conditions to identify combinations that may promote the mesenchymal to epithelial transition in tumour cells.

P58 - GLIOBLASTOMA NETWORK DEPLETE NEURONAL WG AND INDUCES NEURODEGENERATION

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Malignant astrocytic gliomas such as glioblastoma (GBM) are the most common, aggressive and lethal tumor of the central nervous system. These tumors are characterized by diffusely infiltrative growth and great invasiveness, which leads to neurological dysfunction and death despite intensive radio and chemotherapy. Recently, it has been discovered that these types of tumors extend highly functional, ultra-long membrane protrusions (tumor microtubes-TM) that interconnect tumoral cells forming a glial network. This network is responsible for the infiltrative growth and therapy resistance, giving a positive correlation between the length and number of TMs and unfavorable prognosis. The neuronal growth-associated protein 43 (Gap43) is necessary in the formation of these TMs, here we demonstrate that suppression of Gap43 expression specifically in glial cells of a Drosophila Melanogaster brain tumor model prevents the network formation and promotes survival. Moreover, we show that the projections of glial cells that form the network overexpress wg/Wnt receptor frizzled1 but no frizzled2 to deplete wingless to neighboring neurons. As a consequence, wg/Wnt pathway is active in GBM cells which enhance proliferation and invasiveness. On the other side, wg depletion from neurons causes downregulation of this signaling pathway and, as a consequence, synapse loss and neurodegeneration. The results elucidate mechanisms through which glial cells induce neurodegeneration and propose mechanisms to prevent neurodegeneration, tumor expansion and thus, to extend life span and improve life quality.

P59 - GENETIC BASIS OF OPITZ C SYNDROME, AN EXTREMELY HETEROGENEOUS DEVELOPMENTAL DISORDER

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Introduction: Opitz C syndrome (CS, MIM #211750) is an extremely rare genetic disorder characterized by multiple malformations (e.g. trigonocephaly, congenital heart defects), contractures, variable intellectual and psychomotor delay and a high mortality rate. Different patterns of inheritance and genetic heterogeneity have been suggested for this condition. Material and Methods: We studied a cohort of 13 patients (10 unrelated pedigrees) with clear or tentative diagnosis of CS. Patients and parents were analysed by means of whole exome sequencing (WES). Selected putatively-pathogenic variants were validated by Sanger sequencing and, where appropriate, functional analyses were performed to assess pathogenicity. Results: We identified the disease-causing mutation in 8 of the 10 families in 8 different genes sharing demonstrated roles in development and cancer. All these genes were associated with other diseases with phenotypic similarities to CS. For example, MAGEL2 truncating mutations were shared by one of our CS patients and all patients with Schaaf-Yang syndrome reported so far, while a FOXP1 truncating mutation (a splicing mutation provoking exon skipping) in another patient was similar to those causing FOXP1 syndrome. A third patient bore a de novo missense mutation in a novel gene and the Genmatcher tool allowed us to contact other research groups with patients bearing mutations in the same gene. Conclusions: WES is a very powerful approach to identify CS related mutations, since it was successful in 80% of our cases. Genematcher has been an essential tool to connect with other researchers who identified mutations in the same gene in other patients, a necessary way to solve the cause of extremely rare diseases in the context of a high genetic heterogeneity. Our results point to CS as a causally heterogeneous phenotype instead of a specific entity.

P60 - HISTONE ACETYLATION REGULATES THE DEVELOPMENT OF BEHAVIORAL INDIVIDUALITY IN ZEBRAFISH

ROMAN, ANGEL CARLOS*. *FUNDAÇAO CHAMPALIMAUD

Behavioral inter-individual variability arises even in isogenic populations under identical environments, but its underlying mechanisms remain elusive. We found that inbred zebrafish (Danio rerio) larvae develop consistent individual behaviors when swimming freely in identical wells or in reaction to stimuli. We also found that behavioral inter-individual variability depends on the histone 4 acetylation levels, and specifically H4K12 acetylation. More precisely, we found a set of genomic regions whose histone 4 acetylation depends on the distance between the individual and the average behavior. We detected that a complex of Yin-yang 1 (YY1) and histone deacetylase 1 (HDAC1) that binds to and deacetylates these regions is responsible of the alteration of behavioral individuality. These changes were not only maintained at the transcriptional level, but also amplified as most candidate regions were located near genes encoding transcription factors like jun or myc, among others. We suggest that this pathway is responsible for the development of genetic-independent behavioral inter-individual variability.

P61 - THE REGULOME OF REGENERATION

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The ability to regenerate varies greatly not only between species but also between tissues and organs or developmental stages of the same species. Differential activation of the genome, determined by a complex interplay of regulatory elements functioning at the level of chromatin, must be the initial mechanism behind these different regenerative capabilities. Resetting gene expression patterns during injury responses is, thus, shaped by the coordinated action of genomic regions that integrate the activity of multiple sequence specific DNA binding proteins. Drosophila imaginal discs, which show a high regenerative capacity after genetically induced cell death, are a great model to interrogate chromatin function through the regeneration process. Using genome- wide approaches (RNA-seq and ATAC-seq) at different tissue time-points after injury we have identified the regulatory elements and the expression profile dynamics governing the process. Our findings point to a global co-regulation of gene expression and provide evidence for a regeneration program driven by different types of Damage Responsive Regulatory Elements (DRRE). Among them, novel-DRRE are found acting exclusively in the damaged tissue, and cooperating with DRRE co-opted from other tissues and developmental stages. Altogether, our results decipher the regulome of regeneration and suggest the existence of a specific toolkit to drive the regenerative capacity.

P62 - RAG-2 DEPENDENT DNA BREAKS GENERATION IMPACT IN RETINAL NEURONAL DIFFERENTIATION.

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INTRODUCTION. During nervous system development, including retina neurogenesis, DNA breaks have been associated with an early cell death phase in a process not well understood. After decades of controversy, single cell sequencing techniques have finally demonstrated that somatic mosaicism plays a necessary role in the generation of neural diversity. Several genetically-modified mouse model systems defective in DNA double-strand break repair present a dramatic phenotype during neural development, suggesting a possible function of DNA break generation in neurogenesis (Baleriola, Alvarez-Lindo et al, Sci Rep 2016). In addition, LINE-1 transposition has been suggested to act as a necessary source of DNA breaks during embryonic development. In this study we focused on an alternative site-directed source for DNA double strand breaks: RAG-2, a DNA endonuclease involved in the generation of DNA breaks in the immune system. We have analyzed the in vivo requirement of RAG-2 for proper neural-retina development, using the mouse rag2-/- as a model. METHODS. RAG-2 expression in the developing retina was studied by semi-quantitative PCR. Whole mount and dissociated retinas from rag2-/- mice were processed for TUNEL to detect apoptosis and for γH2AX to detect DNA breaks presence. Whole mount retinas were stained with TUJ-1 to analyze the ganglion cell axonal growth pattern. Dissociated retina cells were cultured to determine the effect of RAG-2 deficiency on neuronal differentiation and axonogenesis. RESULTS. RAG-1 and RAG-2, the two subunits of the DNA endonuclease, are present in the embryonic mouse retina thorough the neuronal developmental period, from E12.5 to P2. rag2-/- mice presented 15% less unprocessed DNA breaks, suggesting a functional role of the endonuclease activity. rag2-/- mice displayed a 28% increase in neuronal cell death and defects in axonal pattern and in neurite growth. Surprisingly, the rag2-/- model also displayed

altered microglia maturation. CONCLUSIONS. Our results show that RAG-2 can be responsible of DNA break generation in the developing retina. Its presence plays a critical role in survival and differentiation of embryonic retinal neurons. Further work is required to integrate RAG-2 induced DNA double-strand break generation in coordinated action with DNA double-strand break repair during retinal neurogenesis.

P63 - THE BIRTH OF A NEW GENE CLUSTER IN MAMMALIAN EVOLUTION: EXCITING ORIGIN, INTRIGUING SHARED REGULATION, (UN)KNOWN FUNCTIONS, AND IMPLICATIONS IN HUMAN DISEASES

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In recent years it is becoming clear that genome organisation and architecture made an insightful contribution to gene regulation during embryonic development, being in some cases responsible for the maintenance of gene clusters, through genes being linked by sharing of regulatory sequences, or by using global cluster regulatory landscapes. These genome architectures and its sudden changes may well be linked to morphological evolution, by changing networks of developmental regulatory genes. Among those phenomena, the sudden birth of new gene clusters has been scarcely reported. We wish here to introduce our latest research in these fields; the BGW gene cluster (name to be changed). The cluster suddenly appeared at the origin of Eutherian Mammals, and the initial genes we analysed, ArmcX 1-6, play a key role in mitochondrial dynamics in neurons. Surprisingly, the cluster encompasses not six, but nearly 20 genes poorly analysed. We will show here the true origin of this cluster, Also, we will analyse its maintenance in most eutherian mammals, explore conserved regulatory motifs shared by these genes, and show that they are expressed during mouse embryonic development, namely but not solely in the central nervous system. Finally, as some of the cluster genes are involved in the control of proliferation, apoptosis and neuronal differentiation, we will hypothesise that the origin of the cluster was correlated to the increase in complexity of the central nervous system of Eutherian mammals. CRISP-R transgenic mice of one of the genes excitingly suggest it may be implicated in human autism

P64 - GENETIC STUDY IN A COHORT OF CEALIAC PATIENTS DQB1*2 GENE DOSAGE EFFECT AND THE SUSCEPTIBILITY OF CELIAC DISEASE DEVELOPMENT

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Introduction. Celiac disease (CD) is a systemic and autoimmune disease triggered by the ingestion of gluten. Is characterized by a wide variety of clinical gastrointestinal or highly variable non-gastrointestinal symptoms, specific antibodies and DQ2 or DQ8 HLA haplotype. CD occurs in genetically predisposed individuals; serologic and genetic markers are important for the diagnosis. HLA genotype DQ2 are present in around 92% of celiac disease patients, while 5 to 10% are DQ8 positive. The aim of this study is to characterized the gene dosage effect and the susceptibility of paediatric patients with DQ2 positive: DQA1*0501/DQB1*0201. Methods. Two-hundred pediatric

patients were study and compared with 200 healthy controls from Madrid, Spain. A fully automated assay was established for the extraction of DNA using the MagNA Pure instrument and Luminex HLADQA1/DQB1 typing technology. Results. DQA1*0501/DQB1*0201 genes were present in 92,5% of the patients in contrast with the control group 25,5%; p<0,01. The results of the DQB1*02 gene dosage effect in the group of patients with CD was 50% in comparison with the control group 3% (p< 0,01) Conclusions. This study pretends to enhance the importance of the DQB1*02 gene as a dosage effect in individuals DQA1*0501/DQB1*0201with an important susceptibility to develop celiac disease, probably because a strong antigenic presentation derived from gluten peptides is taking place in this context.

P65 - IDENTIFYING NEW GENOMIC MECHANISMS CONTROLLING AXON GUIDANCE DECISIONS

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Binocular vision relays on the existence of contralateral Retinal Ganglion Cells (cRGCs) that transmit visual information from each eye to the opposite side of the brain and ipsilateral ganglion cells (iRGCs) that carry sensory information from each retina to the same brain hemisphere. The decision of crossing or avoiding the midline that visual axons take at the optic chiasm during embryonic development is a binary, and consequently, simple axonal choice that represents an ideal model to search for new mechanisms controlling axon guidance responses during the formation of neural circuits. Numerous membrane proteins involved in axonal pathfinding have been identified and characterized in the last few decades, but the mechanisms that regulate the expression of specific transcription factors controlling axon guidance decisions are poorly understood. In an attempt to identify new regulatory mechanisms determining axon guidance decisions, we have compared the chromatin status of iRGCs and cRGCs using Cre-transgenic lines specific for these two retinal populations (Slc6a4-Cre for iRGCs Pou4f2-Cre for cRGCs) combined with a third transgenic line (SUN1-reporter line) to selectively label their nuclear membrane. We have differentially isolated nuclei from these two populations of RGCs from mouse embryos at the moment that visual axons are deciding whether or not to cross the midline and performed ATAC-seq (Assay for Transposase Accessible -chromatin) and RNAseq. This approach should allow us to identify differences in transcription, chromatin accessibility and transcription factor occupancy that correlate with midline crossing decisions, potentially revealing new genomic mechanisms regulating this process.

P66 - SERTOLI CELL-SPECIFIC ABLATION OF MIR-17- 92 CLUSTER SIGNIFICANTLY ALTERS WHOLE TESTIS TRANSCRIPTOME WITHOUT APPARENT PHENOTYPIC EFFECTS

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MicroRNAs are frequently organized into polycistronic clusters whose transcription is controlled by a single promoter. The miR-17- 92 cluster is expressed in most embryonic and postnatal organs. It is a potent oncogene associated to several types of cancer and it is involved in several important

developmental processes. In the testis, expression of the miR-17- 92 cluster in the germ cells is necessary to maintain normal spermatogenesis. This cluster is also expressed in Sertoli cells (the somatic cells of the seminiferous tubules), which require miRNAs for correct cell development and survival. To study the possible role of miR-17- 92 in Sertoli cell development and function and, in order to overcome the postnatal lethality of miR-17- 92 -/- mice, we conditionally deleted it in embryonic Sertoli cells shortly after the sex determination stage using an Amh-Cre allele. Mutant mice developed apparently normal testes and were fertile, but their testis transcriptomes contained hundreds of moderately deregulated genes, indicating that testis homeostasis is tightly controlled in mammals and that miR-17- 92 expression in Sertoli cells contribute to maintain normal gene expression levels, but is unnecessary for testis development and function. Researchers must be careful when interpreting transcriptome data, as significant deregulation of hundreds of genes might have no functional consequences.

P67 - MEIS GENE EXPRESSION IS CONTROLLED BY A DEEP EVOLUTIONARY CONSERVED ENHANCER

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During embryonic development, the behaviour of thousand of cells must be tightly coordinated by a precise cellular crosstalk. Despite this complexity, communication between cells is orchestrated by only a few signalling pathways highly conserved across animals. In response to these pathways, different cis-regulatory elements are activated and repressed, controlling, in a spatio-temporal manner, the expression of their target genes. Although these signalling pathways have been extensively studied, our knowledge of the cis-regulatory changes associated with their activation or repression is still limited. Here, we aim to explore this issue using Danio rerio as vertebrate model. In our laboratory, we are currently manipulating different signalling pathways using agonist and antagonist molecules to further profile open chromatin regions in both treated and control embryos. Using this strategy, we have recently identified a retinoic acid (RA) responding enhancer in intron eight of the zebrafish meis2b gene. This gene encodes a TALE homeobox protein with multiple functions during vertebrate development. Sequence analysis of this enhancer reveals a strong binding site for the RA receptor, suggesting a direct control of meis2b by this signalling pathway. Using zebrafish transgenic assays, we have determined that this enhancer promotes expression in the hindbrain, a RA-dependent territory that shows strong meis2b transcription. The intron-exon organization of the Meis genes is extremely conserved during evolution. Indeed, we have found RA-responding enhancers in the same intron of most meis paralogous in zebrafish. We have also validated with 4C-seq data that some of these enhancers contact the promoter of the meis genes. Interestingly, we have also detected open chromatin regions in the equivalent introns in the amphioxus, sea urchin and flies Meis orthologous. We are currently using zebrafish enhancer assays to examine if the introns from these species contain functionally evolutionary conserved enhancers, and to determine the evolutionary origin of the Meis gene expression control by the RA signalling pathway.

P68 - CAULOBACTER CRESCENTUS CDNL IS A NON-ESSENTIAL RNA POLYMERASE-BINDING FACTOR REQUIRED FOR NORMAL GROWTH AND RRNA TRANSCRIPTION, WITH CONDITIONALLY COLD-SENSITIVE MISSENSE MUTANTS

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CdnL is an essential RNA polymerase (RNAP)-binding activator of rRNA transcription in myxobacteria and mycobacteria but reportedly not in Bacillus (1-5). Whether its function and mode of action are conserved in other bacteria thus remain unclear. Because virtually all alphaproteobacteria have a CdnL homolog and none of these have been specifically characterized, we studied CdnLCc of the model alphaproteobacterium Caulobacter crescentus (6). We show that CdnLCc is not essential for viability but its depletion causes slow growth and cell filamentation. CdnLCc is degraded in vivo in a manner dependent on its C-terminus, yet excess CdnLCc resulting from its stabilization did not adversely affect growth. Moreover, CdnLCc protein levels in vivo appear to be subject to cell cycle-dependent proteolytic control, and changes in its levels closely parallel those reported for the key cell division protein FtsZ. We find that CdnLCc interacts with itself and with the RNAP β subunit, and localizes to at least one rRNA promoter in vivo, whose activity diminishes upon depletion of CdnLCc. Interestingly, cells expressing CdnLCc mutants unable to interact with the RNAP were cold-sensitive, suggesting that CdnLCc interaction with RNAP is especially required at lower than standard growth temperatures in C. crescentus. Our study indicates that despite limited sequence similarities and regulatory differences compared to its myco/myxobacterial homologs, CdnLCc may share similar biological functions, since it affects rRNA synthesis, probably by stabilizing open promoter-RNAP complexes.

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P69 - DOWN REGULATION OF RAF KINASE INHIBITOR PROTEIN (RKIP) IS NOT INVOLVED IN THE MALIGNANT PROPERTIES OF V600E BRAF MUTATED MELANOMA CELLS

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Since the incidence of melanoma is increasing yearly, there is a need to understand the mechanisms by which melanocytes acquire malignant phenotype. Nearly half of all melanomas present mutations in BRAF gene. In spite of the specific immunotherapy available for patients who carry these mutations, only 50% of them respond positively to the treatment. We have previously found that expression of RKIP, a natural inhibitor of BRAF activity, was higher in normal melanocytes than in the melanoma cells. The aim of this study was to give insight into the implication of RKIP in melanoma pathogenesis. To do so, shRNA-based silencing of RKIP expression was performed in two V600E BRAF mutated melanoma cell lines using lentiviral particles. We evaluated RKIP protein expression by Western Blotting and RKIP mRNA levels by RTqPCR in both transduced and un-transduced cells. XTT proliferation assays, wound healing assays and matrigel invasion assays were also performed in RKIP silenced and non-silenced melanoma cells to assess the possible role of RKIP in the malignancy of melanoma. Analysis of molecular expression patterns as well as functional analysis did not reveal a clear role for RKIP in the proliferation, migration and invasion capacity of melanoma cells. However changes of NFkappaB1 and NFkappaB2 expression were observed. These findings suggest that specific repression of MAPK pathway activity might not be a good therapeutic strategy for all the V600E BRAF mutated melanoma patients. Further studies are required to clarify the implications of the observed RKIP expression variations in melanoma aggressiveness and its utility to design therapeutic approaches.

P70 - THE GLOBAL TRANSCRIPTIONAL RESPONSE TO LIGHT IN THE BACTERIUM MYXOCOCCUS XANTHUS

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Light is an important environmental signal for most living organisms, which can also cause photooxidative damage. When exposed to blue light, the bacterium Myxococcus xanthus undergoes a photoprotective response that triggers carotenoid synthesis (1, 2). Our previous genetic analysis identified nine regulatory genes and ten structural genes (nine of which are grouped in the carB operon) involved in the response to light. Expression in the light of the key regulatory operon carQRS leads to production of: (i) the extracytoplasmic function (ECF) σ factor factor CarQ, which directly activates expression of carQRS as well as that of the structural gene crtIb; (ii) its cognate, membrane-bound anti-σ CarR; (iii) the antirepressor CarS, which is required to trigger expression of the carB operon. None of the regulatory factors identified so far bears any resemblance with any known photoreceptor (2-4). Our findings indicate that blue light provokes photoexcitation of protoporphyrin IX and generation of singlet oxygen, which somehow is sensed via the transmembrane protein CarF to inactivate CarR, so that CarQ can trigger the transcriptional response underlying light-induced carotenogenesis in M. xanthus (3).

Alternatively, light can be detected via a very novel mechanism involving a form of vitamin B12 and CarH, a transcriptional repressor of the carB operon (2, 4). In order to define the complete regulon linked to the response to light in M. xanthus, we have performed global analyses to compare the transcriptional profiles of the wild type strain with those of a strain lacking CarQ, in the dark and in the light. These transcriptomics data, which have allowed the identification of a new set of genes whose expression changes in response to light, will be presented. 1. Elías-Arnanz M et al Curr Opin Microbiol 14, 128-135 (2011) 2. Padmanabhan et al Annu Rev Biochem 86, 485-514 (2017) 3. Galbis-Martínez et al J Bacteriol 194, 1427-1436 (2012) 4. Ortiz-Guerrero et al PNAS 108, 7565-7570 (2011) FUNDING: Ministerio de Economía y Competitividad (Spain) grant to MEA (BFU2015-67968-C2-1-P, co-financed by FEDER-UE), and Fundación Séneca (Spain) grant 19429/PI/14 to MEA.

P71 - SAEC-C1 AND SAEC-F1, TWO NEW MONOCLONAL EMBRYONIC CELL LINES FROM SEA BREAM (SPARUS AURATA): A NEW TOOL FOR DEVELOPMENTAL STUDIES IN FISH

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Embryonic cell lines constitute an experimental tool for in vitro studies on animal development as well as on more basic cell processes. These cells retain all developmental potentials, allowing the modification of gene expression by a precise control of the environmental conditions. Here we report the initial characterization of two monoclonal embryonic cell lines from Sparus aurata, SAEC-C1 and SAEC-F1. Cells were obtained from 6 hours post-fertilized eggs and cultured in L-15 media, 10% FBS, 15.0 mM NaCl at 22ºC. After passing 20 times, a limit dilution was carried out and single cell-derived cell lines were selected. The capacity of cell lines to form embryoid bodies was evaluated by cultivation in non-adherent plastic dishes as well as the ability to be transfected using EGFP and DsRed plasmids. Moreover, gene expression was analyzed by RT-qPCR. Cell lines actively grow as adherent monolayers, displaying small and round or polygonal morphologies. After cryopreservation and thawing, they show a viability over 83%. Dissociated cells formed clusters and spherical structures indicative of embryoid bodies. Both cell lines were successfully transfected, being the fluorescence detected at 48-72 hours after transfection. The expression of genes associated with stress (hsp70, sod2, cat, gpx4), fatty acids metabolism, (fas, lpl, scd1a and b, ?6fad) immune innate response (saBD, hep, lyz, ppar?, lox, cox2) and apoptosis (Bcl2, Bax) was detected in both lines. Our results confirm that these monoclonal embryonic cell lines could represent an important tool to enlarge our knowledge on cellular processes as well as to perform in vitro studies on genetic manipulation in sea bream. Funded by Junta de Andalucía and MINECO grants (P06-AGR-02129and AGL2013-49027-C3-2-R).

P72 - RIBOSOMAL PROTEIN L14 CONTRIBUTES TO THE EARLY ASSEMBLY OF 60S RIBOSOMAL SUBUNITS IN SACCHAROMYCES CEREVISIAE

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The contribution of most ribosomal proteins to ribosome synthesis has been quite well analysed in the yeast Saccharomyces cerevisiae. From these studies, a comprehensive outline of the timing of in vivo assembly of these proteins within each ribosomal subunit has been deduced. However, few yeast ribosomal proteins still await characterisation. Herein, we show that L14, an essential protein from the 60S subunit, assembles in the nucleolus at an early stage into pre-60S particles. Depletion of L14 results in a deficit in 60S subunits and the appearance of half-mer polysomes. Pulse chase, northern blotting and primer extension analyses indicate that processing of 27SA2 and 27SA3 to 27SB pre-rRNAs is impaired in the L14-depleted strain. As a result, 27SB pre-rRNAs are subjected to rapid turnover and export of pre-60S particles is blocked. All these phenotypes most likely appear as the direct consequence of the reduced pre-60S particle association not only of L14 upon its depletion but also of a set of neighbouring ribosomal proteins located at the central solvent interface of 60S subunits and the adjacent region surrounding the polypeptide exit. These pre-60S intermediates also lack some essential trans-acting factors required for 27SB pre-rRNA processing but accumulate practically all factors that participate in processing of 27SA3 pre-rRNA. We have also analysed the direct interaction between the eukaryote-specific carboxy-terminal extensions of L14 and its neighbouring L16 protein. Our results show a genetic connection between these extensions and a set of assembly factors known as the Mak5 cluster and indicate that the removal of the most distal parts of these extensions cause slight translation alterations in mature 60S subunits. Whether or not the Mak5 cluster might be required for the stably assembly of L14 and L16 into pre-60S particles needs further work.

P73 - THE CUPULIFORMIS GENES ENCODE NEW COMPONENTS OF THE EPIGENETIC MACHINERY IN ARABIDOPSIS

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Most critical developmental and physiological events in any life cycle depend on the proper activation and repression of sets of genes; plants accomplish this by several mechanisms, including epigenetic regulation. A number of Arabidopsis mutants with defects in the epigenetic machinery exhibit pleiotropic phenotypes, including two common traits: incurved leaves and early flowering [1,2], caused by the ectopic and heterochronic derepression of key developmental regulators. Loss-of-function mutations of INCURVATA11 (ICU11) also show these traits; ICU11 is the founding member of a small gene family that we named the CUPULIFORMIS (CP) family. ICU11 and its closest paralog CP2 are nucleoplasmic proteins. Double mutant combinations of icu11 alleles with loss-of-function alleles of genes known to encode components of the epigenetic machinery exhibit synergistic, severe phenotypes, some of which are similar to those of embryonic flower mutants [3,4]. Severe, synergistic phenotypes were observed in the combinations of loss-of-function alleles of ICU11 and CP2: seeds of the icu11 cp2 double mutants and sesquimutants

germinated but seedlings lacked rosettes leaves, skipped vegetative growth and began flowering upon germination, generating small, sterile flowers. By contrast, the icu11 single mutants exhibit mildly hyponastic leaves and early flowering, and the cp2 mutants are indistinguishable from wild type, except for some infertility. The relatively mild phenotype of icu11 alleles, the completely wild-type phenotype of cp2 alleles, and the severe, lethal phenotypes of their genetic combinations, indicate that ICU11 and CP2 are a pair of redundant genes with an essential function. However, ICU11 and CP2 exhibit unequal functional redundancy: the ICU11/icu11-2;cp2-3/cp2-3 plants are phenotypically wild type, but the icu11-2/icu11-2;CP2/cp2-3 plants have a lethal phenotype. RNA-seq analysis showed that icu11 plants mis-express hundreds of genes, including several members of the MADS-box family. We demonstrated that derepression of the floral organ-identity gene SEPALLATA3 (SEP3) causes the leaf phenotype of icu11 mutants. Bisulfite-seq analysis of icu11-1 plants showed no alteration in DNA methylation levels. Instead of affecting DNA methylation, ICU11 and CP2 are required for the deposition of H3K27me3 at the SEP3 locus. Our results thus reveal a novel family of proteins required for deposition of histone epigenetic marks through an unknown mechanism. 1. Goodrich, J., et al. (1997). Nature 386, 44-51. 2. Barrero, J.M., et al. (2007). Plant Cell 19, 2822-2838. 3. Sung, Z.R., et al. (1992). Science 258, 1645-1647. 4. Chen, L., et al. (1997). Plant Cell 9, 2011-2024.

P74 - PREFOLDIN CONTRIBUTES TO GENE EXPRESSION IN HUMAN CELLS

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Prefoldin is a cochaperone, present in all eukaryotes, that cooperates with the chaperonin CCT. It is known mainly for its functional relevance in the cytoplasmic folding of actin and tubulin monomers during cytoskeleton assembly. However, the subunits of this heterohexameric complex have also been found in the nucleus, and are functionally connected with nuclear processes in yeast, plants and metazoa. We have previously showed that four of the canonical prefoldin subunits of the yeast Saccharomyces cerevisiae contribute to RNA pol II-dependent transcription elongation and play a role in chromatin dynamics during this process. Here, we report that depletion of the PFDN5 subunit in human HCT116 cells caused a marked decrease in the proportion of Ser2-phosphorylated RNA polymerase II that is bound to transcribed genes. This type of phosphorylation is a well-known marker of the elongating form of this enzyme and is functionally associated to cotranscriptional mRNA processing. Accordingly, we found that PFDN5 depletion produced defects in co-transcriptional splicing in CTNNBL1, a long human gene commonly used to analyse transcription elongation. Moreover, RNA-seq analysis showed that depletion of the PFDN5 subunit causes general alterations in pre-mRNA splicing.

P75 - MYC TRANSCRIPTIONAL REGULATION DURING METABOLIC CELL COMPETITION

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MYC is a highly conserved and well-known transcription factor implicated in proliferation, growth, differentiation, metabolism and apoptosis of pluripotent cells. Recently, MYC has been reported to be expressed heterogeneously in mammalian embryonic stem cells (ESC). This heterogeneous endogenous expression of MYC triggers cell competition, which is a mechanism whereby cells in a tissue compare their fitness resulting in the elimination of the less fit cells. MYC is a general activator

of cell anabolism, and therefore cell competition promotes the expansion of higher-anabolic cells (winners) at the expense of lower-anabolic cells (losers). This suggest that Myc regulation is essential during spontaneous cell competition, however the transcriptional regulation of Myc in ESCs remains poorly understood. The major aim of our work therefore is to find cis-regulatory elements involved in Myc transcriptional regulation in mouse embryonic stem cells (mESCs). Previous published work and some preliminary data from the Torres laboratory suggest that cis-regulatory regions of Myc in ES cells span close to 200kb surrounding the Myc locus. Focusing on this surrounding region, we have selected Bacterial Artificial Chromosomes (BACs) that cover the region of interest. In parallel, we have derived Myc- KO ESCs. For this, we have derived ES cells from Myc flox/flox; RNApolII-CreERT2 mice and we have induced Myc KO by exposing these ESCs to Tamoxifen. Using these Myc-KO ESCs, we will test the reconstitution of Myc expression from BACs containing the Myc transcriptional unit and different parts of the complete BACs. In addition, we are exploring several cloning strategies to isolate and modify the BAC gene in order to functionally study the putative regulatory elements. We have cloned by digestion and ligation the minimal expression unit of Myc, and BAC modification by recombineering is ongoing to obtain knock-in YFP-MYC fusion protein, which will allow us to follow the MYC protein levels by YFP fluorescence. Using the approaches mentioned above we will expect to obtain a full picture of the cis-regulatory activities in the Myc locus, which will be essential for a better understanding of the biological function and regulation of endogenous cell competition in pluripotent stem cells.

P76 - ROLE OF MIR-138 IN THE MESENCHYMAL MODE OF MIGRATION INVASION AND ANGIOGENESIS IN GLIOBLASTOMA .

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Glioblastoma (GB), the most common central nervous system neoplasm in adults. This neoplasia, which could be defined by its morphological and genetic heterogeneity, shows a high infiltrative capacity. Molecular screening for gene amplification studies had revealed frequent amplification of the EGFR, observed in about 35–70% of glioblastomas.
MicroRNAs which have emerged as important regulators of growth, differentiation, and apoptosis by altering the expression of other genes and play a role in tumorgenesis and cancer progression.
In the present work we show the alteration in miRNA-138 expression (down-regulated) which, through its HIF-1 modulating action, acts as a transcription factor activating in hypoxia situations, modulating changes in the neoplastic cells, intervening in the metabolic processes of glycolysis and through VEGF, participating in processes of angiogenesis.
We propose the development of a new model of study, in cell cultures that allows comparative analysis in cell lines and primary cultures of primary GB with different levels of EGFR amplification: (i) in situations of overexpression and silencing of miRNA-138, (ii) in situations of inhibition of EGFR and HIFmRNA and (iii) different situations of hypoxia.
The expression of miR-138 under normal conditions decreases and under conditions of hypoxia is maintained constant in the cultures. As for the studied VEGF and HIF1a mRNA, it is always seen an increase in their levels both under normal conditions and under conditions of hypoxia.
On the other hand, after transfection of miR-138 overexpression, an increase in the miR-138 values and a decrease in the values of the VEGF and HIF1a mRNA are expected in both normal and hypoxic conditions.

Regarding EGFR silencing, miR-138 levels decrease considerably in cell cultures and mRNAs VEGF and HIF1a increase in cultures in a similar way.
We observed that overexpression of miR-138 causes an increase in apoptosis. In normoxia, in the line U-118 an increase in apoptosis is observed and under conditions of hypoxia a greater increase. As for the U-87 line and HC-444 culture under normal conditions an increase in apoptosis is observed and under hypoxia conditions an increase but less.
Our findings indicate that miR-138 is deregulated in GB and changed their expression levels witch the different grades of GB tumors suggesting a potential role for these molecules in the pathogenesis of cancer. We reaffirm the possibility of miR-138 being able to act modulating the changes in the MMMI and the process of dysplasic angiogenesis in GB.

P77 - NODAL-ENHANCED MICRORNAS CONTROL HEART POSITIONING BY REGULATING PRRX1 ASYMMETRIC EXPRESSION

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Despite their external bilateral symmetry, vertebrates have internal Left/Right asymmetries that are fundamental for optimal organ packaging and function. We have recently shown that two parallel pathways respectively driven by Nodal and BMP on the left and right lateral plate mesoderm (LPM) instruct heart laterality and morphogenesis. While the left determinants, Nodal and its downstream target Pitx2, are exclusively expressed on the left, the right determinants, BMP and its downstream targets Snail1 and Prrx1, are initially expressed in a bilaterally symmetric manner. However, Snai1 and Prrx1 transiently become higher on the right LPM. We have now found that this transient asymmetric L/R expression is achieved by a Nodal- mediated transient upregulation of microRNAs (miRNAs) that attenuate the expression of the right determinants on the left LPM. Thus, the left pathway not only instructs left-handed information but also allows the differential activation of the right-handed pathway that governs heart laterality.

P78 - POTENTIAL NEW ROLE FOR ROS IN THE EPIGENETIC REGULATION OF GENE EXPRESSION

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The generation of Reactive Oxygen Species (ROS) as byproducts of the highly efficient aerobic metabolism constitute an ineludible biochemical side effect that can be extremely harmful for cell viability, due to the irreversible oxidation of lipids, proteins and nucleic acids. Nevertheless, eukaryotic cells can also actively generate ROS as essential components of molecular mechanisms regulating key cellular processes, including proliferation and differentiation, through the oxidation of redox-sensitive proteins such as kinases and phosphatases. In addition, here we propose that eukaryotic cells could use ROS to directly regulate DNA methylation and gene expression patterns through the oxidation of methylated cytosines in CpG Islands (CGI) of target gene promoters. To test this hypothesis, we have used a Protoporphyrin IX-dependent photodynamic treatment as a tool to activate a transient production of non-lethal ROS levels in the feeder-independent E14 mouse embryonic stem cell line. Using this tool, we report that the endogenous production of non-lethal ROS levels promotes a cytosine demethylation of promoter CGIs, suggesting that these oxygen derivatives can be involved in the regulation of gene expression patterns through the dynamic modulation of DNA methylation patterns.

P79 - THE ARABIDOPSIS MAS2 MULTIFUNCTIONAL PROTEIN INTERACTS WITH RNA METABOLISM FACTORS

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NF-kappa B activating protein (NKAP) is a multifunctional protein that acts in splicing and transcriptional repression in animals. The Arabidopsis ortholog of NKAP is MORPHOLOGY OF argonaute1-52 SUPPRESSED2 (MAS2), which is required for 45S rDNA transcription and 45S pre-rRNA processing [1]. In a yeast two-hybrid (Y2H) screen for MAS2 interactors, we identified the Arabidopsis orthologues of yeast ribosome biogenesis factors Ribosomal RNA processing protein 7 (Rrp7) and Nucleolar protein 53 (Nop53): RRP7 and SMALL ORGAN 4 (SMO4; [3]), respectively. Lack of RRP7 function causes nucleolar hypertrophy, altered 18S rRNA maturation, and nucleolar retention of mature and precursor 18S rRNA species. Mutation of SMO4 causes accumulation of 5.8S and 18S rRNA precursors, and nucleolar retention of 25S and 18S, but not 5.8S rRNA species. The RRP7 and SMO4 genes are coexpressed with genes encoding factors required for the 45S pre-rRNA processing and ribosome subunit assembly. The Arabidopsis NUCLEOLIN1 (NUC1) gene encodes a nucleolar protein that participate in 45S pre-rRNA maturation and in the epigenetic control of the 45S rDNA expression [2]. We observed synergistic phenotypes in double mutant combinations of alleles of RRP7 and SMO4 with alleles of NUC1 and MAS2. CAX INTERACTING PROTEIN4 (CXIP4) was the most represented MAS2 interactor found in our Y2H screen. CXIP4 is a nuclear and cytoplasmic protein of unknown function previously identified as interacting with the high-affinity vacuolar calcium antiporter CAX. CXIP4 is a plant-specific protein, exhibiting a conserved zinc knuckle motif, which binds DNA, RNA and proteins. The 30 first amino acids of CXIP4 show 70% similarity to the mammalian splicing factor SREK1-interacting protein 1. CXIP4 is a single-copy and essential gene in Arabidopsis, as shown by lethality of its cxip4-1 T-DNA insertional allele. We constructed two artificial microRNAs designed to target CXIP4 (amiR-CXIP4) to circumvent the lethality associated with the lack of CXIP4 function. amiR-CXIP4.1 plants display pointed and reticulated leaves, a phenotype reminiscent to that caused by hypomorphic or null alelles of genes encoding components of the translational apparatus. Our results indicate that Arabidopsis MAS2 participates in several RNA metabolism pathways, like its human ortholog NKAP. 1. Sánchez-García, A.B., et al. (2015). Plant Cell 27, 1999-2015. 2. Pontvianne, F., et al. (2010). PLOS Genet. 6, e1001225. 3. Zhang, X.R., et al. (2015). J. Integr. Plant Biol. 57: 810–818.

P80 - THE PROMETASTATIC GENES TMPRSS4 AND DDR1 ARE CO-OVEREXPRESSED AND CO-DEREGULATED BY HYPOMETHYLATION IN LUNG CANCER

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Metastasis is the main cause of cancer-related death in non-small cell lung cancer (NSCL) and understanding the mechanisms of this process is critical to reduce mortality. We have previously identified TMPRSS4 as a prometastatic gene in lung cancer whose high expression is associated with poor prognosis. In order to uncover a TMPRSS4-related lung cancer-signature that might shed light about the malignant behavior of this gene in patients, we carried out high throughput co-expression analysis across 6 public databases including lung adenocarcinomas (LUAD) and squamous cell carcinomas (LUSC), and identified a 29-gene signature. Statistical analysis of

differential expression confirmed up-regulation of 19 of these genes in tumors as compared to non-malignant samples, particularly for LUSC. Gene Ontology showed that many genes were related to cell adhesion/migration and interaction with the extracellular matrix. The prometastatic gene DDR1 was one of the genes of the signature, and co-expression with TMPRSS4 was validated in a 187-cancer cell line panel (CCLE) and 34 lung cancer panel (by qPCR). Analysis of the promoter methylation found that both TMPRSS4 and DDR1 were significantly hypomethylated in NSCLC as compared with non-malignant lung in the TCGA and CURELUNG series of patients and that their expression was inversely co-related with their promoter methylation status. To further validate methylation status of TMPRSS4 we used digital droplet PCR in an independent series of lung cancer patients. Moreover, Cox regression and ELASTICNET statistical analyses demonstrated that hypomethylation in both TMPRSS4 and DDR1 was associated with poor prognosis in NSCLC patients. In conclusion we have demonstrated that prometastatic genes TMPRSS4 and DDR1 are co-expressed and co-regulated by hypomethylation in lung cancer, a condition that is associated with bad prognosis. Analysis of methylation status of these genes may constitute a novel biomarker for diagnostic and prognostic purposes.

P81 - P73 IS REQUIRED FOR APPROPRIATE BMP-INDUCED MESENCHYMAL TO EPITHELIAL TRANSITION DURING SOMATIC CELL REPROGRAMMING

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Somatic cell reprogramming to generate induced pluripotent stem cells (iPSCs) occurs in three sequential phases: initiation, maturation and stabilization. The initiation phase starts with a mesenchymal-to-epithelial transition (MET) triggered by BMP signaling and is characterized by the activation of an epithelial gene expression program. The maturation phase is considered the major bottleneck of the process, since cells must activate endogenous expression of the pluripotency core circuitry (Oct4, Sox2, and Nanog), thus only a small number of cells progress to the final stabilization phase to become fully reprogrammed iPS cells. p73 is a member of the p53 transcription factor gene family, which also includes p53 and p63. The members of this family have been involved in the regulation of stem cell biology. Indeed, p53 acts as an important barrier to somatic cell reprogramming. However, p73 role in this process has never been addressed. Therefore, we hypothesized that p73 may play a role in the cellular reprogramming process. To address this, we reprogrammed WT and p73KO-mouse embryonic fibroblast (MEFs) and observed that lack of p73 negatively affects the reprogramming efficiency. Moreover, our data indicates that BMP signaling is impaired in the absence of p73 affecting the MET transition during the initiation phase of reprogramming, and resulting in an altered maturation and stabilization phase. Our results revealed, for the first time, that p73 is necessary for appropriate BMP signaling during the MET transition of somatic cell reprogramming, at least in part, by hindering BMP pathway activation. We report that p73 is a positive modulator of the BMP circuit, enhancing its activation by DNp73 repression of the Smad6 promoter. Collectively, these findings provide mechanistic insight into the mesenchymal to epithelial transition coordination, proposing p73 as an enhancer of MET during reprogramming.

P82 - MIR-146A TARGETS C-MET AND ABOLISHES COLORECTAL CANCER LIVER METASTASIS

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A major complication of colorectal cancer (CRC), one of the most frequent and deadly cancer types, involves the progression through liver metastases. At this stage, very few treatment options are available for patients and the disease remains incurable. Therefore, identification of novel molecular mechanisms that play an important role in liver metastasis is needed. Herein we used a well-established mouse model of CRC liver metastasis (CLM) to identify new regulators of this process. By means of serial transplantation of murine MC38 adenocarcinoma cells transduced with a luciferase reporter gene, we obtained liver metastatic variants that displayed higher clonogenicity in vitro and extremely strong liver colonization ability in vivo when injected intrasplenically. Using these new established cell lines, we performed gene expression arrays as well as microRNA (miR) profiling. Comparative and predictive analyses between the two arrays showed higher expression of c-met and concomitant reduction of miR-146a in the metastatic variants. Interestingly, we identified c-met as a new target for miR-146a that blocks its translation, using luciferase assays with the wild type or mutant versions of the c-met 3'-UTR as well as western blot. Of relevance, overexpression of miR-146a in metastatic clones showed reduced in vitro malignancy and abolished the development of primary tumor and liver metastases. Our results document a new mechanism for c-met regulation in CLM and highlight the crucial role of miR-146a in suppressing tumorigenesis. Our data suggest that miR-146a-targeted therapy might result in effective reduction in liver metastasis. Further investigation with patient's samples, currently in course, is necessary to confirm the role of c-met and miR146a in CRC progression.

P83 - CONTRASTING MATERNAL AND PATERNAL EFFECTS ON THE METHYLOME OF BROWN TROUT DURING DEVELOPMENT

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In the last years the important role that epigenetics plays in fish genome plasticity has been highlighted, with a special focus on changes in DNA methylation (Moghadam en al 2015). However, little is know about how offspring inherit the DNA methylome from parents. In this work we artificially crossed three populations of brown trout (Salmo trutta) in order to examine parental influence on epiloci. We employed a partial factorial mating design to cross 3 males with 3 females from three domesticated brown trout populations (F = Hardy, S = Hardy-Prosper, G = Gournay) to produce 9 families (Morán et al 2016). Genome-wide DNA methylation was studied in the 6 parental trouts and in sixteen alevins of each of the 9 families by means of the Methyl-Sensitive Amplification Polymorphism (MSAP) technique that use two enzymes (MspI and HpaII) that recognize and cleave CCGG target sequences, but cleaving differently when the inner or outer C is methylated at both strands. Differences in the presence/absence MSAP profiles were explored using the R package msap v.3.2.2 (Pérez-Figueroa 2013) and two-way AMOVA using X-AMOVA (http://www.danielwilson.me.uk/xAMOVA.html). The MSAP analyses yielded a total of 150 polymorphic loci. Of these, 80 loci were identified as methyl sensitive loci (MSL), whereas the remaining 70 ones were non-methyl sensitive (NML). A 68% (48 loci) of the NML were classified as polymorphic whilst the proportion of polymorphic loci reached 91% (73

loci) of the MSL. To further assess whether locus-specific methylation on the progeny depends on father or mother, Fisher's exact tests were used to detect candidate loci among the MSL. After statistical adjustment of the resulting P-values according to Benjamini and Hochberg false discovery rate (FDR), 53 statistically significant loci (Adjusted $P < 0.05$) presented a differential distribution of its methylation among trout heirs. Most of them (42), exhibited paternal effect, whereas the remaining one revealed maternal effects. Our results provide striking evidence that the paternal DNA methylation pattern is maintained for paternal effects on offspring methylome suggesting that males sustain higher transgenerational epigenetic transmission than females, as it have been precisely showed in zebra fish (Jiang ant al 2013). However, the existence of sire x dam interactions suggests that genetic variation also may play a role in offspring development and survival.

P84 - MULTIFACTORIAL CONTROL OF THE EXPRESSION OF A CRISPR-CAS SYSTEM IN MYXOCOCCUS XANTHUS BY AN ECF-σ/ANTI-σ PAIR AND THE CARD-CARG GLOBAL REGULATORY COMPLEX

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CRISPR-Cas adaptive immune systems are heritable, adaptive defense systems that protect bacteria and archaea against phages and foreign DNA. Their action is based on small CRISPR RNAs (crRNAs) generated by specific Cas proteins upon processing a long CRISPR RNA transcript. CRISPR-Cas loci must be first expressed to function in vivo and it is essential to know how this crucial first step is initiated and regulated. Our genetic and transcriptomic analyses reveal that expression of a CRISPR-Cas system and production of the corresponding crRNAs in Myxococcus xanthus requires: (i) DdvS, an extracytoplasmic function (ECF) s factor, whose activity is negatively regulated by its membrane-bound anti-s factor, DdvA; (ii) the global regulatory complex formed by CarD and CarG, which regulates various other processes such as light-induced carotenogenesis and fruiting body development. CarD, the first known member of the large CarD_CdnL family of bacterial RNA polymerase (RNAP)-binding proteins, is found only in myxobacteria where it is required for the action of many ECF-s factors (1-3), whereas CdnL is widespread in bacteria, acts on primary sA–dependent promoters (such as those of rRNA genes), and is required for normal growth and cell division (4-5). We mapped several DdvS-dependent promoters, including one in the cas operon whose transcription reads through to enable simultaneous expression of the associated CRISPR array in vivo. To our knowledge, this is the first description of an ECF-s/anti-s pair that, together with a global regulatory complex, coordinates the controlled expression of a CRISPR-Cas system for crRNA biogenesis in bacteria. 1. Elías-Arnanz M et al FEMS Microbiol Rev 34, 764-778 (2010) 2. García-Heras et al PNAS 106, 13546-13541 (2009) 3. Abellón-Ruiz J et al Environ Microbiol 16, 2475-2490 (2014) 4. García-Moreno et al Nucl Acids Res 38, 4586-4598 (2010) 5. Gallego-García et al (2014) PLoS One 9, e108946 (2010); Sci Rep 7, 43240 (2017) FUNDING: Ministerio de Economía y Competitividad (Spain) grants to MEA (BFU2015-67968-C2-1-P, co-financed by FEDER-UE) and to SP (BFU2015-67968-C2-2-P), and Fundación Séneca (Spain) grant 19429/PI/14 to MEA.

P85 - CHARACTERIZATION OF BILE-RESISTANT ISOLATES OF SALMONELLA ENTERICA RECOVERED FROM MURINE GALL BLADDERS

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Bile is a digestive secretion with antimicrobial properties. Bile salts act as detergents on membranes, denature proteins, and cause oxidative damage to DNA. Nevertheless, enteric bacteria are resistant to bile salts and use them as signaling molecules for gene regulation. An outstanding case of bile resistance is found in the pathogen Salmonella enterica, which colonizes the hepatobiliary tract and can establish a chronic infection in the gall bladder. Salmonella survival in the presence of bile can be due to either adaptation or mutation. Adaptation is the increase in resistance to bile salts after exposure to a sub-lethal dose [1]. This phenomenon involves multiple changes in gene expression. An alternative to adaptation is the acquisition of bile resistance by mutation, and it has been speculated that the bile salts themselves might increase the mutation rate since they DNA-damaging agents [2]. To investigate whether survival of Salmonella in the gall bladder involves adaptation or mutation, BALB/c (immunodeficient) and 129Sv (immunocompetent) mice were infected. Subsequently, Salmonella cells were recovered from the gall bladder. These isolates were classified as mutants (showing stable resistance to bile) and non-mutants ('adapted', showing unstable resistance to bile). The majority of isolates showed unstable resistance to bile, indicating that Salmonella survival in the gall bladder occurs mostly by adaptation. Yet, bile-resistant mutants were also obtained. Whole genome sequencing revealed that most mutations mapped in loci involved in membrane structure and in LPS synthesis and transport. This observation supports the view that the bacterial envelope has a crucial role in resistance to bile. [1] S. B. Hernández, I. Cota, A. Ducret, L. Aussel, J. Casadesús. Adaptation and preadaptation of Salmonella enterica to bile. PloS Genetics 8(1): e1002459, 2012 [2] A. I. Prieto, F. Ramos-Morales, J. Casadesús. Bile-induced DNA damage in Salmonella enterica. Genetics 168: 1787-1794, 2004

P86 - THE NON-CANONICAL WNT-PCP PATHWAY SHAPES THE CAUDAL NEURAL PLATE

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The last stage of neural tube (NT) formation involves the closure of the caudal neural plate, an embryonic structure formed by neuromesodermal progenitors and newly differentiated cells that becomes incorporated into the NT. Here we show that as cell specification progresses, neuromesodermal progenitors and their progeny undergo significant changes in shape prior to their incorporation into the NT. This process, which progresses caudo-rostrally requires actin microfilaments and involves apical constriction and planar cell arrangements. The Wnt-PCP pathway is crucial for reorganizing the actomyosin cytoskeleton of cells in the anterior NT, a modification that is critical at early stages of neurulation. Our results demonstrate that the Wnt-PCP pathway also controls polarized tissue folding in the caudal neural plate, regulating apical constriction and cell reorganization without affecting cell differentiation. Indeed, when this pathway is genetically or chemically impaired the polarized cellular behavior and cellular morphology within the entire caudal neural plate is disturbed, producing delays in NT closure. Thus, caudal NT closure is not completed in the most severe cases, deriving into spina bifida.

P87 - SHOT LINKS MT AND ACTIN DURING ACTIN-HAIR FORMATION IN PUPAL WINGS

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Actin and microtubule cytoskeleton requires coordination and communication with other signaling pathways during development. How planar cell polarity interacts molecularly with these cytoskeletal changes to harmonize them at the tissue level is largely unknown. We have recently identified the spectraplackin short-stop (Shot) as a PCP interactor using Drosophila melanogaster as a model organism. Shot is a conserved protein which functions as a cross-linker between actin filaments and microtubules. Here, we present the molecular characterization of Shot isoforms and domains role during PCP establishment relative to actin, microtubules and Flamingo (Fmi).

P88 - REPRESENTATIVE CENTRIOLES DISTRIBUTION (RCD) IS A NEW IMAGING TOOL TO MEASURE CENTRIOLE POLARIZATION.

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Centrioles polarization is a dynamic process that depends on Fz-PCP pathway to occur properly during development. Analysis of centrioles localization requires the development of new imaging tools for proper comparison between WT and mutant genotypes. Here we model centrioles positioning based on their probability density in 2D, using flies as they allow spatial and temporal quantitative analysis of centrioles distribution in cell populations from the same sample. We present the concept of representative centrioles distribution (RCD) which represents the probability of finding a centriole in a specific position of the cell, at different confidence ranges. This RCD is helping us to describe changes in centrioles positioning in different regions of the same Drosophila wing in WT and PCP mutant genotypes. It is also allowing us to explore the correlation between centrioles localization and localized actin polymerization.

P89 - P73 MODULATES EPENDYMAL TRANSLATIONAL PLANAR CELL POLARITY VIA TRANSCRIPTIONAL REGULATION OF MLCK AND NON-MUSCLE MYOSIN II ACTIVATION

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Planar cell polarity (PCP) describes the coordinated orientation of cells in the plane of a tissue and it is an essential feature of animal epithelial sheets. In multiciliated epithelial cells (MCC), individual and coordinated cilia orientation is fundamental for correct functioning. In the brain, where ventricles are lined by multiciliated ependymal cells (ECs), defects in PCP establishment results in cilia dysfunction and hydrocephalus. Ependymal cells display two types of PCP, translational cell polarity (tPCP) and rotational cell polarity (rPCP), which are fundamental for coordinated cilia beating. tPCP is first established at individual level during perinatal development when monociliated radial glial cells (RGCs) transform into multiciliated ECs. Regulation of tPCP in ECs is carried out, at least in part, by the activation of non-muscle-myosinII (NMII), an actin-

binding protein essential for the control of cell adhesion and tissue architecture. Activation of NMII is regulated by the phosphorylation of its regulatory light chains (RLCs) by the kinases Rho-associated protein kinase (ROCK) and myosin light chain kinase (MLCK). However, only MLCK-dependent activation is essential for the establishment of tPCP in ECs. p73 transcription factor belongs to one of the most important gene families in vertebrate biology, the p53 family. Trp73 is a master regulator of CNS development being required for neurogenic niche cytoarchitecture. Here we describe a novel mechanism of p73 regulation of translational PCP establishment in ependymal cells.

P90 - MYOSIN II ACTIVATION AS A HALLMARK AND A VULNERABILITY IN MELANOMA CROSS-RESISTANCE

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Melanoma is at the forefront of personalized medicine with both targeted therapies such as BRAF inhibitors (BRAFi) or combination of BRAFi with MEK inhibitors (MEKi); and immunotherapies such as the immune checkpoint inhibitors anti-CTLA-4 and anti-PD-1. However, resistance to therapy is a persistent problem in melanoma management. Cross-resistance to MAPKi and immune checkpoint inhibitors has been suggested to be driven by common transcriptomic alterations. Here we provide evidence that such transcriptional aberrations are crucially dependent on the ROCK-Myosin II pathway, widely studied in cancer invasion and metastasis. We find that Myosin II activity drives the intrinsic survival advantage observed in both targeted- and immunotherapy-resistant melanomas. We observe increased expression of the non-muscle myosin II regulator myosin light chain 2 (MLC2) and ROCK, LIMK and MRTF in resistant melanomas. High activity of this pathway identifies resistant tumours, suggesting its potential as a biomarker and as an actionable target. We show how different targeted- and immunotherapy-resistant cells can be killed by blocking Myosin II activity directly, blocking ROCK activity or via RNAi against components of the Myosin II complex and/or ROCK1/2. Actomyosin blockade also impairs cell survival in 3D environments. This molecular and cellular adaptation is conserved in tumour-derived cells from patients that are resistant to targeted and immunotherapies. We show that in vivo primary tumour growth and survival at metastatic sites of therapy-resistant tumours are blocked by ROCKi treatment. We propose that an important subset of targeted and immunotherapy-resistant melanomas is intrinsically more susceptible to inhibition of the ROCK-Myosin II pathway, which opens up clinical opportunities for combination therapies.

P91 - INFLUENCE OF THE CAPACITATION IN VITRO ON PHOSPHORYLATION STATE OF PROTEINS AND TUBULIN DISTRIBUTION IN HUMAN SPERM

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One of the most important changes during spermiogenesis affects the cytoskeleton of the spermatozoon with the formation, organization, and regulation of microtubules, important for sperm cells motility. On the other hand, the presence of phosphorylated proteins in the sperm

flagellum is strongly related to the sperm–zona binding capacity, and previous studies has shown that the tyrosine phosphorylated levels increases in a time-dependent manner in human sperm. The objective of this study was to relate the presence of tyrosine phosphorylation influence on the tubulin distribution along the sperm flagellum with the state of capacitation and acrosomal reaction. The samples were obtained from five normozoospermic donors. The capacitation was carried out by swim-up during 1 (C1) and 4 hours (C4). The acrosome reaction in C1 and C4 was induced with calcium ionophore A23187 (R1 and R4). Assessment of the presence of tyrosine phosphorylation (PY20 antibody) and the study of the cytoskeleton (anti-α-tubulin antibody) was performed simultaneously by indirect immunofluorescence. In each physiological condition a minimum of 500 cells were observed by confocal microscopy and the data were analyzed using Student-t distribution.

P92 - EFFECTS OF GRAPHENE-RELATED MATERIALS ON METABOLISM, MITOCHONDRIAL FUNCTION AND CELL FATE IN HUMAN SKIN CELLS

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Graphene-related materials (GRMs) as graphene oxide (GO) and few layer graphene (FLG) are potentially interesting molecules for many biomedical applications such as drug delivery, anti-cancer therapy, etc. Nevertheless, there is still lack of information about their interaction with human barriers and their safety. In this work, we used different approaches to explore the effect of GO and FLG in a skin barrier model, using human HaCaT keratinocytes as model and assaying their effect on the metabolome, mitochondrial function, cell death and motility, using metabolomics, cell flow cytometry, confocal microscopy, a Seahorse XFp analyzer and Raman spectroscopy. We also analyzed the ability of keratinocytes to endocyte FLG and GO. Our results show that low doses of GO and FLG induce short term raises in reactive oxygen species and cytosolic calcium, which are maintained for 1 week. Cells treated with FLG for 24 showed a significant increase in the free cytosolic calcium level up to 60%, accompanied by a 22% increase in NADPH oxidase activity and ROS levels. In parallel, GRMs modified mitochondrial respiration and remodeled the metabolome changing lactate, choline, and pyruvate levels, among others. These alterations are linked to both, an increase in apoptotic and necrotic cell death and to a decrease in cell motility, which, in turn, could compromise wound healing. Deeper research is needed to unveil the safety of these and other GRMs, which is directly correlated with the concentration, physicochemical properties and exposure time. Moreover, the study of GRMs uptake and their subcellular sorting and processing is necessary as a mandatory step for their application in biomedicine.

P93 - CORNEAL ENDOTHELIAL CELL ORGANIZATION UPON TNF STIMULATION IN AN EX VIVO MURINE MODEL

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The corneal endothelium is essential for maintaining corneal transparency. Endothelial barrier integrity is conferred by tight (TJ) and adherens junctions (AJ) physically linked to the actin

cytoskeleton. Aging, mechanical, osmotic and inflammatory stress induced by surgery procedures and eye diseases cause corneal endothelial barrier disruption, loss of corneal transparency and the need of transplant. TNF is a proinflammatory cytokine that mediates allograft rejection and corneal inflammatory diseases. In vitro, TNF induces actomyosin remodelling and disrupts endothelial barrier integrity. Here, we have investigated by high resolution confocal microscopy the unique spatial organization of intercellular junctions, focal adhesions (FA) and actin in murine corneal endothelial cells in vivo. Corneal endothelial cells concentrate perijunctional actin filaments that support tension in the monolayer along straight apical TJ and AJ. In contrast, basolateral AJ acquire a unique wavelike morphology, contain low F-actin content and orientate towards a central cellular domain that contains stellate actin bundles and FA underneath the nucleus. Corneas are cultured in tissue banks for weeks before the transplant. We have develop an ex vivo culture model of murine corneas to test new strategies to preserve this tissue. Long-term cornea culture induces a mild inflammatory phenotype, reorientation of apical perijunctional actin and junctions, and loss of undulation in basolateral AJ. TNF exacerbates this junctional and actin reorganization. Therefore, the corneal endothelial barrier is organized in vivo in a way that cannot be mimicked in vitro so far, so in vivo and ex vivo models are essential to investigate the mechanisms mediating corneal endothelial barrier dysfunction.

P94 - CONTRIBUTION OF CENTROSOME-AND GOLGI APPARATUS- MICROTUBULE NUCLEATION TO EPITHELIAL POLARIZATION

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Proper epithelium integrity and homeostasis requires the establishment and maintenance of apico-basal polarity (1). Microtubule organization along the apico-basal axis as well as centrosome and Golgi Apparatus repositioning at the apical pole are common features of polarized epithelial cells (2). Both the centrosome and the Golgi Apparatus are currently considered the main MT organizing centers (MTOCs) of most eukaryotic cells (3). How these organelles regulate MT nucleation and distribution during apico-basal polarization and differentiation of epithelial cells is still unclear.
To analyze the contribution of either the CTR or the GA, and their crosstalk, to epithelial differentiation we selectively inhibited centrosome-based or Golgi-based MT nucleation in both human mammary gland MCF10A and canine kidney MDCKII epithelial cells. We first treated cells with the new drug centrinone in order to produce centrosome-depleted cells (5). We also generated stable AKAP450 knock-out MCF10A and MDCKII cell lines (through CRISPR technology), which abrogated MT nucleation at GA (5). As models for studying cell polarization under standardized conditions and for mimicking epithelial features, we used MCF10A cell doublets grown in micropatterns and three-dimensional cultures of MDCKII cells. Our results indicate that AKAP450-based MT nucleation plays a role in redistributing both the GA and the centrosome towards the apical pole and, in this way, it controls the initial steps of cell polarization. Severe perturbations of apico-basal axis were also observed in cells lacking centrosomes. Our results will allow to better understand cell polarization, a process whose alterations are relevant to many pathologies including cancer.

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P96 - A RIGHT-HANDED PATHWAY DRIVES HEART LOOPING IN VERTEBRATES

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The majority of animals exhibit external bilateral symmetry. However, there are numerous interior left–right (L/R) asymmetries, including the morphology and position of several internal organs. The first morphological marker of L/R asymmetry is the right-sided looping of the developing heart. During early development, the straight heart tube develops in the midline. We have found that in the zebrafish, L/R differential cell movements towards the midline lead to a leftward displacement of the cardiac posterior pole and the heart tube loops toward the right side (Ocana et al., 2017). This mechanism is controlled by a BMP-mediated EMT process more prominent from the right. As such, BMP mediates a L/R asymmetric activation of Prrx1 in the lateral plate mesoderm (LPM) that produces an asymmetric contribution of right and left cells to the posterior pole of the primary heart tube. This asymmetric behaviour generates asymmetric forces and tension on the right-hand side that promote dextral looping through an actomyosin-dependent mechanism. Here we show that this mechanism is conserved during evolution. Indeed, transient asymmetric distribution of Prrx1 is also evident in the chick LPM, with higher levels on the right-hand side, and downregulation of Prrx1 in the chick embryo induced mesocardia. The asymmetric L/R Prrx1 expression in the chick is also compatible with the formation of an actomyosin-dependent mechanism. This mechanism is also conserved in the mouse, where Snail1 fulfils the role played by Prrx1 in the fish and chick. Thus, a differential L/R EMT more prominent from the right side drives heart looping in vertebrates, although the EMT transcription factor involved can be different in different vertebrate groups.

P97 - THE EPIGENETIC PROFILE OF THE INTERDIGITAL MESODERM FATED TO PROGRAMMED CELL DEATH.

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Digit formation in the developing vertebrate limb is accompanied by a remarkable process of massive cell death that eliminates the interdigital tissue. Most dying cells exhibit an apoptotic appearance but the dying mechanism is not well understood. Bone morphogenetic proteins (BMPs) are upstream triggering signal of interdigital cell death, however in the neighbouring digit ray mesenchyme they are responsible for cartilage growth and differentiation. In addition, both caspases and lysosomal cathepsins are central player of interdigital cell death, although their pharmacological or genetic silencing is not followed by syndactyly or any digit phenotype. We have recently shown that DNA damage is an early sign of interdigital degeneration that precedes caspase activation. This DNA disruption is accompanied by the activation of DNA repair mechanisms and is quickly induced (3hr) by local application of BMPs in the interdigital tissue. Based on all those observations, our hypothesis is that interdigital apoptosis is caused by a cell sensitization to factors that promote growth in the differentiating neighbouring mesoderm. The aim of this study is to explore the potential implication of the epigenetic factors in the establishment of the dying process. For this purpose, we will perform a comparative analysis of the epigenetic profile between the interdigital cells destined to die and the cells that survive and form the digits of the embryonic limb. In this work, we present the results of immunolabeling analysis, in interdigital cells from chick embryos, of different epigenetic modifications such as DNA methylation and changes in histones H3 and H4, which include methylation and acetylation of lysines.

P98 - EVALUATION OF SPANISH POPULATIONS OF POA BULBOSA. SELECTION OF GENOTYPES WITH SPROUTING IN LATE SUMMER

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Poa bulbosa is a cold season geophyte species, entering latency with increasing day length and temperature. Starting again its growth from mid-summer in Mediterranean climate conditions. Entry into latency can be induced by water stress. It also delays entry into vegetation at the end of summer. Its vegetative cycle makes it very interesting for its possible use in mixture with the common grass (Cynodon dactylon) species of warm season and that goes to rest in the cold season. For this reason the compatibility of Poa bulbosa-Cynodon with different irrigation rates for ornamental grasses with low maintenance is being studied. Both species are native and frequent in areas of Mediterranean climate. The use in mixture as a lawn involves exposing the Bulbous blue grass to frequent irrigations in summer, if carried out by sprinkling. This has the effect of sprouting the bulb earlier than if it were in natural conditions. Based on this problem, it was proposed to carry out a test with different sources (populations) of the Mediterranean climate zone to see if there were differences in the latency rupture in any population or individual. The present study aims to know how the Poa bulbosa reacts to the application of irrigation without causing water stress. The behavior of 14 Spanish peninsular populations collected in the field has been studied maintaining the individuality of each plant. The essential methodology was the application of irrigation on alternate days for 4 hours and rainfall of 2 mm / hour from the month of January to November, to know the latent entrance and the influence on the rupture of the same in the different populations and individuals that compose them. In total, 266 individuals belonging to 14 local Spanish populations have been studied. It has been observed that the latency entry occurred in the month of April in all populations. The rupture of dormancy occurred from the beginning of August, continuing until the first two weeks of October. In the month of August germination began 13 populations with a total of 161 individuals. In the first half of September there were 11 populations germinating with a total of 85 individuals. In the second half of September there were 8 populations with 19 individuals. In the first half of October, an individual from a population ended on the 12th. These results allow us to consider the possibility of working with the individuals that germinated at the end of the warm season.

P99 - CENTRIOLES POSITIONING ANALYSES IN DROSOPHILA PUPAL WINGS WITH PCP RELATED PHENOTYPES.

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Correct centrioles positioning is crucial for proper development and functioning of different organs. In flies, we commonly use PCP proteins asymmetric distribution, actin-based hair orientation and more recently centrioles polarization to assess correct planar polarity signaling. Here we present a detailed analysis of centrioles polarization in different PCP genetic backgrounds, including the Fat-PCP pathway, which is also affecting Frizzled-PCP pathway, and common PCP effectors, like Fuzzy, Fritz and DRok. We found that, as expected Fat-PCP pathway is also affecting centrioles polarization similar to what we have previously found with Frizzled-PCP. This phenotype is more dramatically observed when clear hair mis-orientation is observed. Surprisingly, we did not found centrioles polarization defects on DRok loss of function conditions. Cells with multiple actin based hairs presented a representative centrioles distribution (RCD) similar to WT regions in the same wing.

P100 - SEARCH FOR NEW GENOMIC INSTABILITY FACTORS BY OVEREXPRESSING RNA-BINDING PROTEINS

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J.A. Mérida-Cerro, A.G. Rondón and A. Aguilera. Centro Andaluz de Biología Molecular y Medicina Regenerativa CABIMER, Universidad de Sevilla, Seville, 41092, Spain Genome instability plays a central role in genetic diseases like cancer. A significant part of genome instability is caused by transcription, a highly regulated process that in eukaryotes is coupled to mRNA processing and packing into a messenger ribonucleoprotein (mRNP). Accurate mRNP biogenesis is essential for gene expression and to avoid the formation of co-transcriptional R-loops, natural structures formed by a RNA-DNA hybrid and the displaced single-stranded DNA. Accumulation of R-loops impedes replication fork progression due to the collision between the transcription and replication machines, being a major source of genomic instability. However, how R loops cause genome instability is yet unclear. Yra1p is a RNA-binding protein involved in the assembly of an export-competent mRNP. The level of Yra1 is tightly regulated in yeast cells and mutations that increase Yra1 expression impair mRNA export and reduce cell growth. We have recently shown that an excess of Yra1 also causes R-loop dependent genome instability, as seen by an increase in recombination and Rad52 foci that are both RNase H sensitive and have proposed that this is due to the ability of an excess of Yra1 to stabilize R loops. We have asked whether other RNA-binding proteins (RBPs) that inhibit growth when overexpressed could also induce genome instability as a way to identify new proteins with the potential to stabilize R-loops and therefore cause genome instability. As signs of genomic instability, we checked for RNA-DNA hybrid-dependent DNA damage and hyperrecombination. In addition, we tested whether an imbalance of mRNP assembly factors caused by overexpression of RBPs affects transcription and nuclear export. The data will be shown and discussed.

P101 - RRM3 IS INVOLVED IN THE REPAIR OF REPLICATION-BORN DSBS BY SISTER CHROMATID RECOMBINATION

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During DNA duplication, replication forks encounter numerous obstacles that hamper their progression leading to fork stalling. Eventually, forks can collapse into DNA double-strand breaks (DSBs), which are among the most cytotoxic DNA lesions. In general, there are two mechanisms to repair a DSB: Homologous recombination (HR) and Non Homologous End joining (NHEJ). HR is the preferred repair mechanism during replication since the use of the sister chromatid as a template ensures the maintenance of genome integrity. The use of a mini-HO system that induces mainly DNA nicks allows us to study the repair of DSBs originated during replication. Here, we have uncovered an unexpected role for the Rrm3 helicase, known to accumulate at stalled replication forks and promote fork progression, in the recombinational repair of such replication-born DSBs with the sister chromatid, thus avoiding genome instability and its lethal consequences.

P102 - HYDROXYUREA-INDUCED PHYSICAL UNCOUPLING BETWEEN REPLICATIVE HELICASES AND NASCENT DNA WITHOUT COMPROMISING THE COMPETENCE OF FORKS TO RESTART

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During S phase, replication forks can encounter several obstacles that lead to fork stalling, which if persistent may result in fork collapse. To avoid this collapse and preserve the competence to restart, cells have developed mechanisms that maintain fork stability under replication stress. In order to characterize these mechanisms in non-transformed human cells, we performed an iPOND-MS analysis in hTERT-RPE cells under different replication stress conditions. Remarkably, our data show that replisome components, including the CMG helicase, are displaced away from nascent DNA after an acute replication stress while the integrity of CMG is maintained. Analyses of ssDNA in parental and nascent DNA indicate, on one hand, that the helicase continue to unwind the DNA while the DNA synthesis is inhibited, and, on the other hand, that some forks have been reversed. Interestingly, forks are able to restart replication even in the absence of CDK activity, suggesting that previously activated CMG helicases are recycled to maintain the competence to restart.

P103 - CRISPR IN C. ELEGANS AS AN OPPORTUNITY FOR PERSONALIZED MEDICINE

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Diseases driven by genetic mutations range from rare diseases to common pathologies as cancer. Mechanisms of the genetic diseases, and therefore the precise treatment, would vary depending on the specific mutation that alters in one way or another the gene function. Our lab took the CRISPR wave from the very beginning and we are capable of efficiently produce missense mutations and deletions of our interest in C. elegans. Taken advantage of this expertise, we are mimicking mutations on well-conserved splicing factors that are drivers of the rare disease Retinitis Pigmentosa in distinct patients. These mutants display mild to severe phenotypes that can be used to screen for potential pharmacological and genetic modifiers of the disease. Cancer is the other field of interest for our lab, located at an oncologic hospital. We are editing the worm genome to resemble mutations of the splicing factor SF3B1, which is frequently mutated in distinct types of cancer as leukemia, melanoma or breast cancer. This approach can help us to distinguish driver from passenger mutations, to identify other genes involved in the "fatidic combination" that lead to cancer, or to run synthetic lethal screens. Finally, CRISPR and also RNAi are our weapons in the battlefield of chemoresistance. We are investigating and identifying gene activities that provide resistance to chemotherapeutic agents as cisplatin in living animals.

P104 - WT1 IS INVOLVED IN THE REGULATION OF THE BMP4 PATHWAY DURING EPICARDIAL DEVELOPMENT

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Embryonic epicardial cells undergo several mophogenetic changes that are accompanied by a sequential downregulation of early epicardial genes and upregulation of genes associated with a mature epicardium. This morphogenetic switch is completed by the appearance of a mature epicardium that is already present at E16.5. The transformation of the embryonic epicardium not only leads to a change in cellular architecture but is simultaneously accompanied by changes in function and stem cell properties. Wt1 gene expression is one of the main hallmarks of the embryonic epicardium signature and furthermore its expression is dynamically modulated during the course of epicardial development. Here we demonstrated that epicardial maturation is characterized by a dynamic expression of BMP4 signaling. We observed that inhibition of this pathway leads to a series of changes in epicardial cells that resemble the phenotype of mature mesothelial cells. In addition we also demonstrated that the BMP4 pathway is tightly regulated by Wt1. Understanding the molecular mechanisms that take place during heart morphogenesis could constitute the first step in the generation of therapeutic strategies for the treatment of cardiovascular diseases.

P105 - TOLERANCE TO PATERNAL GENOTOXIC DAMAGE PROMOTES SURVIVAL DURING EMBRYO DEVELOPMENT IN ZEBRAFISH

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Spermatozoa are prone to suffer DNA damage during spermatogenesis, storage in the male tract and after ejaculation. Fertilization with DNA damaged spermatozoa (DDS) leads in mammals to pregnancy loss, increase of abortions, birth defects and genetic diseases in the offspring. Mammals have a strong sperm selection mechanism and DNA damage response (DDR) is activated in the zygote upon fertilization in order to guarantee the genomic conformity of the reduced progeny. Fish display a very different reproductive strategy based on the increased quantity of offspring with low surviving probabilities after birth. Previous results from our group showed us a downregulation of apoptotic activity in trout embryos with a defective DNA repairing ability, suggesting that tolerance mechanisms to DNA damage were activated in order to maintain cell survival and progression with development. In this work zebrafish embryos were obtained from control or UV irradiated sperm. Comet assay confirmed genotoxic damage with more than 10% of fragmented DNA in all the treated cells, but irradiation did not affect the fertilization ability. Malformation rates, DNA repair foci (γH2AX and 53BP1), apoptotic activity, and expression of genes involved in DDR were analyzed at different developmental stages. Batches from treated sperm provided a very high rate of multi-malformed larvae, suggestive of alteration of several developmental pathways. The study of embryo development showed a much enhanced repairing activity at mid blastula transition that return to the basal level at later stages. Apoptotic activity detected by Anexin V was similar to that of control embryos. Nevertheless, the study of p53 transcripts and phosphorylated p53, revealed the activation of a DDR, characterized by an intense transcriptional and post-traductional p53 upregulation in progenies from DDS. However,

the downstream pro-apoptotic factor noxa showed a significant downregulation, and the anti-apoptotic gene bcl2 was upregulated, triggering a repressive apoptotic scenario. The repressed apoptotic activity in spite of a clear genomic instability suggests the activation of tolerance mechanisms. It has been recently described that p53 is required in DDT pathways activated after UV irradiation, allowing the progression of some polimerases (eta and iota) able to bypass the stalled forks. Evading replicative arrest, DDT mechanism allows cell survival in damaged cells. Our results suggest that p53 switches DDT pathways allowing the embryo survival regardless the paternal DNA damage. DDT could be an evolutionary mechanism in fish: tolerance to unrepaired sperm DNA could introduce new mutations, some of them potentially advantageous to face a changing environment.

P106 - THE OR AND IR OLFACTORY RECEPTOR NEURONS IN DROSOPHILA: DISTINCT SPIKE AMPLITUDE DYNAMICS IN RESPONSE TO ODOR STIMULATION

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Olfactory responses at the receptor level have been thoroughly described in Drosophila melanogaster by electrophysiological methods. Single sensilla recordings (SSRs) measure neuronal activity in intact individuals in response to odors. However, activity has been mainly described by spike frequency that reflects a fast component of ORN response although another slow component of that response has been reported using the LFP (local field potential, the single sensillum counterpart of the electroantennogram, EAG). Our results showed that odorant stimulation produces two different effects in the fast component, affecting spike frequency and spike amplitude. Spike amplitude dynamics is different in two types of olfactory sensilla, basiconic expressing the OR family of olfactory receptors and coeloconic sensilla expressing the IR family of ionotronic receptors, decreasing or increasing spike amplitude respectively during stimulus response. Moreover, spike amplitude follows the same kinetics as the slow voltage component measured by the LFP, suggesting that both measures spike amplitude and LFP are connected. Signalling was studied in ab2 and ab3 basiconic sensilla and in ac2 and ac3 coeloconic sensilla in response to several odors at different concentrations. Both spike amplitude and LFP kinetics depend on odorant, concentration and neuron, suggesting that like the EAG they may reflect olfactory information. Keywords: Olfaction, Olfactory receptor neurons, OR olfactory receptors, IR olfactory receptors, Drosophila melanogaster, Single sensilla recording, Spike frequency, Spike amplitude Acknowledgements: This work was supported by the Spanish Ministry of Economy and Competitiveness [SAF2013-48759-P], the Principado de Asturias (EQP-06-34, SV-PA-13-ECOEMP-51 and GRUPIN14-012), the University of Oviedo (UNOV-12-MA-11) and FEDER Funds.

P107 - SYNAPTIC ACTIVITY ENHANCES GLUCOSE METABOLISM IN RAT CORTICAL NEURONS TO SUPPORT NEURITE OUTGROWTH

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The formation of neurites is an important process affecting the cognitive abilities of an organism. Neurite growth requires the addition of new membranes, but the metabolic remodeling necessary

to supply lipids for membrane expansion is poorly understood. Here, we show that synaptic activity, one of the most important inducers of neurite growth, transcriptionally regulates the expression of neuronal glucose transporter Glut3 and rate-limiting enzymes of glycolysis, resulting in enhanced glucose uptake and metabolism that is partly used for lipid synthesis. Mechanistically, CREB regulates the expression of Glut3 and Siah2, the latter promoting the normoxic stabilization of HIF-1a that regulates the expression of rate-limiting genes of glycolysis. Glut3 knockdown or the expression of dominant-negative HIF-1a blocks activity-dependent neurite growth in vitro and specific ablation of HIF-1a in early postnatal mice impaired the neurite architecture. These results suggest that the manipulation of neuronal glucose metabolism could be used to treat some brain developmental disorders.

P108 - SOD2 INFLUENCE ON SPERMATOGONIAL STEM CELL DIFFERENTIATION

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Redox regulation by reactive oxygen species (ROS) has a key role in cell signaling because these molecules take part in cell fate. Spermatogenesis involves on the one hand the self-renewal of spermatogonial stem cells (SSCs) and, on the other hand, their differentiation into mature haploid spermatozoa. Uncontrolled ROS levels are assumed to influence sperm production thus, antioxidant enzymes might have a relevant role in spermatogenesis by modulating ROS levels. Superoxide dismutase 2 (SOD2/MnSOD), which constitutes the primary defense of the cell against O2.- generated in the mitochondria and controls H2O2 production, might have a relevant role in spermatogenesis by modulating ROS levels. In order to elucidate the role that SOD2 plays in spermatogenesis, we used C57BL/6J WT as well as Sod2+/- knockdown and Sod2+/++ overexpressing mice to study SSCs differentiation. Previously we had examined redox balance by analyzing the levels of different antioxidant enzymes in the testes of mice from the three genotypes. It was found that in those mice overexpressing SOD2, there was not any increment in catalase production which in fact suggests an accumulation of H2O2. Regarding spermatogenesis, we had also proved that Sod2+/++ mice had an increased number of spermatocytes as well as spermatids and spermatozoa, though no changes in the amount of spermatogonia were found among genotypes. When SOD2 levels of male parents were altered, Sod2+/++ mice exhibited a shorter time between crossbreeding and parturition and they delivered more puppies per litter than Sod2+/- mice. However, no significant differences were found when we analyzed testosterone levels in serum from Sod2+/++ or Sod2+/- adult mice. In order to further investigate the involvement of SOD2 in SSCs differentiation, we established an in vitro culture of both Sertoli cells and SSCs. For that purpose, we sacrificed male mice pups at 9 days of age, and after an enzymatic digestion cells were plated and incubated in 5% CO2 at 37ºC. SSCs were characterized by flow cytometry using the antibody CD90.2 as a SSCs marker. Once we were certain that we were isolating SSCs, we established and maintained the culture in order to assess the ability of SSCs from the three genotypes to recolonize seminiferous tubules and begin spermatogenesis.

P109 - ROLE OF CLATHRIN IN TGF-β SIGNALLING IN LIVER CELLS

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In hepatocytes, the Transforming Growth Factor-beta (**TGF-ß**) is an important tumour suppressor factor, inhibiting proliferation and inducing cell death. However, in proliferating hepatocytes and in liver tumour cells, TGF-ß also regulates other processes that contribute to cancer progression. In hepatocellular carcinoma (HCC) cells, TGF-ß induces both pro- and anti-apoptotic signals and the balance among them decides the cell fate. Anti-apoptotic signals are mediated by transactivation of the Epidermal Growth Factor Receptor (**EGFR**). Recent results have emphasized the essential role of the receptors trafficking regulating signalling events. TGF-ß-receptors can internalize via at least two distinct internalization routes: via cholesterol-rich membrane microdomain **lipid rafts/caveolae**, and via **clathrin-coated vesicles**. It has been proposed that EGFR mainly traffics via clathrin-coated vesicles. Caveolin-1 trafficking has been widely studied, but so far how clathrin trafficking may control the different effects of TGF-ß in liver cells is not known yet. Accordingly, the aim of this work was to analyse the specific relevance of clathrin in the TGF-ß intracellular signaling in liver cells (both mouse hepatocytes and human HCC cell lines).
We induced the knock-down of clathrin heavy-chain (CLTC) through small interfering RNA (siRNA). Clathrin knock-down cells were more sensitive to the apoptotic effects of TGF-ß. By western blot analysis, it was observed that these cells were able to phosphorylate SMADs (TGF-ß canonical signalling), whereas they showed an impairment in the activation of the anti-apoptotic signals, in particular, in the transactivation of the EGFR pathway. In fact, CLCT knock-down cells also showed a significant decrease in the response to EGFR ligands, which indicates that CLCT is required for transducing EGFR signals. Concomitant with the increase in apoptosis, CLTC knock-down cells produced higher reactive oxygen species levels in response to TGF-ß, correlating with higher expression of NOX4, a member of the NADPH oxidase family that is target of both TGF-ß and EGFR pathways, which play opposing roles on its expression. Analysis in a cohort of HCC patients revealed that most of them showed higher CLTC levels in tumoral areas in comparison with the surrounding non-tumoral areas. Furthermore, we detected a positive correlation between *CLTC* and *TGFB1* expression.
In conclusion, we describe a novel role for clathrin in liver tumorigenesis, favouring non-canonical pro-tumorigenic TGF-ß pathways, mediated by the EGFR signaling.

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P110 - PRESENCE OF MELATONIN SYNTHESIS ENZYMES IN THE REPRODUCTIVE TRACT OF BULL

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Melatonin is ubiquitous in nature, and it can be found in many animal tissues. Its blood concentration changes following the light/dark cycle, due to its synthesis in the pineal gland during the night. This circadian variability and the multiple processes it regulates is considered the most important feature of melatonin, but it is also involved in multiple functions independent of the circadian cycle. Its biosynthesis is mediated by the enzymes serotonin-N-acetyl transferase (AANAT)

and hydroxindole-O-methyl transferase (HIOMT). Many studies have reported the presence of melatonin synthesis in extrapineal tissues, such as the male reproductive system. The objective of our study was to test the presence of the melatonin synthesis enzyme in organs of the genital tract of the bull (Bos taurus). Samples were obtained from six bulls slaughtered in an abattoir, discriminating testis, epididymis, vas deferens, ampulla, seminal gland, prostate and bulbourethral gland. We extracted the proteins and carried out Western blot with specific antibodies to detect these enzymes. Our results support, for the first time, the presence of AANAT and HIOMT in the reproductive tract of the bull. The amount of each enzyme varied among tissues, indicating a possible differential activity on the synthesis and contribution to the melatonin detected in seminal plasma (González-Arto et al. 2016, Theriogenology 86:1958-68). We detected the highest levels of both enzymes in the prostate. The band pattern also varied among organs, possibly due to interactions of these proteins with others, or to different postranscriptional modifications that could modulate their activity. The testis showed the typical 38 kDa band and another specific high-molecular weight band, approximately 100 kDa for HIOMT. Some differences were also detected among bulls, possibly due to age or the date of slaughter. Therefore, we can propose that the melatonin synthesis pathway is active in the reproductive system of the bull. The synthesized melatonin could play a paracrine role as much as being secreted to the seminal plasma, possibly by the prostate. These findings emphasize the importance of melatonin in bull reproduction, and they could be applied in reproductive health and in the improvement of assisted reproductive techniques.

P111 - NOTCH/WNT CROSS-SIGNALING REGULATES STEMNESS OF DENTAL PULP STEM CELLS THROUGH EXPRESSION OF NEURAL CREST AND CORE PLURIPOTENCY FACTORS

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Dental Pulp Stem Cells (DPSCs) from adult teeth express neural crest (NC) markers together with core transcription factors associated with stem cell pluripotency, such as Oct4a, Sox2, cMyc, Rex1, Stella/Dppa3, SSEA1/Fut4, Lin28 and Nanog. The possibility to boost the natural stemness features of DPSCs by mild methods that do not involve gene and/or chromatin modification is highly desirable for cell therapy. Canonical Wnt and Notch are two highly conserved developmental signaling pathways that are involved in NC emergence and stem cell self-renewal. We determined that both pathways operate coordinately to regulate the expression of core pluripotency and NC factors in DPSCs. Pharmacological inhibition of the Notch pathway by the ?-secretase inhibitor DAPT for 48 hours abolished the expression of NC and core factors together with a silencing of canonical Wnt signaling and a clear reduction in the stemness potential of DPSCs, as shown by a lower ability to generate mature, fully differentiated osteoblasts and adipocytes. Conversely, pharmacological activation of the Wnt pathway, by either the GSK3-ß inhibitor BIO or human recombinant protein WNT-3A for 48 hours, largely increased the expression of NC and core factors together with an increased efficiency of DPSCs to differentiate into mature osteoblasts and adipocytes. These results show that a short preconditioning activation of Wnt/Notch signaling by small molecules and/or recombinant proteins enhances the stemness and potency of DPSCs in culture, which could be useful to optimize the therapeutic use of these and other tissue-specific stem cells.

P112 - NEW INSIGHTS INTO THE MOLECULAR MECHANISMS OF THE NADPH OXIDASE NOX4 AS A TUMOUR SUPPRESSOR

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The NADPH oxidase (NOX) family has emerged in the last years as an important source of reactive oxygen species (ROS) in signal transduction. NOX4 has been implicated in a variety of physiological processes, including cellular migration, differentiation and senescence, regulation of apoptosis or endoplasmic reticulum stress, as well as pathological processes, particularly fibrosis. We previously found that in liver cells, NOX4 up-regulation is necessary for TGF-β-induced suppressor effects, in particular, for cell death (Caja et al., Cancer Res. 2009). In recent works we found that stable knockdown of NOX4 expression in liver tumour cells increases their proliferative capacity in vitro. Furthermore, NOX4 silencing enhances the tumorigenic potential of human HCC cells in xenografts in mice, resulting in earlier onset of tumour formation and increase in tumour size (Crosas-Molist et al., Free Radic Biol Med 2014). NOX4 could also regulate other cellular processes that occur later in progression and that favour tumour metastasis, such as migration and invasion. In this sense we have found that NOX4 supports epithelial parenchymal structures and that NOX4 loss, a common event in HCC, is accompanied by acquision of efficient amoeboid invasive behaviour. Overexpression of NOX4 in highly invasive cells is enough for maintaining parenchymal structures, increasing cell-substrate adhesion and suppressing actomyosin contractility and invasive behavior in HCC cells (Crosas-Molist et al., Oncogene 2016). The aim of this work was to determine the cellular and molecular mechanisms involved in the regulation of these effects by NOX4 in liver cells. Proteomic analysis to compare WT with either NOX4 silenced or NOX4-overexpressing HCC cells allowed the identification of changes in the levels of proteins that are common targets of the transcription factors c-Myc and Nrf2. Upon this observation, we decided to confirm these results in vitro by immunocytochemistry and western blotting of nuclear and cytoplasmic fractions. Interestingly, we observed that cells where NOX4 has been silenced showed an increased nuclear localization of c-Myc and Nrf2 and this effect was also significant in a reverse way in NOX4 overexpressing cells. In summary these preliminary results suggest that NOX4 would play a suppressor effect through the inhibition of the c-Myc and Nrf2 pathways.

P113 - MOLECULAR CHAPERONES IN SYNAPTOGENESIS AND STRESS

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Environmental changes like heat,cold or luminic stress cause accumulation of ROS and an increase of unfolded proteins,which trigger cell stress and death. In the context of synaptogenesis, stress can lead to changes in synaptic plasticity. Molecular chaperones are proteins involved in the refolding of misfolded proteins under stress situations. Besides this function, we have demonstrated in our study that small chaperones can change synapse number in neurons from Drosophila melanogaster. Furthermore,modulation of chaperone expression levels induces changes in locomotion and circadian rhythm. In this project we explore the role of Small Heat Shock Proteins(sHsp) Hsp23 and Hsp26 in synaptic function, the molecular mechanism that lead to synaptic changes and the proteins involved in the process. Moreover, we examine changes in synapse number under stress situations and the contribution of sHsp to synapse number restoration. Furthermore we explore

the molecular mechanism of activation an inhibition of Hsp23 and Hsp26 through an unknown gene called CG1561. We explore the contribution of Hsp23,Hsp26 and CG1561 to mantain or disrupt the circadian activity. The final goal is to decrypter the mechanisms which regulate behavior, locomotion and circadian activity upon stress through chaperone activity and propose sHsp as targets to modulate the impact of environmental stress in neurons.

P114 - MELATONIN ANTITUMORAL EFFECTS ARE RELATED TO AN IMPAIRED GLYCOLYTIC METABOLISM IN ACUTE MYELOID LEUKEMIA CELL LINE.

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Acute myeloid leukemia (AML) is the most common acute leukemia affecting adults, and its incidence increases with age. It is a clonal disorder of the myeloid line of blood cells characterized by accumulation of immature blast cells in the bone marrow and peripheral blood that interferes with the production of normal blood cells. For these reasons, it is imperative to discover new antitumor treatmentsthat could specifically kill tumoral cells, allowing the survival of normal dividing cells. The antitumor effect of the indolamine melatonin has been already established many years ago, mainly related to an antiproliferative action. However, its cytotoxic effects have recently been demonstrated in a small number of tumor types including AML. This cytotoxic effect of melatonin has been related to changes in the intracellular oxidative state, generating a prooxidant environment inside the cells after treatment. Since cellular metabolism constitutes one of the main sources of intracellular free radicals and it is well known that cancer cells present an altered glycolytic metabolism (Warburg Effect), we hypothesized that melatonin cytotoxic effect in AML could be related to an effect of the indolamine on cellular glycolytic metabolism as it has already been described in Ewing Sarcoma cells.In this regard, we observe that melatonin induces a cytotoxic effect in MOLM-13 AML cells which correlates with an early increase in reactive oxygen species. We evaluate melatonin effect in the hallmarks of Warburg effect and we found a decrease in glucose uptake, lactate dehydrogenase activity and intracellular lactate levels. Evaluation of HIF-1α expression -a glycolytic metabolism master regulator- also revealed a decrease in HIF-1α activation levels after melatonin treatment. Our data suggestthat melatonin could be blocking Warburg effect in MOLM-13 cells, forcing them to attempt to use alternative energy sources,which would be relate to cell death.

P115 - MAGNETICALLY TUMOR-TARGETED POLYETHYLENIMINE-COATED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES IMPAIRS TUMOR VASCULARIZATION AND PROMOTES MACROPHAGE INFILTRATION IN A MOUSE MODEL OF BREAST CANCER

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Endothelial cells line the interior of blood and lymph vessels and form a barrier between the circulating blood or lymph and the organism. These cells are not only crucial for vascular morphogenesis and physiology, but also for tumor vessel formation. Targeting endothelial cell of

the tumor vasculature is therefore a therapeutic approach for controlling tumor vascularization and growth. In previous work, we showed that polyethylenimine (PEI)-coated superparamagnetic iron oxide nanoparticles (SPIONs) could activate macrophage towards an M1 phenotype and impair pancreatic tumor cells invasion. Due to PEI-SPION potential anti-tumor properties, we evaluated their effects on a murine endothelial cell line (SVEC) and on primary human umbilical cord vein endothelial cells (HUVEC). PEI-SPIONs were taken up and displayed similar toxicity in endothelial cells as in other cell lines. Gene expression profiling in SVEC and HUVEC, indicated that PEI-SPIONs trigger a pro-inflammatory response. PEI-SPIONs impaired migration in SVEC and primary HUVEC wound healing assays. These nanoparticles inhibited HUVEC tube formation both directly and indirectly in an M2 macrophage-promoted tube formation assay. PEI-SPION treatment affected HUVEC actin organization probably by impairing Src and Cortactin activation. PEI-SPION altered the expression of some HUVEC surface integrins, although lymphocyte transmigration did not seem affected. To test *in vivo* the antiangiogenic potential of PEI-SPIONs we use the MDA-MB-231 human breast tumor xenograft model. We observed that magnetically tumor-targeted PEI-SPIONs alters tumor vascularization and promotes macrophage infiltration and collagen IV deposition.

P116 - IRF2BPL: A KEY REGULATOR OF NFAT2 EXPRESSION AND OSTEOCLASTOGENESIS.

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Osteoclasts are unique multinucleated bone-reabsorbing cells that play an essential role in bone homeostasis. However, the mechanisms regulating precursor cell differentiation into osteoclasts are largely unknown. Several studies have shown that calcineurin-dependent NFAT2 is the main transcription factor driving the conversion of macrophage-related preosteoclasts into giant multinucleated and functional osteoclasts upon stimulation with RANKL (receptor activator of nuclear factor kappa-B ligand). By contrast, RCAN2, a transcriptional target of NFAT2, has been recently described as a potent inhibitor of the calcineurin/NFAT2-dependent osteoclastogenesis, suggesting that a finely tuned feedback loop mechanism regulates osteoclast differentiation. Our group has recently observed that IRF2BPL (Interferon Regulatory factor 2 Binding Protein Like), a transcriptional regulator with poorly understood functions, regulates negatively Rcan2 gene expression. Here we have investigated the role of IRF2BPL in RANKL-induced osteoclastogenesis. In agreement with its role as transcriptional regulator, IRF2BPL showed a nuclear location in RAW 264.7 macrophage cells as well as RAW 264.7-derived osteoclasts. Remarkably, siRNA-mediated downregulation of endogenous IRF2BPL levels virtually abolished the conversion of RANKL-stimulated RAW 264.7 macrophages into osteoclasts. To gain insight into the mechanism underlying IRF2BPL-dependent osteoclastogenesis, we downregulated IRF2BPL mRNA and protein levels by RNA interference and measured its effect on the signaling cascade driving osteoclastogenesis upon induction by RANKL. IRF2BPL depletion specifically modulated RANKL-induced NFAT2 mRNA and protein levels, while ERK1/2 phosphorylation, IkBa degradation or C-FOS accumulation remain unaffected. In agreement with the decreased NFAT2 levels observed at 24 h upon IRF2BPL depletion, NFAT2-target genes (Trap, Oscar, Mmp9, Rcan1, Rcan2) were also downregulated. Taken together, our results suggest that IRF2BPL plays an important role in osteoclast formation by controlling NFAT2 transcription factor levels during RANKL induced osteoclastogenesis.

P117 - IN VIVO POTENTIATION BY MELATONIN OF THE ANTITUMORAL EFFECT OF CYTARABINE IN EXPERIMENTAL MODELS OF MUTANT FLT3-ITD ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is a group of neoplasms characterized by impairment in differentiation and consequent accumulation of immature myeloid progenitor cells in the bone marrow and peripheral blood that interferes with the production of normal blood cells. It is a heterogeneous disease at both cytogenetic and molecular levels. Abnormal signaling of one of these pathways, the FLT3 pathway, are currently used as biomarkers of poor prognostic and clinical outcome. Flt3 is a type III receptor tyrosine kinase which is found mutated in around 30% of AML. The therapeutic strategy against FMS-like receptor tyrosine kinase 3 Internal Tandem Duplication (Flt3-ITD), the most frequent of such mutations, has largely remained unchanged from the standard cytarabine plus anthracycline combination. Indeed, it should be noted, the chemotherapy treatment presents a high toxicity with known side effects on normal dividing cells and a poor disease-free survival. Novel therapies with lower toxicity are therefore needed to nullify tumor chemoresistance. Melatonin is an indolamine without relevant side effects that has been recently shown to present proapoptotic properties in hematological malignancies. We found a synergistic effect of cytarabine in combination with melatonin in MOLM-13 (Flt3-ITD) cells with decrease in cell viability, which corresponds to an increase in cell death due to apoptosis induction. This pro-apoptotic effect is not appreciated in wild type HL-60 cells. The same synergistic effect seems to be recurrent in blast collected from patient samples. In vivo xenografts using a FLT3-ITD model show a decrease in tumor growth and an increase in the mouse survival after this combination treatment. Taken together, our results open the doors for a new promising approach to the treatment of AML, decreasing drug doses and so its toxicity.

P118 - HUMAN STROMAL DENTAL PULP STEM CELLS GROWN IN NEUROCULT MEDIA INTEGRATES AND DIFFERENTIATES INTO ENDOTHELIAL CELLS BOTH IN VITRO AND AFTER INTRAHIPOCAMPAL GRAFT INTO ATHYMIC NUDE MICE.

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One of the best characterized and well-established sources of mesenchymal-stromal/stem cells is the dental pulp from human molars (DPSCs). DPSCs are used for its osteogenic potential and are actively studied for their possible therapeutic use in bone tissue repair. However, recent findings describe how under specific media culture α-MEM + 10%FBS, these cells are able to differentiate into endothelial vasculature when grafted subcutaneously into immunosuppressed mice. On the other side, when DPSCs cells are cultured in Neurobasal media, they are able to acquire neuronal-like lineage. In the present work, we want to address the phenotypic fate of adult DPSCs cultured in serum-free Neurocult complete media (StemCell) usually used for the expansion of adult Neural Stem Cells (NSC). Our result shows that non-engineered and non-modified adult DPSCs are able to generate neurospheres as well as NSC do. Strikingly and opposite to NSC, human DPSC

cells unexpectedly acquire endothelial markers such as CD31 and VEGF without need of serum addition. When DPSCs cells are grafted intrahipocampally on athymic nude animals, DPSCs are localized as endothelial vasculature positive for human-nestin and human-CD31 markers showing no tumorigenic potential co-existing together with murine vasculature. Our results show that Neurocult culture media allows a fast an easy enrichment of endothelial cells from human DPSCs and these cells keeps their phenotype when grafted into nude mice integrating into existing vasculature. This work has been financed by "Ramón y Cajal" programs RYC-2013-13450 & RYC-2012-11137 and MINECO SAF2015-70866-R.

P119 - GLUT1 OVEREXPRESSION ENHANCES THE ANTIPROLIFERATIVE EFFECT OF MELATONIN IN PROSTATE CANCER CELLS

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The neuroindole melatonin is produced mainly by the pineal gland and also by many other tissues of mammals, and it is also found in a variety of taxa including invertebrates and unicells. It exerts a broad range of biological actions in different tissue targets. Some of these actions are mediated by membrane receptors, but others are receptor independent, for which it has to enter into the cell. Recently, our group demonstrated that facilitative glucose transporter GLUT1 is responsible for melatonin uptake at least in prostate cancer cells. Therefore, melatonin reduces glucose metabolism and cell proliferation in prostate cancer (PCa) by a blockade of glucose metabolism. Thus, the main aim of this work was to confirm whether the overexpression of GLUT1 transporter in PCa cells potentiates the inhibitory effect of melatonin on prostate cancer cells growth. For this aim, androgen-sensitive LNCaP and androgen-insensitive PC-3 cells were stably transfected to overexpress GLUT1. Cell proliferation and cell cycle arrest were studied in GLUT1-overexpressing cells in the presence or absence of melatonin. Results showed that the effect of melatonin on cell growth was significantly higher in GLUT1-overexpressing cells respect to their parental counterparts. In conclusion, GLUT1 overexpression might promote the antiproliferative effects of melatonin at least in PCa cells.

P120 - EXOGEN Q10 COENZYME REMODES GLIOBLASTOMA HUMAN CELL PROTEOME

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Grade IV glioblastoma is the most aggressive brain tumor. It is highly resistant to surgery and to cytotoxic therapy, creating new solid tumors, which are re-vascularized and reproduce pathophysiological characteristics of the primary tumor. Dysfunctional mitochondria are involved in these processes, where we find high levels of reactive oxygen species (ROS) and an imbalance of the redox state, resulting in a metabolic reprogramming of the tumor, increasing the rate of proliferation, invasion, inflammation, angiogenesis, also promote the evasion of apoptosis and induce resistance to chemotherapy / radiotherapy. The objective of the present study is to analyze the effect of the CoQ antioxidant on GBM in order to identify proteins related do oxidative stress that can relay behind migration and invasion, as well as the signal transduction pathways of glioblastoma Cells. The proteomic analysis allowed us to detect 9000 proteins, 195 altered by CoQ, namely, 116 down regulated and 79 up-regulated. Among the altered routes, the

most important are those related to cell motility, stress response and protein phosphorylation. We checked the level of three of them implicated in cell motility upon CoQ treatment: i) MYLK3 is a calmodulin-dependent protein kinase involved in actin contraction, ii) C3G is a Guanine nucleotide-releasing protein that binds to SH3 domain of CRK and GRB2/ASH. C3G plays a role in nerve growth factor (NGF)-induced sustained activation of Rap1 and neurite outgrowth, and iii) OR2T35 is an olfactory receptor type 7TMDs, a G-protein coupled receptor protein signaling pathway. Our results demosntrate that CoQ decreases the total level of MYLK3, C3G and OR2T35. These results, together to changes observed in other proteins in the proteomic study indicate that exogenous CoQ regulates the mitochondrial O2 level by remodeling the proteome of grade IV astrocytic tumor cells in vitro by down-regulating targets involved in cell motility. Acknowledgements: JJCM Ministry of Education, poyect nº PPII-2014-01 0-P and UCLM grants GI20163390.

P121 - EPH SIGNALING CONTROLS MITOTIC SPINDLE ORIENTATION AND CELL PROLIFERATION IN DROSOPHILA OPTIC LOBE NEUROEPITHELIA

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A tight regulation of mitotic spindle orientation is crucial during development and adult tissue homeostasis. It determines cell fate specification and tissue architecture in the context of asymmetric and symmetric cell division, respectively. Two major mechanisms, autonomous and non-autonomous, have been implicated in positioning the spindle during cell division. However, while the intrinsic factors that control spindle orientation have been extensively studied over the past decades, our knowledge about the extrinsic signals that modulate this process is much more limited. Here, we uncover a novel function of the Ephrin-Eph intercellular signaling in controlling mitotic spindle orientation and cell proliferation in the symmetrically dividing neuroepithelial cells of the Drosophila optic lobe. The loss of Eph in this neuroepithelia led to an impairment of the cortical actomyosin network resulting in tissue architecture failures and mitotic spindle misorientation. We found that aPKC activity is mediating the effect of Eph on myosin II (Spaghetti squash, Sqh in Drosophila) to make it fully functional. In fact, a constitutively activated form of aPKC rescued the myosin II localization and spindle orientation defects detected in Eph null mutant neuropithelia. In addition, Eph null brain lobes were bigger than wild type brains. Concomitant with this phenotype, an increase in the rate of neuroepithelial cell proliferation, along with a decrease in apoptosis, was already observed at early larval third instar stages and maintained at late stages in Eph mutants. In these mutants, we found a consistent increase in activated Akt1 levels that could account for the increased proliferation rate. Hence, Eph signaling is a novel non-autonomous mechanism that regulates both spindle orientation and cell proliferation in neuroepithelia.

P122 - CONCOMITANT DELETION OF H-RAS AND N-RAS LEADS TO PULMONARY IMMATURITY, RESPIRATORY FAILURE AND NEONATAL DEATH IN MICE

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The canonical members of the Ras gene family (H-ras, N-ras, and K-ras) encode four protein isoforms (H-Ras, N-Ras, K-Ras4A and 4B) regulating cell proliferation, differentiation or death/survival. Whereas genomic disruption of K-ras4B causes embryonic lethality, H-ras, N-ras and

K-ras4A knockout mice are viable. H-ras and N-ras double KO (DKO) mice are also viable but show increased perinatal mortality suggesting critical physiological roles of those proteins around birth. Our characterization of DKO (Hras-/-;Nras-/-) mice uncovered a significant mortality rate due to respiratory failure during the first two postnatal days. Although DKO mice develop normal lung branching, they show delayed pulmonary maturation, a defect affecting both bronchiolar (Ciliated and Clara Cells) and alveolar lineages (type I and type II Pneumocytes), plus a reduced alveolar space and thicker septa. Clara Cells showed abnormal morphology and lack of proteoglycans production, whereas Ciliated Cells showed incorrect cilia structure. Alveolar cells showed also abnormal phenotypes. Whereas type II Pneumocytes were wrongly localized inside a cell mass instead of surrounding the alveolar spaces, the type I cells did not exhibit typical flat shape, pointing to a defect in differentiation. The delay in lung maturation was further supported by the observation of increased number of precursor cells of alveolar lineage (more RCA/SftpC+ cells), a significant increase in glycogen granules in type II pneumocytes, as well as by higher proliferation rates. Additionally, DKO animals show a higher lung apoptotic rate. Our data uncover important, specific functional roles of H-Ras and N-Ras in lung maturation and neonatal survival that cannot be substituted by K-Ras action.

P123 - COLOURED AEQUORINS FOR SIMULTANEOUS CALCIUM IMAGING IN ORGANELLES

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Advanced imaging of luminescent-fluorescent probes allows observing what is actually happening inside cells dynamically. Intracellular free calcium, involved in a legion of physiological processes, can be detected with aequorin-based sensors, which display low toxicity, steep dependence on calcium, high signal-to-noise ratio and large dynamic range. In addition, they can be molecularly targeted to specific subcellular locations. Cell Calcium homeostasis relies upon intricate exchange between organelles. To dissect this subcellular signalling, one would want to record calcium in different compartments of the same cell simultaneously, i.e. with two colour-shifted aequorins. In this work, we have targeted our red-emitting Redquorin and a green GFP-Aeq (GA) to the mitochondrial matrix or to the cytosol. We have also analysed a series of modifications, including mutation of several Aeq residues and codon humanization. We screened these biosensor variants in transfected live HeLa cells in suspension with an ad hoc protocol in a 96-well microplate luminometer. Apoaequorin was reconstituted with three coelenterazines (native, h or f). Cells were challenged with 100 micromolar histamine to elicit maximal physiological response and with digitonin in high calcium buffer to release all luminescent counts. With this assay, we obtained the global response of each probe that recapitulates the level of expression, folding quality, functional reconstitution, response to intracellular calcium rise and its intrinsic luminescence capacity. Whereas we were unable to discriminate the participation of each of these factors, the method allowed us to evaluate the average behaviour of the probes in cells. The brightest constructs were selected for further characterization under the microscope. As low photon yield is a strong limiting factor in aequorin-based imaging, this approach turned out to be very useful. Selected sensors were expressed in HeLa cells, alone and in pairs, and cells were stimulated to induce cytosolic and mitochondrial calcium rises. Time-lapse images were acquired with an EMCCD camera on an inverted widefield fluorescence microscope, adapted for luminometry.

Images were analysed pixel-by-pixel to discriminate signals originating in different compartments of individual cells. We imaged individual calcium responses and oscillations in two compartments with appropriate biosensor combinations. The pair GA-Redquorin has a great potential to reveal complex patterns of calcium movements that cannot be recognized in cell populations. Funding from Grant BFU2015-69874-R (FEDER-funded) is gratefully acknowledged.

P124 - CARDIAC HIF/VHL SIGNALING REGULATES GLYCOLYTIC AND OXIDATIVE METABOLIC PROGRAMS AND IS ESSENTIAL FOR MYOCARDIAL MATURATION DURING HEART DEVELOPMENT

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Heart development is a complex process that includes changes in cardiomyocyte metabolism to ensure the massive ATP production required for proper cardiac function after birth. We have determined that compact myocardium (CM) exhibits strong HIF1a expression and glycolytic signature until midgestation, contrasting with increased mitochondrial metabolism and low HIF1a levels in the trabeculae. By E14.5, HIF1a levels drop and the CM switches toward oxidative metabolism. Deletion of HIF1a main negative regulator Vhl, in Nkx2.5 cardiac progenitors, results in increased and extended HIF1a expression in the trabeculae leading to loss of metabolic compartments, enhanced glycolysis and decreased mitochondrial number and activity. These metabolic alterations cause myocardial thinning and reduced expression of conduction system genes, resulting in impaired cardiac function. Interestingly, simultaneous deletion of Hif1a and Vhl genes recues the metabolic and structural defects found in Vhl mutants but not their embryonic lethality, suggesting HIF-independent roles of VHL during cardiogenesis. Additionally, Hif1a single mutants do not display cardiac alterations and are viable but show inhibited glycolytic program in the CM and precocious mitochondrial maturation by E12.5, indicating cardiac metabolic adaptations in response to Hif1a loss. Taken together, these findings reveal a new connection between metabolism and cardiomyocyte maturation, with HIF/VHL pathway controlling the dynamics of cardiac bioenergetics during heart development.

P125 - CAPACITATION TIME IS ASSOCIATED WITH GLYCOCONJUGATES DISTRIBUTION IN HUMAN SPERM PLASMA MEMBRANE

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Sperm glycocalyx is extensively modified during capacitation preparing sperm surface for proper gamete recognition. These modifications are associated with specific changes in the content and distribution of surface carbohydrates. Nevertheless, the high heterogeneity of spermatozoa surface carbohydrate composition implies that glycocalyx in most aspects remains unknown. Due to that, the aim of this research was to characterize the redistribution of glycoconjugates in human spermatozoa plasma membrane after different times of capacitation 1h and 4h. Samples were obtained from fifteen normozoospermic donors and spermatozoa were capacitated by swim-up for 1h and 4h in a buffer containing BSA (5mg/ml). Surface carbohydrates were detected by four lectins conjugated to fluorescein isothiocynate: Aleuria aurantia (AAA), Canavalia ensiformis (ConA), Peanut agglutinin (PNA) and Wheat germs (WGA). Capacitation was evaluated using an anti-phosphotyrosine antibody produced in mouse and a secondary antibody against mouse

IgG conjugated to Cy3. A minimum of 100 cells per sample was observed by fluorescence and data were analyzed by Student's t-distribution ($p < 0.05$). Tyrosine phosphorylation results presented that the percentage of spermatozoa with phosphorylation increased as capacitation time increased from 1h to 4h which is useful as capacitation control. After 1h of capacitation results showed a significant increase in binding site on acrosomal domain for AAA and PNA in comparison with non-capacitated spermatozoa, WGA presented dotted fluorescent all over the head and no changes were observed in ConA-binding sites. However, after 4h of capacitation, in addition to changes described above, it was observed a significant increase in ConA-binding sites on acrosomal and postacrosomal region in contrast to non-capacitated spermatozoa. When we compared between two times of capacitation results showed that AAA and ConA-binding sites were the ones that most modified their location. Regarding AAA lectin after 4h of capacitation spermatozoa reduced binding sites on postacrosomal region and increased on acrosomal domain in comparison with 1h capacitated. Concerning ConA lectin results showed the opposite, there was a reduction in the percentage of spermatozoa with fluorescence only in acrosome and a significant increment of those which presented ConA-binding sites in both acrosome and postacrosome in contrast to 1h capacitated. In conclusion, carbohydrates performed a profound redistribution during capacitation suggesting a relevant implication in this processes. Moreover, the redistribution of some sugars is only observed with longer capacitation times. Above all, lectins allow a better understanding of spermatozoa carbohydrates changes during capacitation, providing a useful tool to identify heterogeneity and to improve sperm selection methods.

P126 - BIOENERGETIC ALTERATION OF NON-MELANOMA SKIN CANCER CELLS SUBJECTED TO PHOTODYNAMIC THERAPY

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Non-melanoma skin cancer (NMSC), mainly basal and squamous cell carcinoma (BCC and SCC, respectively), is the most prevalent cancer type. Among the approaches for this disease is Photodynamic Therapy (PDT) which requires light, a photosensitizer compound and molecular oxygen to generate reactive oxygen species that induce cell death. Nevertheless, PDT is not always effective and resistant cells may appear after treatment. While normal differentiated cells depend primarily on mitochondrial oxidative phosphorylation to generate energy, cancer cells change this energetic metabolism to an aerobic glycolysis (Warburg effect), which could influence in the antitumor therapies response. Therefore, we evaluate the potential metabolism changes that occur in resistant NMSC cells to photodynamic therapy. In this context we have used the lines cell from a human squamous cell carcinoma, SCC-13; and from mouse basal cell carcinoma, ASZ, BSZ and CSZ. These cells called parental (P) we subjected to 10 PDT cycles to obtain resistant cells (10°G). We have also employed SCC carcinomas induced in SKH-1 hairless mice exposed chronically to UV, the major responsible in the induction NMSC, and treated with PDT. We have evaluated by immunofluorescence and western blot the expression of the β-subunit of the H+-ATP synthase (β-F1-ATPase), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and pyruvate kinase (PKM2) as metabolic markers in SCC and BCC cells and their relationship with PDT resistance. The bioenergetic signature (β-F1-ATPase/GAPDH), a biomarker from the use of glucose by tumour cells, was diminished in tumour cells with respect to HaCaT (immortalized human keratinocytes used as control). In addition, this biomarker was lower in PDT resistant cells compared to parental cells. We have also founded a relationship with the expression of the tumour suppressor gene p53, which is mutated in approximately the 60% of NMSC. The

bioenergetics signature was lower in those cells with low p53 expression. We have validated in vitro results obtained in SCC-13 cells, in SCC carcinomas induced in SKH-1 hairless mice and treated with PDT. In conclusion, we can indicate that both, SCC and BCC tumour cells show metabolic reprograming which is enhanced in resistant to PDT cells.

P127 - AUXIN BIOSYNTHESIS AND TRANSPORT ARE REQUIRED IN STRESS-INDUCED MICROSPORE EMBRYOGENESIS WHILE THEY DECREASE DURING POLLEN DEVELOPMENT IN RAPESEED AND BARLEY

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Auxin is key regulator of plant growth and development. and it acts as a major coordinating plant morphogenetic signal in the regulation of plant development, by controlling important cellular processes like cell division, expansion and differentiation. During in vivo anther development, after meiosis, microspores develop and follow the gametophytic pathway to produce pollen grains. Isolated microspores can be reprogrammed in vitro by stress, they become totipotent cells, follow embryogenesis and produce doubled-haploid embryos and plants, widely used in plant breeding. The knowledge about the involvement of auxin in these two microspore pathways is very limited. In the present study we analyzed auxin concentration and cellular accumulation, expression of auxin biosynthesis gene TAA1 and auxin efflux carrier gene PIN1 during the two microspore developmental pathways, gametophytic development and microspore embryogenesis in Brassica napus (rapeseed) and Hordeum vulgare (barley). Effects of inhibitors of auxin biosynthesis (Kynurenine), transport (N-1-naphthylphthalamic acid, NPA) and action (a-(p-Chlorophenoxy) isobutyric acid, PCIB) in microspore embryogenesis were also analyzed. During stress-induced microspore embryogenesis TAA1 gene was up-regulated, auxin concentration increased and accumulated in early embryo cells from the first embryogenic divisions. Kynurenin treatments decreased microspore embryogenesis efficiency, indicating that de novo auxin biosynthesis was required in this microspore developmental pathway. PIN1-like gene expression was also induced with microspore embryogenesis, while NPA and PCIB inhibitors impaired embryogenesis initiation and development. These results indicated that polar auxin transport and auxin action were required for microspore embryo progression. In contrast, during gametophytic development auxin levels, TAA1 and PIN1-like expression were high at early microspore development, in tetrads and tapetum, while they progressive decreased during gametogenesis in both pollen and tapetum cells. Findings showed opposite auxin dynamics along the two microspore pathways with different fates. Endogenous auxin biosynthesis, action and polar transport are required for microspore embryogenesis initiation and progression while auxin progressively diminishes during gametophytic development, in both species. Rodríguez-Sanz et al. (2015) Auxin biosynthesis, accumulation, action and transport are involved in stress-induced microspore embryogenesis initiation and development in Brassica napus L. Plant Cell Phys 56: 1401-1417. Supported by project (AGL2014-52028-R) funded by Spanish Ministry of Economy and Competitiveness (MINECO) and European Regional Development Fund (ERDF/ FEDER). YPP is recipient of a grant (PEJ15/ BIO/AI-01S8) funded by Comunidad de Madrid and ERDF/FEDER.

P128 - ABERRANT PRIMARY CILIA FUNCTION AS A CONTRIBUTING FACTOR IN TOWNES-BROCKS SYNDROME

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Townes-Brocks syndrome (TBS) is a rare dominant genetic disorder characterized by a spectrum of symptoms that include limb/digit abnormalities, imperforate anus, dysplastic ears, sensioneural deafness and renal defects including polycystic kidneys. Interestingly, some of these phenotypes overlap with known ciliopathies, an emerging class of disorders resulting from primary cilia malformation and dysfunction. We aim to demonstrate that truncated forms of SALL1 found in TBS patients might interfere with cilia-related proteins that contribute to primary cilia assembly and function. In vitro experiments were conducted on MEFs, HEK293FT, RPE-1 and primary fibroblast cultures from both TBS and healthy donors. Also, CRISPR-Cas9 was employed to make a TBS model cell line. Cells were starved with low-serum medium to induce ciliogenesis and subjected to purmorphamine treatment to activate the Sonic-Hedgehog signaling pathway (Shh). Analysis included immunofluorescence, proteomics, gene expression, and reporter assays. We found that MEFs carrying mutations in Sall1 gene and dermal fibroblasts derived from TBS patients exhibit significantly longer primary cilia in comparison to control cells and they show increased sensitivity to Shh. Moreover, we have used the novel BioID method to identify potential SALL1 interactors related to primary cilia and/or centrosome. We observed that the aberrant complex formed between the wild-type and the truncated forms of SALL1 interferes with core centrosome and cilia related proteins, such are the negative regulators of ciliogenesis CP110 and CEP97. These findings suggest that the etiology of TBS may not depend solely on the classical role of SALL1 as a transcriptional repressor, but that aberrations in primary cilia formation and function might be contributing and causative factors.

P129 - A CD36 ECTODOMAIN MEDIATES INSECT PHEROMONE DETECTION VIA A PUTATIVE TUNNELLING MECHANISM

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CD36 proteins are highly conserved transmembrane receptors implicated in diverse cellular processes such as lipid uptake, cell adhesion and innate immune detection. Despite their widespread functions, the molecular mechanisms by which these proteins act are poorly understood. A subfamily of CD36-related proteins in insects, the Sensory Neuron Membrane Proteins (SNMP1s), was previously shown to facilitate detection of lipid-derived pheromones by their cognate receptors in olfactory cilia of pheromone-sensing sensilla. Genetic studies, in Drosophila melanogaster, have demonstrated that SNMP1 acts together with the extracellular odorant binding protein LUSH and the heteromeric odorant receptor complex, OR67d/ORCO, in mediating neuronal activation by the sex pheromone cis-vaccenyl acetate. How SNMP1 collaborates with OBPs and ORs to produce pheromone-evoked activity in olfactory cilia remains an outstanding question. In this work, we have performed a large-scale in vivo structure/function analysis of SNMP1, comprising a full-protein deletion scan, site-directed mutagenesis of predicted sites of post-translational modification and other evolutionarily conserved residues and also, functional complementation with CD36-related proteins from other species. Structure–activity dissection demonstrates that SNMP1's ectodomain is essential, but intracellular and transmembrane domains dispensable, for

cilia localization and pheromone-evoked responses. SNMP1 can be substituted by mammalian CD36, whose ectodomain can interact with insect pheromones. Homology modelling, using the mammalian LIMP-2 structure as template, reveals a putative tunnel in the SNMP1 ectodomain that is sufficiently large to accommodate pheromone molecules. Amino-acid substitutions predicted to block this tunnel diminish pheromone sensitivity. We propose a model in which SNMP1 funnels hydrophobic pheromones from the extracellular fluid to integral membrane receptors, providing insights into both the molecular basis of pheromone detection in insects and - more generally - the conserved and divergent molecular mechanisms of CD36 protein function.

P130 - SENSING TISSUE DAMAGE BY OXIDATIVE STRESS DURING WING DISC REGENERATION

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Recent work has strengthened Drosophila imaginal discs as a model system for regeneration studies. Evidence is accumulating that oxidative stress drives the cellular responses for repair and regeneration. Drosophila imaginal discs generate a burst of reactive oxygen species (ROS) upon cell death that propagates from dying cells to living nearby tissue and that is necessary for the activation of the Jun N-terminal kinase (JNK) and p38 MAP kinase signaling pathways. Moreover, these pathways are pivotal for the activation of regenerative growth mediated by the cytokines unpaired, which belongs to the IL-6 family. Two key issues arise from these observations. The first is how the ROS are propagated. The second, what is the link between ROS and the stress activated protein kinases p38 and JNK. We found that the Apoptosis signal-regulating kinase 1 (Ask1) operates as a sensor to link oxidative stress and these pathways. In addition, living or surviving cells need Akt/PI3k activity to modulate Ask1 in order to promote epithelial recovery.

P131 - PROGRAMMED MITOPHAGY IS ESSENTIAL FOR THE GLYCOLYTIC SWITCH DURING CELL DIFFERENTIATION

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Retinal ganglion cells (RGCs) are the sole projecting neurons of the retina and their axons form the optic nerve. Here, we show that embryogenesis-associated mouse RGC differentiation depends on mitophagy, the programmed autophagic clearance of mitochondria. The elimination of mitochondria during RGC differentiation was coupled to a metabolic shift with increased lactate production and elevated expression of glycolytic enzymes at the mRNA level. Pharmacological and genetic inhibition of either mitophagy or glycolysis consistently inhibited RGC differentiation. Local hypoxia triggered expression of the mitophagy regulator BCL2/adenovirus E1B 19-kDa-interacting protein 3-like (BNIP3L, best known as NIX) at peak RGC differentiation. Retinas from NIX-deficient mice displayed increased mitochondrial mass, reduced expression of glycolytic enzymes and decreased neuronal differentiation. Similarly, we provide evidence that NIX-dependent mitophagy contributes to mitochondrial elimination during macrophage polarization towards the proinflammatory and more glycolytic M1 phenotype, but not to M2 macrophage differentiation, which primarily relies on oxidative phosphorylation. In summary, developmentally controlled mitophagy promotes a metabolic switch towards glycolysis, which in turn contributes to cellular differentiation in several distinct developmental contexts.

P132 - LUNG REGENERATION AFTER TOXIC INJURY IS IMPROVED IN ABSENCE OF DIOXIN RECEPTOR

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Recent experimental evidences from cellular systems and from both mammalian and non-mammalian animal models highlight novel functions for the aryl hydrocarbon/dioxin receptor (AhR) in maintaining cell differentiation and tissue homeostasis. Notably, AhR depletion stimulates an undifferentiated and pluripotent phenotype likely associated to a mesenchymal transition in epithelial cells and to increased primary tumorigenesis and metastasis in melanoma. In this work, we have used a lung model of tissue regeneration to investigate whether AhR influences the activation of stem-like cells and pluripotency genes considered relevant for restoring tissue architecture and function. AhR-null mice developed a faster and more efficient repair of the lung bronchiolar epithelium upon acute naphthalene injury that involved increased cell proliferation and the early activation of stem-like Clara and Basal cell lineages. Neuroepithelial precursors were overrepresented in basal AhR-deficient lungs and most probably helped to enhance their regenerative competence. Improved renewal of the bronchiolar epithelium in AhR-null mice was coincident with an initial increase in multipotent lung stem Sca1+/CD31-/CD4- cells and with the upregulation of pluripotency inducing factors NANOG and OCT4 prior to naphthalene treatment. The lack of response to Sonic Hedgehog (Shh) repression in AhR-deficient lung shortly after injury may also positively influence tissue repair. These results support a role for AhR in the regenerative response against toxins, and open the possibility of using AhR antagonists to improve tissue and organ repair.

P133 - JNK AND JAK/STAT SIGNALING ARE REQUIRED FOR PROMOTING CELL FATE RE-SPECIFICATION DURING IMAGINAL WING DISC REGENERATION IN DROSOPHILA MELANOGASTER.

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The regenerative process after tissue damage relies on a variety of cellular responses that includes compensatory cell proliferation and cell fate re-specification. The identification of the signaling networks regulating these cellular events is a central question in regenerative biology. Tissue regeneration models in Drosophila have shown that two of the signals that play a fundamental role during the early stages of regeneration are the c-Jun N-terminal kinase (JNK) and JAK/STAT signaling pathways. These pathways have been shown to be required for controlling regenerative proliferation, however, their contribution to the processes of cellular reprogramming and cell fate re-specification that take place during regeneration are largely unknown. Here, through a combination of genetic techniques and surgical excision, we present evidence for a previously unrecognised function of the cooperative activities of JNK and JAK/STAT signaling pathways in inducing cell fate re-specification in imaginal discs. We show that co-activation of these signaling pathways induces both the cell fate changes in injured areas, as well as in adjacent cells. We have also discovered that the Caspase initiator Dronc is key to promote these cell fate changes in response to the activation of JNK signaling. Ultimately these observations provide novel insights to better understand the complex signaling networks that control cell plasticity during regeneration.

P134 - ISOLATION AND CHARACTERIZATION OF BOVINE MESENCHYMAL STEM CELLS DERIVED FROM PERIPHERAL BLOOD AND ENDOMETRIUM

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Mesenchymal stem cells have the ability to migrate to damaged, inflamed and tumor tissues where they proliferate and exert their immunomodulatory properties through the release of cytokines and exosomes. Considering that pregnancy is a proinflammatory condition, we suggest that bovine endometrial mesenchymal stem cells (eMSCs) may contribute to uterine regeneration regulating embryo implantation and pregnancy in cows. eMSCs may originate from a subset of bone marrow MSCs included in peripheral blood mesenchymal stem cells (pbMSC). To analyze these possibilities, we isolated and immortalized (using retroviral vector LXSN-16E6E7 to prevent proliferative arrest), eight eMSCs lines from the uterus of heifers at different estrous cycle stages: (i) one from early luteal phase (ii) four from late luteal phase and (iii) three from follicular phase. In addition, a total of five bovine pbMSCs were isolated from male calves, and maintained during more than twenty passages: two control cell lines (cpbMSC) and three cell lines obtained after mobilization from bone marrow by granulocyte colony-stimulating factor (G-CSF) treatment (bmpbMSC). eMSCs and pbMSCs were characterized to comply the following MSCs criteria: (i) plastic-adhesion and (ii) expression of mesenchymal CD44 and embryonic Pou5F1 markers and lack of CD34 and MHC-II. For a deeper characterization, we also analyzed (iv) Cytokeratin and Vimentin expression by Immunocytochemistry and Flow Cytometry (v) proliferation rate and (v) their ability to migrate in vitro by agarose spot assay. All lines expressed vimentin but not the epithelial marker Cytokeratin, with the exceptions of eMSC-11H from late luteal phase and eMSC-18C from follicular phase, that expressed high levels of both markers. bmpbMSC-81 and eMSC-18D were the lines had a higher growth rate with an average population-doubling time of 2.6±0.75 and 2.6±0.56 days respectively. eMSC-18C showed a significantly higher migration rate than the rest of either pbMSC or eMSC lines with 1007.89±38.26 μm/day 7; being bmpbMSC-84 significantly the most migratory pbMSC line with 606.27±97.62 μm/day. In the present work, the isolation of bovine pbMSC is shown for the first time. Bovine pbMSC and eMSC show characteristics of human MSCs including the high proliferative potency. One of the most remarkable but least understood findings it is the migration ability they may use to reach the uterus from bone marrow and peripheral blood. Subsequent analysis of communication between pbMSC or eMSC and the bovine embryo, through inflammatory or implantation cytokines and through extracellular vesicles, will shed light on the functions of MSC during the implantation process.

P135 - IMPROVED GENERATION OF FUNCTIONAL HUMAN SKIN EQUIVALENTS FROM CRYOPRESERVED SAMPLES

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There is a persistent interest in the development of new procedures to improve the treatment of severe or chronic skin lesions, including large or recurrently infected wounds and burns or ulcers in diabetic and older patients. A major goal in the field is the efficient development of skin equivalent allografts from human biopsies, avoiding the presence of immune responsive elements. Actually, there are several limitations precluding an efficient and rapid development of these skin allografts, mainly related to the immediate availability of starting samples and

the expansion efficiency of primary cell populations. In this sense, the development of new protocols for functional long term storage of skin biopsies and for accelerated expansion of cell populations from this samples can be important achievements in regenerative medicine. Tissue cryopreservation is an effective method for long-term biopsy conservation, facilitating transport, warehousing and manipulation. Here we outline procedures for the maintenance of small skin biopsies in optimal frozen conditions to further use these samples for the subsequent extraction of functional viable cells. We also introduce a patented technology allowing a rapid expansion of these primary skin cell populations for the subsequent generation of artificial skin allografts. This technology is based on the photodynamic, Protoporphyrin IX-dependent generation of non-lethal levels of reactive oxygen species (ROS) in the tissue. In conclusion, here we introduce the use of cryopreserved human skin biopsies as a practical source of primary skin cells, and the use of ROS-dependent protocol to hugely accelerate the proliferation and differentiation of these cell populations.

P136 - IGF-1 ENHANCES THE OSTEOGENIC ACTIVITY OF BMP-6 IN VITRO AND IN VIVO, AND TOGETHER HAVE A STRONGER OSTEOGENIC EFFECT THAN WHEN IGF-1 IS COMBINED WITH BMP-2.

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Bone morphogenetic protein-2 (BMP-2) is widely used in orthopedic surgery and bone tissue engineering because of its strong osteogenic activity. However, BMP-2 treatments have several drawbacks and alternatives are being explored. Since BMP-6 has been demonstrated to be more osteoinductive, its use, either alone or together with other cytokines, might be an interesting option. We have compared the effect of BMP-2, BMP-6, or insulin-like growth factor-1 (IGF-1), either alone or in combination. MC3T3-E1 cells were treated with IGF-1 and/or of BMP-2 or -6 and the expression of osteogenic genes, proliferation and alkaline phosphatase (ALP) activity in vitro were analyzed. The results showed that IGF-1 greatly enhanced the BMP-induced osteogenic differentiation of these cells and that the ALP activity in the cultures was higher when the combination was made with BMP-6 than with BMP-2. Other in vitro experiments showed that the osteogenic effect of these combinations can be modulated controlling the sequential administration of the growth factors. Furthermore, we have tested the osteogenic potential of these treatments in vivo by loading them onto absorbable collagen sponges which were implanted into an ectopic bone formation model in rats. These experiments revealed that only BMP-6 was able to induce bone formation at the used dose and that the addition of IGF-1 contributed to an increase of the mineralization in the implants. Hence, the combination of BMP-6 with IGF-1 might be a better alternative than BMP-2 for orthopedic surgery and bone tissue engineering approaches with potential application through using controlled delivery systems.

P137 - HIPPO SIGNALING RESTRICTS CELL PLASTICITY IN PLANARIANS

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The Hippo pathway plays a key role in regulating cell turnover in adult tissues, and abnormalities are consistently associated with human cancers. Initially related with the control of cell proliferation and death, Hippo inhibition is commonly linked to an expansion of stem cells and progenitors, which leads to larger organs and tumors. To understand the mechanism through which Hippo directs cell renewal and promotes stemness, we studied its function in planarians, a stem cell-based organism that allows analysis of the complex cellular events underlying tissue renewal in the whole organism. We show that hippo inhibition in planarians decreases cell death, produces cell cycle arrest, and promotes the dedifferentiation of post-mitotic cells. Thus, hippo (RNAi) planarians have extensive undifferentiated areas and overgrowths with no effects on size or cell number. We propose an essential role for hippo in restricting cell plasticity, and thus in preventing tumoral transformation.

P138 - GLIAL REGENERATIVE RESPONSE IN ADULT FLIES

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The human central nervous system (CNS) does not normally regenerate upon damage and as a consequence stroke, spinal cord injury or neurodegenerative diseases result in permanent disability. In response to a CNS insult glial cells proliferate and differentiate, enwrap axons and engulf cell debris. This process is known as Glial Regenerative Response (GRR) and it has been reported from flies to fish and mice, implying that it is sustained by a conserved genetic mechanism. However, the genetic network that controls the switch from proliferation to differentiation is not well understood thus, manipulation on these genetic interactions could lead to unwanted outcomes (e.g. cancer). Besides, the correct understanding of this process would allow its genetic manipulation to favor CNS regeneration. The conserved genetic network that governs GRR was discovered taking advantage of Drosophila genetics. This gene network can be manipulated in glia to promote CNS repair in larvae, however, it is not clear whether these genetic manipulations operate once the developmental program is finished. Most of CNS insults occur in the fully differentiated CNS hence, deciphering GRR in adult brains is a need. We have established a new injury paradigm that enables the investigation of GRR in living adults, in addition we have stablished a functional recovery essay of locomotion to validate functional regeneration “in vivo”. Here, we show that the main features of GRR are present in this new paradigm in adult CNS. Currently, we are investigating: (1) a reliable method to measure functional recovery; (2) whether the manipulation of glial genes involved in the GRR may enable neural regeneration in adults; (3) identify novel genes that regulate this process. Key words: Drosophila, CNS, glia, injury, repair, regeneration, screening.

P139 - DISSECTING THE ROLE OF THE NADPH OXIDASE NOX4 DURING LIVER REGENERATION.

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In the recent years, it has been described that the NADPH oxidase NOX4 mediates Transforming Growth Factor-beta (TGF-β)-induced apoptosis (Carmona-Cuenca et al., J Hepatol. 2008) and myofibroblasts activation (Sancho et al., PLoS One. 2012), contributing to the development of liver fibrosis. However, our group has also demonstrated that NOX4 inhibits hepatocyte growth and tumorigenesis and decreases cell migration (Crosas-Molist et al., Free Radic Biol Med. 2014; Crosas-Molist et al., Oncogene 2017), functioning as a potential liver suppressor gene. Considering this background about NOX4 controlling proliferation in the liver, the main objective of this work was to analyse the molecular and cellular mechanisms regulated by NOX4 in liver cells during a physiological proliferative process: liver regeneration. For this purpose, we have used Nox4 -/- mice, where two-thirds partial hepatectomy (PHx) was performed to study liver regeneration. Here we provide evidences that an earlier liver recovery takes place in Nox4 -/- mice after two-thirds PHx, when compared with WT mice. Thus, Nox4 -/- mice were able to recover the liver-to-body weight ratio earlier than WT mice in order to maintain liver homeostasis. Furthermore, from a histological point of view, Nox4 -/- livers were able to re-establish a well-organized parenchyma faster than WT livers and they did not to present the well-known regenerative hepatocellular fat accumulation. In addittion, hepatocytes from regenerating Nox4 -/- livers showed an anticipated entry into the cell cycle, analyzed through Ki67 incorporation. This result was concomitant at the molecular level with an earlier expression of the cell cycle positive regulators Cyclin D1, A2 and B2. Interestingly, Nox4 -/- livers also presented higher and earlier Cdkn1a (p21) mRNA expression levels after PHx when compared with WT livers. Finally, livers from Nox4 -/- mice showed an earlier attenuation of the TGF-β pathway, which is involved in inhibiting hepatocyte proliferation. In conclusion, all these results seem to point out that NOX4 exerts its role as a suppressor of proliferation during liver regeneration, being an important factor regulating the homeostasis of the liver.

P140 - DIOXIN RECEPTOR ADJUSTS LIVER REGENERATION AFTER ACUTE TOXIC INJURY AND PROTECTS AGAINST LIVER CARCINOGENESIS

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The aryl hydrocarbon receptor (AhR) has roles in cell proliferation, differentiation and organ homeostasis, including the liver. AhR depletion induces undifferentiation and pluripotency in normal and transformed cells. Here, AhR-null mice (AhR-/-) were used to explore whether AhR controls liver regeneration and carcinogenesis by restricting the expansion of stem-like cells and the expression of pluripotency genes. Short-term CCl4 liver damage was earlier and more efficiently repaired in AhR-/- than in AhR+/+ mice. Stem-like CK14+ and TBX3+ and pluripotency-expressing OCT4+ and NANOG+ cells expanded sooner in AhR-/- than in AhR+/+ regenerating livers. Stem-like side population cells (SP) isolated from AhR-/- livers had increased ß-catenin (ß-Cat) signaling with overexpression of Axin2, Dkk1 and Cyclin D1. Interestingly, ß-Cat, Axin2 and Dkk1 also increased during regeneration but more notably in AhR-null livers. Liver carcinogenesis induced by diethylnitrosamine (DEN) produced large carcinomas

in all AhR-/- mice but mostly premalignant adenomas in less than half of AhR+/+ mice. AhR-null tumoral tissue, but not their surrounding non-tumoral parenchyma, had nuclear ß-Cat and Axin2 overexpression. OCT4 and NANOG were nevertheless similarly expressed in AhR+/+ and AhR-/- lesions. We suggest that AhR may serve to adjust liver repair and to block tumorigenesis by modulating stem-like cells and ß-Cat signaling.

P141 - DIFFERENTIAL CONTRIBUTION OF SOS1 AND SOS2 IN MOUSE SKIN HOMEOSTASIS AND CARCINOGENESIS

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Ras proteins cycle continuously between active (RasGTP) and inactive (RasGDP) states and this process is controlled by negative and positive regulators. The Sos-family of Ras activators comprises two highly homologous but functionally different isoforms, Sos1 and Sos2. Previous studies documented that Ras signaling is essential for skin homeostasis and carcinogenesis. In addition, Sos upregulation results in development of skin papillomas in mice. Using Sos1-KO, Sos2-KO and Sos1/2-DKO mice, we assessed the functional role of Sos1 and Sos2 in skin homeostasis under physiological and/or pathological conditions. Sos1 depletion resulted in significant alterations of skin homeostasis including reduced keratinocyte proliferation, altered hair follicle and blood vessel integrity in dermis, and reduced adipose tissue in hypodermis. These defects worsened significantly when both Sos1 and Sos2 were absent. Simultaneous Sos1/2 disruption led to severe impairment of the ability to repair skin wounds as well as to almost complete ablation of the neutrophil-mediated inflammatory response in the injury site. Furthermore, Sos1 disruption delayed the onset of tumor initiation, decreased tumor growth and prevented malignant progression of papillomas in a DMBA/TPA-induced skin carcinogenesis model. Finally, Sos1 depletion in preexisting chemically-induced papillomas resulted also in decreased tumor growth, probably to a significant reduction of the underlying keratinocyte proliferation. Our data unveil novel, distinctive mechanistic roles of Sos 1 and Sos2 in physiological control of skin homeostasis and wound repair as well as in pathological development of chemically induced skin tumors. These observations underscore the essential role of Sos proteins in cellular proliferation and migration and support the consideration of these RasGEFs as potential biomarkers/therapy targets in Ras-driven epidermal tumors.

P142 - CABUT AND D-GADD45 AS PUTATIVE MODULATORS OF THE JNK PATHWAY IN REGENERATION

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Drosophila imaginal discs are a well established model system to study regeneration, both after physical damage or cell-death induction. Although little is known about the early signals driving the regenerative process, the activation of the Jun N-terminal kinase (JNK) pathway is likely to play a decisive role. Studies on the gene expression profiles of imaginal discs at different time points after cell death reveal several genes showing an expression burst right after damage and returning to normal levels early in the process. We focus on two of these genes, the transcription

factor Cabut (cbt or dTIEG or TGF-b inducible early gene) and the Drosophila Growth arrest and DNA damage-inducible gene 45 (D-GADD45). Cabut is a crucial downstream mediator of the JNK signalling required during wing disc regeneration. D-GADD45 is a stress sensor involved in DNA repair, apoptosis and cell cycle control. Downregulation of D-GADD45 after cell death blocks the activation of the JNK pathway and severely compromises the regeneration process. Our results confirm Cabut and D-GADD45 as putative modulators of the JNK pathway during regeneration.

P143- C-MYC CONTROLS CELL REPROGRAMMING BY INDUCING AEROBIC BIOSYNTHETIC GLYCOLYSIS

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The Somatic cell reprogramming to pluripotency induces a profound transformation in cellular metabolism. However, the regulation of the metabolic rewiring during cell reprogramming remains unknown. Here, we present data showing that c-Myc controls metabolic remodeling early during cell reprogramming. We found that c-Myc expression induces cell proliferation, and mitochondrial fission through Erk1/2- and Cdk1-mediated Drp1 activation. Combined biochemical, protein antibody microarrays, Nuclear Magnetic Resonance and respirometry analyses show that c-Myc controls the complete adjustment of cellular metabolism during early reprogramming by increasing overall metabolic fluxes. We conclude that c-Myc orchestrates a rewiring of somatic metabolism early in cell reprogramming to induce a tumor-like metabolic state, based on increased glycolytic and anabolic fluxes, which greatly influences cell fate.

P144 - SILENCING REVEALS NOVEL ROLES FOR RAV GENES IN HEADING DATE AND CARPEL DEVELOPMENT IN RICE

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Among the plant-specific RAV family of transcription factors, TEMPRANILLOs (TEM1, TEM2) were previously shown to negatively regulate the floral transition in Arabidopsis thaliana through direct repression of FLOWERING LOCUS T and AtGA3oxidase1/2, encoding major components of the florigen. Here we identify RAV genes from rice (Oryza sativa), and unravel their regulatory roles in key steps of reproductive development. Our data strongly suggest that like TEMs, OsRAV9/OsTEM1 plays a conserved function as repressor of flowering upstream of the floral activators OsMADS14 and Hd3a through a mechanism reminiscent to that underlying floral transition in temperate cereals. Furthermore, OsRAV11 and OsRAV12 acquired a novel function in the differentiation of the carpel, likely downstream of floral homeotic factors. Our data reveal conservation of RAV gene function in the regulation of flowering time in monocotyledonous and dicotyledonous plants, but also uncover new roles in the development of the gynoecium in rice.

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