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Organitzadors:

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Joan Roig (IBMB-CSIC)

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VPS13B links lipid transport to primarycilium formation and function
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Phosphorylation regulates BICD2 and the function of the dynein motor complex in G2/M
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Benedetta Bolognesi (IBEC)

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Jordi Torres (UdL)

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Structural basis for the E3 ligase activity enhancement of yeast Nse2 by SUMO- interacting motifs
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15.45 Short Talk 7
Targeting G-quadruplex of KRAS and cMYC with Polypurine Reverse Hoogsteen Hairpins in human cancer cell lines
Simonas Valiuska (UB)

16.00 Short Talk 8
Autophagy and lysosomal degradation, new safeguards of genomic stability
Caroline Mauvezin (UB)

16.15 ***Sticking to your partner or how to prevent desynapsis during meiotic prophase***
Ignasi Roig (UAB)

17.00 Beer and poster/short talk prizes

18.00 End

Short Talks

Short Talk 1. *Deciphering the SOX11 interactome*

Anna De Bolòs[1], Marta Sureda-Gómez[1], Patricia Balsas[1,2], Marta-Leonor Rodríguez[1], Josep Villanueva[3,2], Virginia Amador[1,2]

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Short Talk 2. *Metastatic recurrence in colorectal cancer arises from residual EMP1+ cells*

Adrià Cañellas-Socias[1,2], Carme Cortina[1,2], Xavier Hernando-Momblona[1,2], Sergio Palomo-Ponce[1,2], Eoghan J. Mulholland[3] , Gemma Turon[1], Lidia Mateo[1], Sefora Conti[4], Olga Roman[1], Marta Sevillano[1,2], Felipe Slebe[1], Diana Stork[1], Adrià Caballé-Mestres[1], Antonio Berenguer-Llergo[1], Adrián Álvarez-Varela[1,2], Nicola Fenderico[1], Laura Novellasdemunt[1], Laura Jiménez-Gracia[5], Tamara Sipka[1], Lidia Bardia[1], Patricia Lorden[5], Julien Colombelli[1], Holger Heyn[5,6], Xavier Trepat[4,7,8,9], Sabine Tejpar[10], Elena Sancho[1,2], Daniele V.F. Tauriello[1,11], Simon Leedham[3,12], Camille Stephan-Otto Attolini[1], Eduard Batlle[1,2,9]

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30-40% of colorectal cancer (CRC) patients undergoing curative resection of the primary tumor will develop metastases in the following years1. Therapies to prevent disease relapse remain an unmet medical need. Here we uncover the identity and features of the residual tumor cells responsible for CRC relapse. Analysis of single-cell transcriptomes of CRC patient samples revealed that the majority of poor prognosis genes are expressed by a unique tumor cell population that we named High Relapse Cells (HRCs). We established a human-like mouse model of microsatellite stable CRC that undergoes metastatic relapse following surgical resection of the primary tumor. Residual HRCs occult in mouse livers after primary CRC surgery gave rise to multiple cell types over time, including Lgr5+ stem- like tumor cells2–4, and caused overt metastatic disease. Using *Emp1* (epithelial membrane

protein 1) as a marker gene for HRCs, we tracked and selectively eliminated this cell population. Genetic ablation of Emp1-high cells prevented metastatic recurrence and mice remained disease-free after surgery. We also discovered that HRC-rich micrometastases were T-cell infiltrated yet became progressively immune-excluded during outgrowth. Treatment with neoadjuvant immunotherapy eliminated residual metastatic cells and saved mice from relapsing after surgery. Together, our findings reveal the cell-state dynamics of the residual disease in CRC and anticipate that therapies targeting HRCs may help avoid metastatic relapse.

Short Talk 3. ***VPS13B links lipid transport to primarycilium formation and function***

Marta Llovera
IRB Barcelona

Cohen syndrome is a rare, multi-systemic, autosomal recessive disorder caused by mutations in the vacuolar protein sorting 13 homolog B (*Vps13b*) gene. Typical features of the disease include developmental delay, intellectual disability, postnatal microcephaly, progressive retinal dystrophy, hypotonia, neutropenia, truncal obesity, and skeletal abnormalities. Curiously, some of these clinical features overlap with those of established ciliopathies, a group of inherited disorders that are caused by defective cilia. Cilia are small, antenna-like structures on the surface of the cells that are particularly important for embryo development and for the proper function of various organs. Interestingly, VPS13B does not encode a ciliary protein and has not been linked to cilia assembly or function. Instead VPS13B is associated with the Golgi and has a proposed role in the transport of lipids. Since lipids are important for building membranes including the membrane that surrounds cilia and that is important for the antenna function of cilia, we speculate that VPS13B mutations may indirectly also affect cilia.
We have used human cultured cells and zebrafish embryos as model systems to test this hypothesis. Our data suggest that disruption of VPS13B function indeed causes defects in cilia both in cultured cells and in zebrafish embryos. Remarkably, we also observed defects in lipid distribution including at primary cilia. Based on this, we propose a model in which lipid distribution defects at the Golgi complex caused by defective VPS13B may alter the composition and/or function of the primary cilium, contributing to some of the distinctive phenotypes in Cohen syndrome patients.

Short Talk 4. ***Phosphorylation regulates BICD2 and the function of the dynein motor complex in G2/M***

Núria Gallisà-Suñé and Joan Roig
Institut de Biologia Molecular de Barcelona, IBMB-CSIC

The dynein complex is the major microtubule minus-end-directed motor of the cell. As so, it is involved in the intracellular movement of a plethora of cellular organelles and structures, such as different types of vesicles, RNA particles, protein complexes and aggregates as well as viruses. It is also key for the correct organization, positioning and function of the mitotic spindle during mitosis and it controls nuclear positioning and movement during cell migration as well as the specialized nuclear movements that take place in neural progenitors during the development of the brain cortex. Dynein is essential in most organisms, and mutations in its components lead to several developmental and neurodegenerative disorders in humans.
The activity of dynein is regulated by a number of adaptors that mediate its interaction with the functionally related dynactin complex, effectively activating the motor while also connecting it to different cargos. The regulation of adaptors is consequently central to dynein physiology, but remains largely unexplored. We now describe that one of the best-known dynein adaptors, BICD2,

is effectively activated through phosphorylation. In the G2 phase of the cell cycle, phosphorylation of BICD2 by CDKs promotes its interaction with the Polo-like kinase PLK1. In turn, PLK1 phosphorylates a single residue in the N-terminus of BICD2 resulting in a conformational change that facilitates interaction with dynein and dynactin, and allows the formation of an active motor complex. We show that BICD2 phosphorylation is central for dynein recruitment to the nuclear envelope, centrosome tethering to the nucleus and centrosome separation in G2/M. Our work reveals adaptor activation through phosphorylation as crucial for the spatiotemporal regulation of dynein activity.

Short Talk 5. ***Ordered chaos in molecular networks***

Laia Ferrer
Institut de Biologia Molecular de Barcelona, IBMB-CSIC

Whether sequential metabolic reactions and other complex step-wise intracellular processes are efficiently executed by proteins dispersed in a crowded environment is a matter of debate. While some researchers do not find necessary that enzymes need be in proximity to efficiently channel the reaction product from one enzyme to the next one as substrate, recent evidence points to the notion that, in many metabolic pathways, sequential enzymes would be organized in protein assemblies. However, the existence of stable interactions holding a large number of proteins all together in a keylock manner to perform a set of complex sequential reactions or processes is rather exceptional. Based on our previous work on single-molecule dynamics of two major yeast chaperones, we decided to test the hypothesis that functional channeling in protein networks could be driven by very transient and somewhat promiscuous protein-protein interactions. Our preliminary data suggest that these protein assemblies would display extremely fast association and dissociation dynamics. I will show our recent data on the enzymes of glycolysis, which suggest that these assemblies are pathway specific and depend on the order-of-function in the biochemical process.

Key words: molecular network, protein cluster, functional channeling, dynamic interaction

Short Talk 6. ***Structural basis for the E3 ligase activity enhancement of yeast Nse2 by SUMO-interacting motifs***

Helena Borràs
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Post-translational modification of proteins by ubiquitin and ubiquitin-like modifiers, such as SUMO, are key events in protein homeostasis or DNA damage response. Smc5/6 is a nuclear multi-subunit complex that participates in the recombinational DNA repair processes and is required in the maintenance of chromosome integrity. Nse2 is a subunit of the Smc5/6
complex that possesses SUMO E3 ligase activity by the presence of a SP-RING domain that activates the E2~SUMO thioester for discharge on the substrate. Here we present the crystal structure of the SUMO E3 ligase Nse2 in complex with an E2-SUMO thioester mimetic. In addition to the interface between the SP-RING domain and the E2, the complex reveals how two SIM (SUMO-Interacting Motif) -like motifs in Nse2 are restructured upon binding the donor and E2-backside SUMO during the E3-dependent discharge reaction. Both SIM interfaces are essential in the activity of Nse2 and are required to cope with DNA damage.

Short Talk 7. ***Targeting G-quadruplex of KRAS and cMYC with Polypurine Reverse Hoogsteen Hairpins in human cancer cell lines***

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Polypurine Reverse Hoogsteen hairpins (PPRHs) consist of two polypurine strands linked by a four-thymidine loop running in antiparallel orientations and forming a hairpin structure through Hoogsteen bonds. The polypurine moieties interact with their polypyrimidine target region, by Watson-Crick bonds and provoke strand displacement(1). G-quadruplex forming sequences (G4FS) are involved in DNA replication, mRNA transcription and regulation, telomere homeostasis and translation of multiple genes. We previously demonstrated the effect of PPRHs on the G4FS of TYMS(2). In the present work, we targeted the complementary strand of G4FS in the highly expressed protooncogenes, *KRAS* and *cMYC*. We designed a battery of PPRHs against specific targets sequences in these oncogenes. For *KRAS*, we designed five different PPRHs: three targeting the promoter, one an intron and one an exon region. For *cMYC*, we designed seven different PPRHs, four targeting the promoter and three against intronic regions. To confirm the specificity of these PPRHs with their target sequences and the consequent strand displacement, we performed Electrophoretic Mobility Shift Assays and strand displacement assays, respectively. For both oncogenes, we performed several analyses in ovarian, neuroblastoma, breast, colorectal, prostate, and pancreatic cell lines transfected with the cationic liposomes Dioleoyl-3-trimethylammonium propane (DOTAP) or Dioleoyl-Pyridinium (DOPY). Among the designed PPRHs against *KRAS* targets, HpKRAS-PR-C and PPRH 1 were the most effective in reducing cell viability in all cell lines tested by 75%, correlating with a decrease of cell growth. *KRAS* PPRHs also significantly reduced mRNA levels(3). Among the designed PPRHs against *cMYC* regions, all reduced cell viability. HpMYC-G4-PR-C and HpMYC-I1-T remarkably reduced the viability in PC3 cells at low concentrations, correlating with mRNA and protein decreases relatively to the control. Our results prove that PPRHs targeting the complementary sequence of G4FS in tumor-related genes can induce cell death and specifically reduce gene expression in different cancer cell lines.

References

(1) V. Noé, E. Aubets, A.J. Félix, C.J. Ciudad, Nucleic acids therapeutics using PolyPurine Reverse Hoogsteen hairpins, Biochem. Pharmacol. (2020) 114371.
(2) Aubets E, J. Félix A, Garavís M, Reyes L, Aviñó A, Eritja R, Ciudad CJ, Noé V. Detection of a G- Quadruplex as a Regulatory Element in Thymidylate synthase for Gene Silencing Using Polypurine Reverse Hoogsteen Hairpins. International Journal of Molecular Sciences. 2020; 21(14):5028.
(3) Psaras AM, Valiuska S, Noé V, Ciudad CJ, Brooks TA. Targeting KRAS Regulation with PolyPurine
Reverse Hoogsteen Oligonucleotides. *International Journal of Molecular Sciences*. 2022; 23(4):2097.

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Short Talk 8. ***Autophagy and lysosomal degradation, new safeguards of genomic stability***

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Mitosis dictates the faithful transmission of the genetic material among generations, which precludes chromosomal instability, a hallmark of cancer. Correct mitotic progression relies on the orchestrated degradation of mitotic factors, and whether autophagy and lysosome-dependent degradation are involved in mitotic coordination remains a controversial question. We recently established that lysosome- dependent degradation is an essential process which prevents chromosomal instability (Almacellas et al., 2020), providing new perspectives in cancer therapeutics.
Our study resulted in three main findings. First, we demonstrated that lysosomes and autophagy are active during mitosis, and that their impairment increased mitotic timing and mitotic errors, thus promoting chromosomal instability. Next, we identified more than 100 novel putative lysosomal substrates in mitotic cells by proteomic approach. Among them, WAPL, a regulatory protein of the cohesin complex, was found to interact with the autophagic adaptor protein SQSTM1 for targeted degradation. Finally, we observed that cells that endured mitotic errors resulted in daughter cells with a toroidal nucleus, a particular perforated nucleus. We characterized the toroidal nucleus as a novel biomarker for the identification of chromosomal instability, inherent in cancer cells. Here, we further explore WAPL recognition by the autophagic machinery, as well as the correlation between the formation of micronucleus and toroidal nucleus for genotoxicity screenings (unpublished data). In all, our results establish a connection between two influential fields in cancer research: autophagy and chromosomal instability. Our findings serve as precedent for the characterization of the regulatory mechanisms, involving autophagy and lysosomes, required for chromosomal stability.

Posters

1. ***Peptidomimetics designed to bind to RAS effector domain are promising cancer therapeutic compounds***

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Oncogenic RAS proteins are important for driving tumor formation, and for maintenance of the transformed phenotype, and thus their relevance as a cancer therapeutic target is undeniable. We focused here on obtaining peptidomimetics, which have good pharmacological properties, to block Ras–effector interaction. Computational analysis was used to identify hot spots of RAS relevant for these interactions and to screen a library of peptidomimetics. Nine compounds were synthesized and assayed for their activity as RAS inhibitors in cultured cells. Most of them induced a reduction in ERK and AKT activation by EGF, a marker of RAS activity. The most potent inhibitor disrupted Raf and PI3K interaction with oncogenic KRAS, corroborating its mechanism of action as an inhibitor of protein–protein interactions, and thus validating our computational methodology. Most interestingly, improvement of one of the compounds allowed us to obtain a peptidomimetic that decreased the survival of pancreatic cancer cell lines harboring oncogenic KRAS**.**

2. ***Polypurine reverse hoogsteen hairpins against SF3B1 and SRSF-1 splicing factors modulate splicing variants of estrogen receptor a in breast cancer***

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Alternative splicing is responsible for generating new isoforms from a single gene. Multiple splice variants for the human estrogen receptor α (ERα) with one or more skipped exons have been identified, and some are translated to proteins with modified functions from the wild-type 66 kDa. Our aim was to assay our technology of Polypurine Reverse Hoogsteen Hairpins (PPRHs) against splicing factors SF3B1 and SRSF-1 in breast cancer cells and to analyze the changes in ERα splicing pattern. Different PPRHs against the *SF3B1* (HpSF3B1-PR1) and *SRSF-1* (HpSRSF-1- Ex3) genes were designed, and their cytotoxic effect was evaluated in MCF-7 cells. Next, we set up the PCR conditions to determine the different ERα splicing variants corresponding to the inclusion/skipping of Exons 3 to 9. Cells were incubated with either HpSF3B1-PR1 or HpSRSF-1- Ex3 for 24h, total RNA was extracted, and RT-PCR analyzed to evaluate ERα splicing variants. Inhibition of SF3B1 affected the splicing variants for ERα Exons 7, 8 and 9, whereas inhibition of SRSF-1 increased the skipping of ERα Exons 5, 7 and 8. Additionally, the splicing pattern of ERα upon transfection of both PPRHs was also evaluated in ex vivo cell cultures from fresh breast tumor samples derived from PDX-476. The levels of different ERα protein isoforms were also determined by Western Blot in cell extracts from both models. Our results indicated that inhibition of either SF3B1 or SRSF-1 splicing factors modulated the ratio of inclusion/skipping for different ERα exons in MCF7 and PDX-C476 cells. This effect was also observed at the protein level with a decreased expression of Erα-66. All together these findings suggest that both splicing factors are involved in the generation of different ERα splicing variants.

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3. Synthesis and characterization of TROPY, a new cationic liposome for the transfection of therapeutic oligonucleotides in cancer cells.

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The use of nucleic acids as gene therapy can be directed to modulate the expression of any gene, and to repair mutations in the DNA responsible for genetic diseases. The limitation in this field is the development of safe and efficient delivery systems. Here we report the synthesis of a new cationic liposome, trioleyl pyridinium (TROPY), connected through a mesitylene spacer, where the pyridinium moiety in the liposome bears an oleyl chain. We evaluated the efficiency of transfection in cancer cells using the PolyPurine Reverse Hoogsteen hairpins (PPRH) as a nucleic acid tool transfected with TROPY. First, we confirmed the interaction of PPRH with TROPY in gel retardation assays. The size of the complexes was 161 nm as determined by Dynamic Light Scattering (DLS). The internalization of the complex was tested in different cancer cell lines: SH-SY5Y (neuroblastoma), 453-WT (breast) and PC-3 (prostate), by fluorescence microscopy, flow cytometry and confocal microscopy. As a model for a therapeutic oligonucleotide, we used a PPRH specifically directed against the antiapoptotic *survivin* gene. The transfection of this PPRH with TROPY decreased the viability of cancer cells by 80% and increased the apoptosis levels to 17% after 18h of incubation. On the other hand, the intrinsic toxicity caused by the cationic liposome alone was less than 10% after 5 days of incubation. Therefore, TROPY can be used as a new cationic lipid vehicle for the efficient delivery of therapeutic oligonucleotides.

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4. Using synthetic microbiology and structural biology to functionalize bacterial flagella.

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The bacterial flagellum is a highly sophisticated organelle primarily evolved for motility and harbors a long filament consisting of up to 30,000 subunits of the proteinicious building block flagellin. We aim to modify naturally occurring structural flagellins, and augment them with enzymatic domains or other functions to create synthetic nanomachines.

To do so, we currently focus on two main research pillars: (1) To understand the molecular and structural basis of proteolytic flagellins, a natural enzymatic flagellar building block found in various bacteria, and to unravel their biological roles in biofilm remodeling and pathogenicity, as well as how they affect the bacterial life style. And (2), we are interested in reprogramming naturally occurring structural (i.e., non-enzymatic) flagellins to encode for enzymatic activity or other functions, and then using such augmented bacteria for biotechnological and biomedical applications.

Present and past funding at the IBMB-CSIC:

- RYC2020-029773-I: Structural Biochemistry of Proteolytic Enzymes. 2022-2027.
- PID2021-128682OA-I00: Redesigning bacterial flagella to harbor functional domains for biotechnology and biomedicine (Flagella 3.0). 2022-2025.
- 2018 BP00163: Post-doctoral fellowship from the Beatriu de Pinós COFUND Program; 2020-2022.

5. New KRAS interactors relevant for its oncogenic activity

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Colorectal cancer (CRC) is the third most common type of cancer worldwide and the second leading cause of cancer deaths. KRAS small GTPases are mutated in 40% of colorectal cancer cases. These oncogenic mutations in KRAS promote an active permanent GTP-bound state, independent of extracellular signals, increasing proliferation, cell growth, and apoptosis avoidance. For this reason, KRAS is a key therapeutic target in CRC, however, efforts to inhibit KRAS have been mostly unsuccessful. In this work, we analyzed how changes in the oncogenic KRAS Serine 181 phosphorylation/dephosphorylation cycle impact the interactions that KRAS establishes with other proteins, affecting tumor properties. We performed a Proximity Dependent Biotin Identification assay (BioID) to identify KRAS interactors in DLD1-KO cells ($KRAS^{wt/-}$) exogenously expressing three different oncogenic KRAS phosphomutant proteins: phosphorylatable (S181), non-phosphorylatable (S181A), and phosphomimetic (S181D). Samples were analyzed by WB and LC-MS/MS.
A total of 379 differentially biotinylated proteins were detected between the three phosphomutants. Biological processes related to autophagy, RNA processing, gene expression, cell cycle, vesicle-mediated transport, and regulation of actin cytoskeleton organization were overrepresented. Cellular components like cell junctions, focal adhesion, lamellipodium, extracellular exosome, spliceosome, and ribonucleoprotein complexes were also overrepresented. This suggests that the oncogenic KRAS Serine 181 phosphorylation/dephosphorylation cycle might regulate these functions through the control of KRAS physical interactions with these proteins. However, further research is needed to validate these interactions.

6. Proteolytic chimeras targeting purine biosynthesis: at the crossroads of antiproliferative and antiviral effects

Mireia Casasampere (IQAC), Hector Carneros (IQAC), Núria Gallisà (IBMB), Alba González (IBMB), Alice Zuin (IBMB), Bernat Coll (IBMB), Jordi Bujons (IQAC), Pablo Gastaminza (CNB), Urtzi Garaigorta (CNB), Fina Casas (IQAC), Gemma Fabriàs (IQAC), Antonio Delgado (UB), Bernat Crosas (IBMB, bcnbmc@ibmb.csic.es)

Background: Targeted protein degradation has emerged as an innovative and versatile approach for the development of novel and more efficient therapies. In a conventional approach, proteolytic targeting chimeras (Protacs) are heterobifunctional molecules that simultaneously bind a target and an E3 ubiquitin ligase, thus inducing ubiquitination and proteasome-dependent degradation of the target.
Purine metabolism exerts a pivotal regulation of multiple cellular functions. Among them, synthesis of nucleotides as building blocks of nucleic acids represents a limiting step in processes demanding high levels of DNA or RNA production. Therefore, blocking key steps in purine synthesis exerts a control in either cell proliferative or viral infection events.

Methods: In the present work we have produced and characterized a series of 11 degrading chimeras targeting a key factor in purine biosynthesis (defined herein as "Target P").
Activity of compounds were tested in human cell cultures. Target P degradation was assessed by immunoblotting. Effect in cell viability was analyzed by MTT. Association of chimeras with their

interactors has been assessed by surface plasmon resonance and by specific cellular ligand-receptor recognition assays. Cell permeability was estimated by quantitation of compounds in medium and cell extracts by UPLC-HRMS. Effect of compounds in SARS-CoV-2 infectivity was analyzed in the Virus Biotechnology Platform, at the CNB (CSIC).

Results: We have observed degradation of Target P by chimeras based on 26S and chaperone recruitment. Linker length has appeared to strongly influence chimera activity. Best behaved compounds exhibit K_D constants around 15 µM.. Testing the antiviral effect of compounds has not revealed so far a neat antiviral effect due to a strong decrease of cell viability.

Conclusions: At the present stage, a series of Target P-degrading chimeras has been developed. Target P depletion has appeared to strongly correlate with decrease of cell viability but not with SARS-CoV-2 infectivity decrease. Further studies are required to determine the potential of these novel compounds as therapeutic tools.

7. *In vitro* and *in vivo* effects of the combination of Polypurine Reverse Hoogsteen Hairpins against Her-2 and Trastuzumab in breast cancer cells

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PolyPurine Reverse Hoogsten hairpins (PPRHs) are formed by two polypurine strands linked by intramolecular reverse Hoogsten bonds, which can bind to polypyrimidine stretches in dsDNA by Watson-Crick. They have been used to silence genes involved in resistance to chemotherapeutic drugs, cancer progression and immunotherapy approaches. In this study, we investigated the effect of PPRHs against *ERBB2* as an approach for breast cancer therapy. The effect of a PPRH against *ERBB2* intron 6 (I6-PPRH) was evaluated both *in vitro* and *in vivo* in MDA-MB-453 cells. The I6-PPRH was transfected either alone or in combination with the antibody Trastuzumab in MDA-MB-453 *cells*, which led to a decrease in cell viability of 32% and 42%, respectively. In the chicken chorioallantoic membrane *in vivo* model, a reduction of 60% in tumor size was observed when this same combination was used. Additionally, the molecular effects of the specific hairpin against *ERBB2* were evaluated both at the mRNA and protein levels.
Our results suggest the potential of the combination of this specific PPRH against *ERBB2* and the antibody Trastuzumab for the treatment of HER2-positive breast cancer tumors. Due to the lack of immunogenicity of the PPRHs, that treatment could also contribute to reduce the length and adverse effects of the anticancer therapy.

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8. Fatal COVID-19 compromises spermatogenesis in man

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The SARS-CoV-2 virus has infected more than 600 million people worldwide in the last two years. SARS-CoV-2 uses the TMPRSS2 protease and ACE2 receptor to infect host cells. Even though it is mainly a respiratory disease, these are expressed in many tissues, such including several testicular cell types. Abnormal levels of sex hormones and a decrease in the sperm quality have been observed in patients during and after recovery from COVID-19. Furthermore, severe damage caused by inflammation has been detected in the testes. In addition, the SARS-CoV-2 has been found in the testis. Thus, the possibility that COVID-19 affects the male reproductive system deserves further research. First, we analyzed the morphology of testis sections from patients deceased by COVID-19 and compared them to control samples of similar ages. Overall, COVID-19 samples displayed various anomalies commonly associated with compromised spermatogeneses, such as vacuolization of the Sertoli Cells, detachment of the germinal epithelium, or thickening of the basal lamina. Next, we studied the presence of different relevant biomarkers of spermatogenic cells, DNA damage, and leukocytes in these samples. A higher fraction of T lymphocytes was detected in the peritubular spaces of COVID-19 samples compared to controls, thus confirming the infiltration of immune cells in the peritubular tissue of the testis. In addition, the seminiferous tubules of COVID-19 samples showed fewer UTF1-positive spermatogonia, which represents the spermatogonial stem cell population from which all sperm derive. Moreover, these presented more DNA damage than control cells, suggesting that COVID-19 could compromise spermatogenesis even after recovery. However, more studies are needed to understand the impact of COVID-19 in spermatogenesis, especially in those patients that have recovered from the infection.

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9. The Unstructured Linker of macroH2A is a Previously Overlooked Functional Domain Involved in Nuclear Conformation, Chromatin Dynamics and Gene Expression

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MacroH2A histone variants are tripartite proteins containing a histone-fold and a globular macrodomain connected by an unstructured linker region. Knock-down of macroH2A drastically modifies nuclear organisation and chromatin conformation causing changes in gene expression. The size and quantity of heterochromatin foci are also affected, which has been shown to be mediated by the poorly characterized linker domain. Specifically, the linker is intrinsically disordered and lysine-arginine rich, resembling the C- terminal tail of H1 histones and the hinge domain of HP1 proteins, functional domains related to phase separation and compaction of DNA. In vitro, the linker causes macroH2A protein aggregation and stabilises DNA at the nucleosome entry-exit site. We

aim to characterise the molecular function and regulation of the linker in the context of chromatin organisation and epigenetic regulation. Using classic knock-down and rescue cell lines, we have shown that the linker domain can regulate the expression of a small gene panel, identified from RNAseq data. From here, we are further characterising the specific aspects of the linker domain important in gene expression with novel rescue cell lines with mutant constructs and identification of linker-specific molecular partners. To expand our knowledge of macroH2As importance in gene expression, we are also looking to identify new direct targets of macroH2A and its linker using cell lines with rapidly degradable macroH2A. After degradation of the protein, we will repeat RNAseq experiments, enriching for nascent transcripts, to identify the most direct and rapidly affected genes, then test the involvement of the linker in their expression. These same cell lines will also allow us to CRISPR screen for upstream regulators of macroH2A, ideally giving us insight into the regulation of macroH2A. Together, this will provide unprecedented understanding of the upstream regulation and the direct downstream effects of macroH2A proteins.

10. Pharmacological targeting of dopamine D_1-D_3 receptor heteromers in the nucleus accumbens

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Several studies found *in vitro* evidence for heteromerization of dopamine D_1 receptors (D1R) and D_3 receptors (D3R), and it has been postulated that functional D1R-D3R heteromers that are normally present in the ventral striatum mediate synergistic locomotor-activating effects of D1R and D3R agonists in rodents. Based also on results obtained *in vitro*, with mammalian transfected cells, it has been hypothesized that those behavioral effects depend on a D1R-D3R heteromer-mediated G protein-independent signaling. Here, we demonstrate the presence on D1R-D3R heteromers in the mouse ventral striatum by using a synthetic peptide that selectively destabilizes D1R-D3R heteromers. Parallel locomotor activity and *ex vivo* experiments in reserpinized mice and *in vitro* experiments in D1R-D3R mammalian transfected cells were performed to dissect the signaling mechanisms of D1R-D3R heteromers. Co-administration of D1R and D3R agonists in reserpinized mice produced synergistic locomotor activation and a selective synergistic AKT phosphorylation in the most ventromedial region of the striatum, in the shell of the nucleus accumbens. Application of the destabilizing peptide in transfected cells and in the shell of the nucleus accumbens allowed demonstrating that, both *in vitro* and *in vivo*, co-activation of D3R induces a switch from G protein-dependent to G protein-independent D1R-mediated signaling determined by D1R-D3R heteromerization. The results therefore demonstrate that a biased G protein-independent signaling of D1R-D3R heteromers localized in the shell of the nucleus accumbens mediate the locomotor synergistic effects of D1R and D3R agonists in reserpinized mice.

11. Antioxidant treatment preserves ovarian reserve from chronic ethanol intake in mice.

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In mammals, oocyte development and maturation are critical processes for female fertility[1]. Lifestyle and diet habits seem to affect these processes significantly, resulting in differences in the

fertility capability among populations[2]. Nevertheless, the genetic mechanisms regulating the follicle reserve are just beginning to be described[3] . Moderate alcohol consumption has been linked to a diminished ovarian reserve in humans.[4,5] Since ethanol causes oxidative stress, which has been associated with a decline in the quality of aging oocytes[6,7], we wondered if a moderate alcohol consumption could damage the ovarian reserve in mice and if treatment with a mitochondrial-targeted antioxidant could revert it. To test this hypothesis, four-week-old C57BL/6JOlaHsd mice were administered for 14 weeks with a water solution containing 0.1% ethanol or supplemented with the 4,632 mg/ml of antioxidant diluted in ethanol or 4,632 mg/ml antioxidant diluted in DMSO. Our preliminary findings reveal a significant negative effect of daily alcohol consumption on the follicles' quality and development. Also, the treatment with the antioxidant could revert these effects on the ovarian reserve and/or even increase the number of primordial follicles in not ethanol-treated mice. Based on our data, we propose that the daily consumption of EtOH, even in small doses, could significantly affect the fertility status of mammalian females. However, an antioxidant treatment could counterbalance the ethanol effect and preserve the ovarian reserve. Also, the consumption of the mitochondrial-targeted antioxidant seems to increase the ovarian reserve so positively affect the fertility status in female mice.

References

1. Jamnongjit M, Hammes SR. Oocyte maturation: the coming of age of a germ cell. Semin Reprod Med. 2005 Aug;23(3):234-41. doi: 10.1055/s-2005-872451. PMID: 16059829; PMCID: PMC1482430.
2. Sharma et al.: Lifestyle factors and reproductive health: taking control of your fertility. Reproductive Biology and Endocrinology 2013 11:66.
3. Ruth, K. S., Day, F. R., Hussain, J., Martínez-Marchal, A., Aiken, C. E., Azad, A., Thompson, D. J., Knoblochova, L., Abe, H., Tarry-Adkins, J. L., Gonzalez, J. M., Fontanillas, P., Claringbould, A., Bakker, O. B., Sulem, P., Walters, R. G., Terao, C., Turon, S., Horikoshi, M., … Perry, J. R. B. (2021). Genetic insights into biological mechanisms governing human ovarian ageing. Nature, 596(7872), 393–397. https://doi.org/10.1038/s41586-021-03779-7
4. Hawkins Bressler L, Bernardi LA, De Chavez PJ, Baird DD, Carnethon MR, Marsh EE. Alcohol, cigarette smoking, and ovarian reserve in reproductive-age African-American women. Am J Obstet Gynecol. 2016 Dec;215(6):758.e1-758.e9. doi: 10.1016/j.ajog.2016.07.012. Epub 2016 Jul 11. PMID: 27418446; PMCID: PMC5124512.
5. Li N, Fu S, Zhu F, Deng X, Shi X. Alcohol intake induces diminished ovarian reserve in childbearing age women. J Obstet Gynaecol Res. 2013 Feb;39(2):516-21. doi: 10.1111/j.1447-0756.2012.01992.x. Epub 2012 Sep 25. PMID: 23002912.
6. Comporti, M., Signorini, C., Leoncini, S., Gardi, C., Ciccoli, L., Giardini, A., Vecchio, D., & Arezzini, B. (2010). Ethanol-induced oxidative stress: Basic knowledge. Genes and Nutrition, 5(2), 101–109. https://doi.org/10.1007/s12263-009-0159-9
7. Sasaki, H., Hamatani, T., Kamijo, S., Iwai, M., Kobanawa, M., Ogawa, S., Miyado, K., & Tanaka, M. (2019). Impact of Oxidative Stress on Age-Associated Decline in Oocyte Developmental Competence. Frontiers in Endocrinology, 10(November), 1–7. https://doi.org/10.3389/fendo.2019.00811

12. Purification, stabilization and characterization of a DNA-binding repressor factor with large disordered regions.

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We are working on a DNA-binding protein encoded in the nucleous, where it bonds to specific DNA regions as a repressor, but that is also imported into mitochondria to presumably bind non-specifically to the mitochondrial DNA. This repressor comprises an HMGbox domain accompanied by large disordered regions, which are probably structured by the binding of the protein to its biological partners and DNA. We have carried out a purification using the NusA fusion protein that improves the expression levels and confers extra solubility. With the pure protein we performed Small-angle X-ray scattering (SAXS) experiments that showed that the isolated protein has a partially folded conformation. We have also performed electrophoretic mobility shift assay (EMSA) experiments to confirm and characterize the protein binding to specific DNA.

13. Study of the replication stress effect on gene expression and chromatin accessibility in colorectal cancer

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Replication stress is defined as the slowing or stalling of the replication fork. It is a source of genomic instability, which is a hallmark of cancer. Cells respond to replication stress by activating replication checkpoint. It promotes a reversible cell cycle arrest, inhibits late origin firing and stabilizes the replisome. However, cancer cells do not respond as non- transformed cells to severe replication stress. Thus, after prolonged replication stress promoted by 10mM hydroxyurea treatment, non-transformed human cells remain arrested in S phase being unable to recover replication. In contrast, HCT116 colorectal cancer cells are able to recover replication by new origin firing and progress into the cell cycle.

This difference is produced because during hydroxyurea treatment, non-transformed human cells activate APC/Ccdh1 during the S phase promoting the degradation of Cyclin A, inhibiting new origin firing, therefore cells remain arrested in the S phase. This APC/Ccdh1 activation is not produced in tumoral cells, so new origin firing is not inhibited allowing cells to recover after long hydroxyurea treatment. This new origin firing is also produced in new replication factories, thus changing the replication timing and altering gene expression.

The present work shows evidence connecting replication stress and gene expression variations. We propose that replication stress could be a source of permanent gene expression changes conferring more heterogenicity to tumour cells.

Therefore this difference can be used as a therapeutic target. As tumoral cells were able to restore replication after severe replication stress by new origin firing, we used roscovitine (CDK2/CDK1 inhibitor) during the release to avoid it. With this new strategy we obtained a synergic effect at low doses of roscovitine that diminishes cell survival, reducing recovery upon severe replication stress.

14. Structural Biochemistry of Immunoglobulin Cleaving Peptidases

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We are employing structural biology and biochemistry to explore and elucidate the enzymatic mechanism and cleavage specificity of a large multidomain zinc dependent metallopeptidase. Notably, this bacterial peptidase exhibits multiple accessory domains that are thought to be key for substrate recognition. As immunoglobulins represent key players of adaptative immune system, such bacterial enzymes are typically considered virulence factors. However, they can be used as valuable tools for biotechnology and biomedicine. Currently, we are using a dedicated proteomic approach and unbiased peptide libraries to elucidate if the peptidase can cleave peptidic substrates, and we have completed our first electron microscopy negative staining experiments to amen if our protein is suitable for Cryo-EM. In this poster, we present and discuss the results achieved and the future approaches we will use to address our objective. These include the expression optimization, the purification strategies, and activity insights. We also discuss the approach we have followed to determine the roles of the accessory domains for substrate recognition, and the biological role of the protease during the health and disease.

15. Differential genomic distribution of human histone H1 variants

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Histone H1 binds to the linker DNA at the nucleosome, participating in the formation of higher-order chromatin structures. Human somatic cells may contain up to seven members of the histone H1 family contributing to the regulation of nuclear processes, apparently with certain subtype specificities. We have explored the functional role of histone H1 variants by shRNA-mediated knock-down of single or multiple H1s. In T47D breast cancer cells, the combined knock-down of H1.2 and H1.4 subtypes (multi-H1 KD) has a strong deleterious effect: coordinately deregulates many genes, promotes the appearance of accessibility sites genome-wide and triggers an interferon response via activation of heterochromatic repeats. Besides, multi-H1 KD translated into more de-compacted chromatin structures at the scale of topologically associating domains (TADs). Profiling of endogenous H1 variants in these cells revealed coexistence in the genome in two large groups depending on the local GC content: H1.2, H1.5 and H1.0 were abundant at low GC regions while H1.4 and H1X preferentially co-localized at high GC regions. Interestingly, above-mentioned chromatin changes upon multi-H1 KD occurred with only slight H1 variant redistributions across the genome. Imaging experiments of H1 variants also support differential genomic patterns revealed by ChIP-Seq data and variant-specific association to particular chromatin environments, such as lamina-associated domains (LADs) or the nucleolus.

16. Structural insights into MraZ conformation and DNA Binding

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Cell division is a fundamental cellular process and the basis of microorganism survival. Thus, the molecular machinery involved in cell division is exposed to a strong evolutive pressure that in bacteria results in highly conserved gene division clusters and regulatory proteins. The division cell wall (dcw) gene cluster of many bacteria is regulated by a sequence-specific DNA-binding protein named MraZ. Whereas the promotor recognition sequence of MraZ is well-known in most organisms, the molecular determinants driving MraZ binding to DNA remain to be elucidated.

Here, we study MraZ protein of the model organism Mycoplasma genitalium to obtain structural insights into its mode of action and mechanism of DNA interaction. We have solved MraZ structure, which conforms a homo-octameric ring, highly similar to the structure previously determined in its close relative, Mycoplasma pneumoniae. Remarkably, MraZ is the unique member of the SpoVT/AbrB DNA-binding proteins that oligomerizes and forms a protein complex with multiple DNA-binding sites. To fully understand the biological role of MraZ, we measured the DNA-binding affinities of MraZ wild type and mutants lacking DNA binding capacity or stable oligomeric state. Besides, we are trying to obtain the structure of MraZ-DNA complex via crystallization methods.

Overall, we expect that exploring MraZ oligomerization and DNA binding may not only shed light into MraZ mechanistic details but also help us describe the biological need that triggered the origin of a supramolecular complex with multiple DNA-binding sites.

17.Salt, pH and Amyloid-beta-peptide aggregation

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Amyloid-beta-peptide (AB) aggregation is considered one of the main hallmarks of Alzheimer's disease. Special attention has been given to the relationship between the changes in pH in the brain and the development of this neurodegenerative disease. Recently, altered endosomal and lysosomal pH values have been observed in AD mouse models. Also, in certain micro-environments, the pH can differ, for example, at the membrane surface, the pH is more acidic, favouring the formation of aggregates. A property that has been associated with the AB antimicrobial activity. In this context, the cells also regulate salt concentration, which forms a repulsion shield that can be modulated to control protein interactions. Moreover, amyloid conformation is pH sensitive. Accordingly, AB aggregation can be reversed by changing the pH. This suggests that, inside the cell, AB conformation could be regulated through pH and salt concentration. In this context, we systematically characterize how AB aggregation and conformation are affected by changes in pH and salt. From a molecular point of view, we aim to shed light on the different toxic and functional effects that this peptide produces on the cell.

Keywords: Amyloid, Alzheimer, endosome, pH, aggregation

18. Enhanced production of immunomodulative MSC-EV by 3D bioreactor culture in chemically-defined medium

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Introduction: There is a growing interest in the clinical therapeutic development of Mesenchymal Stromal Cell-derived Extracellular Vesicles (MSC-EV) due to their regenerative and immunomodulatory potential. For that, we aim for their large-scale production, focusing on 3D-culture. Nevertheless, we need to study if this method impacts MSC-EV functional activity.

Objective: This study aims to test 3D culture of Wharton's Jelly MSC (MSC-WJ) for a scaled-out production of MSC-WJ-EV.

Methods: We lead a comparative study of the production efficiency of EV derived from clinical-grade MSC-WJ cultured either in scaled-up 2D cell culture flasks or scaled-out in 3D microcarriers-based bioreactors using either serum-supplemented (SER) or chemically-defined medium (CHEM). Cell growth, viability and phenotype were monitored for each condition and MSC-WJ-EV were purified by size exclusion chromatography (SEC) after concentration by ultrafiltration or tangential flow filtration. Next, MSC-WJ-EV were characterized in terms of identity, purity, morphology, yield and functional activity.

Results: MSC-WJ expressed a stable phenotype across the different culture settings. Cell recovery was lower in both CHEM conditions as well as in both 3D cultures, although aggregate formation difficulted cell harvest in the latter. Purified MSC-WJ-EV were recovered from each condition, and bead-based flow cytometry confirmed the stable expression of both EV (CD9, CD63, CD81) and MSC (CD44, CD90, CD105) markers in all conditions. A significant decrease in EV size was observed after 3D-CHEM culture with a delay in SEC and confirmed by cryo-electron microscopy (median 117nm (95%CI 111-120nm) in 2D SER and 100nm (95%CI 95-103nm) in 3D CHEM; $P<0.0001$). CHEM-based cultures yielded higher EV protein and RNA quantities relative to cell numbers and culture volumes. In terms of immunomodulatory activity, MSC-WJ-EV derived from 3D cultures showed greater and consistent inhibition of T cell proliferation (60% 2D *vs* 100% 3D positive batches).
Conclusion: While 2D and SER culture conditions yielded higher cell production, 3D-CHEM culture conditions allowed harvests of smaller-sized MSC-WJ-EV with superior immunomodulatory potential.

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Keywords: Bioreactor, Immunomodulation, Production optimization, Chemically-defined