Societat Catalana
de **BIOLOGIA**

XXIII JORNADES DE BIOLOGIA MOLECULAR

Organitzades per la Secció de Biologia Molecular de la **Societat Catalana de Biologia**
amb la col·laboració de la Secció de Biologia i Indústria

INSTITUT D´ESTUDIS CATALANS

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Amb la col·laboració de:

CliniSciences

8:30-9:00 Recollida documentació

9:00 Benvinguda del coordinador de la Secció de Biologia Molecular

9:15-10:00 Conferenciant convidat

Cayetano González (IRB)
Modeling malignant growth in Drosophila.

10:00-10:40 Conferenciant convidat (yeast biology)

Francisco Antequera
(Instituto de Biología Funcional y Genómica, Salamanca)
Nucleosome positioning and genome evolution.

10:40-11:15 Cafè i Pòsters

Sala 1 (stem cells & regenerative biology)

11:15-11:30 Miquel Sureda (UB)
(1) Role of the Wnt-ßcatenin signaling during anterio-posterior axis specification and organogenesis in planarians.

11:30-11:45 Mario Notari (CMRB)
(2) The local microenviroment limits the regenerative potential of the mouse heart.

11:45-12:00 Xavier Roa (UIC)
(3) CPT1C is expressed in human mesenchymal stem cells and promotes cell survival through autophagy pathway and lipid droplets synthesis.

12:00-12:15 Oriol Iborra (IGTP)
(4) Undifferentiated allogeneic porcine iPS cells delivered within engineered constructs are safe but ineffectual in terms of improving cardiac function in aswine model of myocardial infarction.

12:15-12:30 Marta Vila (UAB)
(5) Optimal conditions to derive mESC from single blastomeres.

12:30-12:45 Daniel Vivas (Banc de Sang i Teixits)
(6) Development of an advanced cell therapy product indicated for the treatment of osteonecrosis of the femoral head.

12:45-13:00 Marta Grau (Banc de Sang i Teixits)
(7) Towards the clinical use of mesenchymal stromal cells derived from Wharton's jelly.

Sala 2 (yeast biology)

11:15-11:30 Soraya Játiva (IDIBELL)
(8) PP2A-Cdc55 Downregulation at anaphase onset by Zds1 and separase.

11:30-11:45 Margarita Cabrera (UPF)
(9) Early changes in protein localization during yeast aging.

11:45-12:00 Berta Canal (UPF)
(10) Mrc1 integrates multiple stress signals to prevent genomic instability in S-phase.

12:00-12:15 Clara Suñer (IBMB-CSIC)
(11) Stress induced reprogramming of proteolytic pathways.

12:15-12:30 Joaquin Ariño (IBB-UAB)
(12) In search for the in vivo targets of the S. cerevisiae protein phosphatase PTC1.

12:30-12:45 Stefan Huemmer (UPF)
(13) Crosstalk between U2AF65 and the 5'splice site.

13:00-13:40 Conferenciant convidat (stem cells)

Pia Cosma (CRG)
Wnt activity and dynamics regulates chromatin state in stem cells.

13:40-15:00 Dinar i Pòsters

15:00-15:40 Conferenciant convidat (structural biology)

Eva Estébanez (IBUB)
Crystal structure of human androgen receptor homodimer.

15:50-16:20 Conferenciant convidat (stem cells)

Angel Raya (CMRB)
Redefining cell identity through induced reprogramming.

16:20-16:45 Cafè i Pòsters

16:45-17:00 Arka Chakraborty (IBMB-CSIC)
(14) Protein-DNA interactions in Mitochondrial DNA packaging-Tables turned.

17:00-17:15 Andrea Chicano (UAB)
(15) Cryo-electron tomography study of the three-dimensional structure of metaphase chromatin plates.

17:15-17:30 Verónica Noé (UB)
(16) Usage of repair-polypurine reverse Hoogsteen hairpins as a tool to correct point mutations at the endogenous locus in mammalian cells.

17:30-17:45 Diana Luz Juárez (IDIBAPS)
(17) The role of mitochondrial dysfunction in G2019S LRRK2 mutation carriers in Parkinson's Disease, a study based on a fibroblast model.

17:45-18:00 Amy Curwin(CRG)
(18) Unconventional Protein Secretion.

18:00-18:15 Sébastien Lyonnais (IDIBAPS & IBMB-CSIC)
(19) Molecular 'sponge', RNP granule and quinary interactions. New concepts to describe HIV-1 maturation.

18:15-18:40 Conferenciant convidat —*Biologia i Indústria*—

Immaculada Herrero (MOWoOT)
Què ens cal als investigadors per muntar una empresa?

18:40 Lliurament del premi *Lluís Cornudella* i comiat

18:45 Cerveses i pòsters

- **STEM CELLS & REGENERATIVE BIOLOGY**

1

Role of the Wnt-βcatenin signaling during anterio-posterior axis specification and organogenesis in planarians

Miquel Sureda, UB

The multiple context-dependent roles of the βcatenin-Wnt pathway hinder the understanding of specific roles in embryonic and, especially, in adult tissues. Planarians, flatworms with the striking ability to regenerate and continuously change their size, offer us an ideal system to approach this issue. Planarians plasticity is based in the maintenance on a totipotent population of adult stem cells, which in turn demands the constitutive activation of the intercellular signaling mechanisms (ie. Wnt, Hh, BMP). Previous results demonstrate that *βcatenin-1* is required in planarians to specify posterior identity, and predict the existence of a Wnt-βcatenin activity gradient underlying the specification of the antero-posterior axis. Through the functional study of Wnt-elements and the generation of a βCATENIN-1 specific antibody, we demonstrate that a Wnt-βcatenin1 activity underlies the antero-posterior patterning of planarians from the pre-pharyngeal region to the tip of the tail. However, a *βcatenin-1* activity is also required in the head region to pattern the central nervous system. In addition, we have identified a genetic βcatenin duplication (*βcatenin-4*), which could function as a *βcatenin-1* inhibitor specifically in neural tissues. Finally, we demonstrate that *βcatenin-1* is also required in developing and regenerating organs, particularly during the specification of the germ-line. Altogether, our findings provide the first direct evidence of an antero-posterior nuclear βCATENIN-1 gradient in adult planarians, and uncover novel, context-dependent roles for βcatenin-1 during neural patterning and germ-line specification.

2

The local microenvironment limits the regenerative potential of the mouse neonatal heart.

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Cardiovascular diseases (CVDs) are the leading cause of death in the developed world, yet identifying a cure remains a major unmet medical goal. Although recent investigations have shown that adult human cardiomyocytes (CMs) can be replaced at a low but detectable rate, it is well documented that large injuries to the adult heart culminate in replacement of lost CMs with a collage-rich scar. This results in a progressive deterioration of the heart´s contractile capacity ultimately leading to end-stage heart failure.
Neonatal mice have been shown to regenerate their hearts during a transient window of time of approximately one week. However, experimental evidence for this is not undisputed, as some independent laboratories have failed to detect it. Here, we first confirmed that 1-day old neonatal mice were able to mount a robust regenerative response after heart amputation, whereas the hearts of 9-day old mice underwent massive scarring. Notably, this regenerative ability was lost within 48 hours, as hearts of 2-day old mice responded to amputation with fibrosis, rather than regeneration. We then compared the global transcriptome of 1- and 2-day

old mouse hearts and uncovered that most differentially expressed transcripts encode for extracellular matrix components and structural constituents of the cytoskeleton.
Taken together, our results identify an unexpectedly restricted time window of regenerative competence of the mouse neonatal heart, and suggest that it may be limited by extracellular matrix stiffening.

3

CPT1C is expressed in human mesenchymal stem cells and promotes cell survival through autophagy pathway and lipid droplets synthesis

Xavier Roa, Rut Fadó, Maher Atari, Núria Casals

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Carnitine palmitoyltransferase 1C (CPT1C) belongs to CPT1 enzymes family which control fatty acid entrance to the lumen of mitochondria for its oxidation. Nevertheless, the CPT1C brain isoform has a very low catalytic activity and it is localized in the endoplasmic reticulum. Recently, it has been demonstrated that CPT1C is also expressed in tumor cells, conferring them the ability to survive in hypoglycemic and hypoxia situations.
Given the high similarity among tumor cells and stem cells, in the present work we have studied whether CPT1C is expressed in human adult stem cells and its hypothetical protective role in metabolic stress situation.
At first, we have demonstrated that CPT1C is expressed in human mesenchymal stem cells (hMSC) from dental pulp, and that its expression increases when cells are grown in glucose depleted media. Secondly, we have found that CPT1C over-expression increases survival of human MSC against cellular damage induced by glucose depletion or 2-deoxyglucose (a glycolysis inhibitor), and that CPT1C silencing induces the opposite effect. Thirdly, we show that CPT1C overexpression does not alter the mitochondrial oxidative capacity but increases ATP levels production in glucose depleted conditions. And finally, we demonstrate that CPT1C promotes hMSC survival through the maintenance of the autophagic cycle during glucose deprivation. The significant accumulation of lipid droplets derived from increased autophagy in CPT1C over-expressed cells results in higher ATP levels in a lipolysis dependent mechanism.
CPT1C studies in hMSC can open new therapeutic strategies in regenerative medicine although further research is required.

4

Evaluation of the inflamatory response induced by engineered bioscaffolds of allogeneic porcine iPS implanted in a swine model of myocardial infarction

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The combined use of biomatrices and induced pluripotent stem cells (iPS) is potentially useful to repair myocardial scar after acute myocardial infarction (MI). However, undesired immune responses can drastically hamper outcomes after non-autologous cell transplantation.
Purpose: This work evaluates safety and efficacy of allogeneic transplantation of undifferentiated porcine iPSC with two different engineered constructs under three different conditions: an adipose pericardial graft (iPS+APG), an acellular pericardial scaffold (iPS+PER), or both (iPS+APG+PER), in a swine MI model.
Methods: Fifty-seven swine underwent MI by coronary artery ligation and were distributed randomly into three groups with biomatrices but porcine-iPS-free (APG n=6; Scaffold n=13; APG-Scaffold n=7), and three groups with iPS-enriched constructs (iPS-APG n=9; iPS-Scaffold n=11; and iPS-APG-Scaffold n=11). We then analysed the inflammatory effect by CD3, CD25 immunohistochemistry and cardiac function parameters by using magnetic resonance imaging (MRI) at baseline, 48h post-MI, and 30 days of follow-up, before sacrifice.
Results: immunohistochemistry analysis revealed iPS-APG-PER to display the most extended inflammatory response both at CD3 (P=0.005) and CD25 (P=0.002) levels. In contrast, iPS-PER-treated animals showed the lower inflammatory CD25/CD3 ratio (p=0,006). Moreover, MRI results displayed no significant recovery in cardiac function following iPS transplantation.
Conclusions: After a thorough follow-up, neither signs of adverse effects nor harmful reactions were detected in any of the animals studied, proving the safety of iPS treatment. However, cardiac MRI revealed no significant benefit. Our results showed a local inflammatory reaction only after delivery of iPS within the APG or the APG-PER constructs, suggesting that adipose vascularized tissue reacts against allogeneic iPS. Further improvements are needed in order to enhance the helpfulness of such treatments in cardiac tissue engineering applications.

5

Optimal conditions to derive mESC from single blastomeres

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Mouse Embryonic Stem Cells (mESC) derivation rate is determined by factors such as the genetic background, the culture medium and the presence of modifiers of signaling pathways' activity. This study aims to describe the effect of those variables when mESC are derived from single isolated blastomeres.
First, mESC were derived from whole blastocysts from permissive strain 129S2/Sv x C57BL/6 embryos, non-permissive CBA embryos and non-classified B6CBAF1 embryos using two DMEM-based media: a serum-free medium supplemented with N2B27 and a defined medium supplemented with Knock out Serum Replacement. For both media, one group was also supplemented with 2i, consisting of MAPK inhibitor PD0325901 and GSK3β inhibitor CHIR99021. Next, the same media combinations were used to derive mESC from single blastomeres biopsied from 8-cell stage embryos.
Defined medium allowed a high derivation rate with 129S2/Sv x C57BL/6 and B6CBAF1 blastocysts (74.3% and 77.4%, respectively) and a significantly lower derivation rate with CBA blastocysts (46.9%), indicating that B6CBAF1 behaves as a permissive strain. When 2i was added,

derivation rates were similar for all groups, irrespectively of the media used or the embryo genetic background (75.9%-96.9%).
Derivation rates were low for single blastomeres from the three strains cultured in serum-free medium with or without 2i (0.7%-5.13%). However, when blastomeres from permissive strains were cultured in defined medium, derivation rates (2.9-4.9%) were significantly increased with the addition of 2i (22.9%-24.5%). Although results are preliminary, this increase was not observed with CBA blastomeres (2.2%).
In conclusion, the higher rates of mESC were obtained when culturing single blastomeres from permissive strains in defined medium supplemented with 2i.

6

Development of an advanced cell therapy product indicated for the treatment of osteonecrosis of the femoral head

Daniel Vivas

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Background

Osteonecrosis of the femoral head (ONFH) is a pathologic process whose etiology, pathology and treatment remain unclear but it is known that can lead to femoral head collapse, which is treated by total hip replacement. Since ONFH may be a disease of bone cells, the possibility of stimulating bone regeneration in order to overcome that situation has been raised. Herein we report the development of a mesenchymal stromal cell (MSC)-based therapy, from conception up to a phase I/IIa clinical trial (NCT01605383).

Materials and methods

This endeavor required the design and execution of 1) five preclinical studies, 2) a GMP-compliant bioprocess for cell production and 3) a phase I/IIa clinical trial.

Results and Conclusions

1. The preclinical package included a proof-of-concept and two pharmacodynamic studies in a large animal model of ONFH and two regulatory pharmacokinetic/toxicological studies; one of them *in vitro* and the other one in a small animal model. These studies demonstrated the safety and efficacy of the cell therapy product.
2. A GMP-compliant bioprocess was developed for high titer cell expansion of MSC in a relative short period of time (21 days). The drug product consists of 5cc of bone loaded with $0.3\text{-}1\times10^6$ viable MSC/cc.
3. To date, twenty two patients have been included in the prospective, open-label with blinded assessor, randomized, parallel and single-dose phase I/IIa clinical trial for the treatment of ONFH ARCO grade I-II. Partial data confirms the feasibility and safety of the treatment, and suggests reactivation of neovascularization and osteoblastic activity as well as improving clinical results.

7

Towards the clinical use of mesenchymal stromal cells derived from Wharton's jelly

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The qualities of mesenchymal stromal cells (MSC) are currently being investigated in the clinical setting for the treatment of pathologies in which their multipotentiality and/or immunomodulatory capacity are required. Out of the variety of source tissues from which MSC can be derived, the umbilical cord (UC) offers many advantages as allogeneic cell therapy.
Methods
Comprehensive characterisation of UC-MSC was performed following the methodologies described previously (Oliver-Vila, Irene, et al. Cytotherapy 18.1 (2016): 25-35.), including cytometry, cytogenetics, growth kinetics and in vitro differentiation.
Results
Derivation of UC-MSC from Wharton's jelly was performed successfully from UC fragments (>7g) processed within 80 hours from birth. Cell culture expansion was performed following a two-tiered system of Master Cell Bank and Working Cell Bank compliant with the Good Manufacturing Practice (GMP) regulations.
Genetic stability was assessed by determination of human telomerase reverse transcriptase activity determination, senescence assay and c-myc expression analysis. Also, a karyotype analysis was performed to ensure no genetic abnormalities.
The morphology of the cells and their immunophenotype were checked before and after expansion confirming in both cases their mesenchymal identity.
Multipotentiality of the UC-MSC was proven by specific staining after differentiation into chondroblast, osteoblasts and adipocytes.
Immunomodulation was proven by a potency test performed in cocultures with stimulated peripheral blood-mononuclear cells.
Conclusions
Herein we propose a straightforward derivation and expansion GMP compliant process, in a cost effective and timely manner, resulting in a large number production of safe cells.

8

Downregulation of PP2A-Cdc55 at anaphase onset by Zds1 and separase

Soraya Játiva

Exit from mitosis and completion of cytokinesis require the inactivation of mitotic cyclin-dependent kinase (Cdk) activity. In budding yeast, Cdc14 phosphatase is a key mitotic regulator that is activated in anaphase to counteract Cdk activity. In metaphase, Cdc14 is kept inactive in the nucleolus sequestered by its inhibitor Net1. At anaphase onset, downregulation of PP2A-Cdc55 phosphatase by separase and Zds1 protein promotes Net1 phosphorylation and consequently, Cdc14 release from the nucleolus. The mechanism by which Zds1 and separase

impinge on PP2A-Cdc55 activity remains to be elucidated. Previous results show that Zds1 exert its biological function as PP2A-Cdc55 regulator, by controlling the subcellular localisation of the PP2A regulatory subunit Cdc55. Our previous results suggest that the activity of PP2A-Cdc55 cannot be modulated solely through regulation of its localization, and that an additional regulatory step may be required to control PP2A-Cdc55 activity during mitotic exit. Here we show that Cdc55 regulatory subunit is phosphorylated during anaphase upon PP2A-Cdc55 downregulation. Our results suggest that PP2A-Cdc55 activity is modulated throughout Cdc55 posttranslational modifications in a separase and Zds1-dependent manner.

- **YEAST BIOLOGY**

9

Early changes in protein localization during yeast aging

Margarita Cabrera 1, 2, Liesbeth Veenhoff 1 and Michael Chang 1

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Yeast replicative lifespan indicates the number of daughter cells produced by a single cell. Wild type cells exhibit a limited replicative potential that corresponds to 25 divisions before senescence. During this journey cellular organelles undergo dramatic changes leading lately to cell senescence and death. An early change observed with age is the size increase of the vacuole, the lysosome equivalent in yeast. This process is accompanied by the loss of vacuole acidity that has been shown to trigger mitochondria dysfunction. The normal mitochondria network is disrupted in aged cells, although is not clear if this event is common to the whole population. Other hallmark of replicative aging is genomic instability that is especially important within the rDNA repeats where the formation of ERCs (extrachromosomal rDNA circles) has been reported. Protein oxidation seems to contribute to replicative aging in several organisms. These damaged proteins tend to form aggregates, which become targets of autophagy and chaperones.
A main challenge in the aging field is the application of high throughput methods to study replicative lifespan. The traditional approach used to monitor replicative potential in yeast consists in the isolation by micromanipulation of the daughter cells produced by a single mother cell. Recently several microfluidic systems have been developed to allow an easier and quantitative analysis of the replicative capacity of yeast cells. We combine in this study the Mother Enrichment Program (MEP) system to select aged mother cells with a microfluidic device called CellASIC that allows the visualization of aged cells for long periods.

10

Mrc1 integrates multiple stress signals to prevent genomic instability in s-phase

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Replication and transcription processes are coordinately regulated to prevent genomic instability. Specific DNA structures and proteins isolate replication and transcription processes in highly transcribed regions of the genome. General transcription, in the other hand, takes place all over the genome and is thought to be temporary separated from replication. However, cell adaptation to abrupt environmental changes requires fast and massive reprogramming of the gene expression pattern. This transcriptional activation also occurs during S-phase and poses risk of collision between replication and transcription machineries, requiring a precise coordination of the replication and transcription to prevent genomic instability. We described the existence of a dedicated checkpoint pathway to coordinate both machineries upon osmostress-induced transcriptional outbursts (Duch et al., 2013). In response to osmostress, the same protein that orchestrates the activation of hundreds of osmoresponsive genes, the Stress-Activated Protein

Kinase (SAPK) Hog1, phosphorylates the replisome protein Mrc1 to block DNA replication in order to prevent genome stability. Mutation of the three Hog1 target sites in Mrc1 avoids S-phase arrest leading to Transcription-Associated Recombination (TAR) and the subsequent genomic instability upon osmostress. Interestingly, this mechanism operates independently of the known DNA damage checkpoint pathway, pointing out the necessity of a dedicated S-phase checkpoint to deal with the massive transcription upon osmostress. The aim of this project is to study if Mrc1 is also targeted upon other environmental stresses demonstrating the existence of a universal mechanism to protect genomic integrity upon stress-induced transcriptional outbursts.

11

Stress induced reprogramming of proteolytic pathways

Clara Suñer

Despite much evidence of the involvement of the proteasome-ubiquitin signaling system in temperature stress response, the dynamics of the ubiquitylome during cold response has not yet been studied. Here, we have compared quantitative ubiquitylomes from a strain deficient in proteasome substrate recruitment and a reference strain during cold response. We have observed that a large group of proteins showing increased ubiquitylation in the proteasome mutant at low temperature is comprised by reverses suppressor of Ty-phenotype 5 (Rsp5)-regulated plasma membrane proteins. Analysis of internalization and degradation of plasma membrane proteins at low temperature showed that the proteasome becomes determinant for this process, whereas, at 30 °C, the proteasome is dispensable. Moreover, our observations indicate that proteasomes have increased capacity to interact with lysine 63-polyubiquitylated proteins during low temperature in vivo. These unanticipated observations indicate that, during cold response, there is a proteolytic cellular reprogramming in which the proteasome acquires a role in the endocytic-vacuolar pathway.

12

In search of the in vivo targets of the S. cerevisiae protein phosphatase Ptc1

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Ptc1 is one of the seven isoforms of the type 2C protein phosphatase (Ptc1-7) in the budding yeast Saccharomyces cerevisiae and likely the most relevant, as deduced from the numerous phenotypic defects exhibited by the ptc1 mutant. However, only a few Ptc1 cellular targets, accounting for a limited number of phenotypes, have been so far identified. To understand the molecular basis of Ptc1 function we undertook two complementary approaches. First, we developed a genetic screen in search of mutations in protein kinase–encoding genes able to suppress multiple phenotypic traits caused by the ptc1 deletion. The second approach was based on a high-copy number suppressor screen for three characteristic ptc1 phenotypes (sensitivity to Calcofluor White, rapamycin, and alkaline pH). The combination of both research lines revealed that Ptc1 has a preeminent role in signaling through the CWI pathway and that the key function of Ptc1 is to dephosphorylate and inactivate Mkk1, one of the two MAPK kinases upstream the Slt2 MAPK, to ensure return to basal CWI pathway activity. We show that hyperactivation of the Slt2 module is responsible for many of the phenotypic defects of a strain

lacking Ptc1, including hyperphosphorylation of Cdc28 and arrest in G2-M cell cycle transition, therefore pointing to Mkk1 as a major physiological Ptc1 target in vivo.

13

Crosstalk between U2AF65 and the 5`splice site

Stefan Huemmer, Angel Guerra-Moreno, Elena Hidalgo and Jose Ayte

Splicing of introns from pre-mRNA is one of the essential steps of gene expression in eukaryotes and aberrant splicing is a dominant cause for a variety of diseases including leukaemia and cancer. Introns are excised from the nascent mRNA by highly dynamic multi-component ribonucleoprotein complex, called the spliceosome. The assembly of the spliceosome on pre-mRNA splice sites is initiated by the commitment complex (C-complex) and a key factor in this complex is U2AF65.
To gain further insides into the exact molecular mechanism of U2AF65 during splicing, we have examined the transcriptome of S. pombe in the presence and absence of a functional Prp2, the U2AF65 homolog in S. pombe, by deep sequencing. In accordance with the essential function of Prp2, our data revealed that splicing of the vast majority of the introns is strongly dependent on Prp2. Yet, a significant group of introns was spliced equally well independent of prp2 function. This results could be confirmed by conventional RT-PCR of several candidate genes.
By the use of synthetic genes, in which we exchanged the introns, or placed a Prp2-dependent intron in the position of an independent one, we could determine that not the intron per se, but rather the intron in the context of its surrounding exon sequences provides the features required for Prp2-independent splicing. Combining bioinformatics and mutational analyses, we could reveal, that position -1 of the 5`splice site is absolutely critical for Prp2-dependency in splicing. Thus, an extensive crosstalk between the 5`splice site and U2AF65 at the 3`splice site seems to be required for efficient splicing.

14

Protein-DNA interactions in Mitochondrial DNA packaging-Tables turned

Arka Chakraborty (IBMB-CSIC)

15

Cryo-electron tomography study of the three-dimensional structure of metaphase chromatin plates

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We have used cryo-electron tomography to investigate the chromatin folding in metaphase chromosomes. This technique has allowed us to study chromosome structure in a vitrified and close-to-native state. Our three-dimensional reconstructions show that frozen-hydrated

chromatin emanated from metaphase chromosomes is planar and form multilayered plates, as previously observed in our laboratory using different techniques. Plate thickness measurements show that each single layer is ~10 nm thick, which is equivalent to the nucleosome diameter. These measurements, combined with the observation of many plates contained in the three-dimensional reconstructions, indicate that each plate is formed by a tightly packed single layer of nucleosomes. The same nucleosome organization has been observed in staked layers, but distance measurements in contacting regions between two plates show a thickness of ~16 nm. This distance is smaller than that expected for the sum of two single layers (~20 nm), and indicate that the nucleosomes from both plates are interdigitated and that each layer has an apparent thickness of ~6 nm, as proposed in our thin-plate model for the chromosome internal structure. Plates show dense bars along their cross-sections, which are more or less parallel between them and perpendicular to the surface of the plates. Taking into account the thickness of each layer, these bars could correspond to nucleosomal DNA wrapped around histone octamers. We have observed very large chromatin structures formed by many stacked plates, which have a width equal to the chromosome diameter. All these observations reinforce previous results of our laboratory and support a compact chromosome model consisting of many interdigitated chromatin layers stacked along the chromosome axis. Our results indicate that nucleosomes are oriented with their flat faces approximately perpendicular to the surface of the plate, allowing a lateral interaction between nucleosomes of adjacent layers which gives stability to the overall structure.

16

Usage of repair-polypurine reverse Hoogsteen hairpins as a tool to correct point mutations at the endogenous *locus* in mammalian cells

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Nowadays, the modulation of gene expression by nucleic acids has become a routine tool in biomedical research for target validation and it is also used to develop new therapeutic approaches. Recently, we developed the so-called polypurine reverse Hoogsteen hairpins (PPRHs). PPRHs are hairpins formed by two antiparallel polypurine strands bound by intramolecular Hoogsteen bonds linked by a pentathymidine loop. These hairpins are able to bind to their target DNA sequence through Watson-Crick bonds. We first developed the PPRH technology as a gene silencing tool that show high stability and a low immunogenic profile and we demonstrated their efficacy both *in vitro* and *in vivo*. Since one of the homopurine strands binds with antiparallel orientation by Watson-Crick bonds to the polypyrimidine target sequence, forming a triplex, we had previously reported the ability of PPRHs to effectively bind dsDNA displacing the fourth strand away from the newly formed triplex.
The main goal of the present work was to explore the possibility of repairing a point mutation in mammalian cells using PPRHs as tools. These repair-PPRHs contain different combinations of extended sequences of DNA with the corrected nucleotide to repair the point mutation. As a model we used the *dihydrofolate reductase* (*dhfr*) gene. We had previously demonstrated *in vitro* that PPRHs bind specifically to their polypyrimidine target sequence, opening the two strands of the dsDNA, and allowing the binding of a given repair oligonucleotide to the displaced strand of the DNA. Subsequently, we showed at a cellular level (Chinese ovary hamster cells) that repair-PPRHs are able to correct a single-point mutation in a *dhfr* minigene bearing a nonsense mutation, both in an extrachromosomal location and when the mutated plasmid was stably transfected into the cells. To further evaluate the utility of these molecules, different repair-PPRHs were designed to correct insertions, deletions, substitutions and a double substitution present in a collection of mutants at the endogenous *locus* of the *dhfr* gene. We also describe an approach to use when the point mutation is far away from the homopyrimidine target domain. This strategy consists in designing Long-Distance- and Short-Distance-Repair-

PPRHs where the PPRH core is bound to the repair tail by a 5-thymidine linker. Surviving colonies in a DHFR selective medium, lacking glycine and sources of purines and thymidine, were analyzed by DNA sequencing, and by mRNA, protein and enzymatic measurements, confirming that all the *dhfr* mutants had been corrected. These results show that repair-PPRHs can be effective tools to accomplish a permanent correction of point mutations in the DNA sequence of mutant mammalian cells.

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17

The role of mitochondrial dysfunction in G2019S LRRK2 mutation carriers in Parkinson's Disease, a study based on a fibroblast model

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide. PD has been associated to mitochondrial dysfunction by inhibition of the mitochondrial respiratory chain and increase of oxidative stress. Mutations in the leucine-rich repeat kinase 2 (LRRK2), including G2019S, are the most frequent cause of dominant inherited familiar PD and have been associated to mitochondrial dysfunction and autophagy regulation, however, the ultimate molecular and biochemical mechanism leading to the development of the disease is still unknown.

We hypothesize that mitochondrial dysfunction may underlie PD aetiology and thus the manifestation of clinical symptoms. We aimed to compare the mitochondrial dysfunction phenotype in a group of PD GS2019S-LRRK2-carriers, asymptomatic ($_{LRRK2}$PD-) *vs* symptomatic ($_{LRRK2}$PD+), with respect to healthy controls (C), using established techniques. Secondary objectives include to validate the utility of cultured fibroblasts as an experimental disease model that reproduces the pathophysiology of PD and to associate the presence of potential mitochondrial alterations to the presence of symptoms to search for disease aetiology, putative biomarkers and therapeutic targets.

Methods: Fibroblasts from skin biopsy of G2019-LRRK2-mutation carriers were cultured in glucose or galactose medium, the latter to emulate neuronal metabolism, to measure: mitochondrial membrane potential (MMP) (by flow cytometry), cytochrome c oxidase (COX) activity (by spectrophotometry), ATP levels (by luminometry) and autophagy (by western blot).

Results: MMP and COX activity appears to be significantly affected in $_{LRRK2}$PD groups, with an increase in the percentage of depolarization in $_{LRRK2}$PD- cohort (when exposed to glucose) and a decline of COX activity for $_{LRRK2}$PD+ when compared to $_{LRRK2}$PD- (when exposed to galactose). Conversely, there were no significant differences between groups in ATP levels and autophagy.

Conclusions: G2019-LRRK2-carrying fibroblasts partially reproduce PD pathophysiology reinforcing their use as a PD model in further investigations. Coming efforts are needed to

search for mitochondrial or autophagic parameters differentially altered in LRRK2-carriers depending on the presence of symptoms.

18

Unconventional Protein Secretion

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Cells produce thousands of different proteins with a variety of different roles in the body. Some proteins, for example the hormone insulin, perform roles outside of the cell and are released from cells in a process that has several stages. In the first step, newly-made insulin and many other “secretory” proteins enter a compartment called the endoplasmic reticulum. Once inside, these proteins can then be loaded into other compartments and ultimately transported to the extracellular space. Collectively this process has been termed “conventional protein secretion” in order to distinguish it from another class of secretory proteins that are released from the cell without first entering the endoplasmic reticulum. This process is termed “unconventional protein secretion” and comparatively very little is known about the molecular mechanisms involved. A protein called Acb1 is released from yeast cells in this manner. Previous research identified a novel compartment (named CUPS) that is involved in this process. CUPS form specifically in conditions that promote Acb1 secretion and need to mature into a form that is involved in the release of Acb1 proteins from the cell. Acb1 is only found in the mature CUPS. This maturation process requires some of the proteins that are involved in producing another type of compartment in the cell called a multivesicular body (MVB). These proteins, called ESCRTs, somehow work to shape the CUPS, but in a mechanism that is distinct from that in MVB formation. Therefore MVBs are not involved in the release of Acb1 from the cell and the challenge remains to understand the final steps of CUPS maturation and Acb1 secretion.

19

Molecular 'sponge', RNP granule and quinary interactions. New concepts to describe HIV-1 maturation

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A growing number of studies show that long RNA molecules can control protein population in the cell by assembling dynamic ribonucleoprotein (RNP) granules. These molecular 'sponges' are stabilized by quinary (transient and weak) interactions and concern proteins involved in numerous biological functions. HIV-1 forms by self-assembly through binding of the viral RNA (vRNA) by membrane-bound Gag and GagPol polyproteins precursors. Infectivity requires extracellular budding of the particle followed by its maturation: the ~2400 Gag and ~120 GagPol are cleaved by ~60 viral proteases (PR), which sequentially remodel virus internal architecture. The vRNA is condensed by the NCp7 nucleocapsid released from Gag, while CAp24-self-assembled capsid encapsulates this RNP. We will describe here this molecular choreography, focusing on the RNP formation by NCp7 and using the concepts of RNP granule formation. We describe successive weak-strong-moderate quinary NC-gRNA networks during the sequential processing of the GagNC domain by original in vitro tools such as atomic force microscopy and dynamic light scattering. We finally show how PR is sequestrated within this RNP, accelerating the vRNA:NCp7 complex maturation/condensation within minutes, much faster than previously proposed, which unambiguously links virus maturation with its budding from the cell membrane. We anticipate such findings to stimulate further investigations of quinary interactions and macromolecular machines in crowded environments throughout the broad and flourishing array of RNP granules.

PÒSTERS

- # BIOLOGIA MOLECULAR (GENERAL)

1

Possible role of Gαq in mitophagy

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Heterotrimeric G proteins are well-established mediators of signal transduction pathways downstream of G protein-coupled receptors (GPCRs). There is supporting evidence that demonstrates the novel localization of Gαq at the mitochondria, where it participates regulating fusion/fission processes and mitochondria physiology. Mitochondrial dynamics are closely related to mitochondrial quality and, therefore, cellular quality control. Mitophagy is a mechanism that cells utilize to get rid of damaged mitochondria. For the mitophagy process the interaction between mitochondria and autophagosome via Fis1-TBC1D15/17-LC3-complex and the control of Rab7 activity are needed.

A proteomic analysis showed that Gαq and Gα11 bind to Fis1 and TBC1D17. Here, we found through immunoprecipitation studies the interaction between the active form of Gαq (Gαq-GTP) and Fis1, TBC1D15/17 and Rab7. Additionally, we observed that Gαq overexpression or depletion alters mitophagy process by means of analyzing LC3 fragmentation and accumulation. Interestingly, the interaction between $G\alpha_q$ and Fis1 decreases during the process of mitophagy. Taken together, our results point to a possible role of Gαq controlling the balance between mitophagy and cell death.

2

Dissecting Parkinson's disease genetic complexity by combining gene editing and patient's cells

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Since the discovery of the first genetic variant related to Parkinson's disease (PD), many disease-causing mutations as well as risk loci have been identified. However, the genetic component of Parkinson's disease remains largely unknown. Mutations in LRRK2 gene are the most common cause of familial PD. The disease presentation of patients with LRRK2 mutation is typically clinically indistinguishable from sporadic PD cases, making the onset of disease due to LRRK2 dysfunction clinically relevant. We have successfully derived iPSC lines from patients with idiopathic PD and patients carrying G2019S mutation in the LRRK2 gene and showed that iPSC-derived dopaminergic neurons from PD recapitulate human disease phenotypes such as increased susceptibility to neuronal degeneration, abnormal α-synuclein accumulation, and alterations in the autophagy machinery. Moreover, we have showed that PD patient-specific iPSC capture the increased susceptibility of PD patients to undergo age-related DAn neurodegeneration, indicating that this susceptibility is likely to be encoded in the patients' genome, both for familial (associated to LRRK2 mutations) and sporadic PD. As a follow-up of this study, here we proposed to generate a complementary set of iPSC lines that will allow directly testing the relative contribution of pathogenic mutations and gene susceptibility factors to PD-related DAn neurodegeneration. For this purpose, we derived iPSC lines from non-manifesting carriers (NMC) of LRRK2 mutations, as well as isogenic controls that differ only in the presence or absence of the mutation, by targeted gene edition in our already established LRRK2-PD iPSC lines. Dopaminergic neurons differentiated in parallel from this subset of iPSC lines have been cultured over a long time span and monitored for the appearance of neurodegeneration phenotypes (including reduced numbers of neurites and neurite arborization, accumulation of autophagic vacuoles, and α-synuclein accumulation) after 75 days in culture. Interestingly we found that while PD iPSC-derived DA neurons showed altered morphology and shorter/fewer neurites, DAn derived from NMC show mature morphology and long neurites with complex arborization, similar to those differentiated from Ctrl-iPSC. We have also identified mutation-linked phenotypes such as α -synuclein accumulation whose appearance was delayed in NMC neurons compared to LRRK2-PD neurons. Assays to assess the effects of the LRRK2 mutant on autophagy and synaptic vesicle dysfunctions that are relevant to PD, are being established. The availability of a refined set of PD patient- specific iPSC lines representing symptomatic and asymptomatic cases of familial PD sharing the same pathogenic mutation in LRRK2, as well as isogenic iPSC lines in which the mutation has been edited out, will provide a unique test bed for revealing the specific genetic determinants contributing to or preventing the neurodegeneration in PD.

3

Retinoblastoma-independent effects of the Cdk4/6 inhibitor Palbociclib in primary glioblastoma cells

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Gliomas are the most common primary malignancies in the central nervous system. Glioblastoma cells show high rates of migration and infiltration in the surrounding tissue even in initial grades, which make them very prone to reappear after treatment or surgery. Ccnd1 expression is

frequently amplified in glioblastomas and correlates with high malignance and poor prognosis. Inhibition of the Ccnd1-cdk4 complex by adding Palbociclib strongly decreases the proliferation of glioblastoma cell-lines RB1 positive and intracranial xenografts in mouse models *(Michaud et al, 2010. Cancer Res 70, 3228)*.
Cyclin D1 (Ccnd1) together with its binding partner, cyclin-dependent kinase 4 (Cdk4), is involved in the regulation of proliferation and migration, and abnormal Ccnd1 expression promotes tumor growth and metastasis. Our previous data indicates that Ccnd1-Cdk4 is acting in the control of cell adherence and invasion through paxillin phosphorylation and Ral-GTPase activation *(Fernández et al, 2011. Oncogene* 30, 1936, *NP Fusté et al,* 2016 *Nature Communitacions)*. However, little is known about the effects of Ccnd1 in cancer stem cells and decrase in invasion ability of glioblastomas.
Our objective is to measure the importance of the Ccnd1-dependent activity in cancer stem cells and the control of invasion of those tumors analyzing whether this control is executed through the phosphorylation of paxillin and the activation of Ral-GTPases. Our results indicate that inhibition of Ccnd1-cdk4 complex by Palbociclib affects viability in primary tumors and cancer stem cells (tumorospheres) promoting apoptosis even in Rb deficient cells. We have demonstrated that inhibition of Ccnd1-cdk4 complex in glioblastoma primary cell cultures is able to decrease invasion indicating that Ccnd1 is a regulator of this pathway.
Our data suggests that Ccnd1 is relevant in cancer stem cells viability and in the regulation of invasion capacity of the cells in glioblastomas through the phosphorylation of paxillin and the activation of Ral-GTPases.

4

Caracterització d'un model murí doble knockout PTEN-VDR

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La vitamina D és una hormona que regula l'homeòstasi del calci i fòsfor en l'organisme; i en la mineralització dels ossos. La forma activa de la vitamina D, la $1,25(OH)_2D_3$ o calcitriol duu a terme la seva funció biològica mitjançant la unió al receptor de la vitamina D (VDR). L'element regulador negatiu de la via PI3K-AKT és PTEN que s'encarrega de generar PIP2 a partir de la desfosforilació de PIP_3. PTEN va ser descobert inicialment com un gen supressor tumoral amb un paper en la regulació de la proliferació i supervivència cel·lular. La pèrdua de PTEN promou un augment del creixement cel·lular i la supervivència. PTEN i VDR són dos gens supressors tumorals i relacionats amb el metabolisme de la glucosa.
En el nostre laboratori hem generat un model murí doble knockout induïble PTEN-VDR (PTEN-VDR-KO). S'ha observat una mort prematura dels ratolins PTEN-VDR-KO que no sembla deguda a un augment de les neoplàsies, com s'esperaria a priori, i per això s'ha fet un estudi general d'aquest model murí.
És sabut que la supressió del gens PTEN i VDR produeix alteracions en el metabolisme de la glucosa. Els models PTEN-VDR-KO presenten hipoglucèmies severes i nivells baixos de pèptid C (en efecte, insulina) en sang. Així com també nivells baixos d'insulina excretada pels illots pancreàtics després de tractar-los amb glucosa. Els nivells baixos d'insulina en sang s'explicarien ja que no hi ha glucèmia elevada i l'alliberament d'insulina és depenent de glucosa. Tot aquest

mal funcionament creiem que pot estar degut a que aquests ratolins tenen una major excreció de glucosa en orina, tenint així, algun defecte en els transportadors de glucosa en els ronyons.

5

Candidate deubiquitinating enzyme genes in retinal dystrophies

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Introduction: Post-translational modifications, such as conjugation of ubiquitin, are crucial for the differentiation of specific retinal neurons and may play a role in photoreceptor cell fate. Ubiquitination is dynamic and reversible since the ubiquitin moiety is deconjugated from protein targets by deubiquitinating enzymes (DUBs). Many DUBs are implicated in human pathological disorders from cancer and neurodegeneration to hereditary visual disorders. Besides, knockout or knockdown animal models of specific DUB genes show severe neuronal and eye phenotypes.
Methods: We aimed to characterize the gene expression pattern of several DUBs in the mouse retina. To this end, we detected mRNA and protein localization by *in situ* hybridization and fluorescent immunodetection. As a proof of principle, we also performed *knockdown* of selected DUB genes by morpholino microinjection in zebrafish embryos to study the resulting phenotype.
Results: Several expression patterns emerge, from ubiquitous expression to DUBs mostly detected in the photoreceptor layer or axonal processes, pointing to specific functions for different DUBs in the retina. Zebrafish knockdown morphants of selected DUBs show moderate to severe eye morphological defects, with defective formation of retinal structures: no lamination, no observable plexiform layers nor differentiated photoreceptors. These results support the relevance of some DUBs in the formation and differentiation of the vertebrate retina, making them good gene candidates for inherited retinal dystrophies or other visual disorders.
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6

Differential TGF-ß actions in wild type and PTEN-deficient endometrial epithelial cells.

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TGF-ß is a multifunctional cytokine involved in the regulation of a wide range of biological processes such as proliferation, differentiation and apoptosis. Increasing evidences demonstrate that such variability is attributable to differences in cellular context. During carcinogenesis, TGF-ß is a double-edged sword: it acts as a potent tumor suppressor on normal or pre-malignant

cells but it is a tumor promoter for malignant stages. The molecular mechanisms through which TGF-ß switches from a tumor suppressor to a tumor enhancer are not fully understood.
The PI3K/Akt pathway plays a pivotal role in the regulation of cell survival and proliferation and is frequently altered in human cancers. PI3K/Akt signalling is negatively regulated by PTEN; which is the most frequently mutated tumor suppressor gene in endometrial carcinomas.
Because both TGF-ß/Smad and PI3K/Akt signalling pathways participate in the control of similar cellular processes such as cell proliferation or apoptosis on female uterine tract, a complex interplay between these two pathways is required to coordinate and integrate cellular outcomes. However, the mechanisms of crosstalk between these two pathways are not completely elucidated and several models have been proposed.
In the present work, we have used 3D cultures of mice endometrial epithelial cells to analyse the TGF-ß/Smad and PI3K/Akt regulatory axis. We demonstrate that TGF-ß triggers apoptosis of wild type endometrial epithelial cells in a Smad3-dependent, but Smad2-independent manner. Mechanistically, we show that TGF-ß induced apoptosis is caused by an increase of PTEN transcription, which results in an inhibition of the PI3K/Akt pathway. In contrast, on a PTEN knock-out context TGF-ß-induced apoptosis is completely blocked. These results provide a new Smad3/PTEN regulation axis that can explain differential endometrial epithelial cells response to TGF-ß.

7

A new non-canonical pathway of G(q) protein regulating mitochondrial dynamics

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A novel localization of heterotrimeric G proteins at the mitochondria and their implications on the physiology of the organelle has been recently reported (Beninca et al., 2014; Zang et al., 2010;). In particular, the Gq subfamily is required to keep the proper balance between mitochondria fusion and fission acting at both outer and inner membrane dynamics (Beninca et al., 2014; Sánchez-Fernández G et al., 2014). Together with Gß (that binds to Mfn1), Gαq stabilizes elongated mitochondria and cristae structure. Gq is also necessary for the maintenance mitochondrial membrane potential and the activity of the respiratory chain and mitochondrial ATP synthesis. Surprinsingly, Gq is necessary for the supercomplex assembly at the inner membrane. The molecular mechanism of action of heterotrimeric G proteins at the mitochondria is still unknown. A recent MS-proteomic analysis has helped us to decipher the Gq-interactome ("Gq-mitoproteome"). We have utilized mitochondrial enriched fractions from four different cell lines, among them the Gq/11-MEF knockout, the recover Gq-MEF-knockout, MEFs wild type and NIH3T3 cells, as well as, two different anti-Gq antibodies. The new candidate binding proteins are being analyzed by their capacity to interact to Gq. Among the candidates outer and inner mitochondrial binding partners are present, proteins necessary for mitochondrial protein import, as well as, proteins involved in the respiratory chain response, mitochondrial dynamics and mitophagy. In summary our group postulates a new non-canonical mitochondria-function of heterotrimeric G proteins that involves their translocation to the mitochondria and the interaction with several mitochondrial partners.
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8

Silencing of CD47 and SIRPα by Polypurine reverse Hoogsteen hairpins promotes MCF-7 breast cancer cells death by macrophages

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In the context of tumor immunology, tumor cells have been shown to overexpress CD47, a “don’t eat me” signal directed to macrophages, to escape from phagocytosis by interacting with Signal Regulatory Protein α SIRPα. In the present work, we designed Polypurine reverse Hoogsteen hairpins, PPRHs, to silence both the expression of CD47 in tumor cells and SIRPα in macrophages with the aim to eliminate tumor cells by macrophages in co-culture experiments.
THP-1 cells were differentiated to macrophages with PMA, showing an increase in the mRNA levels of macrophage surface markers (CD14, Mcl-1) and pro-inflammatory cytokines (IL-1β, IL-18, IL-6, IL-8 and TNF-α) as determined by qRT-PCR.
The ability of PPRHs to silence *CD47* and *SIRPa* was evaluated at the mRNA level by qRT-PCR and at the protein level by Western Blot. PPRHs were able to decrease both CD47 expression in tumor cells and SIRPα expression in macrophages at the mRNA and protein levels.
Macrophages were co-cultured with tumor cells in the presence of PPRHs to silence CD47 and/or SIRPα. Watson and Crick- and scrambled-PPRHs were used as negative controls. In the presence of PPRHs, tumor cells were eliminated by macrophages in co-culture experiments, whereas tumor cells survived in the presence of negative control PPRHs. Moreover, the effect produced by the two PPRHs was similar to that observed with a monoclonal antibody directed against the interaction domain of CD47 with SIRPα. We also explored the mechanism involved in tumor cell death by determining the degree of apoptosis. The incubation for 24 and 48 h with the two PPRHs caused an increase in apoptosis.
These results indicate that PPRHs could represent a new approach with immunotherapeutic applications.

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9

Generation of murine models for retinal dystrophy studies

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Introduction: Mutations in over 200 genes are associated to inherited monogenic retinal degenerative 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, to dissect and characterize their precise role in photoreceptor cells.
Methods: We have generated mouse models by the new approach RNA-guided endonuclease CRISPR/Cas9 system. For *CERKL*, we performed a full gene deletion of nearly 100 kb. For *NR2E3*, we aimed to delete some of the functional domains. After zygote injections and embryo transfer, mosaic pups were genotyped to characterize the modified alleles, and were used as founder animals to obtain heterozygous homozygous mice in subsequent matings.
Results: No off-target mutations have been detected in the genetically modified chimaeric mice. Experiments to assess the effect of CERKL and NR2E3 deletions in retinal phenotype are currently being carried out on wildtype, heterozygous and homozygous littermates. Retinal morphology and functionality is being assessed and compared to other knock-out and knock-down animal models. Our preliminary phenotypic analysis of the new genetically modified models show retinal morphological alterations that need further analysis and that probably are dysfunctional. These new genetically modified strains will provide further insights into the role of these two genes in visual disorders.
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10

Severe defects in brain connectivity in RhoE knock-out embryos

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Axon guidance regulation in the developing brain often requires previous migration of some neurons or other axons. Ultimately, this is mediated by axon guidance cues that act through specific receptors to remodel axon cytoskeleton and trigger attractive or repulsive responses. In thalamocortical projections (TCAs), migration of the so-called "corridor cells" is necessary for TCAs to cross the subpallium. Later in development, corticofugal axons have been proposed to help TCAs to reach the pallium in what is known the "hand-shake" hypothesis. Other axon-axon interactions, such as the striatal-thalamic interaxonal interactions have been proposed to help TCAs. Rho GTPases are well-known transducers of the effect of axon guidance cues on

cytoskeleton dynamics. RhoE is an atypical RhoGTPase involved with axon growth and neuron migration. *RhoE* knock-out mice (*RhoE* gene-trapping allele or *gt*) show postnatal lethality, neurodevelopmental delay and impairment of olfactory bulb development. We used this mouse model to study brain axonal connectivity during development. Immunofluorescence and DiI/DiA tracing analysis revealed that *RhoE*$^{gt/gt}$ mice show severe axonal projection defects: TCAs are unable to cross the diencephalon-telencephalon boundary (DTB), striatonigral axons (SNAs) are misguided ventrally and corticothalamic axons are disorganized. Surprisingly, Islet1 staining shows a properly formed corridor at rostral levels, although it is wider at caudal ones. RhoE is expressed in subventricular regions and in striatum mantle and thalamus. However, we propose that TCAs missdevelopment is indeed secondary to SNAs misguidance for which RhoE, through an unknown upstream receptor, is a key signaling regulator. We are currently working on the hypothesis that SNAs exert an attractive/permissive effect on TCAs to cross the DTB. In summary, our results indicate a function of RhoE in the correct development of brain connectivity and an important role of SNAs in TCAs guiding through the DTB.

11

Potential antiparasitic DNA minor groove binding drugs against *Trypanosoma brucei*

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Human African trypanosomiasis (HAT) is a neglected tropical disease caused by parasites belonging to the genera Trypanosama, which circulate in the bloodstream and tissue fluids of their mammalian hosts and are transmitted by tsetse flies in sub-Saharan Africa. There are two subspecies of *Trypanosoma brucei: T. b. gambense* and *T. b. rhodesiense*, responsible for the human diseases sleeping sickness, or HAT, which is fatal if left untreated.

Although there are a few drugs available for HAT treatment (i.e., suramin, pentamidine, melarsoprol, eflornithine monotherapy, and nifurtimox-eflornithine combination therapy), these are far from being ideal due the high toxicity, unacceptable side-effects and the low effectiveness against some trypanosome species.

Kinetoplastid parasites, such as *Trypanosoma brucei* are distinguish by the presence of a mitochondrial AT-rich DNA structure called kinetoplast, constituted by a network of circular DNA (kDNA) commonly arranged in a disk-shaped planar array.

We have previously studied the compounds FR60 and CDIV32, a couple of bisimidazolinium diphenyl compounds discovered by Dr. Dardonville's group at the *Instituto de Química Médica-CSIC* in Madrid, as potential DNA ligands. It is been demonstrated that they have high affinity to bind DNA, specifically the minor groove of AT rich regions, and therefore, it is been suggested as an anti-kinetoplastid activity compound against trypanosomes.

A series of 10 chloro, fluoro, and pyridinyl derivatives of FR60 were tested *in vitro* against wild-type *Trypanosoma brucei* in order to investigate the efficacy and the mechanisms by which the drugs affect the mitochondrial membrane potential using flow cytometry and the cell cycle of trypanosomes using fluorescence microscopy. Hence, potential new compounds and therapeutic strategies against these parasites could be identified. Furthermore, the affinity and kinetics of the compounds to DNA were analysed and compered using surface plasmon resonance (SPR) biosensor method.

12

Effect of Polypurine Reverse Hoogsteen Hairpins against MCL1 in different human cancer cell lines

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Research on nucleic acids as therapeutic agents has increased considerably since the description of several types of nucleic acid-based molecules. Recently, we developed a new type of gene silencing molecules called polypurine reverse Hoogsteen hairpins (PPRHs) as a new approach for gene therapy. These hairpins are constituted by two antiparallel polypurine strands linked by a pentathymidine loop that allows the formation of reverse Hoogsteen bonds between them, and are able to bind to polypyrimidine sequences target on the DNA by Watson-Crick bonds knocking down the expression of specific genes. In this work, we tested the effect of PPRHs in different cancer cell lines using as a target gene MCL1, a bcl-2 antiapoptotic family protein that is highly overexpressed in a variety of human cancer cell lines thus conferring the ability to evade cell death.

We designed three different PPRHs against the promoter, exon 1 and intron 2 of the MCL1 gene. Then, the efficacy of the PPRHs as a silencing tool was tested by cell viability assays in HeLa, PC3, HepG2 and MCF7 human cancer cell lines. We observed a decrease in cell survival over 80% using a concentration of 100 nM. The order of effectiveness of the PPRHs was Intron 2 >Promoter > Exon 1. As negative controls we used a WC-PPRH, a hairpin bound by Watson and Crick instead of Reverse Hoogsteen bonds, which did not cause any effect. We also explored the mechanism involved by determining the degree of apoptosis. The incubation for 24h with the 3 different PPRHs at 100 nM caused an increase in apoptosis. The results obtained confirm the applicability of PPRHs as silencing tools especially in cancer therapy and validate MCL1 as a target in the treatment of cancer.

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13

Alginate/protamine nanoparticles as gene delivery vectors for Polypurine reverse Hoogsteen hairpins

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In the last years a set of techniques to modify the genetic makeup of cells and organisms have been developed. In this context, one of the latest technologies developed for gene silencing and gene repair is the usage of Polypurine Reverse Hoogsteen Hairpins (PPRHs). However, despite all the work in this field, some aspects of these technologies remain unsolved. One of these bottlenecks is the transfection step for which the finding of an efficient, non-toxic and safe delivery vector has become a real challenge.
In this work, we present a new nanoparticle for gene delivery formed by the electrostatic interactions between protamine, alginate and DNA. Electrophoretic mobility assays revealed that the positive charges of protamine compensate the negative charges of the DNA, forming binary nanoparticles with 81 nm of diameter. The addition of alginate reduced the strong interaction between DNA and protamine, without totally reversing their binding and increasing the size of these tertiary vectors, whose diameter ranged from 200 nm to 400 nm. Despite their differences in size, uptake experiments revealed that both vectors were internalized inside the cells.
Finally, transfection efficiency was determined by measuring the effect of two different PPRHs against survivin and *BCL2* genes in the Prostate Cancer Cell line PC3. The reduction of cell viability for the binary protamine/DNA vector was less than 15% for both survivin and *BCL2* PPRHs, pointing out its low silencing effectiveness compared with the cationic liposome DOTAP, used as positive control. The addition of alginate increased the silencing effect by enhancing the endosomal release. The PPRH/alginate/protamine tertiary vector reduced cell viability around 50% for both survivin and *BCL2* PPRHs. These results indicate a promising future for the Protamine/alginate/PPRH vector in gene delivery.
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14

Characterization of a high affinity G-quadruplex binding protein in mitochondrial DNA

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The propensity of the guanine-rich Heavy strand of the mitochondrial DNA (mtDNA) to form G-quadruplex (G4s) has been recently highlighted, suggesting potential functions in mtDNA replication origin definition or negative effects on mtDNA stability. The formation of G4s in mtDNA raises the question of their specific recognition by factors within the pool of proteins associated with the mitochondrial nucleoid. We report here our recent efforts to find high affinity partners for mtDNA-associated G4, as well as a biochemical characterization of one very

interesting candidate. Our data add another strong argument for the formation of G4 structures in nucleic acids found in mitochondria.

- **BIOLOGIA DE LLEVATS**

15

Cdc5 kinase regulation and implication in FEAR network

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Mitosis is a highly conserved and regulated process in eukaryotes. In *Saccharomyces cerevisiae* mitosis exit is regulated by two different pathways: FEAR (or Cdcfourteen Early Anaphase Release) and MEN (Mitotic Exit Network). Both cooperate in the Cdc14 phosphatase release from the nucleolus where Net1 sequesters it. This release promoted by Net1 phosphorylation allows the cell to exit from mitosis.

Net1 is phosphorylated by Clb2-Cdc28 complex and Cdc5 kinase in FEAR. Cdc5 is a polo-like kinase that mediates Cdc14 release in two different ways: through MEN GTPase cascade where it promotes the second wave of Cdc14 phosphatase release leading to the exit from mitosis or acting in the Cdc5-FEAR branch. Cdc5 in FEAR mediates Cdc28 kinase activation avoiding Sic1 inhibitor action, stabilizes the microtubules along with Cdc14 before MEN action and also has been implicated in Cdc14 direct phosphorylation.

Cdc5 direct role in phosphorylating Net1 in a MEN independent and parallel way (probably from FEAR) has to be demonstrated. Some publications support this idea, but nobody has determined the Cdc5-dependent Net1 phosphorylated residues. Also, it has to be demonstrated how Cdc5 is activated by Cdc28.

We have demonstrated that Cdc5 requires Cdc28-dependent phosphorylation in anaphase at T242 and T70 to be active as a kinase and that each residue has a separate contribution to Cdc14 release. Also, we have reduced by mass spectrometry and peptide array, which are the presumably phosphorylated Net1 residues by Cdc5. However, we have still to confirm which residues are *in vivo* phosphorylated by Cdc5.

16

Characterization of new separase-interactor proteins

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Sister chromatid separation at anaphase onset is triggered when the Scc1 subunit of cohesin is cleaved by the protease separase to destroy the cohesin complex. Separase is also involved in the regulation of release from the nucleolus of the budding yeast phosphatase Cdc14. Cdc14 is sequestered and kept inactive in the nucleolus for most of the cell cycle by binding to the nucleolar protein Net1. It has been shown that separase activation at the onset of anaphase is sufficient to promote release and activation of Cdc14. However, neither the mechanism how separase triggers the release and activation of the Cdc14 nor the importance of this mechanism are known.
To advance on the knowledge of this investigation line, we aim to identify proteins that could be involved in this signaling pathways interacting directly or indirectly with separase. Thus, a characterization of this pathways would help us to understand better how separase regulates a large set of proteins during mitosis and how all this processes are simultaniouly regulated.
The main purpose of this project is to validate the functional relationship between separase and Vhs1, Pah1 and Sak1, a set of proteins isolated from a suppressor screening using a thermosensitive mutation in separasase, *esp1-2*. On the other hand, we also want to validate, by immunoprecipitation experiments, the physical interaction found between separase and some separase-interactor proteins, such as Yrb1 and Tub3, by a mass spectometry study done previously in our lab.
The results of this project indicate that the deletion or overexpression of V*HS1*, *PAH1* and *SAK1* could have an impact in cell growth in absence of separese. Besides, Yrb1 and Tub3 interact directly with separase. Thus, separase might regulate the spindle body formation through Yrb1 and Tub3.
All this results help us to understand how separase regulate different biochemical processes that occurs at the same time of cell cycle, in metaphase-anaphase transition, as chromosome segregation, Cdc14 activation, spindle elongation and condensation and resolution of the rDNA.

- **BIOLOGIA ESTRUCTURAL**

17

Methodology used to characterize protein in cell compartments: localization and biological implications

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The importance to describe proteins in their cellular context is crucial in order to better understand the biological process in which they are involved by following their synthesis,

localizations, interactions and degradation during cell life. Our work is focused on the characterization and localization of a DNA-binding protein *in vivo*. The aim of this work is to be able, by using this protein as a target, to follow and to study the DNA changes during the different stages of the cell cycle. We designed a library of clones that incorporate a metal-tag (MT-tag) to the target protein allowing its localization in specific cell compartments by electron microscopy. All this studies were done by using Human Embryonic Kidney (HEK293t) and human cervical cancer - HeLa cell lines. By western blot analysis and proteinase K assay we assessed that all the clones of the library were transfected in the cells and that a construct of the protein of interest with the MT-tag at the C-terminal was processed, indicating its compartmentalization. After 24h there is a decrease in the expression of this protein construct. Cryo and electon microscopy studies are underway at this moment. The application of this methodology in this specific case will be discussed.

• BIOLOGIA REGENERATIVA I CÈLUL·LES MARE

18

Altered functional activity in vmDA neurons derived from Parkinson's disease-induced pluripotent stem cells (iPSC)

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Parkinson's disease (PD) is an incurable, progressive disorder leading to premature invalidity and death. It is clinically characterized by motor impairments, such as tremor, rigidity and bradykinesia, although non-motor features are also important in later stages of the disease. Neuropathologically, PD is characterized by progressive preferential loss of striatal-projecting neurons of the substantia nigra pars compacta, a specific subtype of dopaminergic neurons (DAn) patterned as ventral midbrain (vmDan). Cellular and molecular mechanisms responsible for PD pathogenesis have been proposed based on genetic studies or brain pathology. However, a major impediment to testing PD hypotheses has been the lack of human cell models. Recently, we developed a humanized Parkinson's disease *in vitro* model using iPSC from patients suffering sporadic or familial PD, and age-matched controls, which were used to generate patient-specific dopamine neurons (DAn). We found that patients' DAn show PD-relevant phenotypes such as abnormal alpha-synuclein accumulation, alterations in the autophagy machinery, and increased susceptibility to undergo neurodegeneration upon long-term culture. By taking advantage of this genuinely humanized PD model, here we investigated the emergence of early pathogenic events resulting in functional alterations occurring in DAn differentiated from PD patient-specific iPSC, that would predate neurodegeneration providing valuable information as to ways to prevent, rather than rescue, neurodegeneration in PD patients.

For this purpose, iPSCs from PD patients and heathy individuals (CTRL) have been used to derive neuroepithelial stem cells (NESCs), that were further differentiated into vmDAn, using a previous published protocol that mimics the developing human midbrain based on the dual SMAD inhibition. We first found that under the appropriate cell culture conditions both CTRL and PD-derived DAn differentiate into dopaminergic neurons with a high efficiency (up to 50% off all cells become dopaminergic midbrain neurons) and express vmDA marker, such as Girk2 at 50 days of the differentiation process. Using calcium fluorescence imaging assays, that allows for the interrogation of how human-derived neurons behave complexively in circuits, we then plated paired CTRL and PD NESCs-iPSC to mature in dishes and recorded their activity at early stage of dopaminergic differentiation, as well as at long span culture. Interestingly, we found that after 35 days, the total number of spikes and the total amount of active cells in CTRL and PD neuronal culture, were similar. On the contrary, as the cultures matured, while the number of spikes increased in CTRL DA neurons, the numbers of spikes in PD DA neurons did not, leading to a significant difference between PD and control cultures. After 80 days, we also detected synchronized bursts from CTRL neurons that was never found from PD neurons, suggesting an impairment of network formation in PD-derived neuronal cultures. Analysis of the different subtypes (excitatory/inhibitory neurons) present in our culture that would explain reduction in the amount of spontaneous activity in PD neurons compared to control culture, are underway. We believe that iPSC from PD patients and their dopaminergic neurons will allow the straightforward investigation of the cellular defects associated with Parkinson's disease providing an ideal setting to contribute to the development of new effective therapeutic options in PD.

19

Cell number regulation in planarians

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The control of cell number is a crucial requirement during development still poorly understood. Its control depends on the tight balance between two fundamental cellular process, cell death and cell proliferation. The striking plasticity of planarians, which allows them to regenerate any missing part and to continuously change their body size, offer us an ideal scenario to study this process. In order to achieve our goal, we performed an RNAi screening to detect cellular dynamics alterations. We identified a novel secreted peptide, *Smed-Blitzschnell* (*Smed-bs*), which inhibition produces an increase in the total cell number during planarian homeostasis. This increase is due to a higher rate of proliferation and a decrease of cell dead, while cell differentiation appears normal. Sustained inhibition of *Smed-bs* leads to cell accumulation in specific tissues or organs as in the epidermis and the brain, and eventually to the appearance of overgrowths. The increase of cell proliferation and reduction of cell death also occurs in *Smed-bs* RNAi animals after any proliferative stimulus, like feeding, injury or amputation. *Smed-bs* RNAi regenerating animals show an early differentiation of the missing structures (brain, eyes and chemoreceptors). Our results demonstrate that *Smed-bs* is required for the control of the cell number in planarians. *Smed-bs* would exert a general role in attenuating cell proliferation

and promoting cell death both during homeostasis and during a regenerative response. The sustained unbalance of cell proliferation and cell death during homeostasis induces tumoral transformation.

20

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.

21

Directing stem cell fate on Ti via surface-grafted integrin-binding peptidic ligands

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Endosseous implants' success relies on their ability to reach proper osseointegration. In such process a crucial part is played by the host stem cells, which are recruited to the injured site to exert their paracrine action and ultimately differentiate into osteoblasts, depositing new bone matrix at the peri-implant site. A way to drive recruited cells' behavior is to functionalize the implant surface with extracellular matrix-inspired motifs. Peptidomimetics have emerged as very promising alternatives to full length proteins and amino acids based-ligands as implant coating molecules, due to their high and selective cell-binding activity, low immunogenicity and high stability. In this work we chemically anchored to titanium (Ti) two peptidomimetic molecules, both recently synthesized by us. Our peptidomimetic ligands have high activity and selectivity for only one integrin subtype, either integrin αvβ3 or integrin α5β1, both reported to be involved in bone biology. After proving the covalent binding of the synthetic ligands to Ti, the response of hMSCs on the functionalized surfaces was analyzed: the presentation of integrin-binding motifs enhanced cell adhesion and spreading, regardless of the selectivity of the ligand, compared to the uncoated Ti surfaces. Interestingly, the shape of cells adhered to coated surface in the absence of serum was found to be ligand-dependent and was quantified by morphometric analysis of the actin cytoskeleton. Moreover, a tendency towards higher cell growth on the α5β1-selective mimetic was observed, while differentiation towards the osteoblastic lineage was fostered on the αvβ3-selective one. Furthermore, in vivo testing in a partial thickness calvarial defect in rat revealed increased formation of new bone adjacent to the αvβ3-selective ligand, compared to the α5β1-selective mimetic. The collected data are promising for the development of novel cell-instructive surfaces with tunable cell and tissue response.

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