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amb la col·laboració de la Secció de Biologia i Indústria

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Amb la col·laboració de:

EXCELENCIA
MARÍA
DE MAEZTU

PROGRAMA

8:30-9:00 Recollida documentació

8:50 Benvinguda del coordinador de la Secció de Biologia Molecular

Sessions Matí

Chair: Ángel Raya

Conferenciants convidats

9:00-9:45 Manel Esteller (PEBC-IDIBELL)

Epigenètica del Càncer: Del Coneixement a l'Aplicació

9:45-10:30 Maribel Geli (IBMB-CSIC)

ER contact initiates actin polymerization at endocytic sites

10:30-11:15 Jaume Bagunyà (UB)

Animal Regeneration: before and beyond the hype of stem cells

11:15-11:40 Cafè i Pòsters

Short talks

Chair: Teresa Adell

11:40-12:00 Neus Roca Ayats (UB)

Identification of a novel mutation in the GGPS1 gene by whole-exome sequencing in three sisters with bisphosphonates-associated atypical femoral fractures

12:00-12:20 Oriol Iborra Egea (IGTP)

Mechanisms of action of sacubitril/valsartan on cardiac remodeling: a systems biology approach

12:20-12:40 Laura Pineda i Cirera (UB)

Exploring genetic variation that influences brain methylation in Attention-Deficit/Hyperactivity Disorder (ADHD)

12:40-13:00 Laura Gasa (UIC)

New cyclins as factors of malignancy in lung cancer

Conferenciant convidat

13:00-13:45 Samuel Sánchez (IBEC)

Biohybrid robotic systems: Learning from nature

13:45-15:00 Dinar i Pòsters

Sessions Tarda

Chair: Xavier Gomis-Rüth

Conferenciants convidats

15:00-15:40 Salvador Ventura (UAB)

Novel Therapies for the Transthyretin Amyloidoses

15:40-16:20 Patricia Casino (Universidad de Valencia)

Understanding two-component signalling systems through their 3D structures

16:20-17:00 Cafè i Pòsters

Short talks

17:00-17:20 Joan Ramon Daban (UAB)

Multilayer planar chromatin in metaphase chromosomes explains the structure of chromosome bands, cancer rearrangements and sister chromatid exchanges

17:20-17:40 Arka Chakraborty (IBMB-CSIC)

Universal Trends in Mitochondrial DNA Compaction: A U-turn and A-tract face-off

17:40-18:00 Elena Vizcaya Molina (UB)

The regulome of regeneration

18:00-18:20 Josep Tarragó Celada (UB)

Metabolic adaptation of the metastatic process in colon cancer

Sessió *Biologia i Indústria* / Chair: Ramon Roca

Conferenciant convidat

18:20-19:00 Marc Cusachs (Transplant Biomedicals)

Reptes y oportunitats de l'emprenedoria científica

19:00-19:10 Lliurament del premi *Lluís Cornudella* i comiat

19:10 Cerveses i Pòsters

ABSTRACTS

TALKS

1

ER contact with endocytic sites initiates actin polymerization

Javier Encinar, Fatima-Zahra Idrissi, Patricia Garcia, Isabel María Fernandez-Golbano, Elena Rebollo, Marek K. Krzyzanowski, Helga Grötsch, Maria Isabel Geli

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Oxysterol Binding Proteins Related Proteins (ORPs) are a family of conserved lipid binding proteins, enriched at endoplasmic reticulum (ER) contacts sites. ORPs promote non-vesicular lipid transport to other organelles and work as lipid sensors in the context of multiple cellular tasks, but the determinants of their distinct localization and function are still not understood. Using a combination of Time Resolved Electron Microscopy (TREM) and life-cell imaging in yeast, we demonstrate that the endocytic invaginations associate with the cortical endoplasmic reticulum as they mature, and that this association requires the ORPs Osh2 and Osh3, which bridge the endocytic myosin-I Myo5 to the ER integral-membrane VAMP-associated protein (VAP) Scs2. Using mutations that specifically disrupt the myosin-I/ORP/VAP link, as well as a reticulon mutant with extended cER-free plasma membrane subdomains, we show that ER contact to the endocytic sites has a dual function initiating actin-dependent membrane invagination and promoting vesicle scission. Further, we show that ER-induced actin polymerization requires the localized transfer of sterols by Osh2.

2

Understanding two-component signalling systems through their 3D structures

Patricia Casino
Universidad de Valencia

Two component systems are the major signal transduction pathways in bacteria, that are absent in mammals, thus, they are important targets for the design of new antimicrobials. In order to understand how these system works we have used a combination of functional and structural studies on the basic proteins that constitute these systems, the histidine kinase and the response regulator. This have allowed us to understand the mechanism of autophosphorylation in histidine kinases, the interaction specifity between the histidine kinase and the response regulator for the phosphotransfer reaction as well as the phosphorylation-state of the response regulator and its interaction with DNA. The autophosphorylation reaction includes major conformational changes in the cytoplasmic domains to approach the ATP to the catalytic His for γ-phosphate hydrolysis, catalysis by a conserved His helped by a neighbor acidic residue and by additional residues close by to the nucleotide binding site but also the cis/trans reaction directionality, a feature resulted of the dimeric nature of histidine kinases. The phosphotransfer reaction includes the fine-tune interaction between the response regulator with the histidine kinase for phosphoryl transfer. Finally, the phosphorylation state of the response regulator leads to a dimerization event in a variable set of dimeric conformations that have direct consequences on the mode of interaction with DNA.

3

Identification of a novel mutation in the *GGPS1* gene by whole-exome sequencing in three sisters with bisphosphonates-associated atypical femoral fractures

Neus Roca-Ayats[1], Natàlia Garcia-Giralt[2], Maite Falcó-Mascaró[3], Núria Martínez-Gil[1], Josep Francesc Abril[3], Roser Urreizti[1], Joaquín Dopazo[4], José Manuel Quesada Gómez[5], Xavier Nogués[2], Leonardo Mellibovsky[2], Daniel Prieto-Alhambra[2,6], James E. Dunford[6], Muhammad K Javaid[6], R Graham Russell[6,7], Daniel Grinberg[1], Susana Balcells[1], Adolfo Díez-Pérez[2]

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Atypical femoral fractures (AFFs) are a rare but devastating type of fractures often associated with long-term bisphosphonate (BPs) therapy, the main treatment for osteoporosis and cancer-related bone disease. Unfortunately, the pathogenic mechanisms underlying AFFs remain unclear. Given the low incidence of these fractures, there may be underlying rare genetic causes that might interact with BPs to trigger their occurrence.

We identified three sisters and three additional unrelated patients, all presenting with AFF and previously treated with BPs for more than 5 years. The present study explored their genetic background by whole exome sequencing. Rare non-synonymous mutations shared among the three sisters were selected, considering either a dominant or a recessive inheritance model. We detected 37 rare heterozygous mutations in 34 genes. Among them, a novel mutation was found in the gene encoding geranylgeranyl diphosphate synthase (GGPPS), an enzyme crucial for osteoclast function, which can be inhibited by BPs. Other identified variants, such as those found in the *CYP1A1*, involved in steroid metabolism, and in the mevalonate pyrophosphate decarboxylase (*MVD)* genes, may also contribute to susceptibility to AFF. Pathway analysis among the mutated genes showed enrichment of the isoprenoid biosynthetic pathway (GO:0008299), containing these three genes (p-value 0.0006). Preliminary functional studies of the *GGPS1* mutation showed a reduced enzymatic activity of the mutated protein.

Our results are compatible with a model in which an accumulation of susceptibility variants (including some in relevant genes, notably *GGPS1* and *CYP1A1*) constitutes a possible genetic component of AFF causality and may lead to novel risk assessment tools to personalize osteoporosis therapy.

4

Mechanisms of action of sacubitril/valsartan on cardiac remodeling: a systems biology approach

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Sacubitril/Valsartan, proved superiority over other conventional heart failure (HF) management treatments, but its mechanisms of action remains obscure.
In this study, we sought to explore the mechanistic details for Sacubitril/Valsartan in HF and post-myocardial infarction (MI) remodeling, using an in silico, systems biology approach. Myocardial transcriptome obtained in response to MI in swine was analyzed to address post-infarction ventricular remodeling. Swine transcriptome hits were mapped to their human equivalents using Reciprocal Best (blast) Hits, Gene Name Correspondence, and InParanoid database. HF remodeling was studied using public data available in GEO (accession GSE57345, subseries GSE57338), processed using the GEO2R tool. Using the Therapeutic Performance Mapping System (TPMS) thecnology, dedicated mathematical models trained to fit a set of molecular criteria, defining both pathologies and including all the information available on Sacubitril/Valsartan, were generated. All relationships incorporated into the biological network were drawn from public resources.
An artificial neural network analysis revealed that Sacubitril/Valsartan acts synergistically against cardiomyocyte cell death and left ventricular extracellular matrix remodeling via 8 principal synergistic nodes. When studying each pathway independently, Valsartan was found to improve cardiac remodeling by inhibiting members of the guanine nucleotide binding protein family, while Sacubitril attenuated cardiomyocyte cell death, hypertrophy, and impaired myocyte contractility by inhibiting PTEN. The complex molecular mechanisms of action of Sacubitril/Valsartan upon post-MI and HF cardiac remodeling were delineated using a systems biology approach. Further, this dataset provides pathophysiological rationale for the use of Sacubitril/Valsartan to prevent post-infarct remodeling.

5

Exploring genetic variation that influences brain methylation in Attention-Deficit/Hyperactivity Disorder (ADHD)

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Allele-specific methylation (ASM) is a common epigenetic mechanism that involves SNPs correlating with differential levels of methylation at CpG sites. Epigenetic changes drive lasting changes in gene expression in several tissues, including the brain. As alteration of DNA methylation has recently been linked to ADHD symptoms, we aim at exploring the contribution of ASM to ADHD through a case-control association study using GWAS datasets.
We performed a SNP selection based on two previous studies that identified ASM variants in brain regions of post-mortem human samples. We selected a total of 3,896 SNPs following these criteria: *cis*-associations between SNP genotypes and methylation, correlation of methylation with gene expression (R^2) ≥ 0.2 and tagSNPs for each CpG site (LD, r^2≥0.85). These SNPs were inspected in summary statistics data from the first GWAS meta-analysis of adult and childhood ADHD, performed by iPSYCH and the Psychiatric Genomics Consortium in 20,183 cases and 35,191 controls.
We applied 5% False Discovery Rate (FDR) to determine the top hits (corrected *P*≤ 6.78e-05). We identified eight tagSNPs surviving FDR, which correlate with differential methylation of six CpG sites. Top hits were followed-up to retrieve SNPs located within the same CpG site. By adding other SNPs present in these sites, we reached a total number of 39 candidate SNPs. The six CpG sites are located in the promoter regions of five genes: *PIDD*, *ARTN*, *C2orf82*, *NEUROD6* and *GAL*. Notably, all SNPs associated with differential methylation at *PIDD*, *ARTN* and *C2orf82* are eQTLs for these genes in various brain tissues.
In summary, we followed a systematic approach by employing *cis*-acting ASM variants to pinpoint candidate genes for ADHD. Interestingly, several of the identified variants are eQTLs for genes that are expressed in brain. Our aim is to investigate the contribution of these genes in the development of the ADHD phenotype.

6

New cyclins as factors of malignancy in lung cancer.

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Lung cancer is a leading cause of cancer related deaths and the treatment options for patients with advanced disease are still limited. The mammalian cell cycle is mainly controlled by complexes of Cyclin Dependent Kinases (CDKs) and cyclins, in a tightly regulated process; the loss of this regulation may favor tumor development and progression. Several CDKs and cyclins were identified by the Human Genome Project, but the role of some of these newly discovered members is still unclear. Therefore, we investigated the expression and functional role of these new cyclins in lung cancer. We evaluated, by western blot analysis, the expression of eight new cyclins in a cohort of formalin-fixed and paraffin-embedded (FFPE) lung tumor tissues and compared with paired adjacent non-tumor tissues. Our results show that some of these new cyclins are significantly overexpressed in lung tumors. Accordingly, the expression of the novel cyclins is increased in lung cancer cell lines as compared with normal fibroblasts. The overexpression of these upregulated cyclins enhanced lung cancer cell viability and migration. Furthermore, we found a significant correlation between high expression levels of these cyclins and decreased overall survival of lung cancer patients. Our results suggest that some of these new cyclins are factors of malignancy in lung cancer, and pave the way for the development of new therapeutic strategies and to the establishment of innovative prognostic biomarkers.
This study was funded by Fundació La Marató de TV3 (project number 20131010) and Ministerio de Economía y Competitividad MINECO (grant ref BFU 2013-44189-P).

7

Multilayer planar chromatin in metaphase chromosomes explains the structure of chromosome bands, cancer rearrangements and sister chromatid exchanges

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The structure of chromatin in metaphase chromosomes has been one of the most challenging problems in Molecular Biology since the discovery of the nucleosome. Cytogenetic data obtained in many different laboratories have been used to investigate the three-dimensional organization of chromatin within chromosomes. The orientation angle α of G-bands and R-bands with respect to the chromosome axis has been measured; the mean values obtained for angle α range from 88º to 91º. This orthogonal orientation is observed even for the thinnest bands. The sub-bands produced by mechanical splitting of the original bands and the thin replication bands are also approximately perpendicular to the chromosome axis. These results indicate that in three dimensions bands are disk-like structures that can be very thin and that relatively short stretches of DNA can occupy completely the chromosome cross-section. In sister chromatid exchanges, the resulting connection surfaces are planar; the observed orientation angle α of these surfaces is approximately 90º. The connection surfaces observed in chromosome translocations occurring in different carcinomas and hematological malignancies are also planar and approximately perpendicular to the chromosome axis

(mean values of angle α range from 90º to 92º). These observations place strong geometric constraints on models that attempt to explain chromatin folding in metaphase chromosomes. Most of the models proposed so far do not satisfy these constraints. The thin-plate model, which consists of many stacked layers of planar chromatin orthogonal to the chromosome axis (reviewed in ref. 1), is compatible with all these constraints. Furthermore, this model gives for the first time a consistent explanation of the chromosome structural properties that are used in clinical cytogenetics for the diagnosis of hereditary diseases and cancers. A complete description of this work can be found in ref. 2.

(1) Daban, JR (2011) Micron 42:733.
(2) Daban, JR (2015) Scientific Reports 5:14891.

8

Universal Trends in Mitochondrial DNA compaction: A U-turn and A-tract face-off

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Mitochondrial DNA (mtDNA) is compacted into nucleoprotein structures called nucleoids. Compaction of human mtDNA (h-mtDNA) relies on transcription factor A (TFAM or mtTFA) whereas in yeast (S. cerevisiae) mtDNA is compacted by Abf2p. Both proteins have additional and different activities: TFAM is a transcription factor whereas Abf2p has been related to DNA recombination. They both contain two HMG box domains arranged in tandem, yet they differ in additional motifs that underlie protein-specific DNA binding mechanisms. Despite their differences, they both induce a similar U-turn on the DNA. On the other hand, human and yeast genomes are dissimilar. H-mtDNA is a circular molecule of 16.5 kbp with few intergenic sequences and asymmetric GC content in strands, while y-mtDNA is a 85 kbp linear molecule with intergenic non-coding regions and rich in poly-adenine (poly-A) tracts. The different sequence contents endow different structural and topological properties on the DNAs and thus may impose different regulation strategies on DNA compaction. We will present insights into the molecular basis of mtDNA compaction based on the analysis of these distantly-related proteins and their complexes with DNA. Additionally, we explore U-turns and poly-A-tracts as components of general strategies in mtDNA compaction across species.

9

THE REGULOME OF REGENERATION

Elena Vizcaya[1,2], Cecilia Klein[2], Carlos Camilleri[1], Florenci Serras[1], Roderic Guigó[2] and Montserrat Corominas[1]
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A key issue in regenerative biology is to understand the transcriptional program and regulatory elements that respond to tissue damage. *Drosophila* imaginal discs are epithelia that activate a regenerative response after cell death induction or physical injury. To identity regulatory elements governing tissue regeneration we performed genome-wide analyses (ATAC-Seq and RNA-Seq) at different time points (0, 15 and 25 hours) after cell death induction in the wing imaginal disc. Comparison between control and damaged discs shows more regions of accessible chromatin immediately after cell death, which correlates with higher number of up-regulated genes detected at the same moment. Both ATAC and RNA profiles recover the normal levels at 25h after cell death. Regions of open chromatin can be classified in: 1) specific regions accessible only after cell death; 2) non-specific regions that become more accessible after cell death but are also present in control discs, probably corresponding to developmental regulatory regions. In addition, a fraction of up-regulated genes tend to be located in nearby genomic regions indicating co-regulation of gene expression during regeneration. Individual interactions between accessible regions and clusters of co-regulated have been confirmed by 3C analyses. Selected enhancers have been experimentally validated after different types of damage, such as cell death and physical injury. Finally, among differentially expressed genes we have detected a collection of long non-coding RNAs which points to a potential role of this class of transcripts in the fine-tune regulation of regeneration.

10

Metabolic adaptation of the metastatic process in colon cancer

Josep Tarragó Celada

Metastasis is the main cause of cancer death and its mechanisms are still not enough understood. A better comprehension of the process of how the disseminated tumour cells manage to survive the circulation and initiate new tumours is crucial for developing new therapies. Genetic alterations seem to be the main driving force for tumourigenesis, while when it comes to aggressiveness, less genetic changes are identified. For this reason we hypothesise that the metabolic adaptation, essential for cancer cell growth and survival, could be a key factor of the metastatic process. Colorectal carcinogenesis is commonly driven by the inactivation of the adenomatous polyposis coli (APC) tumour suppressor gene and the activating mutation of the oncogene KRAS. Metastasis frequently occurs in the close lymph nodes and to the liver the portal system and also to the lung, bone or brain. To characterise the metabolic signature of colon cancer metastasis we use the highly-valuable same patient-derived SW480 (primary) and SW620 (metastatic) commercial cell lines together with SW620-LiM1 and SW620-LiM2 (enrichment of SW620 subpopulations with the highest metastatic potential). The four cell lines constitute an excellent model to study the metabolic alterations suffered in the metastatic progression of the KRAS-driven colon cancer cells. Additionally, the SW620-LiM2 specific metabolic features are assessed in other metastatic colon cancer cell lines, COLO201 (ascites metastasis) and T84 (lung metastasis) in order to extrapolate those features. The non-tumour colon cell line NCM460 is used

as reference. Metabolites and metabolic fluxes are measured by spectrophotometry and mass spectrometry-based metabolomics. All this metabolic characterisation is combined with transcriptomics, molecular biology measurements and in vitro clonogenic assays to create a genome-scale metabolic network model that will allow to have a systemic view of the metastatic process and find its possible vulnerabilities.

PÒSTERS

1

Genetic heterogeneity in Opitz C syndrome

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Introduction: Opitz C syndrome (OTCS, 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.

Material and Methods: We studied a cohort of 13 patients (10 unrelated pedigrees) with clear or tentative diagnosis of OTCS. Patients and parents were analysed by means of whole exome sequencing (WES).

Results: We identified the disease-causing mutation in 8 of the 10 families in 8 different genes sharing demonstrated roles in development and cancer. Three of them are still in the final validation stage. All these genes were associated with other diseases with phenotypic similarities to OTCS. For example, PIGT compound heterozygous mutations were found in one of our OTCS patients, similar to what has been described in three unrelated families by others. Interestingly, all four cases identified so far have at least one missense variant that leads to a partial loss of function, suggesting that a minimum amount of the PIGT gene product may be essential for life.

Conclusions: WES is a very powerful approach to identify OTCS related mutations, since it was successful in 80% of our cases. Genematcher has been a very useful 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 OTCS as a causally heterogeneous phenotype instead of a specific entity.

2

Inhibition of *EXTL2* in Sanfilippo C patients' cells using lentivirus-encoded shRNAs as a long-term substrate reduction therapy

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Sanfilippo C syndrome is a rare lysosomal storage disorder caused by mutations in the *HGSNAT* gene, which encodes an enzyme involved in heparan sulphate (HS) degradation. The enzyme deficiency causes the storage of partially degraded HS molecules inside the lysosome. The disease has an autosomal recessive inheritance pattern and is characterized by a severe and progressive neurodegeneration for which no effective treatment exists.
Previously, we demonstrated, on Sanfilippo C patients' fibroblasts, that the use of siRNAs targeting *EXTL2* and *EXTL3*, genes involved in HS synthesis, could be effective as a short-term substrate reduction therapy (SRT). Here, we have used five different lentiviruses encoding shRNAs targeting *EXTL2*, to analyse the effect of a long-term treatment. All the shRNAs caused a notable reduction in the mRNA levels (around 90%) of the *EXTL2* gene sixty days post-infection. Moreover, immunocytochemistry analyses showed a clear decrease of the HS amounts after treatment.
Due to the good results obtained on patients' fibroblasts, now we are using the most effective shRNAs on induced pluripotent stem cells (iPS cells) derived from patients' fibroblasts. We have set up the protocol for the differentiation of those iPS cells into neurons, the most affected cell type in this disease. This protocol includes the synthesis of lentiviruses that encode transcription factor genes involved in the differentiation of iPS cells into neurons, and allows this differentiation to be carried out within a week, which greatly accelerates the assessment of any treatment.
Once we have all iPS cells (infected with shRNAs or not) differentiated into neurons, we are going to perform on them the same assays that we carried out in fibroblasts.
Our results confirm the usefulness of shRNAs as a longterm SRT, becoming a promising approach for a future therapeutic option for Sanfilippo C syndrome.

3

Functional characterization of *Smed-foxK2* during planarian regeneration and homeostasis

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Regeneration has always been one of the most interesting biological phenomena. It is defined as the ability to restore lost tissues or even organs via cell proliferation and remodelling of pre-existing tissues. Knowing the molecular basis that orchestrate regeneration not only contributes to a better understanding of how the cellular machinery works but gives rise to new ideas in the biomedical field. Planarians are Platyhelminthes (flatworms) with great regeneration abilities, being able to restore a whole organism from a very tiny piece thanks to a population of adult pluripotent stem cells (neoblasts). These animals have become a very useful model organism for the study of regeneration. A lot of signalling pathways are involved in the regulation of cell proliferation and differentiation, most of them containing transcription factors. The Forkhead family of transcription factors participates in a variety of cellular processes, from cell cycle regulation to epithelium differentiation. The key role of these forkhead transcription factors explains the severity of the phenotypes associated with their mutations.

Here we describe a homologue of a Forkhead box K2 gene identified in the planarian *Schmidtea mediterranea* that we called *Smed-foxK2* and its role during regeneration and homeostasis. In mammals, FOXK2 has been mainly involved in cancer progression. *Smed-foxK2* is expressed in the cephalic ganglia and throughout the mesenchyme in intact planarians. During regeneration *Smed-foxK2* expression is observed throughout the animal, particularly in the cephalic ganglia. In X-ray-irradiated planarians, the number of *Smed-foxK2*-expressing cells is clearly decreased, suggesting that it is also expressed in neoblasts. Finally, the silencing of *Smed-foxK2* by RNAi results in animals with a reduced blastema and aberrant eye differentiation. Besides, the treated animals show an impaired response to light stimulation, remaining motionless compared with the controls.

4

Functional studies of DKK1 variants in general population

Martínez-Gil, Núria; Roca-Ayats, Neus; Urreizti, Roser; Garcia-Giralt, Natàlia; Van Hul, Wim; Boudin, Eveline; Nogués, Xavier; Mellibovsky, Leonardo; Díez-Pérez, Adolfo; Grinberg, Daniel; Balcells, Susana

The *DKK1* gene encodes a secreted protein that acts as a Wnt pathway inhibitor. Numerous studies have associated the Wnt pathway with bone formation and risk of fracture. Our group has identified two missense variants in DKK1. Firstly, we identified the p.Y74F variant, which cosegregates with the high bone mass (HBM) phenotype in a family and later, we identified the p.R120L variant in another case of HBM. Furthermore, the ExAC database provides a list of several DKK1 missense variants present in the general population whose effects in terms of bone mass are unknown. The p.R120L variant appears in ExAC as the most frequent amino acid change of DKK1 [MAF =0.003]. Our hypothesis was that DKK1 variants found in individuals with HBM could be loss-of-function or hypomorphic, leading to a greater activity of the canonical Wnt pathway due to a lack of inhibition. In a similar way, variants found in the general population could also affect the functionality of the protein and contribute to the population variability of bone mineral density.
To test the functionality of these DKK1 mutant proteins, we performed luciferase reporter assays and western blots. Luciferase reporter assays have been designed to measure the activity of the Wnt pathway in the presence or absence of wild-type or mutant DKK1 protein. Our results showed that in the presence of wild-type DKK1 there was significant decrease in Wnt-dependent luciferase activity. In the presence of p.A41T mutant luciferase activity was significantly different from both the WT-DKK1 and the activated Wnt pathway. The results of the western blot show high levels of all the DKK1 mutant proteins. Thus, the loss of activity seen for the p.A41T mutation would not be attributable to a lack of protein. In conclusion, these studies show that the presence of missense variants can produce an increase in BMD in the general population.

5

Galanin Modulates Opioid Signaling in the Ventral Tegmental Area Through μ Opioid-Galanin Receptor Heteromers

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The neuropeptide galanin has been shown to interact with the opioid system. More specifically, galanin counteracts the behavioral effects of the systemic administration of μ-opioid receptor (MOR) agonists. Yet the mechanism responsible for this galanin-opioid interaction has remained elusive. Using biophysical techniques in mammalian transfected cells we found evidence for selective heteromerization of MOR and the galanin receptor subtype Gal1 (Gal1R). Also in transfected cells, a synthetic peptide selectively disrupted MOR-Gal1R heteromerization as well as specific interactions between MOR and Gal1R ligands: a negative crosstalk, by which galanin counteracted MAPK activation induced by the endogenous MOR agonist endomorphin-1, and a cross-antagonism, by which a MOR antagonist counteracted MAPK activation induced by galanin.

These specific interactions, which represented biochemical properties of the MOR-Gal1R heteromer, could then be identified *in situ* in slices of rat ventral tegmental area (VTA) with MAPK activation and two additional cell signaling pathways, AKT and CREB phosphorylation. Furthermore, *in vivo* microdialysis experiments showed that the disruptive peptide selectively counteracted the ability of galanin to block the dendritic dopamine release in the rat VTA induced by local infusion of endomorphin-1, demonstrating a key role of MOR-Gal1R heteromers localized in the VTA in the direct control of dopamine cell function and their ability to mediate antagonistic interactions between MOR and Gal1R ligands. The results also indicate that MOR-Gal1R heteromers should be viewed as targets for the treatment of opioid use disorders.

6

CANONICAL AND NON-CANONICAL WNT ACTIVATE A COMMON CELLULAR RESPONSE

Aida Villarroel i Mireia Duñach

Wnt factors have crucial roles in development and disease. Wnts interact with transmembrane Frizzled (Fz) receptors and depending on the co-receptor they stimulate canonical or non-canonical pathways. The canonical ligand Wnt3a binds to the co-receptors Fz and LRP5/6 promoting ß-catenin stabilization and transcriptional activation of ß-catenin-dependent genes. In contrast, the non-canonical Wnt5a associates with Fz and with the tyrosine kinase co-receptor Ror2, leading to cellular polarization and migration. Despite these differences, both pathways share a common initial step, Dishevelled (Dvl) recruitment to Fz.

It has been recently described that the activation of non-canonical Wnt induces Stat3 phosphorylation a modification dependent on the tyrosine kinase Fyn. Furthermore, we have shown Stat3 phosphorylation by canonical Wnt as well. In this study, we have aimed to characterize the mechanisms implicated in Fyn activation and Stat3 phosphorylation in both pathways.

Our results indicate that upon Wnt stimulation (canonical and non-canonical) Fyn is recruited to Fz and activated promoting Stat3 phosphorylation. Fz phosphorylation depends on another Tyr kinase, Src that constitutively interacts with LRP5/6 and Ror2. Src is required for Fyn-Fz interaction and for Stat3 phosphorylation.

The effect of this Src/Fyn/Stat3 axis in different steps of both pathways was evaluated. Fyn and Src depletion leads to a similar or augmented response to Wnt5a in terms of Dvl recruitment, Rac1 activation, JNK2 phosphorylation and ß-catenin downregulation. Likewise, the absence of Fyn does not affect the Wnt3a-induced ß-catenin stabilization. However, Fyn is required in both pathways for Stat3 phosphorylation and cell invasion.

These results suggest that, canonical and non-canonical Wnts activate a common cellular response that implicates Fyn, Src, and Stat3 phosphorylation, required for cell invasion. Fyn/Stat3 axis antagonizes canonical and non-canonical Wnt branches involving Fz/Dvl2 and their downstream effects. Therefore, Src and Fyn have a negative role in both pathways to avoid over-activation of Wnt signalling.

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New 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 and *in vitro* cell culture assays are an essential tool to dissect and characterize the precise role of *NR2E3* and *CERKL*, two retinal dystrophy genes.
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 aimed to delete the full locus and intend to investigate 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.

8

Nutrient supply impacts osteocytic specification by regulating a nuclear transcription program

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Osteocytes constitute the most abundant type of cell in mature bone. Osteocytes, besides orchestrate bone homeostasis, play a key role in the regulation of phosphate metabolism, calcium availability and energetic homeostasis through the secretion of multiple soluble factors and hormones. Osteocytes are terminally differentiated osteoblasts which reside in a mineralized extracellular matrix (ECM) where the access to nutrients is low. Nowadays, the factors that regulate this differentiation process remain unknown, and in this context, glucose metabolism has emerged as a central regulator of bone differentiation.
To study the role of glucose in osteocyte differentiation we cultured primary Osteoblast and IDG-SW3 cell line in hypoglycaemic, normoglycaemic and hyperglycaemic culture mediums (1, 5 y 25 mM glucose respectively). AlpI and Alzarin Red staining as well as gene expression analysis of early and late osteogenic genes demonstrate that hypoglycaemic conditions promotes osteocyte differentiation whereas hyperglycaemia hampers differentiation. Moreover, during normal osteocyte differentiation we found increased expression of PGC1-α, a key regulator of energy metabolism, and a reduction in PGC1-ß levels. Bioenergetic analysis of osteoblast and IDG-SW3 differentiated in 1, 5 y 25 mM glucose revealed metabolic adaptations to these conditions. In

general, hypoglycaemia triggers a reduction of respiratory flux and depletion of ATP, despite inducing higher glucose uptake and mitochondrial fusion. In this state of energy deprivation, we found an activation of AMPK pathway, which can phosphorylate and activate the transcription factor PGC1-α. Overexpression of PGC1-α and ChIP studies revealed that PGC1-α is able to act as a coactivator of osteocytic genes, acting as a linker between glucose metabolism and osteocytic differentiation.

9

Inhibition of replicative stress genes *WEE1* and *CHK1* by Polypurine Reverse Hoogsteen hairpins promotes cancer cells death and apoptosis

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Replication stress activates the ATR-CHK1 pathway to produce cell cycle arrest and provide enough time to repair DNA damage. The alteration of this checkpoint can lead to develop illness such as cancer (1, 2). In this study, we investigated the inhibition of genes involved in the ATR-CHK1 pathway using Polypurine Reverse Hoogsteen hairpins (PPRHs). Specifically, we focused on *WEE1* and *CHK1* genes, which prevent the association between CDK1 and cyclin B and cell cycle progression.
The efficacy of various PPRHs directed against different regions of *WEE1* and *CHK1* genes was evaluated. Cells were incubated with specific PPRHs and two negative controls: scrambled-PPRHs and Watson-Crick PPRHs. PPRHs against the promoter (HpWEE1Pr) and intron (HpWEE1I5 and HpWEE1E11) of the *WEE1* gene and PPRHs against intron 1 (HpCHK1I1-C and HpCHK1I1-T) of the *CHK1* gene showed a high decrease in cell viability in different cell lines (HeLa, SKBR3, MCF7 and PC3).
Furthermore, we determined the level of apoptosis at different times to get insight into the mechanism involved in the process of cell death. After 15 h of transfection, the inhibition of *WEE1* or *CHK1* genes by PPRHs showed an increase in apoptotic cells compared to the control as determined by the rhodamine and flow cytometry method. In addition, we performed the caspase 3/7 apoptosis test in cells with *WEE1* silenced for 8 hours, demonstrating an increase in caspase activity.
We conclude that the inhibition of components from the ATR-CHK1 pathway by PPRHs, such us *WEE1* and *CHK1*, has proven to be highly effective and therefore may constitute a possible new approach in cancer therapy.

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Association of the *PLCB1* gene with drug dependence

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Drug dependence is a complex neuropsychiatric disorder that results from the interaction of genetic and environmental factors. MicroRNAs (miRNAs) are very abundant in the central nervous system and seem to play key roles in the drug-induced plasticity of the brain that likely drives the emergence of addiction. Some studies suggest that SNPs located in both miRNAs and miRNA target sites could alter the miRNA-mediated regulation of gene expression that underlie disease and non-pathological phenotypes.
In the present work we have explored the role of miRNAs in drug addiction. With this aim, we selected SNPs in the 3'UTR of target genes that are predicted to alter the binding of miRNA molecules and performed a case-control association study in a Spanish sample of 735 cases and 739 controls. We found an association between rs1047383 in the *PLCB1* gene and drug dependence that was replicated in an independent sample (663 cases and 667 controls). Then we investigated the functional effect of this SNP and rs1047381-rs708910 (in LD with it) in the vicinity on the binding of different miRNAs using a luciferase reporter assay. Hsa-miR-582 was found to downregulate *PLCB1* expression, without showing differences between the two alleles at rs1047381-rs708910. Previous studies had reported that some genes that show altered expression under cocaine also carry variants that confer susceptibility to cocaine dependence. Under the hypothesis that *PLCB1* is involved in the vulnerability to drug dependence, in particular with cocaine dependence, we explored the possibility that *PLCB1* expression is altered by cocaine. We observed a significant upregulation of *PLCB1* in the nucleus accumbens of cocaine abusers and in human dopaminergic-like neurons after cocaine treatment. To our knowledge, this is the first study assessing the effect of SNPs in miRNA binding sites in drug dependence, and we provide additional evidence for the participation of the *PLCB1* gene in the vulnerability to susceptibility to drug dependence.

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RESPONSE project: predicting biomarkers in myelodysplastic syndrome patients treated with azacitidine

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Myelodysplastic syndrome (MDS) is one of the most common bone marrow disorders in the elderly characterized by an ineffective hematopoiesis and strong predisposition to acute myeloid leukemia (AML). Azacitidine (AZA) is the major treatment given to high-risk MDS patients who are ineligible for bone marrow transplantation due to its severe side effects. Only 40% to 50% of AZA-treated patients show hematological improvements and complete responses are limited to 10% to 15%. The treatment is not curative and response is lost over time.

Our aim is to identify response-predicting biomarkers and novel drug targets that would be suitable for improved combinatorial therapeutic approaches. For this, we use genetic loss-of-function screening in available MDS-AML cell lines to identify AZA-sensitivity affecting genes that will be validated in primary patient samples from the first longitudinal study of AZA treatment in 100 patients. As AZA is incorporated into DNA, we will focus on chromatin and transcriptional regulators. Following a standardized protocol, we collect samples at diagnosis, after AZA treatment and, if occurring, at relapse.

Funded by ISCIII, the RESPONSE project applies the same approach to three major cancers and their current best treatment. In addition to MDS treated with AZA, this includes non-small lung cancer and colorectal cancer treated with chemotherapy.

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Immunotherapy of prostate cancer cells using Polypurinecreverse Hoogsteen hairpins against PD-1 and PD-L1

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We are using immunotherapy strategies to provoke cancer cell death by inhibiting the interaction between membrane receptors in macrophages and membrane proteins expressed in cancer cells acting as a “do not eat me” signals. Previously in our lab we tested this methodology using CD47, an anti-phagocytic signal directed to macrophages by interacting with Signal Regulatory Protein α (SIRPα) (1). In that work, we used Polypurine reverse Hoogsteen hairpins (PPRHs) (2) to silence the expression of CD47 in breast cancer cells (MCF-

7) and SIRPα in macrophages (THP-1). These PPRHs were able to decrease both CD47 expression in the MCF-7 cell line and SIRPα expression in macrophages, leading to the elimination of MCF-7 cells by macrophages in co-culture experiments.
Now we are applying the same technology to inhibit the expression and the interaction of PD-1, which is a immunoinhibitory receptor mainly expressed in T cells, and PD-L1, which is overexpressed in various types of cancer, including prostate cancer PC3 cells (3,4). We designed various PPRHs targeting different regions of both PD-1 (promoter, intron 4 and exon 4) and PD-L1 (intron 1 and intron 2) genes. First, we performed cell viability assays in both THP-1 and PC3 cell lines to demonstrate there was no cytotoxic effect caused by the PPRHs themselves. Then, we proceeded to test different combinations of PPRHs against both genes in co-culture experiments and all of them provoked a decrease in cell viability of at least 55%. The most effective combination was the PPRH against intron 2 of PD-L1 with the PPRH against intron 4 of PD-1, producing a PC3 cell death by macrophages of 90% compared to that of the co-culture control. These results corroborate the potential of PPRHs to be used in distinct immunotherapy approaches.

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The role of SUMO E3 ligases on NR2E3 sumoylation

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The orphan nuclear receptor NR2E3 is a retinal transcription factor (TF) that plays an essential role in the development and maintenance of rod photoreceptors by activating rod-specific genes at the same time that represses cone-specific genes. This repression is mediated by the sumoylation of NR2E3: when NR2E3 is sumoylated, it is able to bind and repress the expression of cone genes. Therefore, the identification of SUMO E3 ligases that are able to sumoylate NR2E3 will shed some light on the post-transcriptional regulation of this transcription factor. To this end, our group has approached the characterization of retinal SUMO E3 ligases and investigate their interaction with NR2E3.
Previous work by other authors showed that the SUMO E3 ligase PIAS3 sumoylated NR2E3 *in vivo*, turning it into a repressor of cone-specific genes. However, since suppression of PIAS3 reduces -but does not ablate- sumoylation of NR2E3, other SUMO E3 ligases are also involved in this relevant retinal developmental decision. Several new ligases that presumably act upon NR2E3 were identified using different technical approaches, among them, BRET, BioID, co-localization, immunoprecipitation and co-transfection. Several SUMO E3 ligases that are expressed in the retina are good candidates to sumoylate NR2E3, thereby regulating its activity.

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A novel survivin inhibitor for squamous cell lung cancer treatment

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Squamous cell lung carcinoma (SQCLC) is the second most prevalent non-small cell lung cancer subtype and represents about 30% of newly diagnosed cases. Targeted therapies against key molecular targets of tumor progression have transformed the way of treating certain cancers, increasing the response rate in patients. Unfortunately, these recent advances are not available to treat SQCLC patients. Therefore, current treatment options for SQCLC remain very limited and great efforts should be done to develop new targeted therapies for this malignancy.
In this view, we have performed a gene expression profile comparing tumor samples with their paired healthy lung tissues for the identification of potential therapeutic targets for this disease. After data mining, survivin was finally selected as the most promising candidate from the up-regulated genes. Survivin is a member of the inhibitor of apoptosis (IAP) family and has key roles in regulating cell division and inhibiting apoptosis. Moreover, survivin is highly expressed in most cancers and is associated with chemotherapy resistance and poor prognosis, which makes survivin an attractive molecular target for anticancer targeted therapies. Therefore, in the search for new survivin inhibitors, we have identified a novel tambjamine analogue (tmbj-21), which has shown anticancer properties and to downregulate of survivin at mRNA and protein levels in A549 lung adenocarcinoma cells. This compound has also been studied in the SQCLC cell lines SW900 and H520, showing a sharp decrease in survivin levels and a moderate affectation on XIAP and c-IAP1 proteins, also members of the IAP family. Ongoing experiments with the protein synthesis inhibitor cyclohexamide are trying to elucidate whether tmbj-21 could also regulate survivin levels through protein degradation.
Altogether, these results propose a novel candidate for targeted therapy in SQCLC and identify a new and potent survivin inhibitor with interesting properties for lung cancer treatment.

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Early signals in *Drosophila* imaginal disc regeneration: From ROS to Cytokines

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Countless environmental aggressors could damage the integrity of tissues and organs. In humans, the limited regenerative ability prevents often the recovery of injured tissues, but this is not the case of the fruit fly *Drosophila melanogaster*. *Drosophila* imaginal discs are larval tissues that can regenerate after fragmentation or massive cell death. That is the reason why in our laboratory, we focused towards the understanding of the genetic basis of imaginal disc repair. We concentrated on the initiation of regeneration, and we found that one of the earliest

responses to damage consist in the production of Reactive Oxygen Species (ROS), which propagate from the dying to the nearby living cells, which will drive tissue repair. We revealed that the burst of ROS is essential, because the reduction of those molecules impairs repair. Within the cells, the protein Ask1 (Apoptotic signal-regulating kinase 1) sense ROS and activates both Jun kinase (JNK) and p38 signalling pathways, which are critical for regenerative growth. Although Ask1 was previously associated to apoptosis, we unravelled a novel function related to survival and proliferation. Ask1 inhibition reduces life span after oxidative stress, as well as regenerative ability after cell death. To assume this function, Insulin signalling must attenuate Ask1 activity in living cells. Finally, we described that both JNK and p38 pathways are necessaries for the transcriptional activation of the cytokines Unpaired, which will promote JAK/STAT signalling to drive regenerative growth and recover the missing tissue. Altogether, we demonstrated a new stress-responsive module composed by many signalling pathways conserved through evolution.

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PRIONW: A SERVER FOR THE PREDICTION OF PRION-LIKE DOMAINS AND THEIR AMYLOID CORES

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Prions are a particular type of amyloids with the ability to self-perpetuate and propagate *in vivo*. Prion-like conversion underlies crucial biological processes in a growing number of species but is also connected to several human diseases including Creutzfeldt–Jakob disease and kuru. Yeast prions are the best understood transmissible amyloids. In these proteins, prion formation from an initially soluble state involves a structural conversion, often driven, by specific domains enriched in glutamine/asparagine (Q/N) residues. Importantly, domains sharing this compositional bias are also present in the proteomes of higher organisms, thus suggesting that prion-like conversion might be an evolutionary conserved mechanism. We have recently shown that the identification and evaluation of the potency of amyloid nucleating sequences in putative prion domains allows discrimination of *bona fide* prions. PrionW is a web application that exploits this principle to scan sequences in order to identify proteins containing Q/N enriched prion-like domains (PrLDs) in large datasets. A scan of the complete yeast proteome with PrionW identifies previously experimentally validated prions with a high success rate: a sensitivity of 0.917, a specificity of 0.949, a precision rate of 0.846, an accuracy of 0.941 and a false discovery rate of only 0.154. This website is free and open to all users who can analyze up to 10000 sequences at a time, PrLD-containing proteins are identified and their putative PrLDs and amyloid nucleating cores visualized and scored. The output files can be downloaded for further analysis. PrionW server can be accessed at http://bioinf.uab.cat/prionw/.

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AGGRESCAN3D (A3D): server for prediction of aggregation properties of protein structures

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Protein aggregation has moved beyond being a mostly ignored area of protein chemistry to become a key topic in biomedicine and biotechnology. It underlies more than 40 human disorders, including neurodegenerative diseases -such Alzheimer's and Parkinson's- and non-neuronal related diseases such diabetes type II or some type of cancers. In addition, it is a pivotal factor to take into account while manufacturing protein-based therapeutics like monoclonal antibodies, growth factors or replacement enzymes.
With the aim of anticipate this phenomenon, the present understanding on the molecular determinants of protein aggregation has crystalized in a series of predictive algorithms to identify the aggregation-prone sites of proteins. The vast majority rely on the amino acidic sequence. Therefore they find difficulties to predict the aggregation properties of folded globular proteins, where aggregation-prone sites are often not contiguous in sequence or buried inside the native structure. The Aggrescan 3D server overcomes these limitations by projecting onto the protein structure the experimental aggregation propensity scale from the well-established AGGRESCAN method. In this way, the native neighbouring tendencies modulate the aggregation propensity score for each amino acid to obtain high confident predictions. Using the A3D server, the identified aggregation-prone residues can be virtually mutated to design variants with increased solubility, or to test the impact of pathogenic mutations. Additionally, A3D server enables to take into account the dynamic fluctuations of protein structure in solution, which may influence aggregation propensity, by using the fast simulations of CABS-flex approach. The A3D server can be accessed at http://biocomp.chem.uw.edu.pl/A3D/.

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Smed-fgfr1op2 is required for stem cell maintenance in the planarian Schmidtea mediterranea.

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Regeneration is arguably among the most awe inspiring biological phenomena known to exist which is defined as the ability to replace lost tissues, organs or structures by cell division or remodeling of preexisting somatic tissue, resulting in *de novo* tissues which must integrate in the body of the animal and recover the normal functionality.

Regeneration occurs in widespread contexts and species. Planarians are members of the Platyhelminthes (flatworms) and they have re-emerged as a powerful molecular genetic system

for the investigation of regeneration. Planarians have astonishing regenerative abilities, including whole-body regeneration from tiny pieces, thanks to a unique population of adult pluripotent stem cells called neoblast. However, the signals regulating maintenance/differentiation/migration of stem cells remain mainly unclear. It is well known that the fibroblast growth factor receptor (FGFR) signalling is involved in a variety of signalling cascades in both vertebrates and invertebrates regulating many biological processes, including proliferation, differentiation and patterning.

Here, we studied a FGFR1OP2 homolog (*Smed-fgfr1op2*) required for planarian regeneration. FGFR1OP2 is a novel gene with no clear function yet reported except that it translocates to the FGFR1 locus in myeloproliferative syndrome (EMS). Expression of *Smed-fgfr1op2* was observed in the cephalic ganglia and rather ubiquitously throughout the mesenchyme in intact planarians. In X-ray-irradiated planarians, the number of *Smed-fgfr1op2*-expressing cells in the mesenchyme significantly decreased, suggesting that is expressed in neoblast and not just in differentiated cells. During regeneration, *Smed-fgfr1op2* was observed at the wound region from early stages. Finally, the silencing of *Smed-fgfr1op2* by RNAi resulted in a severe impairment of regeneration showing a completely blocked brain regeneration. Although stem cells seemed to respond initially properly to amputation after silencing the gene, their number decreased with time, suggesting that *Smed-fgfr1op2* could be involved in regulating stem cell maintenance.
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WNT5-ROR2 and SLIT-ROBO-c signals generate a mutually dependent system to position the CNS along the medio-lateral axis in planarians

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The acquisition of bilateral symmetry was a key evolutionary step, allowing for instances the development of a centralized nervous system. However, the developmental signals that allow bilateral symmetric structures to position in relation to the midline are still poorly understood. Planarian plasticity demands continuous positional information to maintain the body proportions and the axis information during regeneration and homeostasis. This ability offers us an ideal context to study the signals required to position the CNS in relation to the midline. Our results demonstrate that Wnt5 and Slit, which are expressed in complementary domains respect to the CNS, are axon repulsive cues in planarians. We identified ROR2 and ROBO-c as WNT5 and SLIT receptors, respectively. Their co-expression in neurons suggests that both signals could cooperate to guide the axonal path in relation to the midline. Furthermore, *ror2* and *robo-c* receptors are also expressed in muscular cells that express *slit* and *wnt5*, respectively, suggesting a regulatory relationship between both signals. We are currently exploring the hypothesis that WNT5-ROR and SLIT-ROBO-c signals could conform a self-regulated system to define their expression boundaries in addition to guide the axonal path. In conclusion, WNT5-ROR2 and SLIT-ROBO-c signals are axon repulsive cues that define the medio-lateral position of the CNS in planarians. Their domains of expression could be mutually regulated, allowing the self-maintenance of the medio-lateral positional information.

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The hippo pathway could target mitochondria in planarians

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Due to its involvement in cancer, the Hippo pathway is a hot topic in Biomedicine and Developmental biology. Although the Hippo pathway was first related to growth control, recent studies show its essential function in cell differentiation and in the control of stemness. For a better understanding of the role of the Hippo pathway, in the last years our lab has studied its function in planarians, flatworms that can regenerate any body part and change their body size depending on environmental conditions. This plasticity is due to an abundant population of adult pluripotent stem cells (neoblasts). We know that inhibition of *Hippo* in planarians does not lead to bigger animals but to the formation of overgrowths composed by undifferentiated cells. Thus, in planarians Hippo has 3 different functions, any of which could underlie the formation of the tumors: it controls the cell cycle, it activates apoptosis and it is required to maintain the differentiated state of the cells that conform differentiated tissues as the central nervous system, the digestive system or the epidermis. In order to understand the specific target genes of Hippo in planarians we performed a comparative analysis of the transcriptome of *hippo* RNAi, *yki* RNAi (the transcription factor that mediates Hippo signal) and control animals. Interestingly, a high proportion of candidate genes were related to mitochondria function. Their functional analysis supports the idea that *hippo* inhibition could promote tumorigenesis through controlling mitochondrial function.

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Regulation of proteasome activity and structural configuration by Rpn5 sumoylation

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Sumoylation is a post-translational modification that regulates a multitude of cellular processes, including cell-cycle progression, replication, protein transport and DNA damage response. In this process, similar to ubiquitylation, SUMO (small ubiquitin-like modifier) is covalently attached to target proteins in a reversible process via an enzymatic cascade. Sumoylation often cooperates with the ubiquitin-proteasome system in its contribution to proteostasis, but very few studies describe sumoylation of the proteasome itself.
In this project, we aim to characterize the sumoylation of the proteasome subunit Rpn5. Performing *in vitro* sumoylation assays, we screened the possible sumoylation sites among Rpn5 sequence and we found that the lysine in position 147 is the principal target for SUMO binding. The second step was to create a strain in which Rpn5 is substituted with the SUMO-Rpn5 chimera, that mimics Rpn5 sumoylation, to better characterize the functionality of a sumoylated proteasome and the effects on cell physiology and viability. Using native gels for *in vitro* activity assays, we observed that SUMO-Rpn5 proteasomes present a decrease in 20S core, a different distribution in RP_2-CP and RP-CP subunits and a decrease in LLVY degradation activity. Moreover, in concordance with native gel results, MS analysis of SUMO-Rpn5 proteasomes revealed a reduced binding to the 20S core of two-fold, comparing with a wild type proteasome, whereas the 19S components are virtually equal. Finally, screening SUMO-Rpn5 strain for different stresses, we

observed an enhanced sensitivity to zinc toxicity, comparing with a wild type strain. We are particularly interested in elucidate the physiological meaning of this phenotype and how proteasome sumoylation affects this stress response. Moreover, we want to go further into the mechanics by which Rpn5 is sumoylated, deciphering which are the factors involved in the enzymatic cascade, the conditions that trigger this modification and the relations with other post translational modification pathways.

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Proteasomal degradation of naturally occurring glutamine-rich peptides

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The ubiquitin proteasome system (UPS) is a central player in eukaryotic protein homeostasis. In a cellular context, ubiquitin activating, conjugating and ligating enzymes act sequentially marking the substrates for their degradation in the proteasome. In this way, ubiquitin serves as a common recognition tag among most of the proteasome substrates.
As part of the UPS system, the proteasome acts as a protein degrading hub for the cell, integrating degradative signals from multiple ubiquitin ligase enzymes. This tag, however, is not imperative for protein degradation. Instead, it has been described that location to the proteasome is sufficient for protein degradation, given that the substrate features an unstructured region able to interact with the inner ATPases of the proteasome.
In this project, we have successfully produced a series of proteasome variants that bypass the ubiquitin tagging system and direct a target protein to the degradation machinery. To do so, we have engineered a proteasome surface to have high affinity for a target protein (TP) which features a glutamine-rich region. These proteasomes show an improved ability to degrade the TP, especially when looking at the presence of potentially toxic degradation intermediates of the protein.
Yeast strains expressing these variants exhibit similar characteristics in terms of growth, morphology and proteasome subunit stoichiometry compared with wild type strains. Additionally, when purified and challenged with fluorogenic proteasome substrates such as LLVY-AMC, these proteasome's proteolytic activity levels are comparable to those of the wildtype counterparts, demonstrating that the increase of TP degradation is not an effect of the global activation of the proteasome but a consequence of the increased affinity.

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'Functional characterization of the Egfr/egr signalling pathway during planarian regeneration'

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Freshwater planarians are capable to regenerate the entire body from small pieces of it. This remarkable ability is due to the presence of a population of adult pluripotent stem cells called neoblasts. The molecular and biological mechanisms that regulate the proliferation and differentiation of the neoblasts are being studied currently.

Previous studies have shown that the epidermal growth factor receptors (EGFR) signalling is necessary for the differentiation of the neoblasts into specific linages. Thus, Smed- egfr-1 is required for the differentiation of the gut lineage whereas Smed-egfr-3, seems to be necessary for neuronal differentiation. More recently, one transcription factor called early growth response 4 (Smed-egr-4), has been characterized as a downstream target of Smed-egfr- 3, and be essential for anterior regeneration, most probably by triggering early brain differentiation. This protein is a zinc finger transcription factor that is known to play a role in proliferation, cell growth, neurogenesis and apoptosis in different organisms, including mammals. We have recently identified another egr in planarians, named Smed-egr-6, which seems to have similar functions during planarian regeneration as the previous one.
Here, we report the function of the above mentioned transcription factors (Smed-egr-4 and Smed-egr-6) on the early wound response and the differentiation of neuronal progenitors. Also, our results suggest that Smed-egfr-3 would regulate the expression of Smed-egr-4 through the activation of ERK. Moreover, Smed-egr-4 seems necessary also to activate ERK through a regulatory loop.
Our results can provide new insights into the role of these genes in planarian regeneration, and to hypothesize new pathways that are necessary for this amazing process.

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TITLE: Human iPSC-derived RPE and photoreceptor precursor cells as a tool for a combined cell-based therapy to restore retinal degeneration

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BACKGROUND: Retinal degenerative diseases are the major cause of blindness and affect millions of people worldwide. They are caused by a progressive death of mainly retinal pigment epithelial cells (RPE) and photoreceptors (PR). Nowadays there is no cure to repair or replace damaged retinal cells so, once RPE and PR cells have degenerated, cell-based replacement therapy is a good approach to restore vision.
OBJECTIVES: To prove that the combined cell-based therapy using RPE and PR cells differentiated from human induced pluripotent stem cells (hiPSC) offers better results than the RPE alone in a rat model of retinal degeneration.
METHODS: We differentiated RPE and PR from hiPSC. We injected subretinally three groups of RCS rats with (i) a combination of PR and RPE, (ii) RPE alone or (iii) cell culture media. The visual function was assessed by electroretinography and the cell integration and survival by histology and immunohistochemistry.
RESULTS: At three months, eyes injected with a combination of PR and RPE responded significantly better to light stimuli than eyes with only RPE cells.
CONCLUSIONS: Our preliminary results might indicate that the combined cell therapy is a better approach to treat retinal degeneration. Furthermore, hiPSC-derived PRE and PR cells
iPSC-derived RPE cells exhibit the same features as the native RPE since they polarize, phagocyte photoreceptors outer segments, function as a barrier and express the specific markers. Similarly, differentiated PR cells show 18% of Rhodopsin + cells (rods) and 95% of M-Opsin + cells (cones).
was in concordance with retinal layers thicknesses, as we observed a higher number of cell rows in the outer nuclear layer. Such effect represents a potentially unlimited source of cell for regenerative medicine and open new opportunities to develop retinal cell models, screening of new drugs, and patient-specific retinal.

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Analysis of the extracellular matrix remodelling during zebrafish heart regeneration

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Cardiovascular diseases are a leading cause of mortality and morbidity in developed countries mainly due to the limitations of human heart to recover after an injury. Though, there are vertebrate organisms, such as zebrafish, that can regenerate their heart after a myocardial injury. Heart regeneration in zebrafish occurs through cardiomyocyte dedifferentiation and proliferation. Although how this process takes place is being highly studied, the role of extracellular matrix (ECM) is poorly understood. It is known that ECM proteins have an important role not only in giving structural support but also in signalling processes as development or tissue remodelling. So knowing the composition of ECM will help to better understand the zebrafish heart regeneration process. In our lab we have established a decellularization protocol of zebrafish ventricles to enrich the content of ECM proteins and better characterize the composition of the zebrafish ventricle ECM and analyse its changes during heart regeneration. In this study we have characterized the composition of ECM proteins in control and regenerating ventricles. Our results show an increase of periostin B and fibrinogens, and an overall decrease in collagens and proteins of cytoskeleton. This changes lead to a stiffening of the whole extracellular matrix contributing in the optimal environment for ventricle regeneration. In conclusion, zebrafish ventricle ECM changes its components and organization to promote regeneration.

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PI3K as a viable therapeutic target for Fibrodysplasia ossificans progressiva (FOP) Abstract

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Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant disease characterized by progressive spontaneous formation of heterotopic bone in muscle, fascia, ligaments and tendons. Currently, there is no approved therapy.

ACVR1 (Activin A receptor type 1), the gene responsible for FOP, encodes a Bone Morphogenetic Protein (BMP) type 1 receptor. This receptor is capable of binding ligands of the TGF-ß family and transduce osteogenic signaling. The most common mutation in patients with FOP (97%) is a single nucleotide mutation, 617G>A, which generates Arg206His mutation (R206H) in the resulting protein. This mutation is in the glycine-serine subdomain (GS) of the receptor and increases the signal transduction of BMP signaling pathways. In addition, the mutation leads to a gain of function by which the Activin A, a protein that signals through Smad 2/3 in a TGF-ß-like manner, becomes recognized by ACVR1 and transduce a signal through Smad 1/5/8, creating a new osteogenic signal. With this scenario, our group is trying to determine whether the non-canonical BMP signaling pathway through phosphatidilinositol 3-kinase (PI3K), which affects both the signaling and the osteogenic differentiation, can be a valid therapeutic target for the treatment of Fibrodysplasia ossificans progressiva. We present the results of this project showing changes in gene expression and protein levels in primary culture of mouse bone marrow mesenchymal stem cells (BMMSCs) expressing the R206H and Q207D FOP mutated forms of ACVR1. We also present the differential signaling in response to BMP2, BMP6 and Activin A and the effects of PI3K inhibition in the osteogenic signaling in mouse BMMSCs and induced pluripotent stem cells (iPSCs) from human FOP patients.

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