SHORT TALKS

ST01. Genetics & Epigenetics

Cellular photosensitization of CNS cells by non-viral vectors for future application in optogenetic neuroprostheses

José David Celdrán López, María José Verdú Polvorinos, Lawrence Humphreys, Eduardo Fernández Jover
University Miguel Hernandez (UMH), Elche, Spain

Visual impairment affects over 314 million people world-wide. Currently, electrode-based prostheses are the most successful in restoring some forms of vision; however, they lack cell specific targeting and can harm the tissue. Optogenetics uses targeted ectopic expression of light-activated proteins (opsins) to control cell specific neural activity with millisecond precision, thus emerging as a promising therapy and does not present the flaws of electrode-based prostheses. Taking this into account, μLED-based optogenetic prostheses could photostimulate tissues with cells expressing opsins. Viruses are commonly used to introduce the opsin genes in neurons; however, they can cause immunogenic reactions. Therefore, it is necessary to find safer alternatives. In this work, we aim to use niosomes and electroporation in central nervous system (CNS) cells to test the suitability of these non-viral vectors for photosensitizing them. Optogenetic plasmids pAAV-Syn-ChrimsonR-tdTomato and pCAG-ChrimsonR-tdTomato (both coding for ChrimsonR protein, a red shifted opsin activated at 590 nm) were delivered in both *in vitro* and *in vivo* experiments. *In vitro* experiments in rat cortical neurons were performed with different formulations of niosomes that delivered the optogenetic plasmids, while the *in vivo* experiments were performed in a retinal degeneration mouse model (rd10) delivering the optogenetic plasmids into the retina by electroporation. Niosomes and electroporation have proven to effectively deliver optogenetic material into CNS cells, but niosome formulations affect both morphologically and electrophysiologically cortical neurons, while electroporation does not produce light-driven visual behavior in rd10 mice. However, non-viral vectors are varied and alternative ways for transfecting cells should be tested.

Keywords: Optogenetics, non-viral vectors, niosomes, electroporation.

ST03. Systems & Synthetic Biology

An integrative approach to characterize the two sides of enzyme-mediated antibiotic escape: resistance and tolerance

Viktoriia Gross[1,2,3], Virgile Andreani[1,4], Imane El Meouche[2], Erick Denamur[2], Gregory Batt[1,4]
[1]*Institut Pasteur, Université de Paris, InBio, Paris, France,* [2]*IAME, UMR 1137, INSERM, Université Paris Cité, Paris, France,* [3]*ABIES, Université Paris-Saclay, Paris, France,* [4]*Inria Paris, Paris, France*

The non-susceptibility of pathogenic bacteria to antibiotic treatments is a major health problem. Bacteria might escape treatments in two ways: being resistant (ability to grow in presence of antibiotic) or by being resilient (ability to survive long treatments). An increasing fraction of commensal and pathogenic *E. coli* bacteria express extended-spectrum β-lactamases and/or carbapenemases. When released by dying cells, these enzymes can degrade the antibiotic in the environment and allow the survival and the re-growth of the resilient bacteria.
Standard *in vitro* approaches are not well suited to observe and characterize these effects. Besides, these tests often fail to predict treatment effectiveness. In urinary tract infections (UTIs), treatment failures are relatively frequent and can be observed even when pathogens are predicted as susceptible to the treatment following standard tests. In contrast, *in vivo* tests are closer to reality. However, they are quite hard and very low-throughput. For these reasons, our main objective is to propose an intermediate experimental setup using low volume bioreactors to help reconcile *in vitro* and *in vivo* results.
To meet this objective, we are developing an experimental platform allowing on-line absorbance measurements, automated dilutions and sampling for systematic off-line measurements of number of live bacteria and of residual antibiotic. We observe a strong impact on resistance and/or resilience of different media and of repeated or delayed treatments. Together with a model of cell responses and population behavior these results will allow to make a quantitative characterization of the phenomena.

Keywords: Antibiotic resistance, bioreactors, modeling.

ST04. Clinical Research, Translational Biomedicine & Personalised Medicine

MIR145 core promoter methylation imprinting-mediated miR-143/145 cluster modulation in bladder cancer progression

Katerina-Marina Pilala[1], Maria-Alexandra Papadimitriou[1], Konstantina Panoutsopoulou[1], Petros Barbarigos[1], Panagiotis Levis[2], Georgios Kotronopoulos[2], Konstantinos Stravodimos[2], Andreas Scorilas[1], Margaritis Avgeris[1,3]
[1]*Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Greece,* [2]*First Department of Urology, "Laiko" General Hospital, School of Medicine, National and Kapodistrian University of Athens, Greece,* [3]*Laboratory of Clinical Biochemistry-Molecular Diagnostics, Second Department of Pediatrics, School of Medicine, National and Kapodistrian University of Athens, Greece*

Owing to its highly heterogenous molecular landscape, bladder cancer (BlCa) is still characterized by non-personalized prognosis and treatment decisions, resulting in lifelong post-treatment surveillance. Motivated by our previous findings on aberrant regulation of miR-143/145 cluster and its clinical significance in improving patients' risk stratification, we studied the epigenetic regulation of miR-143/145 in BlCa. Nucleic acids were extracted from 206 bladder tissues. Genomic DNA underwent bisulfite conversion, followed by PCR amplification of specific MIR143/145 promoter CpGs. Methylation levels were quantified by pyrosequencing of PCR products via PyroMark Q24. miR-143/145 levels were quantified by RT-qPCR, following 3′-terminal polyadenylation of total RNA. Disease progression and patients' survival were used as clinical end-point events. TCGA-BLCA (n = 412) was used as institutionally-independent validation cohort for MIR143/145 gene cluster methylation and transcription. *In silico* analysis unveiled MIR145 promoter as key regulatory region on cluster's modulation. In line with miR-143/145 levels, MIR145 promoter was hypermethylated in tumors compared to normal urothelium, while the MIR145 core promoter emerged as the critical regulatory site controlling cluster's expression. Lower methylation of MIR145 core promoter was associated with aggressive disease phenotype and elevated risk for short-term progression and high morbidity of NIMBC and MIBC, respectively. Multivariate models incorporating MIR145 core promoter methylation with established disease markers displayed superior clinical benefit in BlCa prognostication. Acknowledgements: This study was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH—CREATE—INNOVATE (project code: T2EDK-02196) to the NKUA (KE17358).

Keywords: DNA methylation, epigenetics, prognosis, non-coding RNAs, miRNA.

ST05. Immunology, Microbiology & Infectious Diseases

Verbascoside and its derivatives as potential SARS-CoV-2 inhibitors: an *in silico* study

John Omo-Osagie Uhomoibhi, Saheed Sabiu, Francis Shode, Ademola Kehinde Idowu
Durban University of Technology, Queensburgh, South Africa

Introduction: Polyphenols (PPs) have been studied and recommended for the development of anti-COVID-19 drugs. Its causative agent, SARS-CoV-2 has continued to infect and kill millions of people worldwide. The SARS-CoV-2 variants that emerged recently, both Delta (DT) and Omicron (OM) variants have been classified as the variants of concern (VOC). Our recent studies have shown that Verbascoside (VBS, 1), a natural polyphenolic glycoside has inhibitory potential against the druggable targets of COVID-19. In this study, we aimed to develop potent anti-SARS-CoV-2 inhibitors of its delta and omicron variants using VBS and its 39 derivatives. Materials and Methods: VBS (1) and its 39 derivatives were used for the study. The reference drug was cefoperazone A (CSP). Protein acquisition and preparation were achieved using standard methods before molecular docking followed by molecular dynamics simulations before post-dynamic analysis. Results: VBS (1) and its five derivatives (A-E) gave the best docking scores against DT variant while five derivatives (B, F-I) gave the best docking scores against OM variant. The best binding energies were observed in A and B for Delta variant and G, H, and I for omicron variant. Conclusion: VBS (1), A, and B emerged as the best inhibitors of the DT variant. VBS (1), G, H, and I emerged as the best inhibitors of the OM variant.

Keywords: SARS-CoV-2, COVID-19, Verbascoside, computational modeling.

ST07. Cellular & Molecular Biology

The interaction of DNA nanostructures with hepatic cells

Barbora Smolková[1], Adam Frtús[1], Mariia Uzhytchak[1], Mariia Lunova[1,2], Milan Jirsa[2], Skylar J.W. Henry[3,4], Alexandr Dejneka[1], Nicholas Stephanopoulos[3,4], Oleg Lunov[1]
[1]*Department of Optical and Biophysical Systems, Institute of Physics of the Czech Academy of Sciences, Prague, Czech Republic,* [2]*Institute for Clinical & Experimental Medicine (IKEM), Prague, Czech Republic,* [3]*School of Molecular Sciences, Arizona State University, Tempe, AZ, USA,* [4]*Biodesign Center for Molecular Design and Biomimetics, Arizona State University, Tempe, AZ, USA*

The unique properties of DNA nanostructures (DNs), especially the capacity for self-assembly, enable the production of different 3D structures with precisely scalable size, shape and functionality. Moreover, due to their good biocompatibility and low cytotoxicity, DNs have shown great potential in different biomedical applications, particularly in therapy as vehicles for drug delivery or in diagnostics as imaging components. Despite the recent progress in the field, the precise molecular mechanism of DN-cell interaction remains elusive. In our study we focused on the interaction of differently functionalized DNs with three hepatic cancer cell lines: Alexander, HepG2 and Huh7. We showed distinct kinetics of DN uptake in different cell lines dependent on the

cellular size. Additionally, we analyzed the delivery of modified DNs coated with peptide facilitating the endosomal escape. We observed the formation of so-called protein corona that decreased the efficiency of endosomal escape of DNs in the presence of cell culture medium containing serum proteins. Indeed, the protein corona formation is one of the major challenges in the field of nanotechnology in general. In conclusion, our study offers an important insight for optimization of DN-based delivery systems.

Keywords: Hepatic cancer cells, DNA nanostructures, protein corona, cellular uptake, bio-nano interactions.

ST08. Neuroscience, Psychiatry & Mental Health

Transcriptional adaptation of olfactory sensory neurons to GPCR identity and activity

Luis Flores, Alexis Assens, Leon Fodoulian, Leonardo Marconi, Joël Tuberosa, Madlaina Boillat, Alan Carleton, Ivan Rodriguez
University of Geneva, Geneva, Switzerland

In mammals, chemoperception relies on a diverse set of neuronal sensors able to detect chemicals present in the environment, and to adapt to various levels of stimulation. The contribution of endogenous and external factors to these neuronal identities remains to be determined. Taking advantage of the parallel coding lines present in the olfactory system, we explored the potential variations of neuronal identities before and after olfactory experience. We found that at rest, the transcriptomic profiles of mouse olfactory sensory neuron populations are already divergent, specific to the olfactory receptor they express, and are associated with the sequence of these latter. These divergent profiles further evolve in response to the environment, as odorant exposure leads to reprogramming via the modulation of transcription. These findings highlight a broad range of sensory neuron identities that are present at rest and that adapt to the experience of the individual, thus adding to the complexity and flexibility of sensory coding.

Keywords: Olfactory neurons, adaptation, transcription.

ST09. Cancer Biology & Oncology

The role of APOBEC3C in renal cell carcinoma

Nora Hase[1], Gerald Schwerdt[2], Helge Taubert[3], Marcel Köhn[1]
[1]Medical Faculty, Martin-Luther-University Halle-Wittenberg, Halle, Germany, [2]Julius-Bernstein-Institute for Physiology, Martin-Luther-University Halle-Wittenberg, Saxony-Anhalt, Germany, [3]Molecular Urology, Friedrich-Alexander University Erlangen-Nürnberg, Bavaria, Germany

Our research focusses on clear cell renal cell carcinoma (ccRCC), the most common type of kidney cancer. While prognosis for low stage ccRCC is favorable, metastatic ccRCC is characterized by very low 5-year survival rates due to deficient therapy options. By analyzing the molecular signature of RCC subtypes in several data sets, we observed substantial upregulation of the RNA-binding protein (RBP) APOBEC3C (A3C) in ccRCCs. Moreover, high A3C mRNA levels correlate significantly with poor overall survival.

APOBEC3 proteins belong to a family of Zn-dependent cytidine deaminases binding and putatively editing RNA/DNA substrates. A3C is cytoplasmic and ubiquitously expressed. While the editing function has been investigated *in vitro* and the role of A3C as an antiviral RBP is well described, its cellular role in the cancer context is poorly understood.

To investigate the function of A3C, we generated CRISPR/Cas9-mediated knockout cells in ccRCC-derived cell lines and A3C recovery cell lines. Comparison of these cell lines revealed that the A3C promotes tumor cell growth upon adhesion stress (anoikis resistance) as well as upon FBS depletion (starvation), conditions a growing tumor has to deal with. Additionally, xenograft studies in nude mice provide strong evidence that A3C enhances the subcutaneous growth of ccRCC-derived cells suggesting a substantial oncogenic potential of A3C *in vivo*. Transcriptomic analyses by RNA-sequencing revealed a plethora of pathways affected by deregulated A3C expression, most notably the NFKB-pathway. Luciferase reporter analyses, RIP studies and RT-qPCR demonstrated that A3C activates the NFKB-pathway, putatively by stabilizing factors that regulate the NFKB-pathway positively like CDK6.

Keywords: APOBEC3C, RNA-binding protein, NFKB signaling pathway, clear cell renal cell carcinoma.

ST11. Clinical Research, Translational Biomedicine & Personalised Medicine

Pharmacoprotection of the ovaries during chemotherapy using microRNA technology by gold nanoparticle delivery

Thuy Truong An Nguyen[1], Chrysanthi Alexandri[1], Raphaël Dutour[2], Gilles Bruylants[2], Isabelle Demeestere[1]
[1]Université Libre de Bruxelles – Research Laboratory on Human Reproduction (Medical School), Brussels, Belgium, [2]Université Libre de Bruxelles – Engineering of Molecular NanoSystems Lab (Polytechnic School), Brussels, Belgium

Advances in oncology during the last decades allowed the increase of survival rates. However, several anti-cancer treatments are potentially gonadotoxic. Different options exist to preserve woman fertility but involve invasive techniques. Pharmacological protection appears to be attractive and recently, microRNA-based therapy was considered as a promising tool to prevent chemotherapy-induced ovarian damage. We previously identified let-7a as a potential interesting target to protect mouse ovaries from chemotherapy toxicity considering its profile and function. Replacement miRNA therapy was able to prevent cyclophosphamide-induced apoptosis *in vitro* and follicular development *in vivo* using a mouse ovarian transplantation model. We are now focusing on a clinical applicable delivery system to vehicle this miRNA-mimic based on gold nanoparticles (AuNPs). The main advantage is their ability to be functionalized by adding molecules that could improve safety, stability and specificity. Preliminary results show that AuNPs are non-cytotoxic in human cell lines and mouse ovarian follicle models. Internalization of AuNPs was confirmed by confocal microscopy in both models thanks to fluorochrome functionalization and by transmission electron microscopy. First results about miRNA delivery show increased expression of let-7a compared to negative control after 6 h transfection in human and mouse cell lines. We are currently testing the expression of targeted genes in different transfection conditions, before assessing their effects during co-exposure with

chemotherapy. This project investigates a promising and innovative approach to preserve fertility during anti-cancer treatment and allow us to improve our knowledge of the mechanism of gonadotoxicity.

Keywords: Fertility preservation, microRNAs, nanotechnology, cancer.

ST12. Clinical Research, Translational Biomedicine & Personalised Medicine

CD-64-directed recombinant single-chain antibody fusion protein exhibits cytotoxicity and is a tool for site-specific diagnosis of acute myeloid leukemia

Sikozile Ncembu
University of Cape Town, Cape Town, South Africa

Cancer immunotherapy is a promising innovative and effective treatment for many forms of cancer. Among hematological malignancies, acute myeloid leukemia (AML) remains an unmet medical need as it is mainly treated with chemotherapy, which is associated with serious side effects. Therefore, antibody-based targeted therapy is preferred as it can target and specifically eliminate malignant cells. H22(scFv) ETA' is an immunotoxin consisting of a humanized single-chain fragment antibody (scFv) targeting CD64, which is overexpressed on the surface of AML cells, and a truncated version of Pseudomonas exotoxin A (ETA'), which kills CD64-positive AML cells. CD64 is highly expressed on monocytic blast cells in AML patients but not on normal hematopoietic stem cells, making it a suitable target antigen. H22(scFv) ETA' was recombinantly expressed in *E. coli* Bl21 (DE3) and purified by metal ion affinity chromatography and size exclusion chromatography. The cytotoxic efficacy of H22(scFv) ETA' was assessed by Annexin V bioassay and binding assays using flow cytometry. A diagnostic fusion protein version of H22(scFv) ETA' was constructed in which the toxic component ETA' was removed and replaced with SNAP-tag to generate H22(scFv)-SNAP. SNAP¬tag enables efficient tumor targeting and diagnosis of molecular biomarkers for cancer. This study showed that H22(scFv) ETA' is cytotoxic to AML CD64-positive cancer cells. Specific binding of H22(scFv) SNAP to CD64-positive cell lines HL −60 and U937 was also demonstrated. The current phase of this study is focused on optimizing the productivity of H22(scFv) ETA' and H22(scFv) SNAP and on large-scale production to enable further preclinical/clinical studies.

Keywords: Immunotoxin, SNAP-tag, AML, CD64, process development.

ST13. Biomedical Engineering & Imaging Sciences

Design, fabrication and testing of a new electrode setup for electrical stimulation of cell cultures

Daniel Martín[1,2], Alberto Yúfera[2], Paula Daza[1]
[1]*Facultad de Biología, Universidad de Sevilla, Seville, Spain,* [2]*Escuela Superior de Ingeniería Informática, Universidad de Sevilla, Seville, Spain*

Electrical stimulation is a novel tool to promote the differentiation and proliferation of precursor cells. In this work, we have studied the effects of alternating current electrical stimulation on murine neuronal progenitor cells overexpressing APP (N2a-APP), in comparison with a standard neuronal progenitor cell line, N2a. For this study, we have designed and developed an electrical stimulation system as well as a culture stimulation protocol, and we have tested different electrode setups. The designed stimulation system generates the desired voltage stimulation signal through an STM32 microcontroller and an analog amplification stage. That signal is then applied to the culture through a pair of electrodes. The electrodes designed for this study have a uniform distribution of electric field in space, as well as a pitch of 1 mm between the positive and negative terminals; this allows us to know the exact electric field suffered by the cells and convert it automatically to a value expressed as a voltage divided by a distance (generally, mV/mm). The aim of this work has been to test various electrode setups and materials in order to optimize the biocompatibility, price, convenience, and effectiveness of stimulation. The results of this study show that electrical stimulation promotes differentiation in N2a and N2a-APP, without affecting cell density or viability.

Keywords: Cell differentiation, electrical-stimulation, microelectrodes.

ST14. Obesity, Diabetes & Other Diseases

Finding a receptor isoform selective and thermally stable insulin analogue for better life comfort of diabetic patients

Terezie Páníková[1,2], Katarína Mitrová[1], Lenka Žáková[1], Irena Selicharová[1], Jiří Jiráček[1]
[1]*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences Prague, Czech Republic,* [2]*University of Chemistry and Technology, Prague, Czech Republic*

Insulin elicits its functions through binding to the insulin receptor (IR), which exists in two isoforms. The longer IR-B is predominant in adult hepatocytes (more than 90%), skeletal muscle and subcutaneous fat (both about 70% IR-B), while the shorter IR-A is almost exclusively expressed in the brain, lymphatic tissues, or embryo. We aim to design insulin analogues, which could increase the comfort of diabetic patients. First, such an insulin analogue should bind preferentially to IR-B and therefore enable a more physiological control of internal glucose metabolism. Second, thermally more stable insulin could eliminate the constant need for refrigeration of insulin. We systematically designed dozens of insulin analogues based on the known structures of the insulin–receptor complexes. We have selected one

analogue which has only two amino acid alterations compared to human insulin. The analogue is 4x more thermally stable at 37 °C. It has 3x higher selectivity for IR B than human insulin in competitive binding assay with radioactively-labeled insulin in cell cultures which only express IR-A or IR-B. This analogue is more effective in lowering blood glucose in mice in insulin tolerance test and from our preliminary results it is tissue-specific in mice due to IR-B selectivity. We have filed a patent application for this very promising analogue (PCT/CZ2021/050123).

Keywords: Human insulin analogue, liver selective, thermally stable, diabetes.

ST16. Cancer Biology & Oncology

Investigating the role of aquaporins as transceptors in cancer

Catarina Pimpão[1,2], Inês V. Da Silva[1,2], Graça Soveral[1,2]
[1]*Faculty of Pharmacy, Research Institute for Medicines (iMed.ULisboa), Universidade de Lisboa, Lisbon, Portugal,* [2]*Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal*

Aquaporins (AQPs) are transmembrane proteins that mediate water and glycerol transport across cell membranes, being crucial for body water and energy homeostasis. AQPs are overexpressed in different types of cancer and are involved in tumor progression, cancer cell proliferation and migration, suggesting their potential as novel targets for cancer therapy. Therefore, the identification of AQP modulators with promising anticancer properties can boost cancer therapeutics. Moreover, AQP overexpression in cancer has been correlated with signaling pathways, indicating a novel role of AQPs as transceptors, acting both as transporters and receptors in cell membranes. This new concept can be investigated by studying AQP interplay with signaling pathways and screening novel potent and selective AQP modulators with impact on tumor growth and development. In this work, we developed a human cell model to evaluate AQP activity and modulation. Therefore, HEK-293 T cells with low endogenous expression of membrane transporters and high transfection efficiency, were stably transfected to individually overexpress the AQPs most associated with cancer: AQP3 (water and glycerol channel) and AQP5 (water channel). We have validated both gene and protein expression by qPCR and Western Blot in AQP3- and AQP5-overexpressing cells and confirmed their water and glycerol permeability by epifluorescence microscopy. Through wound closure assay, AQP3- and AQP5-expressing cells revealed a faster cell migration compared to control. This optimized cell platform will enable us to investigate AQPs as transceptors in the modulation of cell biophysical properties, cellular biology and signaling pathways, and will allow the identification of aquaporin inhibitors with anticancer properties.

Keywords: Aquaporins, permeability, cancer modulators, transceptors.

ST17. Obesity, Diabetes & Other Diseases

Identification and characterisation of novel free fatty acid receptor 1 modulators

Cosmin Trif, Sorina-Andreea Anghel, Alexandra-Maria Bġnicġ, Sorin Tunaru
Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

Free Fatty Acid Receptor 1 (FFAR1) is a G-protein coupled receptor (GPCR) expressed in the pancreatic β-cells that enhances glucose stimulated insulin secretion (GSIS) upon ligand binding. Therefore, the discovery of new agonists or positive allosteric modulators (PAMs) might provide new therapeutic strategies for type 2 diabetes mellitus (T2DM). After high-throughput screening targeting FFAR1, we have identified several potential hits. Out of the potential candidates, we have confirmed three molecules that mediate cellular effects in a FFAR1-dependent fashion. We further characterized these compounds by determining their potency, efficacy and selectivity among different FFARs. In order to further investigate their potential involvement in diabetes we assessed their ability to enhance GSIS by performing ELISA on the INS-1E cell line. The identified compounds might be used as novel therapies for T2DM. Furthermore, they can serve as molecular scaffolds for designing more potent agonists. Acknowledgement: *This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS UEFISCDI, project number PNIIIP22.1PED20195179.*

Keywords: FFAR1, insulin, diabetes, GPCRs.

ST19. Immunology, Microbiology & Infectious Diseases

Novel chimeric hepatitis B S/preS1 antigen induces efficient cellular and humoral immune response and production of virus-neutralizing antibodies

Ana-Maria Pantazica[1], Mihaela-Olivia Dobrica[1], Catalin Lazar[1], Catalin Tucureanu[3], Iuliana Caras[3], Irina Ionescu[3], Adriana Costache[3], Adrian Onu[3], Jihong Liu-Clarke[2], Crina Stavaru[3], Norica Branza-Nichita[1]
[1]*Institute of Biochemistry of the Romanian Academy, Bucharest, Romania,* [2]*NIBIO - Norwegian Institute for Bioeconomy Research, Ås, Norway,* [3]*"Cantacuzino" Medico-Military National Research Institute, Bucharest, Romania*

Despite the availability of antiviral therapies and preventative vaccination protocols, Hepatitis B virus (HBV) infection remains a significant global health issue, with more than 800,000 deaths per year due to HBV-associated complications. The current HBV vaccine, based on the small (S) viral envelope protein has significantly contributed to a decrease in HBV infection but was shown to not elicit protective antibody titers in 10% of the vaccinated population. Moreover, the emergence of vaccine-escape HBV variants and data regarding loss of long-term seroprotection have further urged the development of more immunogenic HBV vaccines. In this regard, the preS1 domain of the large (L) envelope protein is of particular interest, due to its critical role in HBV entry. Here, we aimed to develop novel vaccine candidates by incorporating

varying immunogenic epitopes derived from the preS1 domain into the S antigenic loop. We then analyzed the assembly, secretion, and ability to induce HBV neutralizing antibodies of these antigens. We identified one chimeric antigen with superior expression and secretion levels, as well as ability to assemble into SVPs, which was produced in mammalian cells and purified for immunological studies. Analysis of the immune response revealed that the selected chimeric antigen elicited a strong humoral and cellular immune response when compared to the S protein, as well as infection neutralizing antibodies against both wild-type and vaccine-escape HBV variants. Thus, our results suggest that the novel S/preS1 protein is a promising potential vaccine candidate for administration in poor-responders to current HBV vaccines.

Keywords: HBV vaccine, SVPs, antigens.

ST20. Genetics & Epigenetics

A study of the relationship between histone citrullination and methylation in human microvascular endothelial cells

Oskar Ciesielski[1,2], Karolina Górecka[1], Aneta Balcerczyk[1]
[1]Department of Molecular Biophysics, University of Lodz, Lodz, Poland, [2]The BioMedChem Doctoral School of the University of Lodz and Lodz Institutes of the Polish Academy of Sciences, University of Lodz, Lodz, Poland

The co-occurrence of the various histone post-translational modifications on the chromatin template generates the so-called histone code, that is read by proteins modulating chromatin structure and, consequently, gene expression. Several recent research groups have indicated a possible link of the well-known and established histone methylation and the still poorly understood citrullination. The endothelium, considered widely as the largest endocrine organ was shown to be particularly prone to epigenetic changes induced by the environment and most notably diet. Here, we strived to examine whether histone methylation and citrullination are linked in human microvascular endothelial cells. To that end, we used HMEC-1 cells with knock out by specific shRNA histone methylation regulators: demethylase LSD1, but also methyltransferase G9a and Set7/9. We treated the cells with established citrullination inhibitors: BB-CL-amidine and Cl-amidine and performed a cytotoxicity assay with resazurin reduction. Next, we analyzed the histone modification (H3cit, H4R2cit, H3K9me3, H3K4me3, H3K27me3) profile of the treated knockout cells by Western blotting, as well as the expression of heterochromatin-associated proteins: HP1a, HP1g and CAF1. To investigate the possible effects of methylation-citrullination interplay on endothelial cell function, we checked the expression of selected important endothelial genes such as VEGFA, VEGFC, EDN1 and other by qPCR. We found that inhibiting citrullination in the used KO HMEC-1 models induced changes in the epigenetic profile as well as expression of endothelial genes, which shows a possible important link between the two modifications. *The Presented Study was funded by the Polish Ministry of Higher Education under Diamond Grant DI2018 018948*.

Keywords: Citrullination, methylation, histone PTMs, endothelium.

ST21. Obesity, Diabetes & Other Diseases

New players involved in the development of obesity and associated metabolic comorbidities: role of let-7b-5p and miR-191-5p

Carmen Torres Granados[1,2,3], Miguel Ruiz Cruz[1,2,3], Cecilia Perdices López[1,2,3,4], Inmaculada Velasco Aguayo[1,2,3], Manuel Tena Sempere[1,2,3,4], Juan Roa Rivas[1,2,3,4]
[1]Maimónides Biomedical Research Institute of Córdoba (IMIBIC), Cordoba, Spain, [2]Department of Cell Biology, Physiology and Immunology, University of Córdoba, Cordoba, Spain, [3]Hospital Universitario Reina Sofía, Córdoba, Spain, [4]CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Cordoba, Spain

WHO data in 2016 revealed alarming numbers of children and adolescents between 5–18 years old suffering from being overweight or obese, conditions that increase the risk of developing other associated comorbidities in adulthood, including cardiometabolic disorders, consolidating obesity as one of the most threatening public health problems.
In recent years, microRNAs (miRNAs) have become an effective tool in biomedicine due to their applications as biomarkers and therapeutic targets in different pathologies. Previous results of our research group revealed changes in the expression of different circulating miRNAs in obese prepuberal girls, compared with normal weight counterparts. Validation studies in a model of infantile obesity in Wistar rats allowed us to select two specific miRNAs (let-7b-5p and miR-191-5p), as potential targets involved in the development of childhood obesity and associated comorbidities. The follow-up of this project demonstrated the specific blockade of these miRNAs in Wistar rats, using antagomirs (LNA-let-7b and LNA-miR-191), during the prepuberal period, produces a deterioration of the metabolic state. In this context, both treatments produced an increase in body weight and body length, and alterations in glucose homeostasis and energy expenditure, with this effect being more pronounced in the LNA-miR-191 group. In conclusion, our data suggest a protective role for let-7b-5p and miR-191-5p against the development of metabolic complications in obese individuals. The identification of molecular targets and pathways involved in the actions of let-7b-5p and miR-191-5p is currently undergoing in our lab. This could help to define new therapeutic strategies for the management of early obesity and its comorbidities.

Keywords: microRNAs, childhood obesity, metabolic syndrome.

POSTERS

P001. Computational Biology, Bioinformatics & Artificial Intelligence

Characterization of microbial diversity of two tomato cultivars through targeted next-generation sequencing of 16S rRNA and ITS techniques

Abdulsalam Rukayat Abiola, Oluwatosin Ademola Ijabadeniyi, Saheed Sabiu
Durban University of Technology, Durban, South Africa

Despite the fact that the nutritional and economic values of *Solanum lycopersicum* (tomato) are significantly impacted by microbial spoilage, the available data on its microbial community especially during spoilage is limited and have only been primarily characterized by conventional culture-dependent methods. This study used a targeted high-throughput next-generation sequencing method to characterize the microbial diversity of two tomato cultivars (Jam and Round) commonly consumed in South Africa, at different storage intervals (1, 6, and 12 days). The results revealed that on days 6 and 12, spoilage fungi (2 and 12, respectively) and 4 spoilage bacteria species were found on both days in the Jam cultivar, contrary to the 5 spoilage fungi on both days and spoilage bacterial species (1 and 6 respectively) in comparison to day 1 with normal floral. On day 12, *Myroides odoratus* accounted for 60% of the spoilage bacteria while *Fusarium acutatum* accounted for 53% of the spoilage fungi in Jam tomato, which is in sharp contrast to the 50% (*Pantoea agglomerans*) and 87% (*Alternaria alternata*) observed for spoilage bacteria and fungi, respectively in the Round tomato. However, alpha- and beta-diversity metrics showed no significant differences ($\alpha > 0.05$) between the two cultivars as supported by Shannon and Simpson (Alpha-diversity) and non-metric multidimensional scaling (Beta-diversity). Analysis from the study revealed that microbial diversity in the investigated cultivars changed during the storage periods examined, with the Jam tomatoes having higher diversity than the Round tomatoes. Hence, heterogeneity in diversity and abundance is demonstrated between the two cultivars.

Keywords: Microbial diversity, high-throughput sequencing, spoilage organisms, alpha- and beta-diversity.

P002. Cellular & Molecular Biology

Single-fluorophore indicator to explore cellular and sub-cellular lactate dynamics

Camila Aburto[1,2]
[1]*Centro de Estudios Científicos, Valdivia, Chile,* [2]*Universidad Austral de Chile, Valdivia, Chile*

Lactate is an energy substrate and intercellular signaling molecule with multiple bodily functions. Lactate has physiological roles such as neurogenesis, immune response and memory consolidation, and is involved in inflammation, cancer and neurodegeneration. Laconic, the first genetically-encoded lactate FRET indicator has been instrumental to advance the understanding of lactate transport, glycolysis and mitochondrial physiology. However, FRET sensors present challenging sub-cellular targeting and show relatively small fluorescence change, which makes it non-optimal for high throughput screening. We have now developed a single-fluorophore indicator for lactate, CanlonicSF. This indicator exhibits a maximal intensiometric fluorescence response $\Delta F/F0$ of 3.0 and a KD of ~300 μM. The fluorescence is not affected by other monocarboxylates. The lactate indicator was not significantly affected by $Ca2+$ at the physiological concentrations prevailing in cytosol, endoplasmic reticulum and extracellular space, but was affected by $Ca2+$ in the low micromolar range. Targeting the indicator to the endoplasmic reticulum revealed for the first time sub-cellular lactate dynamics. Its improved lactate-induced fluorescence response permitted the development of a multiwell plate assay to screen for inhibitors of the monocarboxylate transporters MCTs, a pharmaceutical target for cancer and inflammation. The functionality of the indicator in living tissue was demonstrated in the brain of *Drosophila melanogaster* larvae. CanlonicSF is well suited to explore lactate dynamics with sub-cellular resolution in intact systems.

Keywords: Lactate transport, GFP-based CanlonicSF, endoplasmic reticulum, high-throughput screening.

P003. Chemistry & Biochemistry

Development of the hydantoinase process

Hovsep Aganyants[1], Haykanush Koloyan[1], Anichka Hovsepyan[1], Artur Hambardzumyan[1], Vehary Sakanyan[2]
[1]*SPC "ARMBIOTECHNOLOGY" NAS RA, Yerevan, Armenia,* [2]*Faculty of Pharmacy, Nantes University, Nantes, France*

The "hydantoinase process" consists of two enzymatic reactions catalyzed by hydantoinase and carbamoylase to convert chemically synthesized precursors into amino acid derivatives. D-hydantoinase catalyzes cleavage of 5-substituted hydantoin to yield corresponding D-carbamoylamino acid. D-carbamoylase hydrolyzes D-carbamoylamino acid into D-amino acid. Therefore, enzymes are promising catalysts for production of unusual precursors essential for drug development that cannot be obtained chemically. D-hydantoinase, which we cloned from *Geobacillus stearothermophilus*, showed activity against D,L-5-methylhydantoin and D, L-5-(2-methylthioethyl)hydantoin. To enlarge the substrate specificity, we performed molecular docking by virtual substitution of amino acids in the protein. We have identified key amino acid residues whose substitution can affect substrate specificity and enzyme activity. This allowed us to design a modified substrate binding pocket, and enabled us to recognize new substrates, including hydantoin precursors of hydrophobic D-phenylalanine and D-tryptophan. In this way, new proteins were generated by site-directed mutagenesis, and enzymatic assays confirmed our expectations. Meantime, the mutant enzymes retained the original thermostability. Thereon, *in silico* approach allowed us to identify two carbamoylases in Pseudomonas sp. and *Sinorhizobium morelense* with appropriate properties. Homology modeling was applied to predict the structure and properties of carbamoylases. The calculated geometric parameters were acceptable for further enzyme improving. The substrate binding sites of carbamoylases were studied by molecular modeling. It was found that the enzymes can exhibit specific activity against carbamoyl-para-hydroxy-D-phenylglycine, carbamoyl-β-alanine, carbamoyl-D-alanine, carbamoyl-D-leucine, carbamoyl-D-methionine, carbamoyl-D-phenylalaine, carbamoyl-D-phenylglycine, carbamoyl-D-

tryptophan, and carbamoyl-D-valine. Genetically engineered constructs are important for the microbial transformation of new chemicals for biomedical applications. *Study supported by the SCS MESCS RA [project No. 21T-2I289].*

Keywords: D-hydantoinase, D-carbamoylase, D-phenylalanine, D-tryptophan.

P004. Immunology, Microbiology & Infectious Diseases

Diagnostics of *Candida* pathogens in Morocco: molecular methods reveal pitfalls of current phenotype-based identification

Islam Ahaik[1,2], Juan Carlos Nunez-Rodriguez[1,3], Samira Bouhdid[2], Alicia Benavente García[1], Ewa Ksiezopolska[1,3], Toni Gabaldón[1,3,4,5], Jamal Abrini[2]

[1]*Institute for Research in Biomedicine (IRB Barcelona), Mechanisms of Disease Program, The Barcelona Institute of Science and Technology, Barcelona, Spain,* [2]*Department of Biology, Faculty of Sciences, University Abdelmalek Essaâdi, Tétouan, Morocco,* [3]*Barcelona Supercomputing Centre (BSC-CNS), Life Sciences Department, Barcelona, Spain,* [4]*Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain,* [5]*Centro de Investigación Biomédica En Red de Enfermedades Infecciosas (CIBERINFEC), Barcelona, Spain*

In the last few decades, Candida infections have become a concerning health problem due to the considerable increase of reported cases, particularly among immunocompromised individuals. In Morocco, the diagnosis of these infections faces many challenges such as the lack of appropriate resources due to relatively low attention in comparison to bacterial infections. Two main phenotypic methods are usually used in Moroccan medical laboratories to identify Candida species: the germ tube test (GT) and the use of selective medium (chromogenic agar). Therefore, it is important to check the reliability of these two identification methods. In this study we compared the results obtained by phenotypic identification and those found using Internal Transcribed Spacers (ITS) sequencing. Ninety-three specimens of Candida species were collected from different clinical laboratories in Tetouan, Morocco. Among them, 39 strains were classified as non-albicans based on the GT test. In addition, some of these strains were re-checked using the selective medium. Interestingly, ITS sequencing showed that 13 out of the 39 strains classified as non-albicans were indeed *C. albicans*, which shows lack of sensitivity for the GT test. Furthermore, most strains (4/6) identified as *C. krusei* based on chromogenic agar were shown to be *C. glabrata* or *C. parapsilosis* using the same molecular technique. Our results suggest that infections by some Candida species may be under- (e.g. *C. albicans*) or over- (e.g. non-albicans infections, *C. krusei*) estimated based on currently used methods. Given the higher precision of molecular methods, its routine use in clinical diagnosis would be advisable.

Keywords: Candida infections, germ tube test, chromogenic agar, ITS sequencing.

P006. Pharmacology, Toxicology & Nutrition

Up-regulation of oxidative stress and inflammation in the brain of albino wistar rats following sub-acute administration of *Synclisia scabrida* root extract

Emmanuel Modo[1], Ijeoma Eke[1], Anthony Nnamudi[1], Utibe Bassey[1], Okon Etim[1], Ekam Akpakpan[1], Wopara Iheanyichukwu[2], Prosper Legborsi[1]

[1]*PAMO University of Medical Science, Port-Harcourt, Rivers State, Nigeria,* [2]*University of Port Harcourt, Rivers State, Nigeria*

Sub-acute neurotoxicity of root extract of *Synclisia scabrida* was evaluated in albino Wistar rats. Thirty male albino rats with average weight of 140 g were randomized into 5 groups consisting of 6 rats in each group. Group 1 was the control while 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg of the root extract were administered to Groups 2, 3, 4 and 5 respectively for 28 days. Malondialdehyde, glutathione, nitric oxide, protein, tumor necrosis factor-α, acetylcholine, catalase and acetylcholinesterase levels were measured in brain homogenates. Histology of the hippocampus and cerebral cortex were evaluated. Root extract of *S. scabrida* was observed to increase malondialdehyde concentration and decrease antioxidant biomarkers when compared with the control. Significantly ($P < 0.05$) increased TNF-α concentration and acetylcholinesterase activity caused by the extract when compared with the control was observed. The concentration of acetylcholine significantly decreased in *S. scabrida*-treated groups in comparison with the control. The histomorphology of the hippocampus and cerebral cortex revealed pyknotic pyramidal neurons in *S. scabrida*-treated Wistar rats relative to the control with normal pyramidal neurons. The study has demonstrated that the root extract of *S. scabrida* induces and upregulates oxidative stress and inflammation in the brain of albino Wistar rats coupled with reduced acetylcholine concentration, hence the extract possesses neurotoxic potential.

Keywords: *Synclisia scabrida*, oxidative stress, antioxidant, inflammation, neurotoxicity.

P007. Immunology, Microbiology & Infectious Diseases

The incidence and current treatment options for multidrug resistant *Acinetobacter baumannii* infections in clinical settings

Raja Waqar Ali[1], Paolo Antonio Grossi[1], Mobeen Haider[2], Imran Nazir[3], Ikram Ahmad[4], Hafza Zahira Manzoor[1]

[1]*Department of Medicine & Surgery, University of Insubria, Varese, Italy,* [2]*Services Institute of Medical Sciences & Hospital, Lahore, Pakistan,* [3]*Aziz Bhatti DHQ Hospital, Gujrat, Pakistan,* [4]*Pakistan Institute of Medical Sciences & Hospital, Islamabad, Pakistan*

Introduction: *Acinetobacter baumannii* is one of the most often isolated bacteria strains in intensive care unit (ICU) patients, and its antibiotic resistance (MDR) poses significant therapeutic challenges. Blood infections caused by multidrug-resistant *Acinetobacter* sp. in ICU patients are a major problem in hospital settings. *Aims & Objectives*: The overall purpose of the study was to investigate the incident and treatment analysis of healthcare-associated infections caused by *A.baumannii*. In addition to exploring the incidence of AB strain infections and treatment success rate of these

infections by administrating cefiderocol, a novel antibiotic, against gram-negative pathogens. *Material & Methods*: The study was conducted at the Department of Infectious Diseases and Medical Bacteriology Laboratory of the Circolo Hospital (ASST SetteLaghi) Varese-Italy. At present we have recruited 15 critically ill patients with either bacteremia or ventilator-associated pneumonia caused by carbapenem-resistant *A.baumannii* who received cefiderocol. All the bacterial isolates of positive MDR-AB strains were included in the present study. *Expected Results & Conclusion*: Thirty-day clinical success was 80% (n = 12). Two patients had expired the death ratio is 13.3% (n = 2). One patient has been referred to another hospital referral ratio was 6.6% (n = 1). The current results showed a good treatment success rate of cefiderocol against MDR *A.baumannii*. However, in the current study report, there are limitations such as the study and more data collection is underway to achieve the required target of clinical samples in the next few months, which will give clear and more precise expected results. Future prospective studies are warranted.

Keywords: Multi-drug resistant bacteria, infection, prevention and treatment.

P008. Cancer Biology & Oncology

Characterization of undifferentiated neuroblastoma tumour cells and their contribution to aggressiveness

Aida Amador-Álvarez[1,2], María A. Gómez-Muñoz[2,3], Ricardo Pardal[2,3], Francisco M. Vega[1,2]
[1]*Department of Cell Biology, Faculty of Biology, Universidad de Sevilla, Seville, Spain,* [2]*Instituto de Biomedicina de Sevilla (IBiS), Universidad de Sevilla/CSIC/Junta de Andalucía, Seville, Spain,* [3]*Department of Medical Physiology and Biophysics, Faculty of Medicine, Universidad de Sevilla, Seville, Spain*

Neuroblastomas are heterogeneous, metastatic tumors originating from the neural crest of the sympathetic nervous system. Intratumor cellular heterogeneity has been observed, including at least two cell populations: undifferentiated stem-like mesenchymal cells and compromised adrenergic cells. The undifferentiated cell population seems to exhibit stem cell properties and has been suggested to contribute to neuroblastoma malignization by evading intensive multimodal therapy and being responsible for tumor relapses and metastasis formation, although the exact mechanism is not clear. The formation of aggressive metastases takes place thanks to a multi-stage process in which cell migration and invasion are essential. Migratory and invasive cancer cells undergo dramatic molecular and cellular changes by reshaping their cytoskeleton. These changes in cell shape can modulate cell phenotype and biological properties and are indicative of the behavior and evolution of the cells, influencing biological processes, such as proliferation, differentiation and stem cell fate.
The aim of this research is to study the specific morphological, migratory and invasive properties of undifferentiated neuroblastoma cells (UNCs). The results obtained show that UNCs present defined, persistent morphological features. These characteristics are concordant with a greater cellular plasticity. In addition, UNCs migrate at a slower speed, but with greater directionality, exhibit increased blebs-mediated membrane actin dynamics, and degrade the extracellular matrix to a greater extent than the rest of tumor cells. These preliminary results show that UNCs are characterized by a particular migratory and invasive behavior, suggesting that they possess significant metastatic features calling for further analysis of their contribution to aggressiveness *in vivo*.

Keywords: Cancer stem cell, metastasis, invasion, migration, neuroblastoma.

P009. Genetics & Epigenetics

DNA topoisomerase II break repair in human cells

Carmen Angulo Jiménez
University of Seville, Seville, Spain

The development of new cancer chemotherapy treatments to specifically attack tumor cells is of significant interest to control this disease, with great impact in public health. Many of these treatments are based on genotoxic agents that promote DNA double strands breaks (DSBs) in tumor cells. DSBs are the most cytotoxic lesions of those occurring in the DNA and can lead to cell death. A prominent target of chemotherapy is DNA topoisomerase 2 (TOP2), an enzyme that relaxes the topological stress generated during DNA metabolism. TOP2 activity is very high in cancer cells, characterized by their high proliferation, therefore, high topological torsional stress. Etoposide (ETP), a TOP2 poison, converts transient TOP2 complexes into DSBs, thus providing a very strong antitumor capacity. However, ETP treatment becomes limiting in cancer chemotherapy due to appearance of secondary leukemias. These secondary leukemias are likely promoted by unfaithful repair of ETP- mediated DSBs. However, the molecular bases of the mechanisms that repair ETP-mediated DSBs are not fully understood. To unveil novel repair pathways that could influence TOP2 DSB repair we have genetically analyzed several factors implicated in Non-Homologous Repair pathway, one of the main DSB repair routes. Our results reveal a new pathway involved in TOP2 DSB repair. This work has important implications for TOP2-based chemotherapy, providing novel insights about TOP2 DSB repair.

Keywords: DNA double strands breaks, topoisomerase 2, etoposide, repair.

P010. Obesity, Diabetes & Other Diseases

Expression of the Prader-Willi syndrome-related genes *Magel2* and *Ndn* and their predicted microRNAs in the hypothalamus of lean and obese rats throughout pubertal development

Álvaro Aranda Torrecillas[1,2], Elvira Rodríguez Vázquez[1,2], Silvia Daza Dueñas[1,2], Manuel Tena Sempere[1,2], Juan Manuel Castellano Rodríguez[1,2]
[1]*Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Cordoba, Spain,* [2]*Molecular Biology, Immunology and Physiology Department, University of Cordoba, Cordoba, Spain*

Prader-Willi Syndrome (PWS) is a rare neurodevelopmental disorder caused by the loss of expression of imprinted, paternally inherited genes at the 15q11q13 locus. Among the endocrine manifestations frequently bound to PWS are hyperphagia, obesity, and alterations in the timing of puberty onset. Emerging data suggest that hypothalamic alterations induced by lack of

expression of *MAGEL2* and *NDN*, two genes located at the 15q11q13 locus, might contribute to the pubertal disorders associated with PWS. However, the regulatory elements that might control these genes and take part in these alterations remain unknown. In this study, we analyzed the expression of *Magel2* and *Ndn* in the hypothalamus of lean and obese rats throughout pubertal development and their potential regulation by miRNAs, which are considered relevant modulatory factors in the central control of puberty. The hypothalamic levels of *Magel2* and *Ndn* mRNA were not altered in lean female and male rats during the pubertal transition or in obese male rats with precocious puberty. In contrast, a significant reduction in the hypothalamic expression of both transcripts was detected in early overfed female rats with advanced puberty. Moreover, such a decline was associated with a rising hypothalamic expression of miR-30b-5p and miR-200b-3p, two predicted miRNAs for *Magel2* and *Ndn* regulation, respectively. Overall, our findings suggest a potential interplay between miR-30b/*Magel2* and miR-200b/*Ndn* in obesity-induced precocious puberty in female rats. However, further studies are required to confirm and extend these preliminary findings.

Keywords: Prader-Willi, puberty, hypothalamus, miRNAs.

P011. Cancer Biology & Oncology

Regulation of ER activity in secretory cancer cells: characterization of the MYRF transcription factor and its interaction partners

Fabiana Arco
European Institute of Oncology (IEO), Milan, Italy

The ability to tolerate endoplasmic reticulum (ER) stress represents a critical adaptive response of tumors that enhances cancer cell survival, angiogenesis and metastatic capacity. This survival strategy is adopted by cancer cells with high secretory activity that adapt their ER function to support the complex folding of secretory proteins. Previous studies conducted in our laboratory demonstrated that the myelin regulatory factor (MYRF), an ER-associated transcription factor, maintains ER homeostasis in human tumors with secretory activity. After trimerization and self-cleavage, MYRF releases the N-terminal fragment which translocates into the nucleus to exert its transcriptional role by regulating the expression of genes encoding secretory proteins. However, the role of C-terminal fragment which remains inserted in the ER is still unclear. To understand the role of this fragment, we first identified its interactors in the ER lumen, including cleavage regulatory factors, using TurboID mediated proximity labeling followed by biotin pull-down and mass spectrometry. Furthermore, to investigate the role of the MYRF C-terminal fragment in controlling ER activity independently of transcriptional regulation, we generated clonal cell lines lacking one of the two critical MYRF functional domains – DNA binding or transmembrane domains - by CRISPR/Cas9-mediated in-frame deletion. MYRF domain-specific mutants will be phenotypically and functionally characterized. Here, we identified MYRF interactors in the ER lumen, potentially critical for its cleavage regulation and function in the ER context, and we are dissecting the role of MYRF functional domains. This work could provide a mechanistic understanding of MYRF in regulation of ER activity in secretory cancer cells.

Keywords: Secretory cancer cells, endoplasmic reticulum, proteomics, biochemistry, stress response.

P012. Cancer Biology & Oncology

Synergistic effect of tamoxifen and enzalutamide in breast cancer cell lines

Nuria Arroyo-Garrapucho[1,2,3], Lorena Misas-Margüello[2], Paula García-Vallés[1,2,3], Jésica Pérez-García[1,2,3], Raquel Seijas-Tamayo[1,4], Rogelio González-Sarmiento [1,2,3], Ana Belén Herrero [1,2,3]
[1]Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain, [2]Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain, [3]Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain, [4]Medical Oncology Service, University Hospital of Salamanca, Salamanca, Spain

Background: Breast cancer (BC) is the leading female cancer worldwide. Tamoxifen, a first-line treatment against BC, is an estrogen receptor (ER) antagonist in breast tissue. However, its prolonged use causes resistance and can cause endometrial cancer due to its agonistic effect in the uterine tissue. Moreover, some subtypes of breast cancer, like triple negative breast cancer, lack targeted therapies. The androgen receptor (AR) is emerging as a novel factor in breast cancer and enzalutamide, an AR inhibitor, is a potential therapeutical option. Thus, we studied the effect of the combination of tamoxifen and enzalutamide in BC cell lines (BCCLs). *Methods*: BCCLs BT549, BT474 and MCF7 were used. MTT and apoptosis assays were performed to test tamoxifen and enzalutamide sensitivity. Synergistic interaction between these two drugs was assessed using the median dose effect analysis of Chou and Talalay, and combination indices were calculated. Western blot and RT-qPCR were used to validate AR and ER status. *Results*: Our results show that tamoxifen and enzalutamide inhibit cell growth and induce apoptosis in both ER+ and ER- BCCLs. Combination of tamoxifen and enzalutamide caused a synergistic effect in all cell lines. *Conclusion*: Our study suggests that there is a synergistic effect between enzalutamide and tamoxifen, which could allow lowering the dose of both drugs, reducing the risk of adverse effects. *Grants*: This study was funded by FIS-FEDER PI20/01569.

Keywords: Breast cancer, tamoxifen, enzalutamide.

P013. Cellular & Molecular Biology

From teeth to bones

Bibiana Badurova[1,2], Romana Zahumenska[2], Tereza Pavlisova[2], Jan Strnadel[2], Maria Janickova[3], Slavomira Novakova[2], Erika Halasova[2], Henrieta Skovierova[2]
[1]Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia, [2]Biomedical Centre Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia, [3]Department of Stomatology and Maxillofacial Surgery, Jessenius Faculty of Medicine and University Hospital Martin, Comenius University in Bratislava, Slovakia

Teeth are highly differentiated organs that are composed of multiple parts such as enamel, dentin, cementum, pulp, and periodontal tissues. Teeth and the surrounding dental tissues are a natural source of stem cells, which are collectively called dental stem cells (DSCs). The potential for clinical use of these cells lies primarily in their excellent proliferative and differentiation properties, thanks to which DSCs can be used as a therapeutic source. Another advantage of DSCs is their non-invasive and affordable

acquisition. In our research, we focus primarily on the osteodifferentiation potential of dental stem cells, which in the future can be applied in the treatment of damaged bones after disease and injuries. The aim of the presented work was to compare the osteodifferentiation potential of dental stem cells, isolated from the patient's dental pulp, cultured in different culture conditions. We differentiated the cells in a commercially-available osteodifferentiation medium. At the same time, we compared the ability of cells to differentiate into osteoblasts/osteocytes or odontoblasts/odontocytes in 2D and 3D culture conditions. We monitored the morphological changes of the cells during osteodifferentiation using light microscopy, then we visualized specific proteins using fluorescence microscopy, and by staining the cells with Alizarin red, which is an indicator of calcium compounds produced by osteodifferentiated cells, we analyzed and quantified the presence of calcifications and the effectiveness of osteodifferentiation. We analyzed the level of expression of specific proteins/genes by Western blot/ qPCR. *This work was supported by GUK 65/2022 and VEGA 1/0310/21.*

Keywords: Dental stem cells, osteodifferentiation.

P014. Cancer Biology & Oncology

Crosstalk between tumour vasculature and ovarian cancer stem cells: the role of L1CAM

Micol Baronio
European Institute of Oncology (IEO), Milan, Italy

Ovarian cancer is the most lethal gynaecologic tumor, mainly due to relapse and chemoresistance, two events fuelled by ovarian cancer stem cells (OCSC). The *tumor microenvironment* (TME) is thought to act as a niche for OCSC, preserving stem cell properties and sustaining tumor recurrence. The crosstalk between TME and OCSC, therefore, offers a source of new targets to overcome the limitations of current treatments. We identified L1CAM, an adhesion glycoprotein, expressed both in tumor endothelium and epithelial cancer cells, as a mediator of the interaction between the vascular TME (vTME) and OCSC. L1CAM expression correlates with cancer proliferation, chemoresistance, metastatic spread, poor outcome, increased vessel permeability and angiogenesis. In addition, endothelial cells express a new isoform of L1CAM (L1-DTM), that is released as a soluble factor. We found that endothelial L1-DTM enhances stem-like properties in OCSC, a process that is mediated by STAT3 signaling. Furthermore, the RNA-sequencing analysis of L1-DTM-treated OCSC revealed a proinflammatory profile, able to support cancer initiation, proliferation and progression. Our approach will explore the possible application of L1-DTM as a druggable target in the crosstalk between vTME and OCSC. In fact, our findings could provide the rationale for exploring novel L1CAM-based therapeutic approaches aimed at the eradication of OC through the elimination of its stem cell compartment.

Keywords: Ovarian cancer, L1CAM, tumor vasculature, stem cells.

P015. Obesity, Diabetes & Other Diseases

Overexpression of the hypoxia inducible factor HIF-2α impairs pancreatic beta-cell function

Alexia Barroso Romero, Carlos Caballero Henares, Álvaro Flores Martínez, David A. Cano González
Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain

HIF transcription factors are the main effectors of the response to hypoxia at the cellular level by activating target genes involved in a variety of biological functions. Three types of HIF alpha subunits have been identified, HIF-1α, HIF-2α, and HIF-3α, HIF1α and HIF2α being the most extensively studied. Several studies have revealed that both impairment and activation of the HIF pathway result in pancreatic endocrine dysfunction, highlighting the crucial role of this pathway for proper endocrine function. HIF2α is overexpressed in beta cells under diabetic conditions suggesting a causal role of this factor in diabetes progression. Here, we aim to directly test this hypothesis by specifically activating HIF2α in the β-cell lineage in mice by Cre/LoxP technology (*Ins-Cre; HIF2dPA mice*). HIF-2α activation specifically in pancreatic β-cells renders β-cells unable to respond appropriately to elevated glucose, leading to severe glucose intolerance in mice. Glucose intolerance is caused by a defect in glucose-stimulated insulin secretion. Although analysis of the islets of Langerhans did not reveal obvious morphological abnormalities, mice with β-cell-specific activation of HIF2α display a decrease in the total area of β cells. Microarray analysis of gene expression in islets of *Ins-Cre; HIF2dPA* adult mice revealed striking changes in genes involved in the glycolysis pathway, vascularization and several signaling pathways involved in beta cell function. Remarkably, we observed that β cells of *Ins-Cre; HIF2dPA* adult mice presented markers characteristic of immature cells.

Keywords: Beta-cell, diabetes, HIF-2alfa, pancreas.

P017. Cancer Biology & Oncology

Deconvoluting the role of ascites in sustaining pro-metastatic chemoresistant CSCs in high grade serous ovarian cancer

Bianca Barzaghi[1,2], Pietro Lo Riso[1], Marta Rosa Sallese[1,2], Alessia Gatto[1,3], Ugo Cavallaro[1,3], Giuseppe Testa[1,2,4]
[1]Department of Experimental Oncology, European Institute of Oncology, Milan, Italy, [2]Department of Oncology and Hematoncology, University of Milan, Milan, Italy, [3]Unit of Gynecological Oncology Research, European Institute of Oncology, Milan, Italy, [4]Centre of Neurogenomics, Human Technopole, Milan, Italy

High Grade Serous Ovarian Cancer (HGSOC) is the fifth cause of cancer death among women due to late diagnosis and recurrence. Growing evidence relates relapse to the persistence of cancer stem cells (CSCs), a model corroborated by the prevalence of concomitant peritoneal ascites. Ascites, indeed, are crucial in supporting the growth of CSCs and their dissemination, defining the metastatic potential of OC. We aimed to investigate the impact of soluble ascitic factors in sustaining CSCs for the identification of pathogenetic mechanisms associated with cancer stemness and

chemoresistance for target discovery and drug development. We showed that culturing 2D cells and CSCs-enriched spheroids from patients' ascites with the supplementation of their ascitic fluid increases proliferation rate, propagability and sphere-forming efficiency, and allows preservation of the metabolic status of fresh samples, lost in standard culturing conditions. Indeed, 2D cells and spheroids cultured in ascitic fluid showed downregulation in cholesterol biosynthesis while upregulated glycolysis resulted only in spheroids, suggesting a phenotype specific for CSCs. Moreover, we found upregulation of ALOX15B, arachidonate 15-lipoxygenase II, involved in the production of fatty acid hydroperoxides, in CSC-enriched spheroids compared to 2D cells, which was particularly evident when using ascitic fluid. These results highlight the importance of recreating the tumor microenvironment in *in vitro* patient-derived models to better capture tumor features, such as its metabolic status. Indeed, supplementing ascitic fluid to 2D cells and spheroids allowed to identify metabolic alterations and putative druggable targets in ascites, highlighting the importance of the microenvironment in OC progression.

Keywords: Ovarian cancer, cancer stem cells, ascites, metabolism.

P018. Cancer Biology & Oncology

Contact percolation promotes collective flocking migration and a pro-inflammatory cytosolic DNA response in early breast cancer

Leonardo Barzaghi[1], Fabio Giavazzi[2], Brenda Green[1], Emanuela Frittoli[1], Andrea Palamidessi[1], Stefano Villa[2], Giorgio Scita[1]
[1]*IFOM, the AIRC Institute of Molecular Oncology, Milan, Italy,* [2]*Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy*

The collective behavior of cells during morphogenesis, wound healing and cancer invasion has been recently described in terms of phase transitions (PT). We previously found that the small GTPase RAB5A promotes a solid-to-fluid PT implicated in breast cancer progression. Carcinoma is heterogenous in both genetic and mechanical features; the impact of such heterogeneity on tissue-level PT is however poorly understood. Here, we address that issue using 2D models of mixed control and RAB5A-overexpressing breast cancer cells. By monitoring the dynamics of mixed monolayers through time-lapse microscopy, we report a flocking transition, characterized by collective and coordinated cellular migration, at a critical tissue composition. This occurs when RAB5A cells reach a threshold of cell connectivity – contact percolation threshold – and form a pervasive network throughout the system. Above this threshold, control cells exhibit an elongated shape and a coordinated mode of motion, similar to RAB5A-overexpressing fluid cells. This transition is mirrored by a transcriptional rewiring of both type of cells towards a pro-inflammatory phenotype, triggered by the hyperactivation of a cytosolic DNA response. Notably, we found that RAB5A cells secrete a variety of pro-inflammatory factors, which contribute to promoting an inflammatory phenotype in control cells. In summary, we outline the percolation of RAB5A cells within mixed populations of breast cancer cells which is dependent upon geometrical arrangements and that drives transcriptional rewiring towards a pro-inflammatory program. This mechano-chemical feedback might be exploited by tumors for local invasion and might represent a valuable marker for the early diagnosis of invasive lesions.

Keywords: Collective motility, breast cancer, mechanobiology.

P019. Cancer Biology & Oncology

Microangiometric study of breast carcinoma and its correlation with immunohistochemical and prognostic factors

Muna Dawoo Bawazeer[1], Ana Fernandez Roriguez[2]
[1]*Hospital Virgen del Rocío, Seville, Spain,* [2]*Universidad de Sevilla, Seville, Spain*

Eighty samples of breast carcinomas were studied in Hospital Virgen Macarena Sevilla. The classic prognostic factors studied were: tumor size, infiltration of lymphatic nodes, histological grade, tumor type and mitotic activity, apoptosis indices, and vascular invasion. A Leitz Diaplan microscope with a 40 X lens was used, and a field area of 0.312 mm^2 was obtained. The microvascularization was estimated with the IMAGE J program. The morphometric study followed a method described by A. Giatromanolaki *et al*. A database was created using the EPINFO v.3.3.2 program. Microvascularization in both tumoral areas (peripheral, internal) was associated with the classic prognostic factors, proliferation rates (ki67/MIB-1), and positive expression of c-erbB-2. Peripheral tumoral microvascularization was associated with negative progesterone receptors, while internal tumoral microvascularization was associated with positive expression of p53. Peripheral tumor microvascularization was significantly higher at the invasive edges of the tumor with a gradual decline towards central areas. In the peripheral areas of the tumor, a correlation was detected between the morphometric parameters representing shape and count of the microvessels with the microvascular density. A decrease in the perimeter, compactness and more regular shape of the microvessels was associated with positive expression of c-cerbB2 and negative expression of estrogen receptors in the inner tumoral area; the microvessel area registered a positive correlation with the count and density of the microvessels, and associated significantly with vascular invasion. High values of parameters representing size of microvessels were associated with high proliferation rates. So the larger caliber of microvessels in the internal tumoral areas indicated the tumors were more proliferative and infiltrating.

Keywords: Cancer, breast carcinoma, angiogenesis, morphometry, immunohistochemistry.

P020. Cardiovascular Disease

Effect of ischemia-reperfusion injury on mitochondria-associated membranes in aged heart

Maria Bencurova, Terezia Lysikova, Peter Kaplan, Peter Raĭay, Zuzana Tatarkova
Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University Bratislava, Slovakia

In cardiovascular diseases, age is a major risk, although medical advances in the last decade have significantly increased life

expectancy. Around 30% of the cell volume of the heart is occupied by mitochondria, which cooperate with the sarco/endoplasmic reticulum (SR/ER) to control various cellular processes. In the heart, Ca^{2+} homeostasis is primarily regulated by SR-mitochondria coupling mediated through IP03R-VDAC1 binding in mitochondria-associated membranes (MAMs). After IR injury, aged hearts have shown less efficient SR Ca^{2+} uptake with a marked reduction in Ca^{2+}-ATPase levels and activity, reaching 71.5% of maximal saturation at higher free Ca^{2+} (0.5–5.00 μM). Entry of Ca^{2+} into aging mitochondria via upregulated (3.9-fold) MAM-enriched VDAC1 stimulates the respiratory chain along with the formation of reactive byproducts such as H_2O_2. This increases peroxidation of lipids, conjugated dienes, and reactive 4-hydroxynonenal, which may inversely modulate SR-Ca^{2+} release in old ischemic hearts. Deprivation of MFN2 protects mitochondrial membrane potential, which stabilizes ATP production during ischemia, but with a negative impact on ER, leading to oxidative stress and impaired redox power mediated by reduced glutathione. Aged hearts were more vulnerable to ER stress-mediated reactive molecule formation with a lower reducing capacity during ischemia. On the other hand, reoxygenation appears to affect Ca^{2+} handling between SR and mitochondria, which can be improved to some extent by pharmacological control of ER stress. Overall, this points to the importance of MAMs in the development and progression of aging-related pathologies, particularly myocardial IR injury. *This work was supported by VEGA 1/0004/19 and UK/34/2022.*

Keywords: Ischemia–reperfusion injury, mitochondria-associated membranes, heart.

P021. Computational Biology, Bioinformatics & Artificial Intelligence

The role of tobacco smoke-induced changes of the microbiome in shaping risk of IBD

Irene Victoria Bermúdez Perez[1,2], Robert Häsler[1,2], Juliana Schramm[3], Susanne Krauss Etschmann[2,3]
[1]*Universitätsklinikum Schleswig-Holstein (UKSH), Kiel, Germany,* [2]*Christian-Albrecht University of Kiel (CAU), Kiel, Germany,* [3]*Deutsches Zentrum für Lungenforschung (DZL), Germany*

Complex diseases like inflammatory bowel disease are influenced by a combination of multiple genes and environmental factors such as smoking. The existing interaction between the host and its microbiome can be affected by the effects of smoking. The objective is to characterize the influence of smoking on the gut microbiome in the context of inflammatory bowel disease risk. A human transcriptome dataset from GEO was used to identify transcripts in the human mucosa that are regulated in response to smoking and that can potentially influence the microbiome. The preliminary results show that there are upregulated genes in smokers that are downregulated in non-smokers, based on heatmap and principal component analysis. Gene analysis was performed showing that most of the differentiated genes correspond to the inflammatory response. It is possible to conclude that smoking has an effect on host transcripts which have the potential to interact with the microbiome. The next steps will be expanding the analysis to smoke-exposed *Drosophila* datasets, integrate different omics and compare the data of model systems and humans.

Keywords: Microbiome, IBD analysis, tobacco.

P022. Cellular & Molecular Biology

Cellular reprogramming for the improvement of protein production using algorithm-predicted transcription factors

Ieva Berzanskyte
MRC Laboratory of Molecular Biology, Cambridge, UK

The Chinese hamster ovary (CHO) cell line is one of the top choices for industrial recombinant antibody production. Decades of engineering and adaptation have improved CHO lines for mammalian protein production; however, the efficiency of large-scale production, especially for complex products, remains unsatisfactory. In this project we aim to improve CHO cell line capabilities for protein production by reprogramming cells towards a plasma cell phenotype – a cell that is highly specialized in antibody production. The direct reprogramming approach we are taking is based on an algorithm named Mogrify, using high-quality transcriptomic data for both the donor cell type (CHO line) and the target cell type (plasma cell), within the context of a background dataset and knowledge of the gene regulatory network provided by databases. Here, we present results from the first step towards reprogramming, which is obtaining transcriptomic data for donor and target cell. A protocol for hamster plasma cell sorting was established, following single-cell RNA sequencing of the sorted cells. New insights into hamster plasma cell markers, compared to model species of mouse, are presented.

Keywords: Reprogramming, antibody, CHO, plasma cells, transcriptomics.

P023. Cancer Biology & Oncology

Role of STK11 as a modulator of the response to immunotherapy in NSCLC

María G. Velasco-Domínguez[1,*], Sofía Boyero-Corral[1,*], Laura Boyero[1], José Francisco Noguera-Uclés[1], Alejandro Castillo-Peña[1], Míriam Alonso[2], Amparo Sánchez-Gastaldo[2], Johana Cristina Benedetti[2], Sonia Molina-Pinelo[1], Reyes Bernabé[2]
[1]*Instituto de Biomedicina de Sevilla IBiS. Hospital Universitario Virgen del Rocío/CSIC/US, Molecular and Translational Research in Oncology, Seville, Spain,* [2]*Oncology Unit, Hospital Universitario Virgen del Rocío/CSIC/US, Seville, Spain*
*These authors contributed equally.

Despite the very promising results obtained with immunotherapy in the treatment of non-small cell lung cancer (NSCLC), only a percentage of patients benefit from this type of treatment. Currently, the application of immunotherapies is not governed by the molecular characteristics of the tumor. As a result, NSCLC patients are treated uniformly according to histology, regardless of other molecular features, despite potential risks associated with treatment without clear benefit. Therefore, it is necessary to identify new and reliable biomarkers of response to treatment in order to apply precision medicine in these patients. Somatic

mutations of the serine/threonine kinase 11 (STK11) tumor suppressor gene are common in NSCLC and have been proposed as a potential mechanism of resistance to anti-PD-1/PD-L1 immunotherapy. To shed light on the contribution of STK11 in the response to immunotherapy, we compared the transcriptome profile in isogenic models of STK11 gain/loss of function in NSCLC. On the one hand, CRISPR-Cas9 STK11 knock-out clones were made using the CRISPR-Cas9 system in H358 and H1781 cell lines. On the other hand, ectopic expression of the wild type STK11 gene or a mutated non-functional version were carried out in the A549 cell line. Experiments were performed in triplicate adopting the criteria of fold change > 2 and < -2 and $P < 0.05$. After data analysis, 14 genes were found to be differentially expressed (12 up and 2 down). Finally, functional enrichment, gene correlations, Kaplan–Meier and immune infiltration analysis were performed, revealing the *IL6* gene as a fundamental piece in the modulation of response to immunotherapy.

Keywords: Biomarkers, NSCLC, immunotherapy, resistance, STK11/LKB1.

P024. Neuroscience, Psychiatry & Mental Health

Evaluating the effect of SARS-CoV-2 infection on dopamine homeostasis

Silvia Breviario[1], Giulia Lunghi[1], Emma Veronica Carsana[1], Gioia Cappelletti[2], Claudio Fenizia[2], Massimo Aureli[1]
[1]*Department of Medical Biotechnologies and Translational Medicine, University of Milan, Milan, Italy,* [2]*Department of Biomedical and Clinical Sciences "L. Sacco", University of Milan, Milan, Italy*

Recent evidence reports that SARS-CoV-2 infection might have a great impact on the brain, by reducing the gray matter thickness and the overall brain size. Moreover, an increasing number of patients, even with mild COVID-19, experiences a wide range of neurological conditions even weeks or months after infection. In particular, the manifested COVID-19-related symptomatology lets us hypothesize an effect of the virus on the dopaminergic tone. To investigate this aspect, human iPSCs were differentiated into dopaminergic neurons and infected with three different SARS-CoV-2 variants (EU, Delta and Omicron). Ninety-six hours post-infection with EU and Delta variants, but not with Omicron, neurons showed a reduced intracellular content and extracellular release of dopamine. In addition, the infected neurons were characterized by reduced protein levels of the tyrosine hydroxylase (TH) together with a reduced mRNA expression of DOPA-decarboxylase (DDC) and the dopamine transporter (DAT) and with a modest increase of the mRNA level of the vesicular monoamine transporter 2 (VMAT2). Moreover, neurons infected with EU also displayed reduced protein expression of the neuronal markers MAP2 and TAU, paralleled by an intense activation of the antiviral intracellular innate immune response (IFITM1, IFITM3 and MxA) and an increase in the neuronal stress marker S100B. Taken together, these preliminary observations lead us to speculate that dopaminergic neurons are affected by SARS-CoV-2 infection with consequences on the dopamine metabolism, explaining some of the neurological symptoms manifested by COVID-19 patients.

Keywords: SARS-CoV-2, dopamine, homeostasis, IPSC-derived neurons, COVID-19, neurological symptoms.

P025. Neuroscience, Psychiatry & Mental Health

Building an *in vitro* platform based on primary astrocytes to scrutinize the molecular mechanisms of rejuvenation-by-reprogramming

Fernando Cala-Fernández[1,2], Pablo Rodríguez[1,2], Francisco M. Vega[1,2], Aida Platero-Luengo[2,3]
[1]*Departamento de Fisiología Médica y Biofísica, Universidad de Sevilla, Seville, Spain,* [2]*Instituto de Biomedicina de Sevilla (IBiS)/HUVR/CSIC/Universidad de Sevilla, Seville, Spain,*
[3]*Departamento de Biología Celular, Universidad de Sevilla, Seville, Spain*

Our goal is to combine cutting-edge partial cell reprogramming on primary cells, lentiviral vectors, fluorescence-activated cell sorting and gene expression analysis to build a robust, unbiased and flexible screening platform to elucidate the molecular mechanisms involved in the reversion of aging hallmarks. The creation of this platform will provide the advantage of automated identification of targets with the potential to improve reprogramming protocols and their translational application for regenerative strategies.

Keywords: Stem cell rejuvenation, reprogramming, iPSC.

P026. Cellular & Molecular Biology

Mechanistic insights into lipid-based protein sorting from the ER

Carlos Calafí, Susana Sabido-Bozo, Auxiliadora Aguilera-Romero, Sofía Rodríguez-Gallardo, Sergio López, Manuel Muñiz
Department of Cell Biology, Faculty of Biology, University of Seville and Institute of Biomedicine of Seville (IBiS)/HUVR/CSIC/US, Seville, Spain

Protein sorting in the secretory pathway is crucial to maintain cellular compartmentalization and homeostasis. In addition to coat-mediated sorting, the role of membrane lipids in driving protein sorting during secretory transport is a longstanding fundamental question that remains unanswered. To address this question, we have investigated in the yeast *Saccharomyces cerevisiae* how a special type of lipid-linked cell surface proteins, the GPI-anchored proteins, are differentially exported from the endoplasmic reticulum (ER). We have shown that ceramide drives the clustering and sorting of GPI-anchored proteins into specialized ER exit sites. Here, we provide a better comprehension of the potential mechanism for this ceramide-based sorting process.

Keywords: GPI, ceramide, ER transport, COPII, P24.

P027. Cancer Biology & Oncology

Development of a new compound for the treatment of acute myeloblastic leukemia, multiple myeloma, and other hematologic malignancies

Rocío Calderón[1], Clara Beatriz García-Calderón[1], Laura Pérez-Ortega[1], Virginia Escamilla-Gómez[1], Paola Hernández-Díaz[1], María De La Rosa-Garrido[1], Beatriz Guijarro-Albaladejo[1], Mayte Medrano[1], Estefanía García-Guerrero[1], Alfonso Rodríguez-Gil[1], Inmaculada Fernández[2], Noureddine Khiar[3], José Antonio Pérez-Simón[1]

[1]*Instituto de Biomedicina de Sevilla, UGC de Hematología, Hospital Universitario Virgen del Rocío and Consejo Superior de Investigaciones Científicas (CSIC), Universidad de Sevilla, Seville, Spain,* [2]*Facultad de Farmacia, Universidad de Sevilla, Seville, Spain,* [3]*Consejo Superior de Investigaciones Científicas (CSIC)*

Multiple myeloma (MM) and acute myeloblastic leukemia (AML) are the two hematologic malignancies with the worst prognosis. Despite continuous therapeutic advances, it is still considered incurable. Hence, there is a need to amplify the currently available therapeutic arsenal. In this regard, our group has investigated the anti-tumor effect of cannabinoids. We show that cannabinoids induce selective apoptosis in cell lines and primary cells from patients with MM, AML, and acute lymphoblastic leukemia (ALL), without causing apoptosis in healthy hematopoietic progenitors. Likewise, we analyzed the signaling pathways involved in this activity, as well as the effect of cannabinoid derivatives (CNB) on metabolic pathways essential for the viability of tumor cells, including glycolysis, the pentose pathway, or ceramide metabolism. Furthermore, CNBs induce stress in the endoplasmic reticulum and mitochondrial damage, although the pro-apoptotic effect is mainly mediated by the activation of parthanatos. In addition, our group has described a powerful synergistic effect with some of the drugs currently used most in the treatment of these diseases, which would prevent resistance to these drugs in monotherapy and the use of lower doses. In this context, this project aims to develop an optimized compound based on previous findings, with the objective of selecting it as the leading compound for the development of regulatory preclinical studies to explore the mechanisms of action of these compounds that justify its powerful anti-tumor effect and its synergy.

Keywords: Hematologic malignancies, cannabinoids, parthanatos, apoptosis.

P028. Biomedical Engineering & Imaging Sciences

Electrospun scaffolds of PCL and gelatin for skeletal muscle tissue engineering

Francisco José Calero-Castro[1], Víctor Perez-Puyana[2], Imán Laga Boul-Atarass[1], Javier Padillo Ruiz[1], Alberto Romero[2], Fernando De La Portilla De Juan[1]

[1]*Instituto de Biomedicina de Sevilla, University Hospital Virgen del Rocío, Seville, Spain,* [2]*Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, Seville, Spain*

Tissue engineering has among its challenges the regeneration of skeletal muscle. It requires the use of tissue scaffolds, which require good biomechanical characteristics and promote tissue organization. Electrospinning is one of the processing techniques often used for the fabrication of nanofibrous structures that allow us to use a combination of synthetic and natural polymers. Moreover, bioreactors that stimulate mechanically and/or electrically could promote the differentiation of skeletal-muscle tissue. Therefore, in this work we have studied the use of electrospinning scaffolds and the effect of mechanical and electrical stimulation for skeletal muscle regeneration. Three electrospinning scaffolds of PCL and gelatin with different degree of fiber alignment were used: random; semi-aligned and aligned. The C2C12 cell line was used. The aligned scaffolds were mechanically and electrically stimulated. Viability, cytoskeleton morphology and functionality of the myoutubes were measured. Random scaffold viability was 80.14 ± 9.36%, semi-aligned scaffold viability was 98.28 ± 0.64% while aligned scaffolds presented a viability of 84.12 ± 12.69%, with no significant difference ($P = 0.171$). There was also no significant difference between the stimulated aligned scaffolds ($P = 0.256$). Myotubes of aligned scaffolds (9.84 ± 1.15 μm) were thinner than those of random (11.55 ± 3.39 μm; $P = 0.001$) and semi-aligned (11.32 ± 2.73 μm; $P = 0.003$) scaffolds. Aligned and mechanically stimulated scaffolds increased thickness (12.92 ± 3.29 μm; $P = 0.000$), nuclear fusion (95.73 ± 1.05%; $P = 0.004$), and actin density (80.13 ± 13.52%; $P = 0.017$) with respect to the control. Random scaffolds and mechanically-stimulated aligned scaffolds presented higher contractility.

Keywords: Scaffolds, biomaterials, electrospinning, tissue-engineering, skeletal muscle.

P029. Immunology, Microbiology & Infectious Diseases

Type 1 regulatory T-cells ("Tr1") as targets of cancer immunotherapy

Elena Carelli[1,2], Ylenia Silvestri[1], Benedetta De Ponte Conti[2], Fabio Grassi[2], Jens Geginat[1]

[1]*Istituto Nazionale Genetica Molecolare Romeo ed Enrica Invernizzi, Milan, Italy,* [2]*Institute for Research in Biomedicine, Università della Svizzera Italiana (USI), Bellinzona, Switzerland*

The role of type 1 regulatory T-cells ("Tr1") in cancer is still incompletely understood. This class of regulatory T-cells secretes a large amount of IL-10 and suppresses tissue inflammation in autoimmunity and inflammatory diseases. Our data demonstrate that Tr1 cells are strongly enriched in different types of human tumors and that, in patients with metastatic melanoma treated with anti-PD-1/PD-L1 antibodies, a higher number of Tr1 cells are associated with a better response to immunotherapy. These findings suggest that Tr1 cells may be a direct target of immunotherapy; however, their role in cancer requires further investigation. In our current research, we are studying tumor infiltrating T-cells from melanoma tumor samples of untreated patients to clarify whether Tr1 cells and IL-10 producing helper T-cell subsets could regulate anti-melanoma cytotoxic responses in an antigen-specific manner, and to identify Tr1 specific factors. To gain a better understanding of the tumor infiltration process, we have also established a mouse model of subcutaneous melanoma and colorectal cancer (CRC) in which we could appreciate an enrichment in tumor-infiltrating Tr1 cells. This preliminary data convinced us to study more in depth the characteristics and

the behavior of Tr1 cells in the tumor, taking advantage of tumor samples from treated and untreated melanoma patients and the B16 melanoma mouse model, in which we can use anti-PD-1 and anti-PD-L1 treatment.

Keywords: Tr1, melanoma, immunotherapy, cancer.

P030. Cellular & Molecular Biology

Identification of microRNAs that provide underlying mechanisms for anti-inflammatory properties of cardiotrophin-1

David Carneros[1,*], Macarena López-Sánchez[2,*], Gema López-Ruiz[1], M. Ángeles Rodríguez-Hernández[1], Francisco Zurita-Díaz[1], María Peña-Chilet[2], Matilde Bustos[1]
[1]*Instituto de Biomedicina de Sevilla, IBIS, Hospital Universitario Virgen del Rocío, Universidad de Sevilla, Consejo Superior de Investigaciones Científicas, Seville, Spain,* [2]*Computational Systems Medicine, Instituto de Biomedicina de Sevilla, IBIS, Hospital Virgen del Rocío, Seville, Spain*
*These authors contributed equally.

Background and Aim: MicroRNAs (miRNAs) are key regulators of metabolic homoeostasis and contribute to the pathology of non-alcoholic fatty liver disease (NAFLD). Cardiotrophin-1 (CT-1), a member of the gp130 family of cytokines, is a hepatoprotective cytokine that modulates fat and glucose metabolism. We have previously reported that the recombinant protein CT-1 (rCT-1) resolves hepatic steatosis in obese mice, suggesting that CT-1 could be a potential therapy for NAFLD. Here, we aimed to clarify the underlying molecular mechanisms. *Methods*: *ob*/*ob* mice were divided into three groups: i) rCT-1-treated mice which received 0.2 mg/kg/day intravenously for 10 days; ii) control mice given saline (vehicle) were allowed access to food *ad libitum*;; iii) control mice given saline and the same amount of food consumed by rCT-1-treated mice (pair fed). Wild-type animals were used. Moreover, miRNA and mRNA paired expression analysis was integrated. *Results*: Our bioinformatic analysis showed that the expression of 12 miRNAs was dysregulated in *ob*/*ob* vs WT mice (6 upregulated and 6 downregulated). The expression levels of these miRNAs were normalized to WT in rCT-1-treated *ob*/*ob* mice. miR-29c-3p, a critical regulator of fibroinflammatory processes in human diseases, was validated by qRT-PCR. Interestingly, integrated miR-29c-3p-mRNA pairs showed downregulated inflammatory genes in rCT-1-treated *ob*/*ob* mice. Our *in vitro* results showed that LPS decreased miR-29c-3p in macrophages (Raw 264.7) compared to untreated cells in clear contrast with rCT-1-treated macrophages stimulated with LPS. *Conclusion*: miR-29-3p could participate in the anti-inflammatory mechanism observed in the treatment with rCT-1.

Keywords: microRNA, cardiotrophin-1, inflammation, liver.

P031. Cancer Biology & Oncology

The transcription factor ZEB1 shapes osteosarcoma aggressiveness by affecting tumour cell differentiation and stemness features

Caterina Cascini[1], Daniele Lecis[1], Laura Botti[1], Chiara Ratti[1], Valeria Cancila[2], Katia Scotlandi[3], Claudio Tripodo[2], Mario Paolo Colombo[1], Claudia Chiodoni[1]
[1]*Molecular Immunology Unit, Department of Research, Fondazione IRCCS Istituto Nazionale Dei Tumori, Milan, Italy,* [2]*Department of Health Sciences, Human Pathology Section, University of Palermo, Palermo, Italy,* [3]*Experimental Oncology Lab, CRS Development of Biomolecular Therapies, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy*

Osteosarcoma (OS) is a mesenchymal bone tumor affecting mainly children and young adults, characterized by a particularly aggressive behavior, with 20% of patients showing lung micrometastasis already at diagnosis. To date, treatment options for OS are still based on multi-drug chemotherapy, and prognosis for metastatic patients is dismal, making it urgent to identify new therapies. We tested the effects on OS cells of targeting ZEB1, a transcription factor known to be critical for the maintenance of mesenchymal and stemness features in cancer. We inhibited ZEB1 expression in a murine OS cell line by lentiviral vectors based on CRISPR-Cas9 technology. We first tested the effects of ZEB1 deficiency *in vitro*, evaluating OS cell stemness potential and osteogenic differentiation. Gene expression profile (GEP) was performed to investigate the transcriptional changes induced by ZEB1 deletion. We also investigated *in vivo* the effect of ZEB1 inhibition. ZEB1 KO clones showed decreased stemness potential and increased Alp staining, both features suggestive of a more differentiated phenotype. Preliminary GEP analysis showed down-modulation of pathways related to cellular proliferation and survival such as MTORC1 signaling, MYC targets, GM2 checkpoint, E2F targets and oxidative phosphorylation in ZEB1 KO clones. *In vivo*, we observed reduced tumor growth in ZEB1 KO clones that were also characterized by a more differentiated morphology. Interestingly, the absence of ZEB1 also affected the immune infiltrate, as tumors derived from ZEB1 KO clones were significantly less infiltrated by pro-tumoural $CD206^{+}$ M2-like macrophages. These results support the hypothesis that ZEB1 is a key factor in OS aggressiveness.

Keywords: Osteosarcoma, ZEB1, mouse models, CRISPR-Cas-9, macrophages.

P032. Cancer Biology & Oncology

Exosomal microRNAs as potential biomarkers of responses to pembrolizumab in non-small cell lung cancer

Alejandro Castillo Peña[1], José Francisco Noguera-Uclés[1], Raquel Chapresto[1], María Ángeles Domínguez-Cejudo[1], Miriam Alonso[1,2], Amparo Sánchez-Gastaldo[1,2], Johana Benedetti[1,2], Reyes Bernabé-Caro[1,2], María Luisa Valdivia Ferreira[1], Laura Boyero[1], Sonia Molina-Pinelo[1]

[1]Institute of Biomedicine of Seville (IBiS)/HUVR/CSIC/Universidad de Sevilla, Seville, Spain, [2]Medical Oncology Department, Hospital Universitario Virgen del Rocío, Seville, Spain

Immune checkpoint inhibitors (ICI) have demonstrated significant clinical efficacy in more than 15 cancer types including non-small cell lung cancer (NSCLC). However, resistance to ICI (anti-PD1/PD-L1) therapies is common, occurring in up to 60% of patients. Our main goal was to investigate whether exosomal microRNAs (exomiRs) may be clinically relevant in the response to pembrolizumab (anti-PD1) in NSCLC patients. ExomiR expression levels after treatment were evaluated using Affymetrix miRNA 4.1 Arrays from 20 patients, 10 of whom were responders (R, show an improvement in the first six months of treatment) and 10 non-responders (NR, the disease progresses before six months). Results were analyzed with Transcriptome Analysis Console. Data were corrected and normalized using Robust Multichip Average method from the oligo package. Subsequently, we evaluated whether the identified exomiRs were deregulated prior to therapy. Finally, target genes of differentially expressed exomiRs were identified to understand their biological basis for response to pembrolizumab. We found that 163 exomiRs were differentially expressed after treatment, and we observed that some of these miRNAs were also differentially expressed at basal level. Their predicted and strong validated target genes were determined and a pathway enrichment analysis was performed. Target genes were enriched in pathways related to lung cancer and the immune system, such as the MAPK signaling pathway or PD-L1 expression and PD-1 checkpoint pathways in cancer. Hence, the data suggest that we could have found an exomiR profile with predictive and/or prognosis value of response to immunotherapy in NSCLC patients. However, data should be experimentally confirmed.

Keywords: Immunotherapy, lung cancer, exosomes, exomiRs, response.

P034. Cancer Biology & Oncology

Establishment of an *in vitro* preclinical platform: patient-derived organoid models with lung and breast cancer

Raquel Chapresto[1], María Ángeles Domínguez-Cejudo[1], Laura Boyero[1], Ana Salinas[1], Ana Gil-Torralvo[2], Reyes Bernabé-Caro[2], Javier Salvador-Bofill[2], Sonia Molina-Pinelo[1]

[1]Instituto de Biomedicina de Sevilla IBiS. Hospital Universitario Virgen del Rocío/CSIC /US, Molecular and Translational Research in Oncology, Seville, Spain, [2]Hospital Universitario Virgen del Rocío/CSIC/US, Oncology Unit, Seville, Spain

Heterogeneity in cancer is regarded as one of the most substantial roadblocks in the development of effective patient-specific therapies. This heterogeneity within and across patients often explains widespread patient therapeutic responses. In this context, patient-derived organoids (PDOs), 3D structures composed of epithelial cells, are changing our understanding of cancer heterogeneity and its implications for personalized medicine. Our aim was to establish and characterize a PDO platform of lung and breast cancer as preclinical tool to research the molecular and cellular mechanisms underlying both tumors. Surgical resections were collected from patients diagnosed with lung or breast cancer. To date, we have successfully achieved an outgrowth efficiency of 70% for both normal and tumor PDOs from patients diagnosed with lung cancer. In the case of breast cancer patients, the efficiency was slightly lower and was set around 50% of tissue samples. Characterization of PDO cultures is essential to validate its predictive potential. To assess whether PDOs represent the tumor of the patient, expression of the main histological markers were evaluated and correlated with the tissue of origin. In parallel, to test the potential that a well-annotated patient-derived organoid biobank has for drug discovery and personalized therapy, we have implemented different protocols to test *in vitro* sensitivity to drugs, to analyze cancer stem cell markers by confocal immunofluorescence on both intact and paraffin-embedded organoid samples and an *in vivo* imaging procedure to better understand tumor heterogeneity and improve the design of effective patient-specific treatment strategies.

Keywords: Organoids, lung cancer, breast, 3D-culture.

P035. Immunology, Microbiology & Infectious Diseases

Dermal trypanosomes: potential cryptic reservoirs for disease transmission in Nigeria

Gloria Chechet[1,2]

[1]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria, [2]Africa Centre of Excellence in Neglected Tropical Diseases and Forensic Biotechnology (ACENTDFB), Ahmadu Bello University, Zaria, Nigeria

At least 19 cases of atypical human trypanosomiasis (a-HT), which is a human infection with animal trypanosomes being thought of as infective only to animals because of innate protection in humans by the Apolipoprotein L-1 (ApoL1) that is trypanolytic to all animal species, has been reported. Although significant progress has been made in reducing the typical human/animal African trypanosomiasis, trypanosome parasites hiding in the skin may undermine efforts to eliminate sleeping sickness or Nagana. Reports have shown that the skin could represent an anatomic reservoir of the human infection that has been overlooked over time because the parasites are primarily known to be blood-dwelling pathogens. We collected 118 human skin snips and 84 pig skin snips in Oyo state, Nigeria, and isolated DNA using an Investigator kit (Qiagen, Germany). Nested PCR was done using ITS-1 generic/gGAPDH and species-specific primers to detect and identify animal trypanosomes. We found 12 and 65 positive human and pig skin snips, respectively, that predominantly had *Trypanosoma congolense* and *Trypanosoma vivax*. The results were authenticated by sequencing. These experiments are currently being repeated to target other genes besides ITS-1 including the determination of G1 and G2 mutations in the human Apolipoprotein-L1 (APOL1) gene. This information is relevant not only in the re-evaluation of diagnostic methodologies and control policies, but also give an insight into the

potential reservoirs that could undermine efforts to eliminate the disease in the near future.

Keywords: African trypanosomiasis, skin, PCR, APOL1.

P036. Immunology, Microbiology & Infectious Diseases

Influence of nanobody scaffolds on protein expression, stability and affinity

Bing Liang Alvin Chew[1], Wan Qi Lee[2], Jun Xi Javier Tan[2]
[1]*Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore,* [2]*School of Life Sciences and Chemical Technology, Ngee Ann Polytechnic, Singapore*

Single variable domain heavy-chain only antibodies (sdAbs), also known as nanobodies (Nbs), are small ~13 kDa protein binders first discovered in Camelids. As compared to conventional antibodies, Nbs are more ideal as biotherapeutics given their small size, better penetration of tissues, higher stability, binding potential to cryptic target regions, and greater bioengineering potential without the use of animals. Nbs have also been raised to trap or induce conformational states of target proteins for structural and drug discovery studies. Given its wide-ranging potential and utility, multiple Nb common structural scaffolds have been reported for various uses. A grafting strategy allows the plug-n-play of hypervariable loops (complementary-determining regions, CDRs) which confer the antibody's specificity and binding affinity to these frameworks. However, the universality of the grafting approach across these scaffolds' sequence context at the transcriptional and translational level remains unclear. Using an alpaca-derived Nb identified against a membrane target protein, this study compares the effect of codon optimization on its native sequence, and grating to a conserved framework, an alpaca framework recognized by a universal anti-Nb antigen-binding fragment (Fab), and a humanized framework on the protein expression in *E. coli*, the stability, and binding of the Nbs.

Keywords: Nanobody framework, antibody, biotherapeutics.

P037. Chemistry & Biochemistry

Characterization and quantification of the lipid profile from mouse large and small intestine

Tania Chica Cid[1], María D. Giraldez[1], Manuel Leon Camacho[2]
[1]*Institute of Biomedicine of Seville, Seville, Spain,* [2]*Instituto de la Grasa (CSIC), Seville, Spain*

Introduction: Altered lipid metabolism is associated with diseases such as colorectal cancer. Thus, the study of lipid profiles in healthy and cancerous intestinal samples to elucidate tumor lipid phenotype is gaining growing attention. *Aim*: To develop an analytical procedure to characterize the lipid fraction from mouse intestinal samples. *Method*:. To optimize the analytical procedure, large and small intestines from C57BL6/J mice were used. We performed lipid extraction with Methyl-tert-butyl ether (MTBE) using 25 mg of tissue as input. Lipid fractions were isolated by solid phase extraction (SPE). Triacyclglycerols, diacylglycerols, monoacylglycerols and free fatty acids were analyzed by gas chromatography and phospholipids by high-performance liquid chromatography. Fatty acids were also analyzed by gas chromatography without the previous isolation step. We used the ANOVA test to compare the lipid composition of large *vs.* small intestine and performed a linear discriminant analysis to identify the most discriminant variables. *Results*: Oleic acid was the most abundant fatty acid identified. The main triacylglycerol compound varied depending on the anatomical location (palmitoyl-dioleoyl glycerol in large and palmitoyl-stearyl-oleoyl glycerol in small intestine). Regarding diacylglycerols, monoacylglycerols and free fatty acids, the principal compound was 1,2-palmitoyl-linoleyl glycerol. Phosphatidylcholine was the most abundant phospholipid. Three fatty acids, six triacylglycerols, two diacylglycerols, cardiolipin and phosphatidylethanolamine showed significant differences between large and small intestine. We obtained a complete separation of both tissues using C17:0, C14:0, C18:2, cardiolipin, phosphatidylethanolamine and distearyl-oleyl glycerol as chemical descriptors. *Conclusions*: We have developed an analytical procedure for identifying and quantifying the lipid profile from mouse intestinal samples.

Keywords: Lipids, chromatography, gastroenterology.

P038. Cellular & Molecular Biology

The role of heme and heme oxygenase-1 in the regulation of replication stress

Patryk Chudy[1], Jakub Kochan[2], Witold Nowak[1], Alicja Józkowicz[1], Wojciech Krzeptowski[1]
[1]*Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland,* [2]*Department of Cell Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland*

G-quadruplexes (G4) are three-dimensional structures of nucleic acids that can form on single-stranded DNA in the replication forks. As a spatial hindrance, G4 can inhibit polymerase progression, leading to replication stress. If stalled forks are not unwound, they eventually collapse, causing double-strand DNA breaks. G4 are stabilized by heme. Previously we described enhanced formation of G4 structures in cells lacking heme oxygenase-1 (Hmox1), a heme degrading enzyme which is located in the proximity of G4 structures. Here, we checked whether Hmox1 protects cells from G4-induced replication stress. We found that an increase in endogenous heme production after treatment of cells with δ-aminolevulinic acid (ALA) more effectively elevated the cellular free heme, than the exposure of cells to exogenous hemin. This effect was stronger in Hmox1-deficient cells. Stabilization of G4 by pyridostatin resulted in a higher proportion of stalled replication forks, as shown using fiber assay. Interestingly, similar replication stress was observed after elevation of free heme by ALA, and again – this effect was stronger in Hmox-1 deficient cells. The boosted DNA repair response was also more pronounced in Hmox1-deficient cells, especially after administration of exogenous hemin. It seems, however, that the activation of DNA repair was not directly related to the accumulation of G4, but rather to the oxidative stress induced by hemin. Finally, the replication stress in Hmox-1 deficient cells led to a reduced rate of cell proliferation and upregulation of type-I interferon response. In summary, Hmox1

protects cells from replication stress induced by free heme, a G4 stabilizer.

Keywords: Heme, heme oxygenase-1, replication stress, cell cycle.

P040. Chemistry & Biochemistry

Regulation of heme oxygenase system in ferroptosis as a novel approach for breast cancer therapy

Valeria Consoli, Valeria Sorrenti, Luca Vanella
University of Catania, Nicolosi, Italy

The term ferroptosis refers to a peculiar type of programmed cell death (PCD) showing characteristic features that differentiate it from other historically well-known types of cellular death such as apoptosis, autophagy, necrosis and necroptosis. Ferroptosis is mainly characterized by extensive iron-dependent lipid peroxidation and mitochondrial dysfunction, together with the rounded morphology of the cell undergoing ferroptotic death. Recently, ferroptosis has been suggested as a potential new strategy for the treatment of several cancers, including breast cancer (BC). In particular, among the BC subtypes, triple negative breast cancer (TNBC) is considered the most aggressive, and conventional drugs fail to provide long-term efficacy. Our study's purpose was to investigate the mechanism of ferroptosis in breast cancer cell lines and reveal the significance of heme oxygenase (HO) modulation in the process. HO's effect on BC was evaluated by MTT tests, gene silencing, Western blot analysis, and measurement of reactive oxygen species (ROS), glutathione (GSH) and lipid hydroperoxide (LOOH) levels. In order to assess HO's implication, different approaches were exploited, using two distinct HO-1 inducers (hemin and curcumin), a well-known HO inhibitor (SnMP) and a selective HO-2 inhibitor. The data obtained showed HO's contribution to the onset of ferroptosis; in particular, HO-1 induction seemed to accelerate the process. Moreover, our results suggest a potential role of HO-2 in erastin-induced ferroptosis. In view of the above, HO modulation in ferroptosis can offer a novel approach for breast cancer treatment.

Keywords: HO-1, heme, ferroptosis, cancer, ROS.

P041. Cancer Biology & Oncology

Targeting translation machinery in hepatocellular carcinoma therapy

Laura Contreras Bernal[1], Jordi Muntané Relat[2], Jesús De La Cruz Díaz[1], Stefano Biffo[3]
[1]Department of Genetic, Institute of Biomedicine of Seville (IBiS)/HUVR/CSIC/University of Seville, Seville, Spain, [2]Department of Medical Physiology and Biophysics, Institute of Biomedicine of Seville (IBiS)/HUVR/CSIC/University of Seville, Seville, Spain, [3]Department of Bioscience, National Institute of Molecular Genetic (INGM), University of Milan, Milan, Italy

Protein synthesis is the major regulator of gene expression. Translation, as well as the signaling pathways that control it, are deregulated in many types of cancer, including hepatocellular carcinoma (HCC). Malignant cells adapt to environmental changes produced into the tumor by regulating protein synthesis to allow cell survival. Furthermore, tumoral cells have a high translation rate to maintain active cellular processes. Indeed, this is known as a cancer hallmark. All this makes protein synthesis a powerful cancer therapeutic target. HCC is the sixth most common cancer and the third cause of cancer-related death worldwide. Sorafenib, a multikinase inhibitor used as a first line of treatment in advanced HCC, is one of the most recommended options in some cases. However, the clinical benefits of Sorafenib are limited because of recurring resistance mechanisms and undesirable side effects. In our laboratory, we investigate the role of protein synthesis in Sorafenib response. We hypothesize that Sorafenib's impact on protein synthesis could mediate its anti-proliferative, anti-angiogenic, and pro-apoptotic properties. Hence, by translational assays like polysome profiles and puromycin incorporation we have shown that protein synthesis is inhibited by this drug. Sorafenib completely inhibits the MAPKs pathway, resulting in the downregulation of some eukaryotic translation initiation factors (eIFs) over time. Indeed, we show that upon Sorafenib treatment, downregulation of eIF2α and eIF4E leads to translation reprograming of a subset of mRNAs associated with cell proliferation (e.g. Cyclin D1) or angiogenesis (e.g. VEGFA). We will further discuss on the clinical relevance of these findings.

Keywords: Hepatocellular carcinoma, liver, Sorafenib, protein synthesis, translation, signaling pathways.

P042. Cellular & Molecular Biology

SLIMP directly connects mitochondrial anabolism to the control of G1-S transition

Luís Filipe Costa Póvoas[1], Lluís Ribas De Pouplana[1,2]
[1]Institute for Research in Biomedicine (IRB), Barcelona, Spain, [2]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

During the process of constructing a *Drosophila melanogaster* model to study human disorders caused by mitochondrial tRNA aminoacylation deficiencies, in addition to the expected mitochondrial and cytoplasmic sequences, a third unexpected sequence was found. This sequence was shown to encode for an SRS-like protein that was termed SLIMP (Seryl-tRNA synthetase-like insect mitochondrial protein). Bioinformatic studies performed by our group suggest that SLIMP originated from a duplication of the mitochondrial Seryl-tRNA Synthetase (SerRS2) early in animal evolution, becoming broadly distributed in invertebrates. This previously unknown protein was also shown to have incorporated non-canonical essential functions in mitochondria and in cell cycle progression, where its depletion led to an accumulation of cells in the G2 phase of the cell cycle and to an increase of E2F mRNA targets. An additional study performed by another group also suggested that SLIMP depletion enabled the G1 to S transition in the absence of E2F, pointing to a potential mechanism independent of the retinoblastoma-E2F pathway. This involvement of a mitochondrial protein in cell cycle progression provides interesting insights into the role of this organelle in cell proliferation. Taking into account all the evidence, our goal is to uncover this potential mitochondrial–nucleus communication mechanism for G1-S transition, independent of the E2F pathway.

Keywords: SLIMP, cell cycle, tRNA, mitochondria.

P043. Immunology, Microbiology & Infectious Diseases

Hepatitis C virus envelope protein E2 antigen design and characterization for vaccine development

Lia Maria Cucos, Teodor Asvadur Sulea, Laurentiu Spiridon, Norica Nichita, Costin Ioan Popescu
Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

With over 58 million individuals infected with Hepatitis C Virus (HCV) worldwide and costly antiviral drugs, the need for a vaccine is even more pressing. The main target in vaccine development is the viral envelope protein E2, which binds to CD81 receptor functioning as an entry factor for HCV. HCV E2 412–423 epitope is involved in CD81 binding and it is targeted by broadly neutralizing antibodies (nAbs). HCV E2 412–423 epitope presents in different conformations due to protein flexibility, and a beta hairpin conformation was found to co-crystalize with a nAb. The objective of our study was to construct and characterize novel HCV E2 derived antigens that elicit a potent neutralizing humoral response by stabilizing the 412–423 epitope in a beta hairpin conformation. To this end, advanced bioinformatics tools were used to predict mutations. A subset of HCV E2 mutants were expressed in HEK 293 T cells and their biochemical and antigenical properties were investigated. A vaccine candidate was expressed in Expi293 cells and the monomeric form was purified. Using ELISA, it was shown that the HCV E2 412–423 epitope was stabilized in a non-linear conformation. Immunization studies are in progress and the immune sera neutralization capacity will be assessed. The presented data suggest that by de-novo antigen design, HCV E2 412–423 epitope can be stabilized in a desired conformation with potential impact on its immunogenicity.

Keywords: Vaccine, HCV, antigen design.

P044. Cancer Biology & Oncology

A transcriptional lineage tracing approach to unravel the genetic and non-genetic determinants of breast cancer chemoresistance

Alberto Dalmasso, Niccolò Roda, Andrea Cossa, Giada Blandano, Pier Giuseppe Pelicci
European Institute of Oncology (IEO), Milan, Italy

Triple negative breast cancer (TNBC) is usually treated with chemotherapy, either in a neoadjuvant or adjuvant setting. Despite the relatively high rate of success, ~30% patients experience tumor relapse after treatment: this is due to the presence of chemoresistant cells. Mutational profiling revealed that most genetic clones survived chemotherapy administration, with none of them outcompeting all the others. Similarly, lineage tracing experiments demonstrated that the clonal composition of breast tumors is largely unchanged upon chemotherapy. These results suggest that most genetic and cellular clones harbor single chemoresistant cells. However, the characteristics and the interclonal heterogeneity of chemoresistant transcriptional phenotypes are still largely unknown. Furthermore, chemoresistance dynamics in distant metastases have not been elucidated yet. The main aim of my project is to dissect the genetic and phenotypic heterogeneity of breast cancer chemoresistance. Specifically, transcriptional lineage tracing will be used to inquire the clonal dynamics and transcriptional changes of resistant clones, from the primary tumor (PT) to distant organs. By leveraging a multi-omics approach, I will investigate different aspects of the chemoresistant phenotype, with the ultimate goal of tailoring novel exploitable therapeutic approaches. By thoroughly analyzing which pathways and genes are differentially expressed within chemoresistant clones, I will identify candidate genes and validate their effective role in promoting primary and metastatic breast cancer chemoresistance. My project will shed light on the genotypic and phenotypic determinants of chemoresistant clones in primary and metastatic breast cancer, to ultimately tailor combinatorial therapeutic approaches to specifically eradicate chemoresistant clones.

Keywords: Chemoresistance, metastasis, multi-omics lineage-tracing, heterogeneity.

P045. Computational Biology, Bioinformatics & Artificial Intelligence

Bioinformatics analysis for the characterization of the mitochondrial genome in difficult to treat hematologic malignancies

Alessandro Davini
European School of Molecular Medicine (SEMM), Padova, Italy

Mitochondria are maternally inherited organelles which play a central role in cellular energy provision. Variations in mtDNA are shown to be involved in cancer pathogenesis as a result of disturbances in energy metabolism and apoptosis. The molecular characterization of the mitochondrial genome can be performed using next-generation sequencing technologies and the small size of the genome allows a depth of coverage suitable for the characterization of even rare and transient events. Several studies have demonstrated the association between mtDNA variants and different types of cancer, but they were limited to few solid cancers. Our aim is to analyze the mitochondrial genome in the context of hematologic malignancies, in order to study the basal state of mitochondria within cancer cells and to evaluate the role of mtDNA variants in the pathogenesis of blood cancers. A first phase of the project will be dedicated to the development of the computational pipeline that will be used to analyze the mitochondrial genome. The pipeline will include the steps of alignment to mitochondrial reference genome, haplogroup assignment, heteroplasmy check, variant calling and differential expression analyses. Whole genome/exome sequencing data of publicly available datasets deriving from patients affected by acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and diffuse large B-cell lymphoma (DLBCL) will be used to extract meaningful information related to the mitochondrial genome in the context of hematological diseases. This study could pave the way to explore new therapeutic strategies, including targeted mitochondrial genome editing and synthetic lethal pharmacologic approaches determining disturbance of mitochondrial metabolism.

Keywords: Mitochondria, NGS, blood, cancer.

P047. Computational Biology, Bioinformatics & Artificial Intelligence

A mechanistic model and machine learning procedure for drug repurposing

Víctor Manuel de la Oliva Roque[1], Carlos Loucera Muñecas[1], Marina Esteban Medina[1], María del Carmen Peña Chilet[1,2,3], Joaquín Dopazo Blázquez[1,2]
[1]*Fundación Pública Andaluza Progreso y Salud, Seville, Spain,* [2]*Institute of Biomedicine of Seville (IBiS)/HUVR/CSIC/US, Seville, Spain,* [3]*Centro de Investigación Biomédica en red de Enfermedades Raras (CIBERER)*

Currently defined molecular pathways can be understood as function-centric encapsulations of the greater biological network. This however does not imply that pathways or the genes which constitute them are completely independent. Identifying intra-pathway interactions has proven to be a complex endeavor, and elucidating inter-pathway interactions even more so. Nevertheless, elucidating the influence that genes can have on pathways which they do not form an implicit part of, perhaps through some upstream regulatory role, can lead to the identification of potential therapeutic targets. This can be particularly attractive in cases where the identified therapeutic target is already a target of a given drug, thus curtailing the need for the expensive and time-consuming process of drug discovery and development. This process is understood as drug repurposing. As attractive as this is, this has historically been mostly through the fortuitous identification of off-target effects. Given the numerous advantages drug repurposing presents, as well as the encouragement given by the successes of various repurposed drugs, more systemic approaches to identifying repurposable compounds have been put forward. Among these, computational approaches leveraging various types of big data have shown promising results. In this work, a collection of models are developed with the goal of identifying known drug targets that can have an influence on known human molecular pathways, even if the target is not part of the currently defined pathway. To this end, a combination of mechanistic modeling of molecular pathway activity and a machine learning approach is applied.

Keywords: Drug repurposing, transcriptomics, machine learning, mechanistic modeling.

P048. Cancer Biology & Oncology

Metabolic impact of glutaminase isoenzyme modulation of glioma cell lines

Juan De Los Santos Jiménez[1,2], Tracy Rosales[3], Bookyung Ko[3], Javier Márquez[1,2], Ralph J. Deberardinis[3], José M. Matés[1,2]
[1]*Departamento de Biología Molecular y Bioquímica, Canceromics lab Facultad de Ciencias, Universidad de Málaga, Malaga, Spain,* [2]*Instituto de Investigación Biomédica de Málaga (IBIMA-Plataforma BIONAND), Malaga, Spain,* [3]*Children's Research Institute, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Many tumors use glutamine for both energy generation and as a biosynthetic precursor. Glutaminases (Gas) catalyze the first step of glutaminolysis by converting glutamine (Gln) into glutamate and ammonia in the mitochondria. In humans, two genes encode for glutaminases: *GLS* and *GLS2*. *GLS* is widely considered as a tumor promoting gene and encodes two isoforms named KGA and GAC, and is usually overexpressed in many tumors. On the other hand, *GLS2* encodes isoforms GAB and LGA, and appears to have more complicated roles, including tumor-suppressive functions in some contexts. In glioma, *GLS2* is commonly silenced and *GLS* is usually overexpressed. We examined the metabolic consequences of inhibiting GLS activity in three glioma cell lines (LN229, T98G and U87MG) by using the clinically relevant inhibitor CB-839, or expressing GLS2, by generating a glioma cell model overexpressing GLS2 (LN229-GLS2), otherwise silenced. Both experimental conditions were analyzed using a metabolomics approach for metabolite levels quantification in an Agilent Quadrupole Time of Flight LC–MS. We also performed stable isotope tracing experiments using U-^{13}C-labeled Gln and ^{15}N-labeled Gln in the amido group to ascertain the metabolic fates of Gln carbon and nitrogen. Briefly, metabolomics and carbon tracing results showed that CB-839 treatment depleted tricarboxylic acid cycle (TCAC) intermediates, while GLS2 maintained those pools, even upon concomitant GLS inhibition by CB-839. The results also showed that GLS inhibition by CB-839 and GLS2 expression had a remarkable effect on nucleotide *de novo* biosynthesis and also affected overall methylation patterns.

Keywords: Cancer, glioma, glutaminase, glutamine metabolism.

P049. Neuroscience, Psychiatry & Mental Health

Protective effect of dietary supplementation with polyphenol-rich *Salicornia ramosissima* extract against brain ischemia

Carmen Del Río[1], Paula García[2], Feifei Ma[2], Marina Romero-Bernal[1], Ana Nájar[1], Mari Luz Cádiz-Gurrea[3], Laura Ramiro[2], Antonio Segura-Carretero[3], Joan Montaner[1,4]
[1]*Neurovascular Research Laboratory, Institute of Biomedicine of Seville (IBiS)/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain,* [2]*Neurovascular Research Laboratory, Vall d'Hebron Institute of Research (VHIR), Barcelona, Spain,* [3]*Department of Analytical Chemistry, University of Granada, Research and Development of Functional Food Centre (CIDAF), Granada, Spain,* [4]*Department of Neurology, Hospital Universitario Virgen Macarena, Seville, Spain*

Stroke is the second cause of death worldwide and a leading cause of disability, with ischemic stroke representing 85% of all cases. The relationship that exists between oxidative stress and the brain damage after suffering a stroke is well known. Regular consumption of polyphenols has been shown to reduce the risk of suffering a cardiovascular event. For this reason, we have investigated the protective effect of *Salicornia ramossisima* (SR), a seasonal Mediterranean saltmarsh plant that, in response to saline stress, synthetizes high amounts of bioactive compounds, including polyphenols. Three different SR extracts (aqueous, hydroalcoholic and ethanolic) were prepared to investigate if dietary supplementation prior to ischemic challenge can prevent the subsequent damage. In this study, we used two animal models of hypoxia/ischemia. First, we evaluated the potential protective effect of each SR extract against hypoxia-reoxygenation in *Drosophila melanogaster* and observed that both ethanolic and

hydroalcoholic extracts protected fruit flies from the deleterious effects of hypoxia. Secondly, we confirmed the protective effect of SR ethanolic extract against brain ischemia using the transient middle cerebral artery occlusion (tMCAO) mouse model. Oral supplementation with SR ethanolic extract for four weeks before artery occlusion reduced infarct volume and lowered the plasma levels of the DNA peroxidant product 8-OHdG. Thus, SR ethanolic extract represent a valuable source of bioactive compounds that show promising disease modifying activities and could be further developed as an effective food supplement to treat neurovascular disorders.

Keywords: Neuroprotection, neurovascular, stroke, polyphenol.

P050. Immunology, Microbiology & Infectious Diseases

Using CRISPR-Cas9 techniques to dissect type I interferonopathies

Adam Desrochers[1], Marc Bienias[1], Tatjana Welzel[2,3], Vanessa Desrochers[1], Mohammedyaseen Syedbasha[4,5], Shintaro Yamada[6], Adrian Egli[5,7,8], Hiroki Kato[6], Jasmin Kümmerle-Deschner[2], Min Ae Lee-Kirsch[1]

[1]Department of Pediatrics, University Hospital Carl Gustav Carus, TU Dresden, Dresden, Germany, [2]Pediatric Rheumatology and Autoinflammation Reference Center Tübingen (arcT), University Children's Hospital, Tübingen, Germany, [3]Pediatric Pharmacology and Pharmacometrics, University Children's Hospital UKBB, University of Basel, Basel, Switzerland, [4]Department of Biomedicine, University Basel, Basel, Switzerland, [5]Applied Microbiology Research, Department of Biomedicine, University of Basel, Basel, Switzerland, [6]Institute for Cardiovascular Immunology, University Hospital Bonn, Bonn, Germany, [7]Clinical Bacteriology and Mycology, University Hospital Basel, [8]ESCMID Study Group for Genomic and Molecular Diagnostics (ESGMD), Basel, Switzerland

A patient presenting symptoms of a type I interferonopathy was determined to have a compound heterozygous mutation in *IFNLR1*, the alpha subunit of the type III interferon receptor. The mutations lead to two truncated INFLR1 variants. The receptor is a heterodimer comprised of the IFNLR1 and IL10RB proteins. The formation of the receptor is catalyzed by IFNLR1 binding interferon (IFN)-λ1, 2, 3 or 4 which recruits IL10RB. Once formed, the receptor phosphorylates STAT1 and STAT2 which form a heterodimer resulting in the expression of interferon stimulated genes. IFNLR1 is unique to this receptor, while IL10RB is also the beta subunit for the IL-10, IL-22, and IL-26 receptors, forming dimers with their accompanying alpha subunits. The receptors compose a part of the IL-10 superfamily which has been associated with both pro and anti-inflammatory activity, with the IL-10 pathway being constitutively anti-inflammatory.

Patient-derived PBMCs were demonstrated to not respond to stimulation with IFN-λ but were able to be stimulated by IFN-α, of the type I interferon pathway. To elucidate the mechanism by which the mutant IFNLR1 proteins may cause disease, CRISPR-Cas9 mediated knockouts of type I, II, and III interferon receptor alpha subunits were made. Overexpression of one of the mutant isoforms of IFNLR1 was demonstrated to cause spontaneous STAT1 phosphorylation, independent of the type I, II and III interferon pathways. Further study of this case of complete IFNLR1 deficiency will provide better insight into the type III interferon pathway and the closely related IL-10 superfamily.

Keywords: CRISPR-Cas9, IFNLR1, interferonopathy, IL-10, IL10RB.

P051. Neuroscience, Psychiatry & Mental Health

Study on the neuroprotective and immunomodulatory effects of human mesenchymal stem cell (hMSCs) secretome in organotypic hippocampal cultures (OHC) from mice with pilocarpine-induced epileptic seizures

Jan Detka, Martyna Strzelec, Maţgorzata Sobociťska, Patrycja Mieszczak, Marta Kot, Marcin Majka

Department of Transplantation, Institute of Pediatrics, Jagiellonian University Medical College, Cracow, Poland

Treatment of drug-resistant epilepsy (DRE) in children still remains a considerable medical challenge. Classic pharmacotherapy has limited clinical efficacy and most notably it often impairs cognitive development of a child. Therefore, novel therapeutic strategies are being sought. Human mesenchymal stem cells (hMSCs) appear to be an effective alternative approach in DRE treatment, due to their clinically-proven long-lasting effect in alleviating epileptic seizure frequency and intensity, and also neuroprotective and pro-cognitive properties. Although the exact mechanisms of hMSC action are complex, the hMSC secretome, rich in trophic factors and cytokines, appears to constitute for a large part of their therapeutic action. We investigated how the hMSC secretome would influence the viability and function of brain cells in organotypic hippocampal cultures (OHC) from NOD SCID mice in pilocarpine-induced epileptic seizures. OHC were cultured in hMSC-conditioned OHC medium (hMSC-CM). The influence of hMSC-CM on OHC was determined by measuring cell viability as well as neuronal, glial and inflammatory markers. The combination of biochemical, molecular and flow cytometry techniques was used. The results that we have obtained so far indicate that the hMSC-derived secretome can influence cell viability and also display some neuroprotective and immunomodulatory properties, thus supporting its key role in the therapeutic action of hMSCs. Further studies will be aimed at identifying key neuroregenerative factors in the hMSC secretome, which may enable development of specific cell-based therapies in the future. *Project supported by National Scientific Center in Poland 2018/31/B/NZ3/01879.*

Keywords: Mesenchymal stem cells (MSC), secretome, epilepsy, organotypic hippocampal cultures (OHC), neuroprotection.

P052. Clinical Research, Translational Biomedicine & Personalised Medicine

The pattern of immune reconstitution determines the survival at three months and the development of chronic graft-versus host disease in allo-HSCT

Paula Díaz Fernández, Izar García-Calvillo Zapatero, Yaiza Pérez García, Itxaso Portero Sainz, Samantha Feijóo Monroy, Valle Gómez García De Soria, Cecilia Muñoz Calleja
Instituto de Investigación H.U. La Princesa, Universidad Autónoma de Madrid, Madrid, Spain

The pattern and quality of immune reconstitution (IR) after transplantation may affect the outcome of hematopoietic allogeneic stem cell transplantation (allo-HSCT). However, there are limited data on the association of the quality of the IR on either the development of graft vs host disease (GvHD) or survival. We therefore aimed to explore the factors conditioning the IR and its impact on survival, and on the development of GvHD. To this end, we prospectively quantified total $CD4^+$ and $CD8^+$ T cells and their subsets in peripheral blood samples from 163 allo-HSCT patients at different time points after transplant. GEE models were used for the statistical analysis. Among the main results obtained, it is worth highlighting a deficient reconstitution of naïve T lymphocytes, which hardly recovered normal counts after one year. Naïve and stem cell like $CD4^+$ T cells were identified among the different lymphoid subsets whose reconstitution was strongly affected by the characteristics of the transplant. The recovery of the absolute number of total lymphocytes and $CD3^+$ T lymphocytes, and probably of the $CD4^+$, $CD8^+$ and naïve $CD8^+$ T cells, determined the survival at 3 months. Finally, a faster restoration of TEM $CD4^+$ T cells was associated with the development of chronic GVHD in the multivariate analysis. In conclusion, we identified IR variables that could be used as biomarkers for both the survival in the first 3 months and the development of chronic GvHD. Also this study can be the basis for therapeutic strategies to recover immune function.

Keywords: Immune reconstitution, Allo-HSCT, immunophenotyping, biomarker.

P053. Cancer Biology & Oncology

The HIF-1-alpha inhibitor PX478 decreases cell proliferation and enhances the radiosensitivity of cancer cell lines

David Diez-Castro[1,2], Pablo Berrocal[2], María Ovejero Sánchez[1,2,3], Juan Manuel Ruiz Robles[2,3], Luis Perez-Romasanta[1,4], Cristina Cigarral García[1,4], Rogelio González-Sarmiento[1,2,3], Ana-Belén Herrero[1,2,3]
[1]*Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain,* [2]*Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain,* [3]*Institute of Molecular and Cellular Biology of Cancer (IBMCC), University-CIC, Salamanca, Spain,* [4]*Oncology Service, Hospital University of Salamanca, Salamanca, Spain*

Background/Objectives: Hypoxia is a common situation in the solid tumor microenvironment. It is associated with resistance to radiotherapy and with poor clinical results. The activation of the Hypoxia-inducible factor (HIF) signaling pathway plays a pivotal role in tumor adaptation to hypoxia. HIF proteins regulate the transcription of several genes implicated in angiogenesis, apoptosis, metastasis or tumoral growth and their overexpression correlates with poor prognosis after radiotherapy. Therefore, HIF inhibitors are used to improve the cell response to radiotherapy. Here, we study the effects of PX478, a first-generation HIF-1-alpha inhibitor, on cancer cell lines under hypoxic and normoxic conditions. *Methods*: HT29 (Colon), MCF7 (Breast), HCC1937 (Breast), VCAP (Prostate), CAL33 (Head and Neck) cell lines were used to test PX478 radiosensitivity through MTT, cell cycle and apoptosis assays. Western blot was used to analyze the expression of HIF-1-alpha. *Results*: Western blot analysis showed that the expression of HIF-1-alpha was partially inhibited by PX478 in a dose-dependent manner. PX478 inhibited cell proliferation and decreased cell survival in normoxia but especially under hypoxic conditions. In addition, the compound increased the radiosensitivity of HT29; results in other cell lines are pending. *Conclusion*: PX478 inhibited the expression of HIF-1-alpha in a dose-dependent manner. This drug had a strong effect on cell survival and increased radiosensitivity, especially under hypoxic conditions. *References*: Albadari N; Deng S, Li W. Expert Opin. Drug Discov. 2019. DOI: 10.1080/17460441.2019.1613370. *Grants*: This project was financed by Gerencia Regional de Salud, JCyL (GRS2171/A/2020).

Keywords: Hypoxia, HIF-1-alpha inhibitor, radiotherapy, PX478.

P055. Neuroscience, Psychiatry & Mental Health

Regulatory T cells in Parkinson's disease: analysis of pathophysiological alterations and its potential therapeutic use as a CAR cell therapy

Nieves Iria Domínguez García[1,2], Estefanía García Guerrero[1], José Antonio Pérez Simón[1], Juan José Toledo Aral[1,2], Alfonso Rodríguez Gil[1,2], Francisco Javier Villadiego Luque[1,2]
[1]*Instituto de Biomedicina de Sevilla-IBiS, HUVR/CSIC/Universidad de Sevilla, Seville, Spain,* [2]*Departamento de Fisiología Médica y Biofísica, Universidad de Sevilla, Seville, Spain*

Parkinson's disease (PD) is characterized by the progressive degeneration of nigrostriatal dopaminergic neurons, alpha-synuclein (aSyn) aggregation and chronic neuroinflammation. Recent studies show that aSyn aggregation leads to T-cell-mediated activation of the immune response, playing a major role in the regulation of the neuroinflammatory response and neurodegeneration. In particular, regulatory T cells (Tregs), which are the main physiological suppressors of the immune system, have been shown to be altered in PD patients, and their adoptive transfer reduces neuroinflammation and protects against dopaminergic degeneration in preclinical PD models. In this work, we carried out a functional characterization of Tregs in murine models of PD. Interestingly, we have found in aged mice carrying the parkinsonian LRRK2 (G2019S) mutation significant alterations in the expression levels of certain markers important for the function and the suppressive capability of Tregs. In addition, with the aim to enhance the immunosuppressive effect of Tregs and to direct them to the damaged nigrostriatal pathway, we have generated CAR-Treg against aSyn aggregates (aSyn-CAR-Treg) that specifically recognize pathological aggregates of human aSyn and promote the activation

of T cells. Moreover, experiments of adoptive transfer in a human-aSyn mouse PD model indicate that the transferred aSyn-CAR-T-cells remain in peripheral blood and are able to infiltrate the damaged nigrostriatal pathway. The identification of functional Treg alterations associated with PD and the preclinical validation of the aSyn-CAR-Tregs outcome would be important steps to design new Treg-based therapies in PD.

Keywords: Parkinson, T-CAR, Tregs, alpha-synuclein.

P057. Cellular & Molecular Biology

How the intriguing dynamics of Cer[EOS] on a molecular level challenge our understanding of the human skin barrier

Ferdinand Fandrei[1], Oskar Engberg[1], Lukáš Opálka[2], Tomáš Havrišák[2], Petra Pullmannová[2], Kateřina Vávrová[2], Daniel Huster[1]
[1]*Institute for Medical Physics and Biophysics, University Leipzig, Leipzig, Germany,* [2]*Skin Barrier Research Group, Charles University, Faculty of Pharmacy, Hradéc Králové, Czech Republic*

The stratum corneum (SC) as the most relevant layer for the barrier function of the human skin protects our body from xenobiotics and excessive water loss. In this study, we investigated the intercellular lipid matrix of the SC with special focus on the molecular behavior of the unique ceramide Cer[EOS]. This molecule is essential for forming the LPP (long periodicity phase) and is reduced in atopic dermatitis patients. Our LPP models consisted of Cer[NS], Cer[EOS], a free fatty acid-mixture (FFA-mix, C16-C24 saturated) and cholesterol at a molar mixing ratio of 0.3:0.7:1:0.45. Utilizing 2H solid-state NMR spectroscopy, we investigated our lipid models at temperatures ranging from 25°C to 80°C. Cer[EOS] was deuterated at 4 different parts, namely the sphingosine chain, the upper and lower part of the ultralong acylchain as well as the linoleoyl moiety and measured in separate mixtures each containing only one deuterated moiety. This data shows, that while most lipids are in a highly ordered crystalline state this was only the case for parts of the ultralong acylchain of Cer [EOS], while the other parts of Cer[EOS] were found in a rather fluid or even isotropic and highly disordered state at skin temperature. This surprising observation provides further evidence for the unique role of Cer[EOS] as a modulator for the barrier properties of the SC and modifies our understanding of the molecular structure and dynamics of the LPP to better understand the barrier properties of the human skin.

Keywords: Skin, lipids, NMR, molecular dynamics.

P061. Genetics & Epigenetics

Human prefoldin modulates co-transcriptional pre-mRNA splicing

Sara Fontalva[1,2], Laura Payán[1,2], Xenia Peñate[1,2], Ildelfonso Casares[3], María De La Cruz Muñoz[1,2], Sebastián Chávez[1,2]
[1]*University of Seville, Seville, Spain,* [2]*Instituto de Biomedicina de Sevilla (IBiS)/HUVR/CSIC/US, Seville, Spain,* [3]*Centro Andaluz de Biología del Desarrollo, Seville, Spain*

Prefoldin is a co-chaperone composed of six different subunits, named PFDN1-6. It is present in all eukaryotic organisms and archaea. It is known for its role in the cytoplasm, folding actin and tubulin monomers during cytoskeleton assembly. While this first discovered function is cytoplasmic, prefoldin has also been found to play nuclear roles. In our lab, we previously reported that prefoldin has a role related to transcription elongation and chromatin dynamics in *Saccharomyces cerevisiae*. In human cells, we found that prefoldin perturbation generates changes in gene expression over the genome. These transcriptional alterations are more acute in long genes with a high number of introns, which is consistent with the co-transcriptional splicing defect detected in prefoldin knockdown cells. We detected genome-wide prefoldin binding to transcribed genes, mainly accumulated in the transcription start site (TSS), following a similar distribution to RNA pol II. Furthermore, its accumulation is correlated with the negative impact of prefoldin-depleted cells on gene transcription. Lack of prefoldin also generates a decrease in the levels of Ser2-phosphorylation of the RNA polymerase II CTD domain, and it is also implicated in the recruitment of the Ser2P kinase CDK9 to the genes. Moreover, splicing factors as PRP19 and U2AF65, which are known to be co-transcriptionally recruited, were also less present in transcribed chromatin in prefoldin knock-out cells. Taken together, these results signify that prefoldin contributes to human gene expression by preserving RNA pol II Ser2-phosphorylation and thereby modulating co-transcriptional splicing.

Keywords: Prefoldin, gene expression, splicing, mRNA, RNA polymerase II.

P062. Cancer Biology & Oncology

Surviving under pressure: oncogenic rewiring of cell density homeostasis

Giuseppe Fragale[1], Nicola Manfrini[2], Rescell Bianan[1], Zeno Lavagnino[1], Maria Grazia Totaro[1], Stefano Biffo[2], Kristina Havas[1]
[1]*IFOM ETS – The AIRC Institute of Molecular Oncology, Milan, Italy,* [2]*INGM – Fondazione Istituto Nazionale Genetica Molecolare, Milan, Italy*

While the accumulation of growth-induced pressure leads to growth arrest in healthy tissues, stiffness has been shown to correlate with cancer progression and metastasis. One consequence of increased mechanical stress is volume loss, which has the potential to strongly affect cytoplasmatic density, defined by cell water content vs dry mass ratio. Although we do not yet have a full understanding of how density is set, we do know that it is an alterable parameter. Still, it remains unknown how cells regulate their density, whether oncogenic signaling impacts upon its regulation and whether changes in cytosolic density could be beneficial for cellular fitness under certain conditions, such as acute mechanical stress. First, we asked whether protein biosynthesis can be tuned by rheological alterations. We acutely altered cell volume using osmotic shocks and mechanical stress. Then, we used single particle tracking to infer density, together with puromycin incorporation, polysome profiling and RIBO-HALO assays to quantify translation rates upon either osmotic stress or compression in healthy and cancer cells. We observed that both osmotic and mechanical stresses illicit transient alterations in cellular rheology with different adaptation times. In line with this, 1D compressive stress also resulted in increased intracellular density and concordant decrease in protein translation. Preliminary experiments suggest an initial biophysical regulation followed by a signaling-driven adaptive

response over mTORC1. Finally, preliminary data obtained in isogenic cell systems suggest that oncogenic HRASV12 mutation affects both rheological properties as well as the translation rate upon acute rheological alterations.

Keywords: Cancer, protein translation, ribosomes, mechanobiology, cell signaling.

P063. Cellular & Molecular Biology

Mechanotransduction in 3D hepatic cancer cell culture

Adam Frtús[1], Barbora Smolková[1], Mariia Uzhytchak[1], Mariia Lunova[2], Milan Jirsa[2], Yuriy Petrenko[3], Alexandr Dejneka[1], Oleg Lunov[1]
[1]*Institute of Physics, Czech Academy of Sciences, Prague, Czech Republic,* [2]*Institute of Clinical and Experimental Medicine (IKEM), Prague, Czech Republic,* [3]*Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic*

Traditional 2D monolayer cell culture on stiff glass-like dishes represents a cheap, easy-to-operate and well-established system for analysis of various cellular functions. However, 3D cell culture offers more physiologically-relevant and close to *in vivo* conditions model, creating a suitable 3D mechanical environment for cell growth and function. Liver cells, such as hepatocytes are surrounded by a functional and specific extracellular matrix (ECM), that contains cross-linked proteins and carbohydrates, including collagen, elastin, laminin, and fibronectin. The dynamic structure of ECM serves as a support for cells and is involved in tissue differentiation and homeostasis. An increase in stiffness of the liver ECM is associated with pathological events, such as cirrhosis and/or cancer progression. Therefore, we designed a soft 3D-engineered collagen-I-based scaffold (3D CS) to study pathophysiological development of hepatocellular carcinoma (HCC) cells. We revealed that hepatic cancer cell lines Alexander and HepG2 changed size and morphology and rapidly decreased their proliferation rate under the physical constraints of 3D CS. We identified YAP-mTOR pathways as a downstream effector of mechanotransduction of 3D cell culture. Moreover, we found, that cells cultured in CS exhibit metabolic rewiring, changing glycolysis rate. Cells adapted their mitochondrial activity and function under growth in 3D CS. In conclusion, our 3D CS liver-like cell culture model offers a useful model for future studies of mechanically-regulated cellular functions of liver tumor cells.

Keywords: Mechanotransduction, 3D cell culture, cytoskeleton, cell plasticity, mitochondria.

P064. Cancer Biology & Oncology

Targeted DNA damage boost with anti-CD19 antibody-drug conjugates in combination with PARP inhibitors in diffuse large B-cell lymphoma

Stefania Fusani
European Institute of Oncology (IEO), Milan, Italy

Diffuse Large B-cell Lymphoma (DLBCL) is the most common non-Hodgkin lymphoma subtype and the standard R-CHOP therapy is effective in 60% of cases; overexpression of the oncogene MYC is a frequent feature of the remaining patients that present relapsed or refractory disease. Since MYC overexpression is usually associated with overactivation of the DNA damage response (DDR), targeting DDR could be a promising strategy in DLBCL. The efficacy of this approach could be further enhanced by combining DDR inhibitors (DDRi) with DNA damaging agents. Antibody-drug conjugates (ADCs) are novel drugs that target specific antigens expressed on the surface of cancer cells for the delivery of cytotoxic payloads, minimizing the effects on healthy tissues. As reported in the literature, MYC-driven cancer cells mainly depend on PARP activity in DNA repair; that is why we started testing the FDA-approved PARP inhibitor Talazoparib in DLBCL cell lines. In addition, we tested a CD19-ADC that binds CD19-positive cells leading to the formation of DNA crosslinks. Our *in vitro* preliminary studies revealed an enhanced anti-proliferative effect with strong synergism of Talazoparib-CD19-ADC combination compared to the cytotoxic activity of both single agents. While both drugs mainly induce a G2/M phase arrest in cell cycle, the combination significantly increases the fraction of cells arrested in the BrdU-negative S-phase; furthermore, Western Blot and Alkaline Comet Assay showed increased DNA damage induction after the combinational treatment. These data suggest a mechanistic interaction between the two compounds and confirm that DDR inhibition significantly enhances the efficacy of the CD19-ADC.

Keywords: DNA damage, lymphoma, DDR, Antibody-drug-conjugates.

P065. Immunology, Microbiology & Infectious Diseases

Role of Akt isofoms in IBD development

Teresa García-Prieto[1,2], Manuel Fresno-Escudero[1,2]
[1]*Universidad Autónoma de Madrid, Madrid, Spain,* [2]*Centro de Biología Molecular Severo Ochoa, Madrid, Spain*

Inflammatory bowel disease (IBD) is characterized by a hyperactivated and uncontrolled state of inflammation in the intestinal tract involving epithelium damage. Multiple factors are involved in the development of the disease, from genetics to microbiota and the diet or medicaments. One of the main features of the progression of the disease is the dissembly of the intestinal epithelial barrier, for which tight junction proteins (TJPs) on the intestinal epithelial cells (IECs) and their maintenance is the main player. Our study focusses on the differential role that Akt1/Akt2 have on TJPs and so on the barrier and disease progression. Our results shown that Akt1 overexpression or Akt2 chemical inhibition have the same effect; an increase on TJP pro barrier molecules which have a functional role as there is a decrease on the permeability and an increase of the resistance of the barrier. On the other hand, Akt1 inhibition and Akt2 overexpression showed the opposite results: an increase of anti-barrier proteins and so a decrease of the barrier function. Moreover, Akt1 inhibition on mice intestinal organoids presents a dissembly and disorganization of the structures that mimics IBD. The same Akt1 and Akt2 inhibitors given to IBD mouse models proved that Akt1 inhibition enhances the development of the disease, while the inhibition of Akt2 ameliorates the symptoms and disease progression. Those results suggest that a balance between Akt1 and Akt2 is necessary to maintain the functionality of the

barrier and that Akt2 inhibition can be considered a new target for IBD treatment.

Keywords: Akt, IBD, tight junctions, epithelial barrier, organoids.

P066. Cancer Biology & Oncology

Whole exome sequencing in patients with polyposis and a family history of polyposis and/or CRC

Paula García Vallés[1,2,3], Luis Antonio Corchete[1,3], Carlos Gutiérrez Cerrajero[1,2,3], Paloma Martín-Bejarano Soto[1,2,3], Jésica Pérez García[1,2,3], Óscar Javier Blanco Múñez [1,4], Antonio Velasco Guardado[4], Ana Belén Herrero[1,2,3], Rogelio González Sarmiento[1,2,3]

[1]Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain, [2]Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain, [3]Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain, [4]Medical Oncology Service, University Hospital of Salamanca, Salamanca, Spain

Background: Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer death. Most CRC arises sporadically from acquired somatic genomic alterations. About 25% of CRC cases have a family history of CRC with no obvious associated genetic cancer syndrome. Only 5% are associated with hereditary cancer syndromes caused by inherited germline mutations. This study aims to characterize the molecular alterations of patients with polyposis and a family history of polyposis and/or CRC with no detected mutations in high penetrance genes. *Methods*: We selected 7 patients with polyposis and a family history of polyposis and/or CRC. Genomic DNA from formalin-fixed paraffin-embedded (FFPE) polyps and normal adjacent tissues was extracted using the QIAamp DNA FFPE tissue kit (Qiagen) and whole exome sequencing (WES) was performed. The sequencing library was prepared using SureSelect V6-Post (FFPE) kit. Multiplexed paired-end sequencing was performed on NovaSeq6000 system (Illumina). *Results*: We identified 40 pathogenic or likely pathogenic variants and 139 variants of unknown significance (VUS) in the polyps (absent in the normal tissues). We found a total of 8 common VUS mutations in *PABPC1* (c.1477C>T; p.R493C), *RGPD8* (c.4288G>A; p.G1430R), *SLC9B1* (c.1318A>T; p.K440*), *CROCC* (c.1387G>T; p.D463Y), *DDX11* (c.1102C>T; p.P0368S), *PARP4* (c.3176A>G; p.Q1059R), *PCDHB10* (c.1336G>A; p.D446N), and *UGT2A2* (c.922G>A; p.G308R). *Conclusions*: WES has become an increasingly useful approach for detection of novel cancer related genes. Further studies are required to find the potential role of these genes in the development of CRC and/or polyposis. *Grants*: Study funded by Fundación Mutua Madrileña.

Keywords: WES, polyposis, CRC.

P068. Cancer Biology & Oncology

Molecular mechanisms of paclitaxel resistance in head and neck cancer

Nerea Gestoso-Uzal[1,2,3], Carmen Sicilia-Navarro[1], Sonia Carretero-Domínguez[1,2,3], Nuria Arroyo-Garrapucho[1,2,3], Luis Antonio Corchete[2,3], Juan Luis García[1,2,3], Ana Belén Herrero[1,2,3], Rogelio González-Sarmiento[1,2,3]

[1]Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain, [2]Biomedical Research Institute of Salamanca (IBSAL), University Hospital of Salamanca-USAL-CSIC, Salamanca, Spain, [3]Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain

Background: Taxanes are cytotoxic agents used in head and neck squamous cell carcinoma (HNSCC) treatment. The study of the mechanisms of tumor resistance to these drugs is essential to optimize and personalize the therapy of these patients. The main aim of this study was to generate paclitaxel-resistant HNSCC cell lines and identify the molecular alterations involved in this resistance. *Methodology*: First, we performed the molecular characterization of two HNSCC cell lines, CAL33, derived from a tongue SCC, and 32,816, established from an oropharyngeal SCC HPV+. Paclitaxel sensitivity was tested by MTT cell viability assay and annexin V assay. We generated paclitaxel-resistant cell lines by growing in step-wise increases in cytotoxic drug. We confirmed the establishment of paclitaxel-resistant HNSCC cell lines by repeating the MTT viability assay and the annexin V assay. Finally, we characterized these cell lines by karyotyping, aCGH and expression microarrays. *Results and discussion*: We successfully established two paclitaxel-resistant HNSCC cell lines. We found differences in the karyotype between the CAL33 drug-resistant cells and the parental cell line, identifying a tetraploidization in the paclitaxel resistant cells that could be responsible for resistance acquisition. In 32,816 drug-resistant cells we detected a differential loss in 7q by aCGH. Cyclin-dependent kinases CDK6 and CDK4, located in the deleted region, were under-expressed and this alteration could be involved in the mechanism of resistance to paclitaxel. *Conclusions*: Our results suggest that paclitaxel resistance is determined by cytogenetic alterations. 7q deletion could be a biomarker of paclitaxel resistance in head and neck cancer.

Keywords: Paclitaxel resistance, head and neck cancer, cytogenetic alterations, CDK dysregulation.

P071. Cardiovascular Disease

Changes in high-density lipoproteins in patients with subarachnoid haemorrhage

María Rosa Pezzotti[1,2,*], Ángela González Díaz[1,*], Zaida Ruiz De Azúa López[3], Olivier Meilhac[4,5], Juan Ureña[1,2], Rosario Amaya Villar[3], Antonio Castellano[1,2], Lourdes María Varela[1,2]
[1]*Institute of Biomedicine of Seville, IBiS, University Hospital Virgen del Rocío/CSIC/University of Seville, Spain,* [2]*Department of Medical Physiology and Biophysics, University of Seville, Spain,* [3]*NeuroCritical Care Unit, University Hospital Virgen del Rocío IBiS/CSIC/University of Seville, Spain,* [4]*Université de La Réunion, INSERM UMR-1188 DéTROI, France,* [5]*CHU de La Réunion, France*
*These authors contributed equally.

Background: Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating disease with high rates of morbidity and mortality. There is increasing evidence that leukocyte-endothelial cell interactions play a critical role in inflammation after aSHA, leading to poor outcomes. High-density lipoproteins (HDLs) are biological molecules that exert anti-inflammatory effects, mainly by regulation of the expression of cell adhesion molecules in the endothelium. However, HDLs undergo changes in protein content that have been associated with loss of functionality in many diseases, but the role of HDL in the inflammatory response after aSAH has not been studied. *Methods*: Plasmatic HDLs were isolated from patients with aSAH and their anti-inflammatory activity and protein composition were analyzed. *Results*: We observed that patients with aHSA had lower levels of HDL in plasma. HDL isolated from patients with aSAH lost the ability to prevent the expression of VCAM-1 in TNFα-stimulated endothelial cells (HUVEC). The proteomic analysis showed that HDL from patients with aHSA presented an altered composition compared to HDL from healthy subjects. Ten proteins (AGT, APOH, C3, CRP, ITIH4, LRG1, SAA1, SAA2, SAA4, SELL, SERPINA3) were overexpressed in patients, while three (APOA4, APOC2, ITIH1) were lower compared to controls. *Conclusions*: We show a decrease in HDL functionality associated with changes in its proteome in patients with aSAH. The study of HDL in the pathophysiology of aSAH is needed, and HDL can be considered a novel therapeutic approach for the treatment of the inflammatory response that participates in the onset of aSAH.

Keywords: HDL, aneurysmal subarachnoid hemorrhage, inflammation, VCAM1, proteomics.

P072. Neuroscience, Psychiatry & Mental Health

Microglial influence during development of aqueductal ependymal cells in AQP4-KO mice

Lourdes González Vinceiro[1], Francisco Mayo León[1,2], Laura Hiraldo González[1], Claudia Calle Castillejo[1], María Del Reposo Ramírez Lorca[1,2], Miriam Echevarría Irusta[1,2]
[1]*Institute of Biomedicine of Seville (IBiS), Virgen del Rocio University Hospital (HUVR)/Spanish National Research Council (CSIC)/University of Seville, Seville, Spain,* [2]*Department of Physiology and Biophysics, University of Seville, Seville, Spain*

Aquaporin 4 (AQP4) is the water channel most widely expressed in the central nervous system. Its location in ependymal cells, glia limitans and pericapillary astrocyte foot processes is crucial for proper cerebrospinal fluid homeostasis. Stenosis of Silvio's aqueduct occurs in 10% of the offspring of AQP4 knock-out (AQP4-KO) mice, causing premature death of the animals due to the development of congenital hydrocephalus. Transcriptomic analysis of the periaqueductal tissue from mice at postnatal day 11 (P11) revealed in AQP4-KO animals, that did not develop hydrocephalus, an overexpression of genes corresponding to a microglial subpopulation positive for CD11c marker, suggesting a possible protective effect for these cells. Here, we described the time course followed by this microglial subtype by immunofluorescence assays against CD11c and IBA1 in the aqueduct of AQP4-KO and wild-type (WT) animals at P03, P5, P7, P11 and P20. To establish a relationship between these microglia and underlying ependymal abnormalities in AQP4-KO mice, we developed a microglial depletion model by injecting the PLX5622 compound into these animals. Through FACS (Fluorescent Activated Cell Sorter), we isolated aqueductal ependymal cells (CD24+) from P11 WT and AQP4-KO animals, untreated and PLX5622 treated, and performed a transcriptomic study. Finally, we measured the apical area of the ependymal cells of animals under these conditions and found that it is reduced in AQP4-KO treated mice compared to the untreated ones, pointing to a beneficial effect of CD11c microglia in preventing ependymal disorganization and aqueductal closure, and therefore in avoiding the development of hydrocephalus.

Keywords: Aquaporin 4, microglia, ependyma, hydrocephalus, postnatal.

P073. Computational Biology, Bioinformatics & Artificial Intelligence

Natural variation in 3D tissue organization

Carmen M. Gordillo-Vázquez, Jesús A. Andrés-San Román, Antonio Tagua, Ana M. Palacios-Barea, Pedro Gómez-Gálvez, Laura Morato-Concejero, Valentina Annese, Luis M. Escudero
Institute of Biomedicine of Seville (IBiS)/HUVR/CSIC/US and University of Seville, Seville, Spain

Natural variation adds diversity to life and is the raw material for evolutionary processes. Although tissue development is controlled by precise morphogenetic programs, it is also subject to natural variation. However, uncontrolled variation during morphogenesis can be detrimental for the tissue function. Here, we study the stochasticity of epithelial morphogenesis to understand the causes and consequences of natural variation in tissue development by using Madin-Darby Canine Kidney cysts as a model. To this end, we have developed a deep learning -based 3D segmentation tool to quantify geometrical and morphological parameters in a high number of samples. Importantly, this method is also able to describe, in a realistic way, the 3D packing of curved epithelial organization. Our results show how natural variation in epithelial organization obeys a number of constraints defined by the limits of certain parameters. We found that these constraints vary with the age of the cysts and can be altered by varying the culture conditions, as culturing cysts under hypoxic conditions. We think that our approach can serve as a reference to study how geometrical and biophysical constraints drive self-organization of tissues.

Keywords: Natural variation, cystogenesis, organogenesis.

P074. Omics (Proteomics, Transcriptomics, Metabolomics, Metagenomics)

A novel and more efficient biosynthesis approach for human insulin production in *Escherichia coli* (*E. coli*)

Kamini Govender[1], Tricia Naicker[2], Johnson Lin[3], Sooraj Baijnath[2], Anil Amichund Chuturgoon[1], Naeem Sheik Abdul[1], Taskeen Docrat[1], Hendrik Gerhardus Kruger[2], Thavendran Govender[4]

[1]School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa, [2]Catalysis and Peptide Research Unit, School of Health Sciences, University of KwaZulu-Natal, Durban, South Africa, [3]School of Life Sciences, University of KwaZulu-Natal, Durban, South Africa, [4]Department of Chemistry, University of Zululand, KwaDlangezwa, South Africa

Insulin has captured researchers' attention worldwide. There is a rapid global rise in the number of diabetic patients, which increases the demand for insulin. Current methods of insulin production are expensive and time-consuming. A PCR-based strategy was employed for the cloning and verification of human insulin. The human insulin protein was then overexpressed in *E. coli* on a laboratory scale. Thereafter, the optimisation of human insulin expression was conducted. The yield of human insulin produced was approximately 520.92 (mg/L), located in the intracellular fraction. Human insulin was detected using MALDI-TOF-MS and LC–MS methods. The crude biosynthesised protein sequence was verified using protein sequencing, which had a 100% similarity to the human insulin sequence. The biological activity of human insulin was tested *in vitro* using a MTT assay, which revealed that the crude biosynthesised human insulin displayed a similar degree of efficacy to the standard human insulin. This study eliminated the use of affinity tags since an untagged pET21b expression vector was employed. Tedious protein renaturation, inclusion body recovery steps, and the expensive enzymatic cleavage of the C-peptide of insulin were eliminated, thereby making this method of biosynthesising human insulin a novel and more efficient method.

Keywords: Biosynthesis of human insulin, diabetes, *E. coli*.

P075. Genetics & Epigenetics

Regulation of RNA polymerase II arrest is a key event in the repair of DNA lesions

Esperanza Granado

Universidad de Sevilla and Instituto de Biomedicina de Sevilla (IBiS)/HUVR/CSIC/US, Seville, Spain

Transcriptional stress is the consequence of either accidental or deliberate blockage of RNA polymerase progression. Nucleosomes, RNA secondary structures, other transcribing polymerases, the replication machinery and several types of DNA damage can become a barrier for elongating RNA polymerases. Transcriptional stress can affect gene expression but can also result in the formation of DNA double strand breaks (DSBs), the most cytotoxic lesions occurring in the DNA. These breaks can promote cell death and genome instability, often associated with cancer. Gene expression is therefore one of the main endogenous sources of chromosome breakage. However, the cellular mechanisms that regulate transcription to prevent genome instability remain unclear. In this study we have identified those transcription factors that participate in the resolution of RNAPII pausing and arrest, their role in preventing genome instability and their impact in DNA repair. Our results reveal that regulation of RNA polymerase arrest is a key event in the repair of DNA lesions.

Keywords: Transcription, DNA repair, RNAPII, arrest.

P077. Clinical Research, Translational Biomedicine & Personalised Medicine

Effect of cannabinoid derivatives in the lymphoid metabolism: role in CAR T cell therapy

Beatriz Guijarro-Albaladejo, Belén Sierro-Martínez, Laura Pérez-Ortega, Virginia Escamilla-Gómez, José Antonio Bejarano-García, Paola Hernández-Díaz, María Dolores De La Rosa-Garrido, Maribel Lara-Chica, Alfonso Rodríguez-Gil, José Antonio Pérez-Simón, Estefanía García-Guerrero

Instituto de Biomedicina de Sevilla, UGC de Hematología, Hospital Universitario Virgen del Rocío and Consejo Superior de Investigaciones Científicas (CSIC) y Centro de Investigación Biomédica en red Cáncer (CIBERONC), Universidad de Sevilla, Seville, Spain

Our group has reported that several cannabinoid derivatives exert a potent antitumor effect in multiple myeloma and acute leukemia. On the other hand, cells of the immune system express CB2 cannabinoid receptor. In lymphocytes, the proapoptotic effect of cannabinoids (CNBs) depends on their activation/proliferation status. CNBs block metabolism, specifically glycolysis, and cause early mitochondrial damage. In this thesis, we consider deepening lymphocyte metabolism and CB2 expression in the different lymphocyte populations, as well as in the study of the role of CNBs in lymphocyte viability and regulation of the immune response. These findings will serve as a platform to evaluate the efficacy of CNB to enhance the antitumor immune response. In addition, we consider evaluating the metabolism and signaling pathways involved in the activation of CAR T lymphocytes. It would be important to know these aspects of the biology of the CAR T cell that would perhaps justify the loss of long-term response when used as a substrate to generate the T CAR lymphocytes of patients with myeloma. Furthermore, we will evaluate CB2 expression in CAR T cells in an attempt to evaluate the potential role of CNBs to prevent or treat the toxicity of these therapeutic approaches or to use CB2 antagonists to increase their antitumor effect. We will also evaluate the efficacy of different compounds to try to improve the quality of the CAR T cell product in myeloma patients, as it has been described that less differentiated CAR T cell products can achieve better responses.

Keywords: CAR-T, metabolism, cannabinoids, cellular therapy.

P078. Cancer Biology & Oncology

Identification of compounds that cause synthetic lethality with ATRX deficiency in gliomas

Alba Guillén Benítez, Paula Aguilera, Andrés J. López-Contreras
Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), Consejo Superior de Investigaciones Científicas (CSIC) - Universidad de Sevilla - Universidad Pablo de Olavide, Seville, Spain

ATRX (alpha-thalassemia mental retardation X-linked) is a member of the SWI/SNF2 family of chromatin remodeling proteins and is a central player in the maintenance of genome integrity. Among human cancers, ATRX mutations have been described in at least 15 different types of cancers, being especially common in gliomas. Gliomas are the most common and aggressive brain tumors, with a poor prognosis. Therefore, a successful strategy to treat gliomas has yet to be developed, for which a better characterization of the pathways altered is crucial. Thus, we aim to elucidate the molecular mechanisms by which ATRX prevent/suppress gliomas in order to develop new potential treatments. To this end, our laboratory performed a screen with an FDA-approved library to identify compounds that cause synthetic lethality with ATRX deficiency. Seven drugs showed a toxicity of at least 1.5-fold higher in the ATRX KO cells compared to the ATRX WT cells. The aim of this project is to validate these seven hits in glioma cell lines, and to identify the molecular mechanisms involved in the vulnerability of ATRX-deficient cells upon treatment with these compounds. Ultimately, the aim is to identify new possible therapeutic opportunities for glioma that exploit specific vulnerabilities of ATRX-deficient tumors.

Keywords: ATRX, gliomas, cancer, synthetic lethality.

P079. Cellular & Molecular Biology

Inhibition of HRD1 leads to death of glioblastoma cells T98G and neuroblastoma cells SH-SY5Y and induction of HRD1 expression

Jaroslava Gužiková[1], Monika Líšková[1], Mária Brodũanová[2], Peter Raĭay[1]
[1]*Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia,* [2]*Biomedical Center Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia*

Endoplasmic reticulum (ER) stress caused by the accumulation of misfolded proteins in the ER or in response to various conditions is involved in the mechanisms of many diseases. In tumor cells, ER stress is caused by their high degree of translation and metabolism as well as the tumor microenvironment. To cope with ER stress, cells activate the unfolded protein response (UPR), a dynamic signaling network that directs homeostasis restoration by arresting translation and increasing the expression of ER-specific chaperones. UPR also results in the activation of ER-associated degradation that involves re-translocation of misfolded proteins from the ER to the cytoplasm and their degradation by the proteasome. Depending on the level of stress, apoptosis may also be initiated. The E3 ubiquitin ligase HRD1 (HMG-CoA reductase degradation protein 1) in complex with SEL1L is essential in the re-translocation of misfolded proteins from the ER to the cytoplasm and their labelling with ubiquitin. The aim of this study was to examine the impact of inhibition of HRD1 enzyme activity on the response of T98G and SH-SY5Y cells. We found that inhibition of HRD1 by LS-102 leads to the death of both cell types, SH-SY5Y cells showing higher sensitivity than T98G cells. Using Western blot analysis, we have observed LS-102-induced increase of HRD1 expression in both T98G and SH-SY5Y cells. The expression of SEL1L and HSP60, mitochondrial chaperones supporting tumor cell survival, was unaltered. Our results indicate that cells compensate for inhibition of HRD1 via its increased expression. *Supported by grant UK/60/22.*

Keywords: ER stress, UPR, HRD1.

P080. Cancer Biology & Oncology

Invasive potential of colorectal cancer through tumor budding in 3D

Tariq Haddad[1], Luuk Van Den Dobbelsteen[1], Alessandro Lugli[2], Shannon Van Vliet[1], Femke Simmer[1], Inti Zlobec[1], Iris Nagtegaal[1]
[1]*Radboud University Medical Center, Nijmegen, The Netherlands,* [2]*University of Bern, Bern, Switzerland*

Background & objectives: Pseudobudding (PsB) relates to Tumor Budding (TB) but occurs via gland disruption. Hypothesizing that tumor invasive potential is better reflected by TB rather than the reactive process of PsB, we characterize TB and PsB to elucidate their underlying biological processes. *Methods*: Cases containing TB and PsB were selected from pathology archives. Sections (80-90 μm) of formalin-fixed paraffin-embedded CRC tissue were cut and stained for Pan-cytokeratin and DAPI. Sections were imaged via confocal microscopy. Tissue microarrays (TMAs) of regions with TB and PsB were created and RNA spatial transcriptomics was performed. *Results*: For sections where the invasive margin contained TB, both single cells and cell clusters appear isolated from the core tumor in 3D. These features exhibit migratory and invasive characteristics associated with epithelial-mesenchymal transition (EMT), as evidenced by complete loss of cell-to-cell adhesions and shape shifting through elongation of the entire cell. In contrast, PsB is characterized by cellular debris surrounding a discontinuous epithelial lining. Undivided cells within the damaged area are observed as disassociated with lagging remnants of cell-to-cell adhesions. This may be due to disruption of the extracellular matrix by the inflammatory response. Differentially-expressed genes are determined via RNA spatial transcriptomics. *Conclusion*: Our findings show that TB and PsB are independent features within the invasive tumor margin which represent two separate biological processes. Considering the strong predictive capacity of TB as a marker of poor prognosis in CRC, TB scoring within routine diagnostic practice benefits from avoiding areas with a strong inflammatory reaction and PsB.

Keywords: Tumor budding, 3D, colorectal cancer.

P081. Cardiovascular Disease

Mouse perivascular adult Bmi1$^+$ cardiac progenitors are required for salvage response after acute myocardial infarct

Guillermo Albericio, Marina Higuera, Miguel Ángel García-Brenes, Diego Herrero, Alejandra Cordero, Susana Cañón, Susana Aguilar, Carmen Mora, Antonio Bernad
National Center for Biotechnology (CNB-CSIC), Madrid, Spain

Purpose: It has been confirmed that adult mammals generate new mature cardiac cells postnatally, but the source of these *de novo* generated cells, however, is still under debate. Previously, we identified a population of Bmi1$^+$ adult cardiac progenitor cells (B-CPC) that moderately contribute in homeostasis to the main cardiac cell lineages, showing a significant bias towards the endothelial lineage. Similar to other adult stem cell niches, B-CPC demonstrate a close relationship with cardiac endothelium (CE), being confined to low-Radical Oxygen Species (ROS) CE domains. Here, we aimed to achieve a deeper understanding of the B-CPC microenvironment and potential under damage conditions. *Methods*: Using an acute myocardial infarction (AMI) model, we evaluated the B-CPC contribution to cardiac repair and the consequences of genetically lineage-specific depletion of Bmi1$^+$ cells. On the other hand, we generated a B-CPC immortalized cell line to design co-culture experiments in order to define the functional effects in the niche-like interaction between B-CPC and CE. *Results*: Damage condition distorted the 3D organization of B-CPC, provoking their proliferative activation and endothelial differentiation. Interestingly, depletion of B-CPC provokes a deficient salvage response after AMI, inducing a dilated cardiomyopathy dominated by an inefficient revascularization. Moreover, we demonstrated a functional crosstalk between the cardiac progenitor/endothelial niche, identifying several factors that could be directly involved. *Conclusions*: Our findings demonstrated the relevant role of the B-CPC population in cardiac repair, proposing its interaction with CE the key on the switch from a quiescent to proliferative state observed in their response after damage.

Keywords: Bmi1, cardiac progenitor cell, vascular niche, myocardial infarct.

P082. Chemistry & Biochemistry

The effect of oxidized phospholipids on pore-forming activity of Bax

Vandana Singh[1], Matej Horvath[1], Karolina Honzejkova[2], Petra Riegerova[1], Radek Sachl[1]
[1]*J. Heyrovsky Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic,* [2]*Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Prague, Czech Republic*

Maintaining a balance between apoptosis and cellular proliferation is of fundamental importance for the health of multicellular organisms, and imbalances are often associated with many diseases. A number of proteins participate in the regulation of apoptosis, the key control point being represented by the Bcl-2 family comprising both pro- and anti-apoptotic proteins. Pro-apoptotic Bax is located in the cytosol, however upon apoptosis initiation translocates to mitochondrial outer membrane (MOM), where it participates in membrane permeabilization and subsequently cytochrome c release – events that represent the so-called ′point of no return′. It was shown that phospholipids (OxPl) within the MOM oxidized during the early onset of apoptosis play an active role in Bax association, however the mechanism is not entirely clear. In this study, we analyzed the effect of OxPl 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine (PazePC) and 1-palmitoyl-2-(9′-oxo-nonanoyl)-sn-glycero-3-phosphocholine (PoxnoPC) on Bax association with the mitochondria-like model membrane and its pore-forming activity. Our study shows that the type of OxPl present in the mitochondria-like model membrane has a significant effect on Bax pore forming activity. These results present further factors, which affect a crucial point in the progress of apoptosis. *This work was supported by the Grant Agency of Charles University (Grant No. 1160120).*

Keywords: Apoptosis, bax, membranes, leakage.

P083. Computational Biology, Bioinformatics & Artificial Intelligence

Benchmark data and software for assessing genome-wide CRISPR-Cas9 screening pipelines

Raffaele M. Iannuzzi[1], Ichcha Manipur[2], Clare Pacini[3], Fiona M. Behan[3], Mario R. Guarracino[2], Mathew J. Garnett[3], Francesco Iorio[1,3], Aurora Savino[1]
[1]*Human Technopole, Milan, Italy,* [2]*Institute for High Performance Computing and Networking (ICAR), Naples, Italy,* [3]*Wellcome Sanger Institute, Cambridge, UK*

Genome-wide recessive genetic screens using lentiviral CRISPR-guide RNA libraries are widely performed in mammalian cells to functionally characterize individual genes and for the discovery of new anti-cancer therapeutic targets. As the effectiveness of such powerful and precise tools for cancer pharmacogenomics is emerging, reference datasets for their quality assessment and the validation of the underlying experimental pipelines are becoming increasingly necessary. Here, we provide a dataset with a paired R package with metrics for the assessment of a novel experimental pipeline upon the execution of a single calibration viability screen of the HT-29 human colon cancer cell line, employing a commercially available genome-wide library of single guide RNAs: the Human Improved Genome-wide Knockout CRISPR (HIC) Library. This dataset contains results from screening the HT-29 in multiple batches with the HIC library and results from several levels of quality control tests on the resulting data. The data and the accompanying R package can be used as a toolkit for benchmarking newly established experimental pipelines for CRISPR-Cas9 recessive screens, via the generation of a final quality-control report.

Keywords: CRISPR-Cas9, data analysis, bioinformatic pipelines, pre-processing quality-control.

P084. Cancer Biology & Oncology

Study of drugs' effectiveness in NOMO1 knockout cell lines

Diego Iglesias Corral[1,2,3], Jorge Úbeda Garrido[2], David Díez Castro[1,2], Abel Martel Martel[1,2,4], Juan Luis García Hernández[1,3], José Perea García[1], Ana Belén Herrero Hernández[1,2,3], Rogelio González Sarmiento[1,2,3]
[1]Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain, [2]Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain, [3]Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain, [4]Medical Oncology Service, University Hospital of Salamanca, Salamanca, Spain

Background: Colorectal cancer (CRC) is one of the most frequent and deadliest tumors worldwide. The early-onset subtype (EOCRC, patients under 50 years) has become especially relevant due to poor prognosis, rapid incidence increase during the last few decades, and lack of targeted therapies. In the search for new EOCRC biomarkers, we found the region 16p13, which includes *NOMO1* (*Nodal modulator 1*), to be more frequently deleted in patients below the age of 50. In this study we tested whether the loss of *NOMO1* modifies cell response to drugs. *Methods*: *NOMO1 knockout* (KO) and *wildtype* (WT) HCT-116, HT-29 (CRC cell lines) and HS-5 (non-tumor cell line) were used. The effect of 5-Fluorouracil, Cisplatin, Oxaliplatin and Irinotecan was tested on both WT and KO cell lines. MTT assays were performed to assess cell viability and cell cycle assays to check cell cycle status. Apoptosis assays were used to evaluate drug-induced cell death. *Results*: All 4 drugs inhibit cell viability and induce cell death in a time- and dose-dependent manner. Treatment with 5-Fluorouracil, Oxaliplatin and Irinotecan showed no significant differences between the WT and KO cell lines. However, KO cell lines treated with Cisplatin showed higher proliferation rates and a reduction in cell death when compared with the WT. Cell cycle analysis showed a WT G2/M cell cycle arrest. *Conclusions*: Loss of *NOMO1* does not affect cell response to 5-Fluorouracil, Oxaliplatin or Irinotecan. *Grants*: Study funded by PI20/01569.

Keywords: Cell culture, colorectal cancer, NOMO1.

P085. Chemistry & Biochemistry

Analysis of the complex formation between the supramolecular carrier (Congo red) and selected tyrosine kinases inhibitor (sorafenib)

Daniel Jankowski[1,2], Marta Kaczor-Kamińska[1], Zuzanna Wadowska[1,2], Leszek Konieczny[1], Małgorzata Lasota[1,2]
[1]Chair of Medical Biochemistry, Jagiellonian University Medical College, Cracow, Poland, [2]SSG of Targeted Therapy and Supramolecular Systems, Jagiellonian University Medical College, Cracow, Poland

Supramolecular structures are increasingly used as drug carriers for cancer therapy. An example of such compounds is Congo red (CR). The incorporation of a drug into a carrier can decrease its toxicity, enhance targeted transport into the cell, and increase the apparent solubility of the drug in water. The aim of this work was analysis of the complex formation between the supramolecular carrier such as Congo red and selected tyrosine kinase inhibitor (sorafenib). Electrophoresis, DLS, and UV–VIS spectroscopy were used for the analysis. The optimal carrier-drug molar ratio was determined and a preliminary evaluation of complex formation was made. Supramolecular systems can provide targeted delivery of therapeutic agents and may in the future be used in targeted anti-cancer therapy. *We acknowledge the financial support from the Polish Ministry of Education and Science through the (SKN/SP/5606/2022) grant "Student Scientific Group create innovations"*.

Keywords: Supramolecular carrier, kinase inhibitors, CongoRed.

P087. Immunology, Microbiology & Infectious Diseases

Metabolic changes of *in vitro* expanded NK cells

Marek Jedliïka[1,2], Tereza Feglarová[1], Eva Mašínová[1,2], Lucie Sládková[1,2], Jana Szabová[1], Veronika Švubová[1,2], Kateřina Kuželová[3], Tereza Kozánová[2,3], Jan Frïi[1,4]
[1]Department of Modern Immunotherapy, Institute of Hematology and Blood Transfusion, Prague, Czech Republic, [2]Charles University in Prague, Faculty of Science, Prague, Czech Republic, [3]Department of Proteomics, Institute of Hematology and Blood Transfusion, Prague, Czech Republic, [4]Cellular and Molecular Immunoregulation Group, International Clinical Research Center of St. Anne's University Hospital Brno, Brno, Czech Republic

NK cells are one of the key immune cell subsets responsible for malignant or virus-infected cells surveillance and removal. Because of their high cytotoxic potential and low risk of GvHD, NK cells are a promising tool in immunotherapy of haemato-oncological disorders. During transition from steady state to highly proliferating and activated state, NKs switch their metabolism towards glycolysis in order to support high energetic and proteosynthetic demands linked with effector functions. However, this transition is further challenged after adoptive transfer of immunotherapeutic cells to patients. The major aim of the project is to understand the metabolic demands of NK expansion during the immunotherapeutic product preparation and later changes after adoptive transfer to patients. In order to clarify the molecular mechanisms linking metabolism and cell cytotoxicity, we have expanded primary NK cells with metabolic modulators and performed cytotoxicity, gene expression and metabolic analysis. Our results showed that the expanded NK cells have higher killing ability compared to freshly isolated cells. Furthermore, metabolically-modulated NK cells sustain changed metabolic and expression profiles after 14 days of expansion. The changes in gene expression included effector molecules such as Granzyme B and IFNγ in a case of 2-deoxyglucose and metformin. Our data confirm that this ability to change metabolism in the long term opens a window for metabolic intervention during expansion and for further direction towards a superior phenotype of immunotherapeutic NK cells. Our research thus suggests possible modulators in regulation of cytotoxic functions of NK cells, and thus helps in understanding the regulation of NK cell cytotoxic machinery.

Keywords: NK, immunotherapy, immunometabolism, cytotoxicity.

P088. Genetics & Epigenetics

Confirmation of the causative role of the MIR204 heterozygous variant in a patient with retinal dystrophy and co-occurrence of OCA2 disease causing variants in one family of Czech origin

Jana Jedlickova[1], Marie Vajter[2], Marek Fichtl[2], Bohdan Kousal[2], Lubica Dudakova[1], Petra Liskova[1,2]
[1]*Department of Pediatrics and Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic,* [2]*Department of Ophthalmology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Czech Republic*

MicroRNAs are short (20–24 nt) non-coding RNAs that are involved in post-transcriptional regulation of gene expression. *MIR204* (MIM* 610942) is expressed at relatively high levels in the retinal pigment epithelium. The *OCA2* (MIM* 611409) gene is an integral melanosomal membrane protein, which plays an important role in control of pigmentation. Iris coloboma, retinal dystrophy, and glaucoma were diagnosed in the proband and 3 other family members from three generations, suggesting an autosomal dominant inheritance. In addition, the spouse of the proband, as well as one of his four offspring, were clinically referred with albinism. The purpose of this study was to identify the molecular genetic cause of these two ocular traits running in the family. Combination of next-generation and Sanger sequencing was used to search for pathogenic variants. The causative variant NR_029621.1:n.37C>T in *MIR204* was identified in 4 affected family members. This variant was previously found in one family with a similar phenotype. Another missense variant was found in *OCA2* connected with mild phenotype of ocular albinism (two individuals) and also complex intragenic rearrangement of *OCA2* gene connected with more severe phenotype (two individuals). Identification of a disease-causing variant in *MIR204* supports its causative role in retinal dystrophy and highlights its importance as a master regulator of ocular development.

Keywords: miRNA, genetics, next-generation sequencing.

P089. Cancer Biology & Oncology

Empty spiracles homeobox genes *EMX1* and *EMX2* act as tumor suppressors in sarcomagenesis by regulating the WNT pathway

Manuel-Pedro Jimenez-García[1,2], Antonio Lucena-Cacace[3], Daniel Otero-Albiol[1,2], Eva-María Verdugo-Sivianes[1,2], Amancio Carnero[1,2]
[1]*Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío (HUVR), Consejo Superior de Investigaciones Científicas (CSIC), Universidad de Sevilla (US), Seville, Spain,* [2]*CIBER de Cancer (CIBERONC), Instituto de Salud Carlos III (ISCIII), Madrid, Spain,* [3]*Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan*

The *EMX1* and *EMX2* genes are two transcription factor homeobox genes involved in cell proliferation, migration, and differentiation processes, during brain development and neural crest migration. The *EMX* genes' potential as tumor suppressor genes has been suggested in some cancers. *EMX1*/*EMX2* act as tumor suppressors in sarcomas by repressing the activity of stem cell regulatory genes. EMX protein downregulation induced the malignance and stemness of cells both *in vitro* and *in vivo*. In murine knock-out *Emx* gene models, 3MC-induced sarcomas were more aggressive and infiltrative, had a greater capacity for tumor self-renewal, and exhibited higher stem cell gene expression than wild-type models. These results were reproduced in different subtypes of sarcoma. It was preliminarily suggested in other cancers that EMX2 is an inhibitor of Wnt-1 and negatively correlates with the WNT pathway. We demonstrated the canonical WNT pathway is one of the mechanisms explaining the interactions of *EMX1*/*2* and stem cell genes in sarcoma. The WNT-*EMX1*/*2* relationship was validated *in silico* with sarcoma patient datasets, *in vitro* in primary derived sarcoma cell lines, and *in vivo*, and *EMX* expression was found to negatively regulate the WNT pathway. In conclusion, our data establish the relationship among the WNT pathway, stem cell genes and the *EMX* genes, because *EMX* represses WNT signaling and activation of the WNT pathway bypasses *EMX*-dependent stemness repression and induces sarcomagenesis. Additionally, *EMX* genes negatively regulate cancer stem cells, acting as tumor suppressors in sarcoma, suggesting the relevance of the WNT/b-catenin/stemness axis as a therapeutic target in sarcoma.

Keywords: EMX, WNT-pathway, sarcoma, cancer, cancer stem cells.

P090. Clinical Research, Translational Biomedicine & Personalised Medicine

Quantitative analysis of triiodothyronine and thyroxine in the pharmaceutical preparation of thyroid powder

Mihaela Jovanovih[1], Ruža Frkanec[2], Leo Frkanec[3]
[1]*Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia,* [2]*Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia,* [3]*Rudjer Boskovic Institute, Zagreb, Croatia*

This research aimed to develop and validate a fast, specific and reliable RP-HPLC method for simultaneous quantitative analysis of triiodothyronine (T3) and thyroxine (T4) in pharmaceutical formulation of desiccated porcine thyroid extract. Thyroid disorders affect an estimated 200 million people worldwide. The complexity of pathophysiology and inadequate response to classical levothyroxine therapy opens up the need for new pharmaceuticals, such as thyroid extracts. The problem in their use is the unresolved issue of content that differs between manufacturers, but also between individual series of the same manufacturer. Until now, a small number of studies were engaged in this topic. Furthermore, RP-HPLC is finding its place in healthcare systems and has an important role in clinical laboratories as a powerful analytic tool. A simple, sensitive and accurate method for the quantitative analysis of T3 and T4 was developed. T3 and T4 were successfully separated and quantified using anthraquinone as an internal standard. The method was validated by examining the accuracy, precision, specificity, linearity, limit of detection and quantification. The method was used for analysis of T3 and T4 in the pharmaceutical formulation of desiccated porcine

thyroid extract. The obtained results revealed that samples within the shelf life correspond to the declared content values, while the analysis of samples outside the shelf life showed a decreased amount of T3 and T4. It was demonstrated that the method is applicable for the quantitative determination of T3 and T4 content in commercially available pharmaceutical preparations as well as for monitoring the stability of such preparations.

Keywords: Thyroid powder, T3, T4, RP-HPLC, validation.

P091. Chemistry & Biochemistry

Pyrazinone derivatives from *Streptomyces* sp. Pv 4-95 after heterologous adpA expression

Anna Kachor[1], Stepan Tistechok[1], Marc Stierhof[2], Maksym Myronovskyi[2], Andriy Luzhetskyy[2], Oleksandr Gromyko[1]
[1]*Ivan Franko National University of Lviv, Lviv, Ukraine,* [2]*Saarland University, Saarbrücken, Germany*

The discovery of new antibiotics and the construction of overproducers are important in biotechnological research, especially in the production of clinically useful antibiotics. Actinomycetes are one of the main producers of a vast array of natural products essential for human health. However, the ability of actinomycetes to produce bioactive compounds has been underestimated due to the presence of biosynthesis gene clusters that are not expressed or are expressed at low levels under standard conditions. Overexpression of the *adpA* in streptomycetes can unlock silent clusters of biosynthetic genes. In view of this, the aim of our work was to activate cryptic gene clusters in *Streptomyces* sp. strain Pv 4–95 by integrating the pleiotropic transcriptional regulator AdpA. The $adpA_{gn}$ gene in the recombinant plasmid pTESadpA$_{gn}$ was integrated into the chromosome of *Streptomyces* sp. Pv 4–95 by intergeneric conjugation with *Escherichia coli* strain WM6026. As a result, the exconjugate strain Pv 4-95FL containing the integrative plasmid pTESadpA$_{gn}$ was obtained. *Streptomyces* sp. Pv 4-95FL demonstrated antibiotic activity against *Staphylococcus aureus* ATCC 25923 compared with the wild type Pv 4–95. Two new mass peaks were detected in the crude extract of Pv 4-95FL strain. These compounds were identified as flavacol and a new 3-β-hydroxy-flavacol derivative from the pyrazine group by NMR spectroscopy. We suggest that the presence of heterologous AdpA has no direct effect on the synthesis of flavacol and 3-β-hydroxy-flavacol in the *Streptomyces* sp. strain Pv 4–95. However, AdpA affects the synthesis of precursors by increasing their amount, which then condenses into the resulting compounds.

Keywords: Flavacol, 3-β-hydroxy-flavacol, *Streptomyces*.

P092. Genetics & Epigenetics

Dual role for the orthologue of HECA, Headcase, in hemocyte progenitor maintenance in the *Drosophila* lymph gland

Bayan Kharrat[1,2], Nikolett Virág[1,3], Enikű Sutus[1], Erika Gábor[1], Rita Sinka[4], Ferenc Jankovics[5], Viktor Honti[1]
[1]*Drosophila Blood Cell Differentiation Group, Institute of Genetics, Biological Research Centre, Szeged, Hungary,* [2]*Doctoral School of Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary,* [3]*Department of Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary,* [4]*Department of Genetics, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary,* [5]*Laboratory of Drosophila Germ Cell Differentiation, Institute of Genetics, Biological Research Centre, Szeged, Hungary*

Due to the high level of homology in mechanisms regulating blood cell formation between *Drosophila melanogaster* and mammals, the fruit fly is regarded as an excellent model for studying hematopoiesis. The main hematopoietic organ of the *Drosophila* larva, the lymph gland, contains hemocyte progenitors in its medullary zone (MZ), differentiated hemocytes in its cortical zone (CZ), and a group of niche cells called the posterior signaling centre (PSC) that controls the balance between the two zones. In a screen performed to find new regulators of hematopoiesis in *Drosophila*, we isolated Headcase (Hdc), the orthologue of the HECA human tumor suppressor. We found that Hdc plays a cell-autonomous role in the MZ, and a non-cell autonomous role in the PSC to maintain progenitors through the regulation of signaling pathways. The results of our genetic interaction studies show that in the niche, Hdc exerts its effect via the insulin/mTOR, Hedgehog (Hh) and Decapentaplegic (Dpp) pathways. In addition, in both the PSC and the MZ, silencing *hdc* leads to ROS generation, which in turn triggers the premature differentiation of hemocyte precursors. To isolate the partners of Hdc, we generated HA-tagged Hdc isoforms, and using LC–MS/MS, we identified a list of potential interacting partners, which are being validated with genetic and physical interaction experiments. We hope that our results will lead to a better understanding of the role of Hdc in the hematopoiesis of *Drosophila*, which in turn will give us more insight into the role of its orthologue, HECA, in mammals.

Keywords: Hematopoiesis, *Drosophila*, HSCs.

P093. Cancer Biology & Oncology

ERN1-dependent impact of glucose and glutamine deprivation on homeobox gene expression in ERN1 knockdown U87 glioma cells

Daria A. Krasnytska, Olena O. Khita, Dmytro O. Minchenko, Yuliaa M. Viletska, Oleksandr H. Minchenko
Department of Molecular Biology, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

Introduction: Glucose and glutamine supply as well as endoplasmic reticulum stress are important factors of tumorigenesis. The homeobox genes encode transcription factors that play important

roles in normal development and are also connected to carcinogenesis. *Objective*: The aim of the current research was to study the impact of glucose and glutamine deprivation on homeobox gene expression in ERN1 knockdown U87 glioma cells to evaluate the role of the ERN1 signaling pathway in their regulation. *Materials and methods*: The expression level of the *PAX6*, *PBX3*, *PBXIP1*, *MEIS1*, and *MEIS2* genes was studied by real-time qPCR in control U87 glioma cells (transfected by vector) and cells with ERN1 knockdown (transfected by dominant-negative constructs of ERN1) after exposure to glucose and glutamine deprivation condition for 24 h. *Results*: It was found that glucose deprivation down-regulated the expression level of *PAX6*, *MEIS1*, and *MEIS2* genes in control glioma cells, but did not significantly change *PBX3* and *PBXIP1*. Crucially, ERN1 knockdown significantly modified the expression of all studied genes in cells treated by glucose deprivation conditions. Under glutamine deprivation, the expression levels of *PBX3* and *MEIS2* genes were down-regulated, at the same time the expression of *PAX6* and *PBXIP1* genes were up-regulated. Furthermore, ERN1 knockdown significantly modified the impact of glutamine deprivation: it removed the up-regulation of *PAX6* and *PBXIP1* gene expression and enhanced the sensitivity of the *PBX3* gene to this experimental condition. *Conclusion*: The exposure of U87 glioma cells to glucose and glutamine deprivation affected the expression of most studied homeobox genes preferentially through the ERN1 stress signaling pathway.

Keywords: Glioma, homeobox genes, nutrient deprivation, endoplasmic reticulum stress.

P094. Cellular & Molecular Biology

Improvement of a transformation-associated recombination (TAR) method for direct cloning of large-size natural product biosynthetic gene clusters in *Actinobacteria*

Olena Kurylenko, Yuriy Rebets
Explogen LLC, Lviv, Ukraine

In the last few decades, actinobacteria and the *Streptomyces* genus in particular have been a major source for the discovery of novel natural products with a variety of biological activities. These compounds are produced by secondary metabolism. Genes encoding biosynthetic pathways for bacterial natural products are grouped together into biosynthetic gene clusters (BGCs). The size of a typical actinobacterial BGC is more than 20 kb and sometimes reaches 200 kb, which makes cloning of BGCs for heterologous expression a very challenging task. In most cases, the genomic library approach is used which is rather random and costly. Taking advantage of the highly efficient recombination capacity of *Saccharomyces cerevisiae*, TAR (transformation-associated recombination) cloning is an innovative approach useful for selective isolation of large chromosomal regions. However, PCR using gene-specific diagnostic primers is the only possible method to identify the clone with the desired gene cluster among thousands of colonies. Therefore, the TAR cloning technique requires improvement by introducing the ability for direct selection of clones with the desired gene cluster. We have optimized the TAR cloning approach by constructing a system for direct selection of colonies with the cloned BGC of interest. The efficacy of the constructed TAR cloning system was tested by cloning several lanthipeptide-encoding BGCs from *Amycolatopsis sulphurea* and *Streptomyces spp* 16–6. The cloned BGCs will be directly introduced into *Streptomyces* host strains and tested for the ability to produce the corresponding compounds.

Keywords: Biosynthetic gene clusters, *Actinobacteria*, transformation-associated recombination.

P095. Neuroscience, Psychiatry & Mental Health

Non-cell-autonomous circadian regulation of brain function in *Drosophila melanogaster*

Blanca Lago Solis, Emi Nagoshi
University of Geneva, Geneva, Switzerland

Nearly all aspects of behavioral, cognitive, and emotional states and physiological processes exhibit circadian (near 24-h) rhythms in most organisms including *Drosophila melanogaster*, in which only approximately 150 neurons and 1800 glia cells contain circadian clocks. Therefore, clock neurons presumably input the day-of-time information to non-clock cells in other regions of the brain to modulate their function and output in a circadian fashion. However, the underlying mechanisms are still poorly understood. Here, we addressed this question by focusing on the mushroom body (MB), a non-clock cell-containing region of the *Drosophila* brain that constitutes the center of associative learning and sleep regulation. By conducting a circadian RNA-seq analysis of the mushroom body neurons, we identified a large number of genes rhythmically expressed, including neurofibromin 1 (Nf1) tumor suppressor gene and cAMP-dependent Protein kinase a catalytic subunit 1 (Pka-c1) gene. Their rhythmic expression is under the control of circadian clocks since it was abolished in period null mutants. Subsequently, taking advantage of calcium and cAMP imaging as well as behavioral analysis, we showed that circadian clocks drive the rhythms of the excitability of MB neurons via NF1-cAMP/PKA signaling, eliciting higher mushroom body activity during the day than at night and thereby, promoting daytime wakefulness. Altogether, our work demonstrates the widespread non-cell-autonomous control of rhythmic brain function by circadian clocks and its implication in sleep.

Keywords: Circadian rhythms, clock neurons, sleep, *Drosophila melanogaster*, mushroom body.

P096. Cancer Biology & Oncology

Antitumor effect of selected platelet-derived growth factor receptor (PDGFR) inhibitor (CP-673451) and its complex with the drug delivery system of supramolecular ribbon-like structures on bladder cancer cells

Małgorzata Lasota[1,2], Grzegorz Kasparek[1,2], Daniel Jankowski[1,2], Sylwia Sanakiewicz[1,2], Anna Wiśniewska[3]
[1]Chair of Medical Biochemistry, Jagiellonian University Medical College, Krakow, Poland, [2]SSG of Targeted Therapy and Supramolecular Systems, Jagiellonian University Medical College, Krakow, Poland, [3]Chair of Pharmacology, Jagiellonian University, Medical College, Krakow, Poland

Targeted therapy seems to be a requirement for cancer pharmacotherapy progress. For this reason, the technique of drug

administration is extremely important in its target transport. One of the most interesting and innovative compounds that can serve as a drug carrier are supramolecular structures formed by self-association, e.g. Congo Red. This compound is known as a dye. However, its supramolecular structure and the ability to react with anti-cancer compounds is undoubtedly an innovative approach in targeted therapy. The aim of the work was to investigate the prevalence and significance of PDGFR expression in bladder cancer cell lines with different malignancy potential. To analyze the emerging complex between selected PDGFR inhibitor (CP-673451) and the investigated carrier, along with *in vitro* evaluation of the effects of the drug itself and the drug in a complex with the carrier on bladder cancer cells. The results of the conducted experiments revealed the expression of PDGFR on tested cell lines. The experiments revealed that the investigated compounds inhibited the proliferation and migration of bladder cancer cells in a dose and time dependent manner. FACS analysis showed independently that tested compounds induced apoptosis. Conducting further research on the role of PDGFR in bladder cancer will contribute to a deeper understanding of the biology of this disease, which may in the future allow the use of targeted, more effective methods of anticancer therapy.

We acknowledge the financial support from the Polish Ministry of Education and Science through the (SKN/SP/5606/2022) grant "Student Scientific Group create innovations".

Keywords: Cancer, tyrosine kinases, inhibitors, carriers.

P097. Cancer Biology & Oncology

Fasting-mimicking diet coupled with escape mechanism inhibition on the way towards novel low-toxic cancer treatments

Samuel Lazzeri[1,2], Valter Longo[1]

[1]*IFOM ETS – The AIRC Institute of Molecular Oncology, Milan, Italy,* [2]*European School of Molecular Medicine (SEMM), Milan, Italy*

The vulnerability of cancer cells to nutrient deprivation makes fasting an intriguing approach in cancer treatment. Fasting, or fasting-mimicking diet (FMD), can reduce the capability of cancer cells to adapt and survive conventional therapies while promoting protection and regeneration in normal cells, an effect described by our laboratory as differential stress resistance. This is attributable to the incapability of cancer cells to reprogram their metabolism and functions in response to nutrient scarcity, and simultaneously to the ability of non-transformed cells to adopt a stress-resistant and low division mode, while cancer cells cannot. FMD is based on severe caloric restriction, low content in proteins and carbohydrates, and relatively high fat content, and it can imitate the beneficial effects of fasting while being more tolerated by patients. Interestingly, in recent years, FMD cycles have been applied in cancer treatment in combination with conventional therapies, showing very encouraging results. We observed that FMD activates peculiar survival/growth pathways in cancer cells, including the PI3K/AKT/mTOR, the ERK/MAPK and the CDK4/6 signaling pathways, which we hypothesize may be cellular escape mechanisms shared by several types of malignancies. FMD may force cancer cells to become heavily dependent on these and few other pathways, which we can investigate by combining experimental and bioinformatics approach. The impairment of these starvation escape mechanisms using well-established inhibitors may underlie innovative and effective low-toxic cancer treatments, inasmuch as FMD was shown to prevent hyperglycemia and other detrimental side effects caused by these types of drugs, which previously limited their use in the clinic.

Keywords: Fasting, cancer, escape pathways.

P098. Obesity, Diabetes & Other Diseases

Leptin resistance consequences on adiposity and inflammation in epididymal adipose tissue

Sara León Álvarez

University of Castilla-La Mancha, Toledo, Spain

Leptin is an adipokine released mainly by white adipose tissue (WAT). This hormone has a metabolic control function. It inhibits orexigenic neurons´ activity and increases that of the anorexigenic ones in the hypothalamus, thus decreasing appetite and increasing energy expenditure. The starting hypothesis of this thesis is that resistance to this hormone, induced centrally by an antagonist called Superactive Leptin Antagonist (SLA), would cause a lack of functionality that leads to a phenotype of inflammation and an increase in adiposity in WAT. In this way, we would observe a phenotype very similar to the one in obesity. It is in this context that the aim of this work was to study the inflammatory response, as well as the adipocyte size in a model of leptin resistance in epididymal adipose tissue (EAT), as adipocyte dimensions are related to tissue functionality and inflammation. The obesity profile caused by leptin resistance has been confirmed in this study of EAT in 21 Wistar rats treated with PBS or SLA intracerebroventricularly using mini osmotic pumps for 21 days. This was evidenced by an increase in adipocyte size and in the expression of pro-inflammatory genes (IL-1B, CCL5) and proteins (TLR4, NFκB and P-JNK), as well as in a decrease in the expression of anti-inflammatory genes (Arg-1 and IL-10). In this manner, this study allows us to further our understanding of the impact of central leptin resistance on adipose tissue.

Keywords: Leptin resistance, WAT, inflammation, obesity.

P099. Cardiovascular Disease

Utility of genetic screening in non-syndromic thoracic aortic disease

Helena Llamas Gómez, Julia Rodríguez Ortuño, María Luisa Peña Peña, Alejandro Adsuar Gómez, David Grimaldos Parra, Pablo Martín Marín, José Eduardo López Haldón

Virgen del Rocío University Hospital, Seville, Spain

Background: Non-syndromic aortic thoracic disease (nsHTAD) is an autosomal dominant disorder with high mortality rate if undetected. Familial evaluation could be useful to identify high-risk patients early. The aim of our study was to assess the yield of genetic screening in a cohort of patients with suspected nsHTAD. *Results*: We studied 51 index cases suspected of nsHTAD. Mean age 54,1 ± 15,1 years, 64% male. 61,5% suffered from high blood pressure and 42,4% had smoking history. Thirty-three patients (63,5%) presented with acute aortic dissection (6 postmortem diagnosis). Family history of aortic aneurysm or dissection was identified in 15 cases (29%). Genetic cause of the disease was identified in 7 families (13,7%). Pathogenic

mutations were found in genes SLC2A10 (2 patients), GAA, TGFBR1 and 2, and MYH11 (2 patients). One of the patients had 22q11.2 deletion. *Methods*: Clinical and genetic data was collected from patients with suspected nsHTA. Bicuspid aortic valve cases were excluded. Genetic study was performed with next-generation-sequencing (at least 30 related genes). All first degree relatives were offered evaluation. *Conclusions*: Genetic screening in suspected nsHTAD is a useful tool for early detection of the disease in family members at risk and for the prevention of future complications.

Keywords: Non-syndromic, aortic, genetic.

P101. Neuroscience, Psychiatry & Mental Health

Developing a novel tool for imaging mitochondrial protein import in real time

Mario Lopez-Manzaneda[1,2], Jaime De Juan-Sanz[1]
[1]*ICM Institut du Cerveau, Paris, France,* [2]*Universidad de Sevilla, Seville, Spain*

Neurons have a unique cellular architecture imposed by their central function in neurotransmission. Maintaining such exceptional geometries and the associated function is metabolically expensive. Most of the energy used by neurons is produced under the form of ATP by mitochondria during oxidative phosphorylation (OXPHOS). For that reason, preserving a functional mitochondrial pool in the appropriate compartments throughout the neuron's life is a major biological challenge. Mitochondrial biogenesis plays a key role in this process. It relies on preexisting organelles that contain their own genetic information. However, it is also heavily dependent on the nuclear genome, which encodes virtually all of its over 1500 protein components. These proteins are synthesized on cytosolic ribosomes and transferred into the organelle in a process termed as mitochondrial protein import. Although the machineries involved in mitochondrial protein import have been well characterized, little is known about the mechanisms that regulate mitochondrial protein import; and virtually nothing is known about how this process adapts to metabolic demands, local cues and external challenges in mammalian cells, and particularly in neurons. In our laboratory we have engineered an Inducible Green Fluorescence Protein (GFP) Split System that allows for the first time the dynamic study of mitochondrial protein import in neurons. This biosensor explores the major import route in charge of transferring two-thirds of all mitochondrial proteins (mitochondrial presequence pathway). Combining the use of this original tool with high-sensitivity collection optics, we can start to gain unprecedented information about mitochondrial protein import and its regulation by synaptic activity with single-neuron resolution.

Keywords: GFP, neurons, mitochondria import.

P102. Immunology, Microbiology & Infectious Diseases

Yeast cell wall protein scw10 proteolytic processing

Mateja Lozanĭiħ, Antonija Grbavac, Ana Matiĭeviħ, Dora Hrestak, Renata Tepariħ, Vladimir Mrša
Laboratory for Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

The Scw4, Scw10, and Scw11 proteins form a group of putative glucanases located in the yeast *Saccharomyces cerevisiae* cell wall. In the previously published study, Scw10 protein was shown to be up to 10-fold more abundant in several clinical isolates compared with laboratory yeast strain, suggesting its possible role in yeast virulence. Its paralog Scw4 undergoes complex proteolytic processing. Scw4 is processed by the Kex2 protease and subsequently by yapsin proteases, which affects the biological activity of Scw4 and its ability to bind to cell walls. The proteolytic processing or binding of Scw10 has not been thoroughly investigated. It has also been shown that both Scw4 and Scw10 can form non-covalent and covalent bonds with the wall that differ from previously known protein-wall bonds. Although these bonds resemble those of Pir proteins, both Scw4 and Scw10 lack the typical Pir binding sequence. In this study, the processing of Scw10 and its potential impact on its biological function are investigated. In addition, the role of several putative Scw4/Scw10 binding sequences in the formation of a covalent bond to cell wall structures is explored.

Keywords: Yeast, cell wall, Scw10.

P103. Genetics & Epigenetics

UBASH3A is involved in the pathogenesis of podocytopathies

Mirela Loziħ
University of Split School of Medicine, Split, Croatia

Podocytes play a key role in preserving the function of the filtration barrier of the glomerulus. They synthesize the glomerular basement membrane (GBM) and demarcate the slit diaphragm with their processes. In most human glomerulopathies, podocytes respond to damage by rearrangement of the actin cytoskeleton, resulting in loss of processes and detachment from the GBM with consequent podocytopenia. The highly permeable glomerular capillaries become segmentally sclerosed by multiplied mesangial matrix and that part of the glomerulus loses its blood supply and filtration function. Over time, focal/diffuse glomerulosclerosis leads to progressive renal failure. In the spectrum of podocytopathies, minimal change disease (MCD) is at one end, and focal segmental glomerulosclerosis (FSGS) at the other. Determining the molecules and regulatory mechanisms that maintain the cytoskeleton provides a better insight into podocyte physiology and potential targeted therapy in various glomerulopathies. In our previous study, we confirmed the expression of *UBASH3A* in fetal and adult kidneys using immunofluorescence and RT-qPCR. Based on the peripheral localization of UBASH3A in immature glomeruli, we concluded that it is expressed in podocytes. Our current aim was to determine the spatial expression of UBASH3A in subjects with podocytopathies. Percutaneous renal

biopsies of 10 subjects with either FSGS or MCD were immunofluorescently stained and compared with controls to determine whether the level and localization of UBASH3A is altered. In contrast to healthy kidneys, no positive punctate signal was found in podocytopathies, indicating the involvement of UBASH3A in their pathogenesis. We postulate UBASH3A dysfunction can lead to filtration barrier damage and proteinuria.

Keywords: UBASH3A, FSGS, MCD, immunohistochemistry, podocytopathies.

P106. Immunology, Microbiology & Infectious Diseases

Measurement of total and species-specific Torquetenovirus (TTV) viremia as a predictive marker of immune system function

Hafza Zahira Manzoor[1,2]
[1]*University of Insubria, Varese, Italy,* [2]*Ospedale di Circolo, Varese, Italy*

Background: The TT virus (TTV) was first identified in 1997 by representational difference analysis of sera from non-A to non-G posttransfusion hepatitis patients, and it has since undergone extensive research as a potential addition to the list of viruses that cause hepatitis. TTV is a DNA genome of approximately 3.8 kb, an extremely high degree of genetic heterogeneity (at least 29 TTV species have been identified so far), a remarkable ability to produce chronic infections with no associated clinical manifestations, and a high prevalence in populations worldwide regardless of age, sex, and socio-economic status. *Objective and goals*: The goals of this project are: (i) To get knowledge and precise understanding of how and how much immunity modulates TTV replication. (ii) To expand our knowledge in the TTV/immune system interplay by investigating TTV and its genetic species as candidate surrogate markers to infer immune depression level, immune-reconstitution process, graft and/or infections risk, and, finally, the overall clinical outcome in adult patients receiving immunomodulant drugs. *Methodology & expected outcomes*: the purposed study will include clinical samples of BAL (Bronchoalveolar lavage) and nasopharyngeal which will be collected from patients who are admitted to the clinical & ICU wards in the hospital of Pavia and Varese region. These clinical samples will be analyzed to assess the presence of TTV by means of biomolecular and genetic lab techniques such as PCR and NGS, which will aid us in identifying new biomarkers which will help physicians in the management of critically ill patients.

Keywords: Viremia, immune system, TTV, NGS, microbiology.

P108. Pharmacology, Toxicology & Nutrition

(-)-Oleacein and (-)-oleocanthal, two phenolic compounds present in Extra Virgin Olive Oil, inhibit angiogenesis

Ana Dacil Marrero[1,2,3], Juan Ortega Vidal[4], Sofía Salido[4], Joaquín Altarejos[4], Ana R. Quesada[1,2,3], Beatriz Martínez Poveda[1,2,5], Miguel Ángel Medina Torres[1,2,3]
[1]*Department of Molecular Biology and Biochemistry, University of Málaga, Malaga, Spain,* [2]*Institute of Biomedicine of Málaga (Ibima)-Plataforma BIONAND, Malaga, Spain,* [3]*CIBER de Enfermedades Raras (CIBERER), Spain,* [4]*Department of Organic and Inorganic Chemistry, University of Jaen, Jaén, Spain,* [5]*CIBER de Enfermedades Cardiovasculares (CIBERCV), Spain*

Phenolic compounds in the Mediterranean diet contribute to many of the health-related benefits accounted in this dietary choice. (-)-Oleocanthal and the less studied (-)-oleacein, are two phenolic compounds present in Extra Virgin Olive Oil that have shown anti-tumoural effects both *in vitro* and *in vivo*. Among their effects on cancer, they could inhibit tumor cell migration and invasion, key processes also in angiogenesis, the process by which *de novo* blood vessels are formed. Herein, we explored the anti-angiogenic potential of (-)-oleocanthal and (-)-oleacein in a comparative study in *in vitro* experiments on endothelial cells, and in two *in vivo* models. (-)-Oleocanthal and (-)-oleacein affected endothelial viability in the micromolar range, as well as the formation of tubule-like structures by these cells, and their migration. Interestingly, only oleacein inhibited cell migration and induced apoptosis significantly. Regarding cellular signaling, both compounds were able to reduce the activation of the AKT and ERK1/2 pathways, which are related to survival and proliferation, respectively. Finally, both compounds showed anti-angiogenic activity in a zebrafish model of regeneration and in the chicken chorioallantoic membrane. Altogether, these results support the anti-angiogenic potential of (-)-oleocanthal and (-)-oleacein, and suggest that (-)-oleacein exerts more potent effects on endothelial cell migration and induction of apoptosis. Thus, we propose these two phenolic compounds, with a special focus on (-)-oleacein, as new candidates for clinical use as anti-cancer and anti-angiogenic agents. *[Grants: PID2019-105010RB-I00 and RTI2018-098560-BC22 (Spanish Government), UMA18-FEDERJA-220, and PY20_00257 (Andalusian Government and FEDER). Funds from BIO 267 (Andalusian Government). CIBERER, CIBERCV].*

Keywords: Angiogenesis, Mediterranean diet, cancer, phenolic.

P109. Cardiovascular Disease

Saraf implication in vascular remodeling

Marta Martín Bórnez[1,2], José Sánchez-Collado[3], Antonio Ordoñez-Fernández[1], Juan Antonio Rosado[3], Tarik Smani[1,2]
[1]*Group of Cardiovascular Pathophysiology, Institute of Biomedicine of Seville, University Hospital of Virgen del Rocío/University of Seville/CSIC, Seville, Spain,* [2]*Department of Medical Physiology and Biophysics of the University of Seville, Seville, Spain,* [3]*Department of Physiology, Institute of Molecular Pathology Biomarkers, University of Extremadura, Caceres, Spain*

Orai1 and STIM1, molecular components of Store-Operated Calcium Entry (SOCE), have been associated with vascular smooth muscle cell (VSMC) proliferation in vascular remodeling. Nevertheless, the role of SARAF (SOCE Associated Regulatory Factor), a regulatory protein involved in STIM1 inhibition, has not been firmly established in vascular remodeling. The objective of this study was to examine the role of SARAF and Orai1 in VSMC proliferation and neointima formation after balloon injury of rat carotid arteries. To this end, experiments were conducted in an animal model of rat carotid angioplasty to characterize neointima formation. VSMCs isolated from rat coronary artery were also used to examine cell proliferation. The formation of neointima after balloon injury of rat carotid arteries was confirmed by hematoxylin and eosin staining of tissue sections up to 3 weeks after surgery. Injured arteries showed significantly higher expression of SARAF, STIM1 and Orai1 compared to control tissues, corroborating the presence of these regulatory proteins in the neointima layer. Proximity ligation and co-immunoprecipitation assays revealed that SARAF interacts with Orai1 in the neointima. Furthermore, selective silencing of SARAF and Orai1 by small interfering RNA (siRNA) inhibited VSMC proliferation. In conclusion, our data suggest that SARAF is involved in VSMC proliferation and neointima formation after vascular injury.

Keywords: SARAF, Orai1, store-operated calcium entry, vascular remodeling.

P110. Cancer Biology & Oncology

Classification of variants of uncertain significance in BRCA1 through functional assays

Paloma Martín-Bejarano Soto[1,2,3], Diego Iglesias-Corral[1,2,3], Eva M Sánchez-Tapia[1,2,3], Teresa Martín-Gómez[1,4], Rosario Vidal-Tocino[1,4], Rogelio Gónzalez-Sarmiento[1,2,3], Ana-Belén Herrero[1,2,3]
[1]*Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain,* [2]*Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain,* [3]*Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain,* [4]*Medical Oncology Service, University Hospital of Salamanca, Salamanca, Spain*

Background: Hereditary breast and ovarian cancer are frequently associated with mutations in BRCA1, a tumor suppressor that plays an essential role in DNA double-strand break repair by homologous recombination (HR). This mechanism is necessary to maintain genome integrity. Thousands of mutations are classified as variants of uncertain significance (VUS), since their effect on protein function has not been determined. In this study, we used different functional assays to quantify the HR efficiency of VUS identified in the Genetic Counseling Unit of Salamanca in the last 21 years. *Methods*: VUS were re-analyzed using ClinVar, VEP and Varsome databases. QuickChange II Site-directed mutagenesis kit was used to generate plasmids containing the studied VUS. A HeLa-derived cell line carrying HR reporter cassettes was transfected with the BRCA1 variants and HR proficiency, determined by the reconstitution of the green fluorescent protein, was analyzed by flow cytometry. *Results*: Thirty-nine previously identified VUS were re-analyzed and 16 were still classified as such. Eight variants (~50%) decreased *BRCA1*-mediated HR in the HeLa system. The identified pathogenic mutations are being introduced into the genome of HCC1937 (*BRCA1* −/−) for further functional characterization using lentiviral vectors. *Conclusion*: The HR-based functional assays are effective methods to analyze VUS as BRCA1 and have allowed the re-classification of 16 VUS as benign (8) or pathogenic (8). *Grants*: This project was funded by CSI264P20.

Keywords: Breast cancer, VUS, BRCA1, functional assays.

P111. Neuroscience, Psychiatry & Mental Health

Functional analysis of cholinergic neuromodulation of chandelier cells from single-cell to circuit

Emilio Martínez-Márquez, Santiago Reyes-León, Guadalupe Asensio-Gómez, José Luis Nieto-González, Pablo García-Junco-Clemente
Instituto de Biomedicina de Sevilla (IBiS, HUVR/CSIC/Universidad de Sevilla), Departament de Fisiología Médica y Biofísica, and CIBERNED

Chandelier cells (ChCs) are GABAergic cortical interneurons that innervate the axon initial segment of pyramidal neurons, controlling the cell firing output. They are abundant in the association cortical areas, where acetylcholine inputs are essential for normal cognitive performance. Located at the layer1/layer2 boundary, they extend their dendrites towards layer1, suggesting that may receive inputs from other cortical areas and deep nuclei, such as basal forebrain, which projects the strongest cholinergic innervation to layer1, implying a plausible role for ChCs as circuit switches. Our interest lies in studying the role of ChCs in the control of cortical networks, with a special focus on its presumed cholinergic modulation. We have identified cortical ChCs using a mouse model expressing td-Tomato under control of precise Cre- and Flp- dependent promoters. Using immunohistochemical and electrophysiological techniques, we have described the existence of cholinergic neuromodulation of ChCs through specific nicotinic receptors. To clarify its role in the regulation of the prefrontal cortical circuitry, we performed *in vivo* 2-photon imaging experiments in awake animals using GECIs, showing that ChCs present a collective behavior during arousal. We used DREADDs to modulate their activity to uncover its influence in the control of the excitatory network. Our results demonstrate that prefrontal ChCs are a subpopulation of fast-spiking interneurons modulated by cholinergic inputs activated during

arousal states in awake mice, with a prominent role in the control of the pyramidal neurons.
We are grateful to C. Cabrera-Romero for excellent technical assistance. *Supported by: RyC-2016-19,906, PRE2019-087729 (MCI/AEI/FSE,UE), VI PPIT-US and PGC2018-095656-B-I00 (MCI/AEI/FEDER,UE).*

Keywords: Chandelier, neuromodulation, 2-photon electrophysiology, prefrontal cortex.

P112. Cardiovascular Disease

A novel CB2 agonist-nanosystem as a selective delivery system for atherosclerosis treatment targeting VCAM-1

Lucía Martín-Navarro Díaz[1], Mª Dolores Herrera González[2], Carmen Mª Claro Cala[3], Josefa Álvarez Fuentes[1,4], Lucía Martín Banderas[1,4]
[1]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Seville, Seville, Spain, [2]Department of Pharmacology, Faculty of Pharmacy, University of Seville, Seville, Spain, [3]Department of Pediatrics, Radiology and Pharmacy, Faculty of Medicine, University of Seville, Seville, Spain, [4]Institute of Biomedicine of Seville (IBiS), Virgen del Rocío University Hospital/CSIC/ University of Seville, Seville, Spain

Introduction: Endothelial dysfunction and the inflammatory process in atherosclerosis are related with the cannabinoid receptor type 2 (CB2). This receptor is characterized for its anti-inflammatory properties. CB2 agonists are a new potential treatment. However, cannabinoids are highly lipophilic and show low bioavailability. *Objective*: To develop novel CB2 agonist-nanoplatforms as selective delivery systems for atherosclerosis treatment targeting VCAM-1 in the vascular endothelium. *Methods*: NPs were produced by nanoprecipitation method using a mixture of PEGylated PLGAs and containing JWH-133, a potent CB2 agonist. MTT assays and NP cell uptake assays were performed in human endothelial cells (HUVEC). $ApoE^{-/-}$ mice were fed HFD (42% kcal) for 20 wk, and treated i.p. with free or encapsulated JWH-133 for 8 wk. *Results*: NPs were spherical, 170 nm in diameter, had a negative surface charge (−26 mV) and high encapsulation efficiency of JWH-133 (EE = 99%). NPs were successfully functionalized with VCAM-1 binding peptide (BP). Cell viability studies indicated low or non-toxicity for blank and JWH-133 loaded-NPs on HUVEC cells. NP cell uptake was studied in TNFα stimulated cells, resulting in increased recruitment and cell uptake of functionalized NPs vs. non-functionalized. Biodistribution studies showed an important NP accumulation in heart and aorta of $ApoE^{-/-}$ mice. Animal treatment with NPs 15% JWH-133 vs. free JWH-133 drastically reduced lipid deposition in the aortic sinus and downregulated intercellular atherogenic proteins in the aorta. *Conclusion*: VCAM-1 BP functionalized JWH-PLGA NPs reduced plaque development and lipid deposition by decreasing inflammation, showing more potent antiatherogenic effects than free drug. *Acknowledgments*: Research co-financed by the *FEDER Program 2014–2020 (US-1263053)*, and the *PPIT-US 2021*.

Keywords: Atherosclerosis, PLGA nanoparticles, CB2, inflammation, VCAM-1.

P113. Cellular & Molecular Biology

Role of ribosomal protein eL15 in ribosome biogenesis in *Saccharomyces cerevisiae*

Sara Martín-Villanueva[1], José Fernández-Fernández[1,2], Jorge Pérez-Fernández[3], Jesús De La Cruz[1,2]
[1]Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain, [2]Departamento de Genética, Universidad de Sevilla, Seville, Spain, [3]Departamento de Biología Experimental-Genética, Universidad de Jaén, Jaen, Spain

Ribosomal proteins play important roles in ribosome biogenesis and function. These roles have been analyzed for most ribosomal proteins in the model eukaryote *Saccharomyces cerevisiae*. Additionally, the timing of their *in vivo* assembly has been investigated. However, few ribosomal proteins still await functional characterization. Herein, we have analyzed the contribution of ribosomal protein eL15 to ribosome biogenesis. This ribosomal protein is essential for growth. We show that depletion of eL15 results in a severe shortage of 60S ribosomal subunits. Examination of pre-rRNA maturation by northern blotting, primer extension and pulse-chase analyses indicate that processing of 27SA to 27SB pre-rRNAs, as well as processing of 27SB to mature rRNAs, is impaired upon depletion of eL15. As a result, export of pre-60S particles from the nucleus to the cytoplasm is blocked. These phenotypes most likely appear as the direct consequence of the reduced pre-60S particle association not only of eL15 upon its depletion, but also of a subset of neighboring ribosomal proteins and trans-acting factors either involved in the processing of 27SA3 or 27SB pre-rRNAs. These factors likely do not have a direct role in the pre-rRNA processing reactions but a structural role in the formation and rearrangement of nucleolar pre-60S intermediates. We will also discuss the possible hierarchical assembly of eL15 together with its nearest neighbor eL8 and eL36 ribosomal proteins during the early stages of 60S ribosomal subunit maturation.

Keywords: Ribosome, genetics, yeast, proteins, molecular.

P114. Neuroscience, Psychiatry & Mental Health

Ependymal cell differentiation and ciliogenesis are impaired in an AQP4-KO mouse model

Francisco Mayo[1,2], Lourdes González-Vinceiro[1], Laura Hiraldo-González[1], Claudia Calle-Castillejo[1], Mª Reposo Ramírez-Lorca[1,2], Miriam Echevarría-Irusta[1,2]
[1]Institute of Biomedicine of Seville (IBiS), Virgen del Rocio University Hospital (HUVR)/Spanish National Research Council (CSIC)/University of Seville, Seville, Spain, [2]Department of Physiology and Biophysics, University of Seville, Seville, Spain

Since the discovery of aquaporins 30 years ago, many studies have characterized their ubiquitous distribution in all organisms. In the mammalian central nervous system (CNS), aquaporin 4 (AQP4) is the most widely expressed member of the family of water channels, contributing to proper fluid homeostasis through various mechanisms. Although its expression in the CNS begins postnatally (undergoing a 10-fold increase between postnatal day 7 and day 14, P7-P14), its role in the neural development process is poorly understood. Interestingly, a small proportion of AQP4 knockout mice

offspring have been reported to suffer from congenital hydrocephalus, produced by cerebral aqueduct stenosis during this period. However, most of their littermates are prevented from developing the disease by activating molecular mechanisms that still remain unknown. In this study, a transcriptomic analysis of the cerebral aqueduct tissue was performed in P11 that showed relevant changes in ependymal cell function and ciliary development as a consequence of the absence of AQP4. The defective levels of these genes in non-hydrocephalus AQP4-KO animals were further assessed by individual qPCRs. Finally, electron microscopy approaches were carried out to evaluate abnormalities within the ependymal surface lining the cerebral aqueduct (by scanning electron microscopy) and the ultrastructure of the cilia (by transmission electron microscopy). In conclusion, we propose that postnatal AQP4 expression plays a significant role in ciliary formation during the fate of ependymal cells.

Keywords: AQP4, CNS development, congenital hydrocephalus, ependymal cells, ependymal cilia.

P116. Cellular & Molecular Biology

The association of specific genetic polymorphisms with clinical picture severity and the outcome in COVID-19 patients

Neven Meseldzic[1], Faruk Šehiħ[1], Una Glamoĭlija[1], Maja Malenica[1], Tanja Dujiħ[1], Besim Prnjavorac[2], Damir Marjanoviħ[3], Tamer Bego[1]
[1]*Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina,* [2]*General Hospital Tesanj, Tesanj, Bosnia and Herzegovina,* [3]*International Burch University, Sarajevo, Bosnia and Herzegovina*

In late 2019, a new coronavirus named SARS-CoV-2 appeared in China and caused unusual viral pneumonia known as COVID-19. SARS-CoV-2 interacts with ACE2 receptors and releases its RNA into the epithelial cells, where it replicates and is released for further infection into neighboring cells. Our study examined the influence of the rs2285666 polymorphism of the *ACE2* gene on the development of the severe clinical picture and its association with the biochemical and hematological parameters in COVID-19 patients. The study included 195 COVID-19 patients who had been recruited at General Hospital in Tešanj. DNA was extracted from whole blood and stored at −20°C. Genotyping was performed using the Applied Biosystems QuantStudio5 RT-PCR System. Our results did not show a statistically significant difference in genotype distribution between COVID-19 patients with mild and moderate ($P = 0.245$), mild and severe ($P = 0.828$), and moderate and severe clinical picture ($P = 0.716$). Patients with mild clinical picture had significantly lower D-dimer values compared to patients with moderate ($P < 0.001$) and severe ($P < 0.001$) clinical picture, as well as significantly lower CRP values compared to moderate ($P = 0.001$) and severe ($P < 0.001$) clinical picture. Also, the results showed that in the group of patients with a mild clinical picture, carriers of the mutated T allele showed an association with significantly higher values of the lymphocyte count ($P = 0.036$) compared to carriers of the C allele. In the group of patients with a severe clinical picture, carriers of the mutated T allele showed an association with significantly higher values of leukocyte count ($P = 0.008$) and granulocyte count ($P = 0.033$).

Keywords: SARS-CoV-2, COVID-19, ACE2, rs2285666 polymorphism.

P117. Obesity, Diabetes & Other Diseases

In vivo and *in silico* analysis of the antidiabetic activity of *Clerodendrum volubile* P. Beauv leaf extracts

Olorunfemi Molehin[1], Olusola Elekofehinti[2], Omotade Oloyede[1]
[1]*Ekiti State University, Ado-Ekiti, Nigeria,* [2]*Federal University of Technology Akure, Akure, Nigeria*

Clerodendrum volubile leaf is commonly used in traditional medicine for the management of various diseases including diabetes in Nigeria. This study sought to propose the possible mechanism underlying the antidiabetic effect of *C. volubile* leaves in streptozotocin (STZ)-induced diabetic rats using *in vivo* and *in silico* methods. Fifty Male Wistar rats ($n = 5$) were divided into ten groups. Diabetes induction in rats was by a single intraperitoneal injection of STZ (65 mg/kg body weight) while *C. volubile* extract was administered orally to diabetic and non-diabetic animals, at doses of 50,100 and 200 mg/kg body weight respectively for 14 days. Also, the interaction of compounds identified from *C. volubile* (HPLC-DAD) on Takeda-G-protein-receptor-5 (TGR5), peroxisome proliferated activated receptor gamma (PPARγ) and dipeptidyl-peptidase 4 (DPP-4) were also investigated through molecular docking. Administration of *C. volubile* extract significantly reduced the elevated plasma glucose level, improved kidney functions, attenuated oxidative stress by decreasing malondialdehyde levels, enhancing superoxide dismutase, catalase and glutathione peroxidase activities, reinstated the lipid profiles and restored pancreatic histological integrity in diabetic rats. Rutin ranked highest among all the compounds identified in *C. volubile* with −8.3 kcal/mol binding energy with TGR5–7.9 kcal/mol (PPARγ) and − 9.5 kcal/mol (DPP-4). Gln77, Arg125, Tyr240, GlGlu343, Tyr 662 are among residues enhancing rutin binding to these proteins. The results revealed that *C. volubile* possess antidiabetic effects through the modulation of TGR5, PPARγ and DPP4. Rutin might be the lead compound responsible for the antidiabetic effect of *C. volubile*, justifying the use of this plant in traditional medicine.

Keywords: *Clerodendrum volubile*, diabetes, antioxidant, antidiabetic, molecular docking.

P118. Neuroscience, Psychiatry & Mental Health

The activity of the electron transport chain prevents abnormal and permanent microglial activation

Bella Mora-Romero[1,2], Nicolás Capelo-Carrasco[2,3], Juan J. Pérez-Moreno[2,3], José López-Barneo[2,3], Marisa Vizuete[2,3], Javier Vitorica[2,3], Alicia E. Rosales-Nieves[1,2], Alberto Pascual[1,2]
[1]*Consejo Superior de Investigaciones Científicas (CSIC),* [2]*Instituto de Biomedicina de Sevilla/HUVR/CSIC/US, Seville, Spain,* [3]*Universidad de Sevilla, Seville, Spain*

Microglia are the brain innate immune cells with relevant functions during development, in adult maintenance, and in the progression of brain diseases. Previous data have suggested that, similar to monocyte-derived cells, microglial activity depends on a metabolic switch from aerobic (dependent on the mitochondrial oxidative phosphorylation system –OXPHOS–) to anaerobic

glycolysis. However, we and others have shown that microglial activity is correlated with the transcriptional upregulation of OXPHOS, suggesting their requirement for normal microglial activation. To investigate the metabolic changes induced by microglial activation, we have combined *in vitro* and *in vivo* models with global transcriptomics and targeted metabolomics, with microglial stimulation. We have found a full reorganization of the cellular metabolism by either physiological or pathological microglial activation. As expected, glycolysis and OXPHOS were upregulated, with a concomitant activation of the one carbon by folate mitochondrial pathway (m1CP). Mitochondrial complex I (CI) has been linked to the m1CP, and therefore we generated a mouse model (MGcCI) where the essential subunit of the CI, Ndufs2, was conditionally deleted in microglia. Interestingly, this model performed faster phagocytosis of non-functional neurons during development. Moreover, three-month-old mice presented activated microglia as determined by morphometric or transcriptional analysis, characteristic of excessive activation of immune pathways. Microglial activation was also associated with GFAP accumulation in astrocytes, decreased locomotor activity, sudden weight loss, and early death. Our results have wide implications in the fields of neurodevelopmental diseases and neurodegeneration and highlight the relevance of brain mitochondria not only for neurons but also for microglia.

Keywords: Microglia activation, metabolism, mitochondria.

P119. Cellular & Molecular Biology

Musashi-2 overexpression enhances atrophy phenotypes *in vivo* by miR-7 repression

Nerea Moreno[1,2], María Sabater-Arcis[1,2], Manuel Pérez-Alonso[1,2], Ariadna Bargiela[3], Rubén Artero[1,2]
[1]*Translational Genomics Group, BIOTECMED, University of Valencia, Burjasot, Valencia, Spain,* [2]*INCLIVA Biomedical Research Institute, Valencia, Spain,* [3]*Neuromuscular Research Unit, Neurology Department, Instituto de Investigación Sanitaria la Fe, Valencia, Spain*

Myotonic Dystrophy type 1 is a neuromuscular genetic disease primarily caused by the expression of toxic CUG repeat DMPK transcripts. However, the origin of muscle wasting in DM1 is not fully understood, and several contributing factors, particularly hyperactivated autophagy, have been proposed to explain excessive catabolism. miR-7, a natural repressor of autophagy, is downregulated in DM1 muscles. We found that the reason is Musashi 2 (MSI2), a protein upregulated in patient-derived myoblasts and biopsy samples that naturally represses miR-7 biogenesis. To demonstrate the relevance of MSI2 overexpression in DM1 muscle dysfunction, we used an *in vivo* DM1 model to overexpress MSI2 in skeletal muscles. We report that adeno-associated (AAV9)-mediated transduction of Msi2 in the HSALR mice downregulated miR-7 and altered the expression of atrophy-related genes. In addition, msi2 overexpression enhanced DM1-like phenotypes related to muscle atrophy, reducing the median fiber size and shifting fiber area distribution. Furthermore, more severe muscle weakness and increased central nuclei were observed after AAV9 administration. Taken together, excessive MSI2 levels repress miR-7 maturation and contribute to muscle pathology in DM1. Therefore, we propose MSI2 as a new therapeutic target to treat muscle dysfunction in DM1.

Keywords: Myotonic Dystrophy type 1, Mushashi 2, miR-7, muscular atrophy, HSALR mice.

P120. Cellular & Molecular Biology

A new therapeutic target to modulate the SIRT1/PARP1 axis in a model of retinitis pigmentosa *in vitro* and *in vivo*

Seyed Mohamadmehdi Moshtaghion[1], Estefanía Caballano Infantes[1], Patricia Gallego Fernandez[1], Álvaro Plaza Reyes[1], Lourdes Valdés Sánchez[1], Berta De La Cerda Haynes[1], María José Marín Sainz[1], Pablo Peñalver[2], Juan Carlos Morales[2], Francisco J. Díaz Corrales[1]
[1]*Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Seville, Spain,* [2]*Institute of Parasitology and Biomedicine "López-Neyra" (IPBLN), Granada, Spain*

Retinitis pigmentosa (RP) is a rare genetic disorder and the most common cause of hereditary blindness in adults. RP is characterized by the progressive loss of the photoreceptors (rod cells). In this line, loss of photoreceptors in RP is associated with a type of caspase-independent programmed cell death known as parthanatos. The long-lasting oxidative stress in the retina of RP patients may cause DNA oxidative damage and subsequently produce an exacerbated activation of DNA repair mechanisms, such as the nuclear recruitment of poly (ADP-ribose)-polymerase 1 (PARP1). PARP1 increase could exacerbate poly (ADP-ribose) (PAR) production. Then, PAR-polymers act as a cell signaling mechanism to release and translocate the apoptosis-inducing factor (AIF) from the mitochondria to the nucleus, producing chromatin condensation, large-scale DNA fragmentation, and finally leading to cell death. Sirtuin 1 (SIRT1), an NAD+ dependent histone-III deacetylase, can inhibit PARP1 activation. Here, we study the role of SIRT1 activation in the 661 W photoreceptor precursor cell line exposed to hydrogen peroxide (H2O2) and the rd10 mouse as *in vitro* and *in vivo* RP models. In this sense, we treat the 661 W cells and the rd10 mice with the new SIRT1 activator PIC-OCT. Our results show PIC-OCT as a potent SIRT1 activator that suppressed PAR-polymers levels and nuclear translocation of AIF, increasing cell viability of 661 W cells after H_2O_2 exposure and preserving retinal function in the rd10 mice. In conclusion, our results suggest that PIC-OCT can protect photoreceptors in both *in vivo* and *in vitro* RP models through the inhibition of parthanatos.

Keywords: Retinitis pigmentosa, oxidative stress, PARP1, SIRT1, Parthanatos.

P121. Cancer Biology & Oncology

Understanding bladder cancer through mouse models

Ester Munera-Maravilla[1,2,3], Cristina Segovia[4], Mónica Martínez-Fernández[5], Sandra P. Nunes[1,2,6], Carolina Rubio[1,2,3], Víctor G. Martínez[1,2,3], Mercedes Pérez-Escavy[1,2,3], Iris Lodewijk[1,2], Miriam Pérez-Crespo[1,2], Marta Dueñas[1,2,3], Cristian Suárez-Cabrera[1,2,3], Jesús M. Paramio[1,2,3]

[1]Molecular Oncology Unit, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Madrid, Spain, [2]Biomedical Research Institute I + 12, University Hospital 12 de Octubre, Madrid, Spain, [3]Centro de Investigación Biomédica en red de Cáncer (CIBERONC), Madrid, Spain, [4]Epithelial Carcinogenesis Group, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain, [5]Genomes & Disease Lab, CiMUS (Center for Research in Molecular Medicine and Chronic Diseases), Universidade de Santiago de Compostela, Santiago de Compostela, Spain, [6]Cancer Biology and Epigenetics Group – Research Center, Portuguese Oncology Institute of Porto (CI-IPOP), Porto, Portugal

Several informative *in vitro* and *in vivo* models have been used to study bladder biology and have the potential to improve our understanding of bladder cancer (BC) progression, as well as its diagnosis and treatment. In this study, we have developed and characterized four genetically engineered mouse models of muscle-invasive BC (MIBC) generated by a double knockout (DKO; *Pten*, *Trp53*) or quadruple knockout (QKO; *Pten*, *Trp53*, *Rb1*, *Rbl1*) of tumor suppressor genes through the delivery of adenoviruses that express Cre recombinase in selected cell types (basal or luminal cells of the urothelium) using specific promoters. Molecular and transcriptomic analyses of tumors developed from these models revealed strong similarities to the human sarcomatoid subtype of BC, and some differences among the mouse models developed. Tumors originating in QKO mice were enriched in genes involved in the cell cycle and inflammation and showed more abundance in inflammatory infiltrates and necrosis, while in DKO mice these genes were related to angiogenesis and epithelial-mesenchymal transition and showed fewer immune infiltrates and necrosis. Additionally, tumors originating from basal cells (K5 positive) had higher metastasis capacity than tumors originating in luminal cells (K20 positive) in both DKO and QKO models. We then extended the methods to develop transplantable murine MIBC cell cultures that allow serial transplantation in immunocompetent syngeneic hosts. The subcutaneous syngeneic mouse models showed conservation of the parental primary tumor features. In conclusion, the development of different mouse models provides us with a useful tool for preclinical studies and different frameworks to study BC.

Keywords: Bladder cancer, mouse models.

P122. Computational Biology, Bioinformatics & Artificial Intelligence

Characterization of the pro-inflammatory profile of post-COVID-19 condition patients: a machine learning approach

Francisco Muñoz-López[1], María Nevot[1], Cristian Tebé[2], Gemma Lladós[3,4], Sergio Espàña[3,4], José Ramón Santos[3,4], Cristina López[3,4], Cora Loste[3,4], Ruth Toledo[4], Marta Font[4], Anna Chamorro[4], Roger Paredes[1,3,4], Bonaventura Clotet[1,3,4], Lourdes Mateu[3,4,5], Marta Massanella[1,5,6]

[1]IrsiCaixa-AIDS Research Institute and Germans Trias i Pujol Health Research Institute (IGTP), Badalona, Spain, [2]The Bellvitge Biomedical Research Institute (IDIBELL), Hospitalet de Llobregat, Spain, [3]Infectious Diseases Department, Germans Trias i Pujol Hospital, Badalona, Spain, [4]Fight Against AIDS Foundation (FLS), Germans Trias i Pujol Hospital, Badalona, Spain, [5]University of Vic–Central University of Catalonia (UVic-UCC), Vic, Spain, [6]Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, CIBERINFEC, Madrid, Spain

During the past three years, over 600 million confirmed COVID-19 cases have been reported. A remarkable portion (8–15%) of these patients present persistent symptoms at least three months after infection, some of them even lasting over a year. This heterogeneous set of symptoms, which have an impact on the everyday life of the patients, has been named as post-COVID-19 condition (PCC) or long COVID. Several hypotheses have been proposed to understand the cellular and molecular processes underlying this disease, the ones involving the immune system being the most promising. The aim of this project is to fully characterize a set of PCC patients coming from KING Cohort extension, at both the clinical and immunological level. This work specifically focuses on the study of pro-inflammatory molecules. To this end, plasma samples from 81 PCC patients were used to perform a 30-plex cytokine panel (Luminex). Plasma from 17 uninfected individuals and 59 COVID-19 recovered patients were used as controls. Mann–Whitney tests and machine learning methods (random forest, decision tree, AdaBoost) were used for cytokine analysis. The results suggest that PCC patients have a slight dysregulation in some markers such as EGF, HGF and eotaxin. These alterations were only detected by machine learning methods, unlike traditional statistical hypotheses tests, showing the potential of this approach to find small or complex alterations.

Keywords: Post COVID-19 condition, pro-inflammatory molecules, machine learning.

P123. Computational Biology, Bioinformatics & Artificial Intelligence

tRNAstudio: facilitating the study of human tRNAs from deep sequencing datasets

Marina Murillo Recio, Lluís Ribas De Pouplana, Adrián Gabriel Torres
IRB Barcelona – Institute for Research in Biomedicine, Barcelona, Spain

Transfer RNAs (tRNAs) are non-coding RNAs that bring amino acids to the ribosome and are thus essential for protein synthesis. Alterations in tRNAs and in the enzymes responsible for tRNA biogenesis, modification and processing are related to complex diseases such as cancer, diabetes and neurological dysfunctions. To date, their molecular role in disease is currently poorly understood. High-throughput sequencing of transfer RNAs (tRNA-Seq) is a powerful approach to characterize the cellular tRNA pool. However, the presence of tRNA modifications, the sequence similarity between different tRNAs, and the large number of tRNAs encoded in the genome impair the interpretation of results and downstream analysis of tRNA-Seq datasets. Therefore, processing tRNA-seq datasets require strong bioinformatics skills that are frequently not available in experimental laboratories. To overcome this challenge, we present tRNAstudio, a user-friendly automated pipeline designed to analyze tRNA-Seq datasets that has been packaged into a graphical user interface (GUI). tRNAstudio GUI can be implemented locally upon running a few simple bash commands. The output obtained includes files with extensive graphical representations and an interactive html report to help interpret the data. Users can extract information on tRNA gene expression, post-transcriptional tRNA modification levels and tRNA processing. This work brings bioinformatics closer to experimental laboratories and will help in expanding the knowledge of the role of tRNA biology in physiological and pathological scenarios.

Keywords: tRNAs, small RNA-seq, alignment, pipeline, GUI.

P124. Chemistry & Biochemistry

E2 glycoprotein oligomerisation strategy as multivalent vaccine design against Hepatitis C Virus

Raed Nachawati, Alina-Veronica Ghionescu, Costin-Ioan Popescu
Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

Hepatitis C virus (HCV) is an enveloped, single-stranded RNA virus belonging to the *Flaviviridae* family that represents a major cause of liver disease, including liver cirrhosis and hepatocellular carcinoma. Globally, it is estimated that 58 million people are chronically infected with HCV. While remarkable progress has been made in recent years in the field of direct-acting antiviral agents for the treatment of HCV infection, no vaccine against HCV is currently available. The envelope glycoprotein E2 is the principal target of neutralizing antibodies and it has been used as the basis for antigen design for vaccine development. The majority of vaccine design strategies focused on momoneric antigens but with limited success. Recently, it was reported for the HCV E2 antigen that multivalent antigen presentation may enhance the potency and breadth of neutralization of the humoral immune response. Herein we present a new strategy to assemble oligomeric HCV E2 structures to be used as multivalent vaccine candidates. Naturally occurring olimerization motifs were fused to the HCV E2 ectodomain (405–661) and the constructs were expressed in HEK293T cells. The recombinant proteins were purified by affinity chromatography and their identity and oligomerization state were evaluated by SDS-PAGE and Western Blot. We further constructed protein modules which may enhance the oligomerization status and moreover generate bioluminescence enabling nanoparticle *in vivo* monitoring in biodistribution studies. The new protein designs we are developing may allow the assembly of protein nanoparticles to be tested as vaccine candidates against HCV.

Keywords: HCV, E2 glycoprotein, vaccine, nanoparticle.

P125. Cellular & Molecular Biology

Characterization and structural elucidation of a thermoactive and detergent-tolerant alkaline metallokeratinase produced on chicken feathers by *Bacillus* sp. NFH5

Nonso Nnolim, Lupho Kokwe, Uchechukwu Nwodo
University of Fort Hare, Alice, South Africa

Robust green technological developments have advanced the goal of a circular economy by minimizing waste generation in recent times. The study was undertaken to explore the keratinolytic potentials of chicken feather-degrading bacteria from South African soil. Keratinolytic SSN-01 isolated from a municipal dumpsite was molecularly identified as *Bacillus* sp. NFH5 and the nucleotide sequence deposited in GenBank, with accession number MW165830.1. Extracellular enzyme production and thiol group generation by *Bacillus* sp. NFH5 peaked at 120 h with 1879.09 ± 88.70 U/mL and 9.49 ± 0.78 mM, respectively. Glutamic acid (4.44%), aspartic acid (3.50%), arginine (3.23%), glycine (2.61%), serine (2.08%), and proline (2.08%) were relatively higher in concentration compared to other amino acids detected in the protein hydrolysate. Keratinase (KerBAN) activity was highest at pH 8.0 and 90 °C but was inhibited by both EDTA and 1,10-phenanthroline. In addition, the keratinase-encoding gene (*kerBAN*) (band size: 1104 bp) nucleotide sequence was submitted to GenBank (accession number OK033360). The *kerBAN* translation product had 362 amino acid residues, with molecular weight and theoretical isoelectric point of 39 kDa and 8.81, respectively. The KerBAN structural homology model was adequately validated to be of good quality using indicator parameters, including the Ramachandran plot and the QMEAN Z-score. The findings from this study highlight the significance of *Bacillus* sp. NFH5 in the bio-recycling of recalcitrant keratinous wastes to protein hydrolysates – potential dietary supplements for livestock feeds. The properties of KerBAN underscore its application potential in green biotechnological processes.

Keywords: Bio-recycling, feedstuff, hydrolysate, keratinase.

P126. Cardiovascular Disease

Proteomic approaches for the identification of novel cardiovascular biomarkers: a proof of concept

Clara Noguera Navarro[1], Esteban Orenes Piñero[2], Silvia Montoro García[1]
[1]*HiTech, Sport & Health Innovation Hub-Universidad Católica de Murcia, Murcia, Spain,* [2]*Instituto Murciano de Investigación Biosanitaria Virgen de la Arrixaca, Murcia, Spain*

Bioactive peptides have been defined as protein-derived short peptides that are released by hydrolysis processes such as dry-curing ham. Evidence suggests that bioactive peptides reduce hypertension and might affect other metabolic pathways which should be confirmed to validate its beneficial effects in humans. The objective is to identify novel biomarkers of the cardiovascular status with promising proteomic techniques. Nine volunteers (49 ± 6 years old, 75% male) consumed dry-cured pork ham enriched in characterized antihypertensive peptides for 28 days. Protein quantification from all citrated plasma samples was performed using the Pierce BCA Protein Assay Kit followed by SDS-PAGE. The digested proteins were analyzed with MS/MS using Sequest and X! Tandem software. Peptide and protein identifications was validated though Scaffold Software. Then, immunohistochemical approaches (ELISA) were used to confirm these results. The extended proteomic study identified changes in proteins such as Akt, MMP-8, MAPK, ApoB, ApoA2 and ApoA1, which are all related to cardiovascular mechanisms of action. Then, these results were confirmed by immunohistochemical techniques ($P < 0.05$). In addition, parameters such as total cholesterol, LDL-col and insulin were significantly decreased ($P < 0.01$), and HDL-col significantly increased ($P < 0.005$) after the dry-cured ham intake. Thus, this study suggests the potential use of proteomic approaches for the identification of novel potential targets which could help in the prevention of cardiovascular diseases as well as other pathologies. In addition, hypocholesterolemic properties of bioactive peptides were significantly demonstrated, although further research is still needed.

Keywords: Biopeptides, cardiovascular, proteomic, lipid profile, novel biomarkers.

P127. Cancer Biology & Oncology

Epigenetic modulation of solute carrier family 22 members in lung cancer

José Francisco Noguera-Uclés[1], Laura Boyero[1], Ana Salinas-Villegas[1], Alejandro Castillo-Peña[1], Ana Artero-Castro[1], Sofía Boyero-Corral[1], María G Velasco-Domínguez[1], Sonia Molina-Pinelo[1,2]
[1]*Institute of Biomedicine of Seville (IBiS)/HUVR/CSIC/ Universidad de Sevilla, Seville, Spain,* [2]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain*

Epigenetics has emerged as an interesting field to explore in the context of cancer. One of their mechanisms, DNA methylation, is responsible for genomic imprinting, a phenomenon that results in unequal genetic expression of homologous paternal and maternal alleles. The regulation of imprinted genes is broadly dependent on promoter methylation marks, frequently associated with both oncogenes and tumor suppressors. Therefore, aberrations in the DNA methylation state of imprinted genes could play a crucial role in lung cancer. In our study, two imprinted genes, the solute carrier family 22 member 18 (*SLC22A18*) and its antisense version (*SLC22A18AS*), showed a hypomethylated state in adenocarcinoma and squamous cell carcinoma patient tissue compared to non-tumor tissue, which leads to its gene overexpression. The high expression of *SLC22A18* and *SLC22A18AS* was significantly associated with a worsening of the disease progression, supporting their classification as oncogenes in lung cancer. *In vitro* assays with ademetionine, methyl-group donor, changed the expression profile of both genes in a panel of 10 lung cancer cell lines with both histological subtypes represented. After 24 h of treatment, a significant down-regulation of *SLC22A18* and *SLC22A18AS* was observed. The silencing of both genes imprinted evidenced their oncogenic role, due to impaired cell proliferation, being significant in almost all tested cell lines with the exception of the H520 cell line, which showed a relative decline of about 10%. Our results suggest that modulation of the methylation status of imprinted genes could be a novel therapeutic approach for lung cancer patients, with diagnostic and prognostic consequences.

Keywords: NSCLC, SLC22A18, SLC22A18AS, genomic imprinting.

P129. Genetics & Epigenetics

Resistance of yeast cells to the anti-cancer drug 5-fluorouracil is leveraged by regulating levels of RNA exosome complex subunits and cofactors

Ana Novaïħ[1], Marina Oskomiħ[1], Bojan Žunar[1], Michael Primig[2], Igor Stupareviħ[1]
[1]*Laboratory of Biochemistry, Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia,* [2]*Irset (Institut de Recherche en Sante, Environnement et Travail), Université de Rennes, INSERM, and EHESP, Rennes, France*

The RNA exosome is a conserved complex that catalyzes $3' \rightarrow 5'$ degradation and processing of RNA molecules in the nucleus and cytosol of eukaryotic cells. This complex was shown to be involved in the cellular resistance to the anti-cancer drug 5-fluorouracil (5-FU) in yeast *Saccharomyces cerevisiae* and human cells. 5-FU is an anti-metabolite that is incorporated into DNA and RNA molecules and interferes with various cellular processes, which is why it is widely used for the treatment of colon and breast cancer. However, the development of resistance to 5-FU can be a major obstacle to 5-FU-based therapies, and the molecular mechanisms underlying such resistance remain poorly understood. In this work, we show that expression of the RNA exosome catalytic subunit Rrp6 is regulated by 5-FU treatment in two evolutionarily distant yeasts: *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Furthermore, we found that artificially up- or down-regulating the levels of RNA exosome complex subunits and cofactors through overexpression or gene deletion influences the resistance of yeast cells to 5-FU. Given the high degree of conservation of the RNA exosome complex and its cofactors, these results might contribute to elucidation of general molecular mechanisms which underlie 5-FU resistance.

Keywords: 5-fluorouracil, RNA exosome, yeast, cancer resistance.

P130. Chemistry & Biochemistry

Comparative analysis of biological properties of *Moringa* (*Moringa oleifera*) and soybean (*Glycine max*) seed proteins

Adenike Blankson, Helmina Akanya, Evans Egwim, Chiemela Chinma, Bright Odey
Federal University of Technology Minna, Minna, Nigeria

Protein concentrates especially from plants are good protein supplements in human diet. However, the utilization of protein concentrate from *Moringa oleifera* seed has not been fully exploited. This comparative study evaluates the quality of protein concentrates from seeds of *Moringa* and soybean. Protein concentrates prepared by alkaline isoelectric precipitation were incorporated into the diet of eight groups of weanling albino experimental rats. Groups 1, 2 and 3 were fed diets containing 10%, 15% and 20% Moringa protein concentrate respectively, groups 4, 5 and 6 received a diet containing 10%, 15% and 20% soybean protein concentrate each. Group 7 (positive control) ingested rat chow, while group 8 (negative control) was placed on a protein-free basal diet. Proximate composition, average daily feed intake and weight gain were determined according to standard procedures. Packed cell volume (PCV) was determined using a hematocrit centrifuge and concentration of serum enzymes was determined using a semi-auto analyzer. Histological examination of major organs was carried out according to standard methods. The concentrates were evaluated for biological value (BV), net protein utilization (NPU) and true digestibility (TD). Protein contents of *Moringa oleifera* and soybean were 68.62% and 73.76% accordingly. Feed intake for all the groups was insignificantly the same and PCV was within normal physiological range. However, the biochemical indices indicated that albumin concentration in the protein-free and 10% *Moringa* groups was significantly lower ($P < 0.05$). There was no tissue damage or organ lession after the 21-day feeding trial. But a significant difference was observed in the BV, NPU and TD of graded levels of both *Moringa oleifera* and soybean protein concentrates. Protein concentrate from seeds of *Moringa oleifera* was thus found to possess a similar quality to soybean and may substitute for soybean concentrate in most uses.

Keywords: Protein quality, *Moringa* protein concentrate, alkaline isoelectric precipitation, plant protein.

P131. Chemistry & Biochemistry

Expression and enzymatic characterization of a novel organic solvent-tolerant alpha amylase from locally isolated *Bacillus lichenisformis*-FAO.CP7 of cocoa pod flora

Frank A. Ogundolie[1,2,3], Adeyemi O. Ayodeji[1,4], Tolulope P. Saliu[1], Olatomide A. Fadare[5], Joshua O. Ajele[1], Naresh G. Kumar[2]
[1]*Enzymology and Enzyme Technology Laboratory, Department of Biochemistry, Federal University of Technology Akure, Nigeria,* [2]*Microbial and Molecular Biology Laboratory, Department of Biochemistry, Maharaja Sayajirao University of Baroda, India,* [3]*Department of Biotechnology, Baze University Abuja, Nigeria,* [4]*Department of Biochemistry, Joseph Ayo Babalola University Ikeji, Nigeria,* [5]*Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria*

Global demand for alpha-amylase due to its wide application in various industries, such as medical, food, and textiles among others, has led to a continuous quest for new sources of this enzyme with desired properties for production to meet industrial need. In this study, a gene coding for Alpha-amylase from a locally isolated *Bacillus lichenisformis*-FAO.CP7 strain (Accession no:MN150530.1) was cloned, using pET22b vector and expressed in BL21(DE3) strain of *Escherichia coli* which was cellularly disrupted using 1 mM IPTG and 0.6% glycine for induction and extracellular secretion respectively. The recombinant protein was purified using Ni-NTA affinity chromatography and biochemically characterized. The purified recombinant enzyme gave a purification fold and specific activity of 116.56 and 410.54 U/mg respectively with a molecular weight of 57.5 kDa using 12% SDS-PAGE. The optimal pH and temperature of the enzyme were 5.0 and 65 °C respectively. The thermal inactivation rate constants (kd) value of the enzyme was 0.003 and it followed first-order kinetics at 333 k. The kinetic parameters Km and Kcat of the enzyme for soluble starch were 0.28 mg/mL and 1080 s^{-1}. The enzyme showed enhanced activity in the presence of 5 and 10 mM Zn^{2+}, Ca^{2+}, K^{+}, Mg^{2+} but was inhibited by Ni^{2+}, Pb^{2+}, $Co^{2+}$and Hg^{2+}. The activity of the enzyme was enhanced in the presence of organic solvents such as methanol, ethanol, n-hexane, acetone and toluene at 10% and 20% (v/v) concentration. The properties observed in this study for alpha-amylase from *B. lichenisformis*-FAO.CP7 suggests its promising application in various biotechnological industries.

Keywords: Alpha-amylase, extracellular expression, organic solvent tolerant, *Bacillus licheniformis*, cocoa pod.

P132. Cancer Biology & Oncology

Targeting VRK1 and ROCK kinases in undifferentiated neuroblastoma cells

Mónica Ojeda-Puertas[1,2], Aida Amador-Álvarez[1,2], María A. Gómez-Muñoz[2,3], Ana Colmenero-Repiso[2,3], Ricardo Pardal[2,3], Francisco M. Vega[1,2]
[1]Departamento de Biología Celular, Facultad de Biología, Universidad de Sevilla, Seville, Spain, [2]Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/ Universidad de Sevilla, Seville, Spain, [3]Departamento de Fisiología Médica y Biofísica, Universidad de Sevilla, Seville, Spain

Neuroblastoma is the most common extracranial solid tumor occurring in childhood and is characterized by poor outcome in advanced stages, with high frequency of metastasis, relapse and resistance to therapy. It is thought that undifferentiated neuroblastoma cells, frequently named as "neuroblastoma cancer stem cells (NBCSC)", are the main cell population responsible for these processes. Protein kinases are one of the best targets for molecular cancer treatment, as they are central in malignant signal transduction and they normally are druggable molecules. In this work we are studying two independent protein kinases: Vaccinia- related kinase 1 (VRK1) and Rho Associated Coiled-Coil Containing Protein Kinase (ROCK). VRK1 is a member of a serine/threonine kinase family that phosphorylates various molecules and transcription factors implicated in chromatin condensation, DNA repair and cell cycle progression. On the other hand, ROCK is also a serine/threonine kinase that acts as downstream effector of some Rho GTPases, regulating contractility, cell adhesion and motility. Although some data from our laboratory and others place VRK1 and ROCK as possible targets in neuroblastoma, their function in the malignant NBCSC has not been explored. In the preliminary work presented, we are using available specific inhibitors of these kinases in undifferentiated neuroblastoma cells to analyze their function in proliferation and differentiation. Our goal is to understand the biology of the NBCSC and determine their suitability as molecular targets to impact in this cell population.

Keywords: Neuroblastoma, VRK1, ROCK, differentiation, proliferation.

P133. Cancer Biology & Oncology

Identification of a potential therapeutic agent against KSHV-associated malignancies

Michael Okpara, Adrienne Edkins
Biomedical Biotechnology Research Unit (BioBRU), Department of Biochemistry and Microbiology, Rhodes University, Makhanda, South Africa

Kaposi's sarcoma-associated herpes virus (KSHV) is the etiologic agent for primary effusion lymphoma (PEL), multicentric Castleman's disease (MCD) and Kaposi's sarcoma (KS). KSHV contributes to the incidence of 1.5 million new infection-related cancer cases annually. As it stands, none of the current treatment options for KSHV-associated malignancies is clinically approved. Consequently, more effective therapeutic interventions are needed. The aim of this study was to identify a potential therapeutic agent against KSHV-associated malignancies. In this study, a high-throughput screening assay based on cell viability and anti-KSHV activity was developed to screen 400 compounds in the Medicines for Malaria Ventures Pandemic Response Box. A non-cytotoxic inhibitor of KSHV lytic replication (which is critical for tumourigenesis) was identified as a hit compound. Viral DNA replication, gene expression and viral re-infection assays were used to show that the identified hit compound inhibited the KSHV DNA replication process, reduced the expression of KSHV immediate-early and late lytic genes (including KSHV ORF57, an important mediator of viral RNA nuclear export) and inhibited the production of infectious KSHV. Taken together, our data suggest that the hit compound is a potential therapeutic agent against KSHV-related malignancies.

Keywords: KSHV, malignancies, cancer, MMV.

P134. Immunology, Microbiology & Infectious Diseases

Plasmodium infection and Artemether-Lumefanthrine chemotherapy modulate mitochondrial electron transport protein and mitochondrial biogenesis in mice

Oludele Olanlokun, Oluwakemi Oloke, Olufunso Olorunsogo
University of Ibadan, Ibadan, Nigeria

Introduction: Malaria is a fatal disease and it has claimed many lives as a result of multidrug resistance. Chronic infection by *Plasmodium* in the liver has caused organ failure with attendant complications. Mitochondria are cellular organelles affected by this infection. There is paucity of information on the effect of *Plasmodium* infection on mitochondrial biogenesis, homeostasis and the electron transport system. *Materials and Methods*: Mice infected with resistant (ANKA strain) *P. berghei* were treated with Artemether lumefanthrine and the controls were treated with vehicle for four days after parasitemia was confirmed. Total RNA was isolated from liver samples and cDNA conversion was performed. PCR amplification for PINK1, PGC-1α, NADH-oxidoreductase and cytochrome oxidase genes were performed and the amplicons were resolved on 1.5% agarose gel. In-gel amplicon bands images captured on camera were processed and quantified using image-J software. All graphs were plotted as mean ± SEM using graph-pad prism. *Results*: *Plasmodium* infection increased PGC-1α, but decreased PINK1 and cytochrome oxidase expression in the infected control compared to the treatment group, while there was no significant difference in cytochrome oxidase expression among the two. *Conclusion*: *Plasmodium* infection affects electron transport system and mitochondrial biogenesis in mice.

Keywords: Chemotherapy, malaria, mitochondrial dynamics, oxidative phosphorylation.

P135. Cellular & Molecular Biology

Site-specific functionalization of nanobodies as bioreagents for diagnostics

Sandra Folarin Oloketuyi[1,2,3], Joachim Jose[4], Giulio Bernardinelli[5], Ario De Marco[3]
[1]*Institute of Microbiology and Microbial Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria,* [2]*Christian Doppler Laboratory for Innovative Immunotherapeutics, Vienna, Austria,* [3]*Laboratory of Environmental and Life Sciences, University of Nova Gorica, Slovenia,* [4]*Institut für Pharmazeutische und Medizinische Chemie, Westfälische Wilhelms-Universität Münster, Münster, Germany,* [5]*Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden*

Nanobodies are high-affinity binders that open new opportunities for therapeutic and diagnostic applications owing to their distinctive properties such as their small size, inexpensive recombinant expression in eukaryotic and prokaryotic systems, amenability to genetic manipulation, high solubility, and physicochemical stability. Functionalization of nanobodies allows site-specific customization of binders for a wide range of applications without disrupting their antigen binding specificity. The possibility of site-specific functionalization of nanobodies using SpyTag-SpyCatcher chemistry, hemin-utilizing G-quadruplex DNAzymes conjugation and *Escherichia coli* surface display system was demonstrated and utilized. Nanobodies were selected by biopanning against different biomarkers (toxic microalgae, human epidermal growth factor receptor 2 (HER2), and C-reactive protein) to recover specific binders as biocapture elements and functionalized using the three strategies listed above. These approaches provide ways to generate inexpensive immunoreagents ready to use for diagnostics and monitoring of biomarkers through colorimetric, fluorometric, and electrochemical reactions.

Keywords: Nanobodies, affinity, site-specific functionalization, diagnostics.

P137. Cellular & Molecular Biology

Genomics data gap in Africa: implications for global disease burden

Olabode Omotoso[1], John Teibo[2], Festus Atiba[2]
[1]*University of Ibadan, Ibadan, Nigeria,* [2]*University of São Paulo, São Paulo, Brazil*

Beyond public health, diseases present individuals and communities with unprecedented social, economic, and financial hardship. The critical effects of these burdens however have galvanized global efforts to tackle the diseases albeit in an unequitable fashion. Africa has gradually been left out, with most of the decisions on drug and vaccine production being made in the high-income countries. Africa has a high population trend and one of the most diverse population genetics. Yet only a small percentage of African genomic data is currently available to contribute to global disease prevention efforts. With recent developments in sequencing technologies, genomic data are now bedrock to the advances in disease prevention strategies, artificial intelligence, machine learning, vaccine development, polygenic risk score prediction, and genetic based testing. It is expedient that African genomics is prioritized for equity of medical research in global efforts to tackle disease burdens. Without this, there are grave implications for global health. For instance, variants from uncharacterized genetic mutations could hamper disease eradication (as observed in COVID-19) and low- and middle-income countries been reservoirs for emerging pandemics. The next evolution of genomic data is encoded in Africa. It is waiting to be mined and utilized to unravel hidden traits for alleviating diseases that continue to plague global health. Therefore, bridging the genomic data gap in Africa is an important call to action for a multidisciplinary approach that would improve research capacity through adequate funding and collaborations with new and emerging (genomic) leaders as well as stakeholders in the omics field.

Keywords: Genomics, precision medicine, disease burden, public health, collaboration.

P138. Cancer Biology & Oncology

The cancer clockwork

Sara María Ortega-Campos[1,2], Eva María Verdugo-Sivianes[1,2], José Ramón Blanco[3,4], José Manuel García-Heredia[1,2,5], Amancio Carnero[1,2]
[1]*Instituto de Biomedicina de Sevilla (IBiS)/HUVR/CSIC/US, Seville, Spain,* [2]*CIBERONC, Instituto de Salud Carlos III, Madrid, Spain,* [3]*Hospital Universitario San Pedro, Logroño, Spain,* [4]*Centro de Investigación Biomédica de La Rioja (CIBIR), Logroño, Spain,* [5]*Department of Vegetal Biochemistry and Molecular Biology, University of Seville, Seville, Spain*

The circadian clock is a very complex molecular mechanism that regulates the expression of a large number of genes in the organism and allows the adaptation of cellular activities to the daily light–dark cycles. Disruption of the circadian rhythm can lead to various pathologies, including cancer. Thus, disruption of the normal circadian rhythm at both genetic and environmental levels has been described as an independent risk factor for cancer. Besides, circadian genes have been proposed to have a tissue-dependent and/or context-dependent role in tumorigenesis and may act both as tumor suppressors and oncogenes. Finally, circadian genes seem to play an essential role in the course of the different hallmarks of cancer. Hence, a deeper understanding of the molecular mechanisms underlying the circadian rhythm would be helpful to identify new prognostic markers in tumorigenesis as well as potential therapeutic targets. In this work, we have performed a bioinformatical approach to the genetics of circadian alterations in cancer.

Keywords: Circadian clock, cancer, bioinformatics, hallmarks.

P139. Chemistry & Biochemistry

Biocompatible systems as a promising approach for eradicating HIV reservoirs *ex vivo*

Francisco José Ostos Marcos[1,2], Marta Martínez Santa[1,3], José Antonio Lebrón Romero[3], Francisco García Moscoso[4], Eva Bernal Pérez[3], Manuel López López[5], María Luisa Moyá Morán[3], Tania Isabel Lopes Da Costa[4], José María Pedrosa Poyato[4], Carmen Gasca Capote[1], María Reyes Jiménez León[1], Ezequiel Ruiz-Mateos Carmona[1], Mohammed Rafii-El-Idrissi Benhnia[1,2], Pilar López Cornejo[3]

[1]Clinical Unit of Infectious Diseases and Microbiology, Institute of Biomedicine of Seville (IBiS)/Virgen del Rocío University Hospital/CSIC/University of Seville, Seville, Spain, [2]Department of Medical Biochemistry, Molecular Biology, and Immunology, School of Medicine, University of Seville, Seville, Spain, [3]Department of Physical Chemistry, Faculty of Chemistry, University of Seville, Seville, Spain, [4]Department of Physical, Chemical and Natural Systems, Pablo de Olavide University, Seville, Spain, [5]Department of Chemical Engineering, Physical Chemistry and Materials Science, Faculty of Experimental Sciences, University of Huelva, Huelva, Spain

Nowadays, for most people living with HIV, combination antiretroviral therapy (cART) can effectively suppress HIV replication resulting in undetectable plasma viral load in blood. Nevertheless, anatomical HIV reservoirs persist despite cART. In this sense, novel immunotherapeutic approaches are being developed to eradicate HIV. Noticeably, Metal–Organic Frameworks (MOFs) and liposomes are promising carriers for the prevention and treatment of HIV infection. A triple combination of antiretroviral drugs (cART) consisting of bictegravir/nevirapine + tenofovir + emtricitabine, was encapsulated in each of the systems, and drug release experiments were also carried out. The cytoxicity of both carriers were evaluated in different non-primary cell lines related to the immune system as well as in Peripheral Blood Mononuclear Cells (PBMCs), isolated from healthy donors. To gain further information about their biocompatibility, *in vitro* hemolytic effect and platelet aggregation assays were performed. The results showed a good encapsulation efficiency of the drugs and a slow controlled release, which could increase the therapeutic effect in HIV-infected patients. A high biocompatibility of the systems in all *in vitro* experiments was observed. Therefore, cART-loaded into MOFs or liposomes could provide a further effective strategy to decrease the DNA quantity of HIV and clear the HIV reservoirs in CD4 cells from infected patients.

Keywords: Metal–organic frameworks, liposomes, antiretrovirals, HIV infection, infectious diseases.

P140. Clinical Research, Translational Biomedicine & Personalised Medicine

Development and application of a LC-MS/MS method for quantification of tryptophan and related metabolites in several biosamples of IBD patients

Mhmd Oumari[1], Na-Mi Kim[1], Danielle Harris[1], Julia Kugler[1], Hang Xiang[1], Stefan Schreiber[1,2], Philip Rosenstiel[1], Konrad Aden[1,2]

[1]Institute of Clinical Molecular Biology, Christian-Albrechts-University and University Hospital Schleswig-Holstein, Kiel, Germany, [2]Department of Internal Medicine I, Christian-Albrechts-University and University Hospital Schleswig-Holstein, Kiel, Germany

Tryptophan metabolism is severely disrupted in IBD patients, resulting in the breakdown of tryptophan through activation of the kynurenine pathway. However, it is still not clearly explained how tryptophan degradation directly affects the pathophysiology of IBD. We aimed to i) establish a quantitative LC–MS/MS method to trace the metabolic breakdown of tryptophan in several biosamples of IBD patients and ii) identify molecular pathways that causally impact tryptophan metabolism. We have developed a Multiple Reaction Monitoring (MRM) method for the detection and quantification of TRP and almost all TRP-metabolites in several biosamples of IBD patients in a single LC–MS/MS run. To directly trace the metabolic conversion of tryptophan into its downstream metabolites and study the exact amount of TRP degradation avoiding the interference of regular TRP in our study, we have further established a method to track ^{13}C-labled TRP and its ^{13}C-metabolites. By using our established LC–MS/MS method, we could show that selective inhibition of JAK–STAT signaling directly mediates restoration of tryptophan metabolism and thereby provides a novel immunometabolic mechanism of action in the therapy of IBD.

Keywords: IBD, tryptophan, kynurenine, JAK–STAT signaling.

P141. Chemistry & Biochemistry

Investigation of histidine residues of the growth hormone secretagogue receptor by solid-state NMR

Emelyne Pacull, Sabrina Stecher, Peter Schmidt, Holger A. Scheidt, Daniel Huster

Institute for Medical Physics and Biophysics, Leipzig, Germany

In the last two years, our structural knowledge about Growth Hormone Secretagogue Receptor (GHSR), a G Protein-Coupled Receptor, has expanded greatly. Crystal and cryo-electron microscopy structures of GHSR in complexes with various ligands and different G-proteins revealed distinct conformations and binding mechanisms. In parallel, biophysical investigations, using techniques like solid-state NMR, gave insight into the dynamic properties of the highly mobile GHSR in a native-like membrane environment. The uniformly and site specific (transmembrane (TM) domains, loops, C-terminus) ^{13}C-labeled GHSR was especially characterized in regards to its dynamics. The mechanisms of GHSR interactions with ghrelin or inverse agonist are now investigated at a single residue level. Here, we monitor the

changes in chemical shift of the three native histidines using ^{13}C-^{13}C DARR NMR. These histidines provide low spectra complexity and are located in sensitive receptor sites, namely the helix 6 (TM6), known to undergo large motion upon ligand binding and the extracellular loop 2, involved in binding events. Here, the histidines were ^{13}C labeled through an established cell-free expression system where the labeled GHSR is expressed in the precipitated form and subsequently functionally reconstituted into DMPC bicelles. Three single histidine deficient mutants, H186A, H258Y and H280Q, were designed to allow the assignment of the NMR signals. Additionally, the well-described A204E mutant was expressed to characterize the effect of the loss of constitutive activity on the histidines in the GHSR. Upon ligand binding, the two helical histidines showed characteristic downfield shifts indicative of structural alterations in the molecule upon outward movement of TM6.

Keywords: ssNMR, GHSR, GPCRs, ghrelin.

P142. Clinical Research, Translational Biomedicine & Personalised Medicine

Prognostic impact of pre-operative cell-free DNA (cfDNA) levels in muscle invasive bladder cancer

Maria-Alexandra Papadimitriou[1], Panagiotis Levis[2], Georgios Kotronopoulos[2], Konstantinos Stravodimos[2], Margaritis Avgeris[1,3], Andreas Scorilas[1]

[1]Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece, [2]First Department of Urology, "Laiko" General Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, [3]Laboratory of Clinical Biochemistry – Molecular Diagnostics, Second Department of Pediatrics, School of Medicine, National and Kapodistrian University of Athens, "P. & A. Kyriakou" Children's Hospital, Athens, Greece

Bladder cancer's (BlCa) high heterogeneity hinders disease prognosis, leading to lifelong invasive post-treatment monitoring and highlighting the need for modern minimally-invasive precision medicine approaches. Herein, we have unveiled pre-operative cell-free DNA (cfDNA) concentration's clinical utility in ameliorating patients' outcome. cfDNA was isolated from pre-operative serum samples of 190 patients and quantified by two assays: *in-house* qPCR (*LEP* gene) and direct fluorometric (Qubit HS-dsDNA) assays. cfDNA fragment profiling ($n = 11$) was performed by capillary electrophoresis. The clinical endpoints used for the survival analysis of non-muscle-invasive bladder cancer (NMIBC;TaT1) and muscle-invasive bladder cancer (MIBC;T2-T4) were patients' recurrence/progression and metastasis/death, respectively. Clinical benefit was conducted by decision curve analysis. cfDNA profiling by capillary electrophoresis highlighted the significantly increased cfDNA fragment sizes in advanced disease stages ($P = 0.018$). Quantification of cfDNA by both Qubit/qPCR displayed highly consistent results (rs = 0.960; $P < 0.001$). Elevated cfDNA concentration was associated with MIBC and significantly higher risk for short-term metastasis [Qubit: Hazard Ratio (HR) = 3.016, $P = 0.009$; qPCR: HR = 2.918, $P = 0.004$] and inferior survival (Qubit: HR = 1.898, $P = 0.042$; qPCR: HR = 1.888, $P = 0.026$) of MIBC patients. cfDNA-fitted multivariate models offered superior risk-stratification and clinical benefit for MIBC prognosis. Conclusively, elevated pre-operative cfDNA levels are associated with adverse post-treatment outcome in MIBC, supporting modern non-invasive disease prognosis and management. *This research was: supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship-Number: 6123); co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH-CREATE-INNOVATE (project code: T2EDK-02196) to the NKUA (KE17358).*

Keywords: cfDNA, ctDNA, liquid biopsy, prognosis, bladder cancer.

P143. Cancer Biology & Oncology

Comparison of the effect of sorafenib (BAY 43-9006) on the survival and growth of human bladder cancer cells lines RT4 and T24

Maksym Patena[1,2], Grzegorz Kasparek[1,2], Patryk Obajtek[1,2], Anna Wiśniewska[3], Małgorzata Lasota[1,2]

[1]Chair of Medical Biochemistry, Jagiellonian University Medical College, Cracow, Poland, [2]SSG of Targeted Therapy and Supramolecular Systems, Jagiellonian University Medical College, Cracow, Poland, [3]Chair of Pharmacology, Jagiellonian University, Medical College, Cracow, Poland

Bladder cancer is the second most frequently diagnosed cancer of the genitourinary system in Europe, characterized by frequent recurrences and a high risk of progression. Research shows that overexpression of the PDGF receptor with tyrosine kinase activity may be responsible for the invasion and progression of this tumor. The aim of our study was to investigate the effect of sorafenib on the growth, survival and migration of bladder cancer cell lines with different malignancy potential (RT4, T24). We also assessed the toxicity of the tested compound on human uroepithelial cells (SV-HUC1) and fitted a logistic model of the dose effect relationship of the tested inhibitor. Sorafenib showed a cytostatic and cytotoxic effect, clearly inhibited the growth of investigated bladder cancer cell lines, induced mainly apoptosis in them and significantly reduced their migration. Multitargeted tyrosine kinase inhibitors such as sorafenib represent a promising class of therapeutic agents for the treatment of bladder cancer. *We acknowledge the financial support from the Polish Ministry of Education and Science through the (SKN/SP/5606/2022) grant "Student Scientific Group create innovations".*

Keywords: Sorafenib, cancer, bladder.

P144. Pharmacology, Toxicology & Nutrition

Spatiotemporal signalling and location of fractalkine receptor CX_3CR1 and its natural genetic variants CX_3CR1-V249I/T280M and CX_3CR1-A55T

Alba Paz Castro[1], Pablo Blázquez[1], Andrea G. Silva[1], Rocío A. De La Fuente[1], Michelle Halls[2], Marián Castro[1]
[1]*Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), University of Santiago de Compostela, Santiago de Compostela, Spain,* [2]*Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Australia*

Chemokine fractalkine (CX_3CL1) acts through its only known human receptor CX_3CR1, a key player in inflammation/immunity and neuron–microglia communication. We aimed at investigating the signaling and trafficking of CX_3CR1 and two natural receptor variants associated with disease. CX_3CR1 interacted with GRK2 and β-arrestin 1 and 2 in response to fractalkine in HEK293 cells. While the CX_3CR1-V249I/T280M variant displayed increased efficacy of interaction with these receptor signaling partners, the CX_3CR1-A55T variant was much less efficient than the wild-type (WT) receptor at promoting receptor/β-arrestin interaction. CX_3CR1 overexpressed in HEK293 cells interacted with GRK2, and β-arrestins 1 and 2 in response to fractalkine, while CX_3CR1-A55T was much less efficient than the WT receptor at promoting receptor/β-arrestin interaction. CX_3CR1-WT stimulated ERK activity both in the cytosolic and nuclear compartments upon fractalkine stimulation in HEK293 cell as monitored by FRET-based ERK biosensors. Cytosolic and nuclear ERK were both inhibited by pertussis toxin treatment, indicating the involvement of Gi/o protein. While the cytosolic ERK signaling was completely abolished by downregulating β-arrestin, the nuclear ERK signaling was only partially dependent on β-arrestin. Moreover, nuclear ERK signaling was fully dependent on dynamin function, opposite to cytosolic ERK that was fully independent. CX_3CR1-WT, CX_3CR1-V249I/T280M and CX_3CR1-A55T internalized in response to fractalkine in HEK293 cells. However, the three receptors show slight differences in their internalization and subcellular trafficking profiles. Our results suggest different signalosomes contribute to compartmentalized ERK signaling by CX_3CR1 with possibly distinct cellular consequences. The altered traffiking and engagement of signaling partners by $CX_{3C}R1$ variants might have etiopathological implications.

Keywords: CX3CR1, fractalkine, CX3CR1-V249I/T280M, CX3CR1-A55T, spatiotemporal- signaling.

P146. Immunology, Microbiology & Infectious Diseases

Metabolomic and immunological profiles associated with cardiovascular events in people living with HIV

Alberto Pérez-Gómez[1], Carmen Gasca-Capote[1], Laura Tarancón-Díez[2], María Reyes Jiménez-León[1], Joana Vitallé[1], Sara Bachiller[1], Isabel Gallego[1], Adrià Curran[3], María Ángeles Muñoz-Fernández[2], Joaquim Peraire[4,5,6,7], Anna Rull[4,5,6,7], Francesc Vidal[4,5,6,7], Luis Fernando López-Cortés[1], Ezequiel Ruiz-Mateos[1]
[1]*Clinical Unit of Infectious Diseases, Microbiology and Parasitology, Institute of Biomedicine of Seville (IBiS)/Virgen del Rocío University Hospital/CSIC/University of Seville, Seville, Spain,* [2]*Immunology Section, Laboratory of ImmunoBiology Molecular, Hospital General Universitario Gregorio Marañón (HGUGM), HIV-HGM BioBank, Madrid, Spain,* [3]*Infectious Diseases Department, Hospital Universitari Vall d'Hebron and Departament de Medicina, Universidad Autónoma de Barcelona, Barcelona, Spain,* [4]*Infection and Immunity Research Group (INIM), Universitat Rovira i Virgili, Tarragona, Spain,* [5]*Infection and Immunity Research Group (INIM), Institut Investigació Sanitària Pere Virgili (IISPV), Tarragona, Spain,* [6]*Infection and Immunity Research Group (INIM), Hospital Universitari de Tarragona Joan XXIII, Tarragona, Spain,* [7]*Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III (ISCIII), Madrid, Spain*

Approximately 90% of people living with HIV (PLWHIV) have an undetectable viral load and a non-transmissible infection, with a significant increase in their life expectancy due to successive improvements in antiretroviral treatments (ART). However, many PLWHIV exhibit a low-grade chronic inflammation state whose origin is a chronic activation of the innate immune system. It is associated with comorbidities, common in aged people, known as non-AIDS events (NAEs), which are the main causes of morbidity and mortality in PLWHIV. One of the main examples are cardiovascular diseases (CVD) with a 1.5 to 2-fold increased relative risk compared to the general population. We previously reported that T-lymphocyte and monocyte activation phenotypes were associated with the development of CVD, specifically acute coronary syndrome (ACS). However, the mechanism of action is still unknown. In this scenario, we have frozen and stored peripheral blood samples close to the time point and from six to 12 months before suffering an ACS in an accurate clinical well-characterized cohort of patients treated with ART. In this cohort, we aim to analyze whether a specific metabolomics profile in plasma identified by non-targeted mass spectrometry and innate immune system and T-cell activation measured by multiparametric flow cytometry are associated with ACS events. This would allow identifying an immunometabolomic profile that could predict the development of CVD, and facilitate the design of immunotherapeutic strategies to reduce the incidence of ACS events in this population, one of the most prevalent causes of premature death in the world.

Keywords: CVD, ACS, PLWHIV, profiles, immunometabolomics.

P147. Cancer Biology & Oncology

Chemoresistance in acute myeloid leukemia: insights from clonal evolution at multi-omics level

Giulia Perticari, Simone Frascolla, Sara Milovanovic, Chiara Caprioli, Andrea Cossa, Thalia Vlachou, Pier Giuseppe Pelicci
European Institute of Oncology (IEO), Milan, Italy

Acute Myeloid Leukemia (AML) represents the most common hematological malignancy in adults. Patients display frequent relapse rates and high levels of intratumour heterogeneity (ITH), which is renowned to drive drug resistance and tumor relapse. An integrated analysis of multiple sources of ITH is still missing, and might be informative for understanding the pathogenesis of chemoresistance and for design of appropriate therapeutic strategies. In this work, I will exploit a PDX model of chemo-resistant human AML in order to characterize genetic, epigenetic, and transcriptional clonal-evolution of leukemic blasts upon chemotherapy treatment. I will couple a lineage tracing approach with the analysis of expressed mutations and gene expression profiles of single cells using the in-house developed SCM-seq (Single Cell and Molecule sequencing), and perform parallel bulk DNA methylation analyses. This multi-omics strategy will allow us to dissect the contribution of diverse sources of ITH to the development of an adaptive phenotype of chemoresistance, and eventually identify putative druggable targets.

Keywords: Single-cell, multi-omics, lineage-tracing, leukemia.

P148. Cancer Biology & Oncology

Chromosomal fragile site FHIT loss in colorectal cancer: impact and therapeutic opportunities

Elena Pietrini, Lucía Simón-Carrasco, Andrés Joaquín López-Contreras
Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), Consejo Superior de Investigaciones Científicas (CSIC) – Universidad de Sevilla – Universidad Pablo de Olavide, Seville, Spain

Common Fragile Sites (CFSs) are genomic regions prone to break upon conditions of replicative stress, thus, they are usually altered in cancer. *FHIT* (Fragile Histidine Triad Diadenosine Triphosphatase) is a long gene spanning over 1,6 Mb located in the *FRA3B* locus, one of the most unstable CFS in the human genome. It codes for a small protein that hydrolyses diadenosine triphosphate (Ap3A). *FHIT* is frequently lost in cancer, particularly in digestive cancers (stomach and colorectal). I aim to investigate if the loss of *FHIT* in cancer is a driver event for tumorigenesis, investigating it using a novel mouse model with the full deletion of the *FHIT* gene generated in our laboratory. Moreover, I investigate whether the lack of *FHIT* could increase the sensitivity of the cells to certain drugs and thus be exploited as a synthetic lethality strategy for cancer treatment, in particular in colorectal cancer. To this end, I have generated isogenic *FHIT* KO and WT clones of two colon cancer cell lines (DLD1 and RKO) to perform drug screenings using high content microscopy. I have performed a preliminary screening using a nucleotide compound library with 269 nucleotide analogues. In the future, I will perform drug screening using an FDA-approved drug library.

Keywords: FHIT, colorectal cancer, drug screening, common fragile sites.

P149. Biomedical Engineering & Imaging Sciences

Modeling retinitis pigmentosa using iPSC-derived retinal organoids

Álvaro Plaza Reyes, Berta De La Cerda Haynes, Lourdes Valdés Sánchez, Félix Andújar Sánchez, Míriam Ruiz Ballester, Sofía M. Calado, Ana B. García Delgado, Francisco J. Díaz Corrales
Fundación Progreso y Salud and Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Seville, Spain

Retinitis pigmentosa is a set of chronic eye diseases of genetic origin and degenerative nature that are characterized by a progressive degeneration of the light-sensitive structure of the eye, the retina. Although an increasing number of disease genes have been identified, the exact cellular mechanisms of RP remain largely unclear, mostly due to the lack of relevant animal models. For that reason, retinal organoids derived from induced pluripotent stem cells of patients provide a powerful platform for deciphering disease mechanisms and an advantageous tool for preclinical testing of new treatments. Here, we derived iPSC from an RP patient carrying a mutation in eyes shut homolog (EYS), which is responsible for one of the most frequent types of autosomal recessive RP. Using these patient-derived iPSC we then generated retinal organoids (ROs) that were used to interrogate disease mechanisms and evaluate potential phenotypic alterations when compared to ROs derived from healthy donors. Although both healthy iPSC and mutant EYS iPSC managed to form ROs, transcriptional analysis and flow cytometry examination determined that the proportion of photoreceptor precursors in mutant EYS ROs was considerably reduced. Moreover, after in-depth examination of cell architecture, we found that the presence of mutant EYS resulted in a reduction of neuroretinal thickness during early stages of RO development and reduced numbers of rod precursor cells, even at later time points. Altogether, our study shows that mutations in EYS affect the structural maintenance of the photoreceptor layer, which results in defective retinal development and incomplete photoreceptor maturation.

Keywords: Retinitis pigmentosa, retinal organoids, disease modeling, EYS, iPSC.

P150. Neuroscience, Psychiatry & Mental Health

Three-dimensional study of the synapses of the human entorhinal cortex

Sergio Plaza-Alonso[1,2,3], Nicolás Cano-Astorga[1,2,4], Javier de Felipe[1,2], Lidia Alonso-Nanclares[1,2]
[1]Laboratorio Cajal de Circuitos Corticales, Centro de Tecnología Biomédica, Universidad Politécnica de Madrid, Pozuelo de Alarcón, Spain, [2]Instituto Cajal, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain, [3]Programa de Doctorado en Biología, Universidad Complutense de Madrid (UCM), Madrid, Spain, [4]Programa de Doctorado en Neurociencia, Universidad Autónoma de Madrid (UAM), Madrid, Spain

The Entorhinal Cortex (EC) is a brain region located in the middle temporal lobe. It mediates the flow of information through the hippocampal formation to the neocortex, acting as an interface between sensory areas, the hippocampus and association cortices. This network is essential for memory formation, consolidation and retrieval. A circuit like this comprises the connection of millions of neurons though a common element: the synapse. In this work, we have used Focused Ion Bean / Scanning Electron Microscopy (FIB/SEM) to study the synaptic organization of layer V and layer VI of the human EC. FIB/SEM analysis enabled us to 3D reconstruct 5402 synapses from 3 human autopsies. We then used EspINA software to analyze synaptic density, proportions of asymmetric (excitatory) and symmetric (inhibitory) synapses, spatial distribution of synapses, synaptic size, synaptic shape and postsynaptic targets. This study represents the first detailed description of the synaptic organization of layers V and VI from the human EC. Intriguingly, our results emphasize that synaptic organization is layer-specific in this region. In this sense, we found differences in the size of synapses between layers, being bigger in layer V. This layer also presented more synapses established on dendritic shafts, whereas in layer VI, synapses were predominantly formed on dendritic spine heads. The characterization of the synaptic organization is crucial to better understand how brain networks actually work. Thus, data presented here are essential to fully understand one of the most important circuits that shape human brain, both in health and disease.

Keywords: Human brain, electron microscopy, FIB-SEM, entorhinal cortex, ultrastructure.

P151. Obesity, Diabetes & Other Diseases

Bioactive peptides derived from *Lupinus angustifolious* reduce obesity, MAFLD and dysbiosis in C57Bl6/N mice fed a high-fat diet

Eduardo Ponce-España[1,2], Guillermo Santos-Sánchez[1,2], Iván Cruz-Chamorro[1,2], Ana Isabel Álvarez-López[1], Iván Parralejo-Ayala[1,2], José María Fernández-Santos[3], Francisco Millán[4], María Carmen Millán-Linares[4], Patricia Judith Lardone[1,2], Ignacio Bejarano[1,2], Antonio Carrillo-Vico[1,2]
[1]Instituto de Biomedicina de Sevilla (IBiS)/HUVR/CSIC/Universidad de Sevilla, Seville, Spain, [2]Departamento de Bioquímica Médica y Biología Molecular e Inmunología, Universidad de Sevilla, Seville, Spain, [3]Departamento Citología e Histología Normal y Patológica, Universidad de Sevilla, Seville, Spain, [4]Grupo de Proteínas Vegetales, Instituto de la Grasa - CSIC, Seville, Spain

Obesity is characterized by an excess of body fat and is the main risk factor for other pathological conditions such as cardiovascular diseases, type II diabetes, and metabolic-associated fatty liver disease (MAFLD). Many researchers have highlighted the importance of the microbiota in the development of these diseases. Our group has recently described the capacity of a lupine protein hydrolysate (LPH) as an antioxidant, anti-inflammatory and hypocholesterolemic both in preclinical and clinical models. Due to oxidative stress, inflammation and dyslipemia are key factors in the development of obesity-related pathologies. The aim of this study was to evaluate the role of LPH in obesity and MAFLD in an obese mouse model. C57BL6/N mice were fed a standard diet (SD) or a high-fat diet (HFD). HFD mice were randomly classified into two groups and treated with LPH or vehicle for 12 weeks. Mice were weighed weekly and stool samples were collected before euthanasia. Blood, adipose tissue, and livers were extracted and snap frozen. HFD mice had higher body weight, larger adipose deposits, biochemical features of dyslipemia and liver damage. LPH reduced body and adipose weight and improved serum markers. Transcriptomic analysis of the liver revealed changes in hepatic expression of genes related to MAFLD in HFD-fed mice. LPH supplementation rescued some gene expression, such as CD36, to SD group levels. Finally, we detected significant changes in the microbiota of the HFD group that were counteracted after LPH consumption, alleviating the dysbiosis. Therefore, we conclude that LPH could be a potential therapy against metabolic diseases.

Keywords: Obesity, MAFLD, microbiota, lupin, biopeptides.

P152. Cancer Biology & Oncology

Plasma extracellular vesicles promoted lung cancer pre-metastatic niche formation through endothelial modulation

Francesca Pontis[1], Ilaria Petraroia[1], Patrizia Ghidotti[1], Mattia Boeri[1], Paola Suatoni[1], Ugo Pastorino[1], Fabio Maiullari[2], Roberto Rizzi[2], Claudia Bearzi[2], Gabriella Sozzi[1], Orazio Fortunato[1]

[1]*Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy,* [2]*Istituto Nazionale Genetica Molecolare INGM "Romeo Ed Enrica Invernizzi", Milan, Italy*

Introduction: Lung cancer is the deadliest cancer worldwide, mostly as a consequence of metastatic dissemination. Tumor-derived extracellular vesicles (EVs) favor cancer cell dissemination and outgrowth inducing pre-metastatic niche (pMN) formation in distant sites. So far little is known about the role of plasma EVs in pMN formation; we aim to elucidate their role in pMN formation and identify new prognostic biomarkers for early stage patients. *Methods*: Plasma-EVs were obtained by ultracentrifugation from 20 early stage patients who survived at 5 years (ESA-EVs) and 20 patients who died within two years (ESD-EVs). EVs were characterized following MISEV guidelines. 2D and 3D-bioprinting co-cultures models were used for *in vitro* experiments. EV miRNA cargo was analyzed by Nanostring and digital PCR. *Results*: Platelet and endothelial markers (CD42a and CD31) are enriched in ESD-EVs compared to ESA-EVs. Plasma-EVs are mostly taken up by endothelial cells followed by fibroblast and macrophages. ESD-EVs significantly increased endothelial VCAM1, CXCR4 and CXCL1 compared to ESA-EVs (in 2D and in 3D models). 3D-bioprinted co-culture (fibroblast, endothelial and epithelial cells) experiments confirmed ESD-EV-mediated endothelial activation, also showing fibroblast (α-SMA, IL6, CXCL12) and epithelial (E-cadherin and EpCAM) phenotype modulation. ESD-EVs induced macrophage M2 polarization (increase of *IL10* and *CD206*). Nanostring revealed 3 miRNAs (MiR-1307, miR450 miR-199a) enriched in ESD-EVs versus ESA-EVs. MiR-1307 ($P = 0.01$) and miR-199a ($P = 0.09$) enrichment was confirmed by digital PCR. *Conclusion*: Our findings highlighted the involvement of lung cancer plasma EVs in pMN formation through endothelial activation and stromal cell modulation. EV-miR-1307 could be useful as a prognostic biomarker for early stage lung cancer patients.

Keywords: Extracellular vesicles, lung cancer, endothelial.

P153. Obesity, Diabetes & Other Diseases

pH-dependence of glucose-dependent activity of beta cell networks in acute mouse pancreatic tissue slices

Sandra Postic[1], Marko Gosak[2,3], Wen-Hao Tsai[4,5], Johannes Pfabe[1], Srdjan Sarikas[1], Andraz Stozer[2], Dean Korosak[2], Shi-Bing Yang[4,5], Marjan Slak Rupnik[1,2,6]

[1]*Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria,* [2]*Institute of Physiology, Faculty of Medicine, University of Maribor, Maribor, Slovenia,* [3]*Faculty of Natural Sciences and Mathematics, University of Maribor, Maribor, Slovenia,* [4]*Taiwan International Graduate Program in Molecular Medicine, National Yang Ming Chiao Tung University and Academia Sinica, Taipei, Taiwan,* [5]*Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan,* [6]*Alma Mater Europaea – European Center Maribor, Maribor, Slovenia*

Extracellular pH has the potential to affect various aspects of pancreatic beta cell function. To explain this effect, a number of mechanisms have been proposed, involving both extracellular and intracellular targets and pathways. Here, we focus on reassessing the influence of extracellular pH on glucose-dependent beta cell activation and collective activity in physiological conditions. To this end, we employed mouse pancreatic tissue slices to perform high-temporally resolved functional imaging of cytosolic $Ca2^+$ oscillations. We investigated the effect of either physiological H^+ excess or depletion on the activation properties as well as on the collective activity of beta cells in an islet. Our results indicate that lowered pH invokes activation of a subset of beta cells in substimulatory glucose concentrations, enhances the average activity of beta cells, and alters the beta cell network properties in an islet. The enhanced average activity of beta cells was determined indirectly utilizing cytosolic $Ca2^+$ imaging, while direct measuring of insulin secretion confirmed that this enhanced activity is accompanied by a higher insulin release. Furthermore, reduced functional connectivity and higher functional segregation at lower pH, both signs of reduced intercellular communication, do not necessary result in impaired insulin release.

Keywords: Insulin network, pH, calcium.

P154. Cancer Biology & Oncology

Functions of PARP family proteins during the response to fractionated dose ionizing radiation in human colorectal cancer cells

Rimvile Prokarenkaite[1,2], Vaidotas Stankevicius[1], Linas Kunigenas[2], Kestutis Suziedelis[1,2]

[1]*Vilnius University Life Sciences Center, Vilnius, Lithuania,* [2]*National Cancer Institute of Lithuania, Vilnius, Lithuania*

Poly ADP-ribose polymerases (PARPs) are enzymes that catalyze the transfer of ADP-ribose to target proteins. PARP proteins participate in various cellular processes, including DNA repair and transcription regulation. It has been noticed that due to the functions performed, PARP proteins are a great target for the development of anti-cancer drugs. Previously, we demonstrated upregulated expression of PARP9/12/13/14 genes after exposure to fractionated ionizing radiation. The understanding of PARP

protein family functions is increasing but remains insufficient. Since some PARP proteins are already targeted in existing cancer treatments, further analysis could be used to improve the effectiveness of radiotherapy. For that purpose, we chose to generate human colon carcinoma PARP knockout cell sublines using CRISPR/Cas9-mediated genome editing. To determine the possible molecular functions of PARP12/13/14 genes we generated DLD1 and HT29 PARP knockout cell sublines. The viability of PARP13 knockout HT29 cells following fractionated dose irradiation in 3D cultures was significantly lower than survival of wild-type cells. Further genome-wide expression analysis revealed genes associated with molecular processes regulating the response to fractionated dose ionizing radiation.

Keywords: PARP, colorectal cancer, radiobiology.

P155. Neuroscience, Psychiatry & Mental Health

Sodium/potassium ATPase activity and protein expression exhibit an age-dependent profile in mice with altered ganglioside composition

Borna Puljko[1], Mario Stojanoviħ[1], Katarina Iliħ[2], Nikolina Maľek Hrvat[3], Marija Heffer[4], Marta Balog[4], Kristina Mlinac Jerkoviħ[1], Svjetlana Kalanj Bognar[1]
[1]Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia, [2]BRAIN Centre, Department of Neuroimaging, Institute of Psychiatry, Psychology, and Neuroscience (IOPPN), King's College London, London, UK, [3]Institute for Medical Research and Occupational Health, Zagreb, Croatia, [4]Department of Medical Biology and Genetics, Faculty of Medicine, University of Osijek, Osijek, Croatia

Gangliosides, membrane glycosphingolipids particularly abundant in nervous tissue, have an important role in signaling events affecting neural development and pathogenesis of various neurological and neurodegenerative diseases. Gangliosides undergo extensive interactions with membrane proteins affecting their function. One of them is Na^+/K^+-ATPase (NKA), a membrane ion transporter with many vital roles including the stabilization of the resting membrane potential and regulation of ion homeostasis. The aim of this study was to investigate the effect of gangliosides on NKA activity and protein expression using the *ST8sia1 null* mouse model with impaired ganglioside synthesis. Wild type (WT) and *null* mice littermates of different ages (1 and 6 months old) were sacrificed and their brains neuroanatomically dissected. NKA activity was spectrophotometrically determined in cortical homogenates. Protein expression was analyzed by Western blotting. The results revealed a difference in both the activity and protein expression between the different age groups, with the activity being unchanged in infant mice and statistically lower in adult *null* mice compared to their WTs. Protein expression was found to exhibit a diametrically opposite profile, with NKA expression being higher in infants, yet statistically lower in adult *null* mice compared to WT mice. These results clearly demonstrate the imminent impact of gangliosides on NKA and are consistent with the fact that ganglioside composition changes during brain development and aging. A change in NKA function due to altered ganglioside composition may contribute to the development of neurodegenerative or neurological diseases by causing changes in ion homeostasis.

Keywords: Sodium/potassium ATPase, gangliosides, lipid rafts.

P156. Cellular & Molecular Biology

Modification of proliferation and apoptosis in breast cancer cells by exposure of antioxidant nanoparticles due to modulation of the cellular redox state induced by doxorubicin exposure

Patricia Ramírez Noguera[1], Laura Denise López Barrera[1], Roberto Díaz Torres[2], Joselo Ramón Martínez Rosas[1]
[1]Departamento de Ciencias Biológicas, Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Cuautitlán Izcalli, Mexico, [2]Departamento de Ingeniería y Tecnología, Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Cuautitlán Izcalli, Mexico

There is a growing interest in finding ways of reducing oxidative stress in tissue during doxorubicin treatment. A promising approach is the use of an antioxidant molecules. Glutathione (GSH) is one of the primary endogenous antioxidants at the cellular level and is associated with various events, such as proliferation, apoptosis, and redox state regulation. It is synthesized exclusively in the cell cytoplasm and once used and in its oxidized state, it cannot be incorporated into the cell, and thus it must be synthesized to maintain the levels in an optimal state. Moreover, GSH is recognized as a fundamental antioxidant molecule for cellular protection from toxins, both endogenous and environmental, including several anti-cancer cytotoxic drugs. Transporting GSH and other agents into cells to reduce the toxic effects of anti-cancer drugs requires the use of innovative delivery systems. Nanotechnology in cancer treatment represents a novel alternative to deliver agents to cells due to the physicochemical properties of many different nanoparticles. Chitosan (CH), a natural polymer, has been used to create nanoparticles (NPs), which are ideal delivery systems. In this report, we investigated whether the use of chitosan-carrying- glutathione nanoparticles (CH-GSH-NPs) can modify proliferation and apoptosis, and reduce cell damage induced by doxorubicin on breast cancer cells. Considering the results, CS-GSH-NPs represent a novel delivery system offering new opportunities in pharmacy, material science and biomedicine. *This work was supported by PAPIIT UNAM Project number: IN 214321.*

Keywords: Breast cancer, nanoparticles, chitosan-glutathione, doxorubicin.

FEBS Open Bio **13** (Suppl. S1) (2023) 3–72 DOI: 10.1002/2211-5463.13608

P157. Cellular & Molecular Biology

POLq and BRCA2 cooperate to prevent replication-associated ssDNA gaps

Miguel Ángel Ramírez-Otero[1], Yodhara Wijesekara Hanthi[1], Joanna Loizou[2,3], Vincenzo Costanzo[1]
[1]*IFOM ETS – The AIRC Institute of Molecular Oncology, Milan, Italy,* [2]*Comprehensive Cancer Centre, Medical University of Vienna, Vienna, Austria,* [3]*Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria*

POLq participates in alt-NHEJ, which acts as a back-up mechanism to repair double strand breaks in the absence of homologous recombination. It has been shown that *POLQ* and *BRCA2* are synthetic lethal; however the molecular mechanism behind the genetic interaction remains largely unclear. Importantly, functions of POLq outside alt-NHEJ and during replication are poorly understood. Here, using a new POLq inhibitor, we uncover a major role of POLq to prevent ssDNA gaps formed during DNA replication. We observed that POLq inhibition in *BRCA2* deficient cells, unlike WT cells, increased RPA accumulation during S-phase suggesting the presence of ssDNA gaps. We detected such gaps using the DNA fiber assay with S1 nuclease. We observed that *BRCA2*-deficient cells accumulate ssDNA gaps on nascent DNA and the inhibition of POLq increases the number of ssDNA gaps observed as shorter IdU-labeled tracks. The persistence of POLq-induced ssDNA gaps was associated with broken forks observed by EM, and an increase in the gH2AX signal during S-phase observed by IF. POLq-induced gH2AX accumulation was reduced when MRE11 was genetically or pharmacologically inhibited. Moreover, knockdown of NBS1, a component of the MRN complex, or CtIP, known to stimulate the MRE11-endo activity, also reduced gH2AX foci formation during S-phase. Our results suggest that POLq and BRCA2 cooperate to avoid the accumulation of ssDNA gaps during replication. Importantly, unprotected ssDNA gaps can be subjected to unscheduled MRE11 activity, generating dsDNA breaks. SsDNA gaps are a vulnerability of *BRCA2* deficient or mutated tumors that can be exploited therapeutically.

Keywords: POLQ, BRCA2, replication, ssDNA-gaps.

P158. Genetics & Epigenetics

Study of the roles of Pol5 and Mybbp1a in ribosome biogenesis

Ana Ramos-Sáenz[1,2], María Del Carmen Mota-Trujillo[3], Jorge Pérez-Fernández[3], Jesús De La Cruz[1,2]
[1]*Instituto de Biomedicina de Sevilla/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain,* [2]*Departamento de Genética, Universidad de Sevilla, Seville, Spain,* [3]*Departamento de Biología Experimental-Genética, Universidad de Jaén, Jaen, Spain*

Ribosomes are the cellular organelles that perform translation. Ribosome biogenesis is a very conserved process in which the so-called trans-acting factors guarantee speed, directionality, and accuracy of the process. In the yeast *Saccharomyces cerevisiae*, more than 300 different protein factors have been identifed, among them Pol5. This protein is nucleolar and a homolog of the human tumor suppressor Myb-binding protein 1A (MYBBP1A), mutations of which are normally associated with different kinds of tumors in humans, most often in kidneys. We have shown that Pol5 is a ribosome assembly factor required for the normal production of 60S ribosomal subunits through its role in pre-rRNA processing and ribosome assembly. Like Pol5, MYBBP1A is nucleolar and is supposed to have a similar role, but how it participates in this process remains unclear.
We have recently shown that the heterologous expression of MYBBP1A in yeast causes a dominant negative phenotype that is related to an interference with the 60S ribosomal subunit biogenesis. Additionally, the subcellular distribution of the human protein is similar to that of the yeast protein as shown by microscopy and fractionation experiments. Currently we are trying to determine the molecular reasons for this negative effect. Clarifying the function of Pol5 and MYBBP1A in the synthesis of ribosomes in yeast can help in further understanding the role of MYBBP1A in human cells.

Keywords: Ribosome, rRNA, nucleolus.

P159. Genetics & Epigenetics

DNA methylation and protein expression of APC gene in prostate cancer

Irena Abramoviħ[1,2,3], Lucija Škara[1,2,3], Dora Raos[1,2,3], Ivan Pezelj[4], Tomislav Kuliš[2,5], Monika Ulamec[2,3,6,7], Nino Sinčiħ[1,2,3]
[1]*Laboratory for Epigenetics and Molecular Medicine, Department of Medical Biology, School of Medicine, University of Zagreb, Zagreb, Croatia,* [2]*Scientific Group for Research on Epigenetic Biomarkers, School of Medicine, University of Zagreb, Zagreb, Croatia,* [3]*Scientific Centre of Excellence for Reproductive and Regenerative Medicine, School of Medicine, University of Zagreb, Zagreb, Croatia,* [4]*Department of Urology, University Clinical Hospital Centre "Sestre Milosrdnice", Zagreb, Croatia,* [5]*Department of Urology, University Hospital Centre Zagreb, Zagreb, Croatia,* [6]*Ljudevit Jurak Clinical Department of Pathology and Cytology, University Clinical Hospital Centre "Sestre Milosrdnice", Zagreb, Croatia,* [7]*Department of Pathology, School of Dental Medicine and School of Medicine, University of Zagreb, Zagreb, Croatia*

Prostate cancer (PCa) represents a malignancy with high incidence and prevalence rates which are expected to rise further in the following years. In the search for new biomarkers, DNA methylation has been recognized as a key event in PCa development and progression. Therefore, in the present research, DNA methylation of the *APC* gene in liquid biopsies and tumor tissue of PCa patients, as well as its protein expression, were investigated. Liquid biopsy samples (blood and ejaculate) and prostate tissue samples were taken from 42 patients with early-stage PCa and 55 with benign prostate hyperplasia (BPH). The degree and pattern of DNA methylation were investigated using pyrosequencing, while protein expression of *APC* was analyzed using immunohistochemistry. In blood and seminal plasma of prostate cancer patients compared to BPH patients, there was no significant difference in cfDNA methylation of the *APC* gene. Moreover, data in the literature suggesting DNA hypermethylation in tumor tissue compared to surrounding healthy tissue or BPH tissue have not been confirmed. Analysis of *APC* protein expression showed that *APC* has higher expression in tumor epithelia than epithelia of surrounding healthy tissue or BPH tissue. In tumor stroma, *APC* had lower expression compared to the stroma of surrounding healthy tissue or BPH tissue. Based on these results,

cfDNA methylation of *APC* does not have potential as a biomarker for prostate cancer and its differentiation from BPH. Changes in *APC* protein expression have potential as prostate cancer biomarkers for immunohistochemistry purposes since their expression in tumor epithelium and stroma differs from surrounding healthy and BPH tissue.

Keywords: Liquid biopsy, prostate cancer, DNA methylation, biomarker.

P160. Chemistry & Biochemistry

Nutritionally-relevant concentrations of indicaxanthin and a mixture of plant sterols inhibit the extrinsic-pathway of eryptosis induced by cigarette smoke extract

Ignazio Restivo[1], Alessandro Attanzio[1], Mario Allegra[1], Luisa Tesoriere[1], Guadalupe García-Llatas[2], Antonio Cilla[2]
[1]*Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo, Italy,*
[2]*Nutrition and Food Science Area, Faculty of Pharmacy, University of Valencia, Valencia, Spain*

The cell death program of red blood cells (RBCs), called eryptosis, is characterized by activation of caspases and scrambling of membrane phospholipids with externalization of phosphatidylserine (PS). Excessive eryptosis is implicated in many inflammatory pathologies and is associated with endothelial cell injury and thrombosis. It has recently been reported that cigarette smokers have high levels of circulating eryptotic erythrocytes, and a possible contribution of eryptosis to the vaso-occlusive complications associated with cigarette smoke has been postulated. In this study, we demonstrate how the phytochemical indicaxanthin (IND) and a mixture of plant sterols (MPS), at blood concentrations reached after ingestion of four fruits of *Opuntia ficus-indica* (L.Mill) or a drink enriched with MPS, inhibit eryptosis induced by whole cigarette smoke extract (CSE) or its particulate fraction (pCSE). Isolated RBCs were exposed for 4 h to CSE or pCSE (10%–20%). Compared to untreated RBCs, exposure to CSE or pCSE caused an increase of the levels of PS outsourcing, ceramide production, cleaved forms of caspase 8/caspase 3 and phosphorylated p38 MAPK. When RBCs were treated with CSE or pCSE in the presence of IND from 1 to 5 μM or 22 μM MPS, a significant dose-dependent reduction of the measured hallmarks of apoptotic death was evident. The mechanism of inhibition of the CSE-induced eryptosis by IND and MPS is currently under investigation in our laboratory. The present data prompt the formulation of a new supplement containing IND and MPS to possibly counteract vaso-occlusive complications in smokers.

Keywords: Biochemistry, phytochemicals, nutraceutics, indicaxanthin, sterols.

P161. Immunology, Microbiology & Infectious Diseases

Selection of lactic acid bacteria strains for use as biocontrol agents

Clara Rey Valenzuela, Sara Rodríguez Sánchez, Pilar Fernández-Pacheco Rodríguez, Ramón Arias Simarro, Susana Seseña Prieto, María de los Llanos Palop Herreros
Analytical Chemistry and Food Technology Department, University of Castilla la Mancha, Toledo, Spain

Lactic acid bacteria (LAB) have traditionally been used as starter cultures to produce fermented products, although recently new applications have emerged as biocontrol agents. For this purpose, it is necessary to carry out a thorough characterization study to select the best strains for their use. Once selected, it will be necessary to determine their implantation capacity. The response to technological stress of thirty-nine strains of different species of lactic acid bacteria (Microbiology group collection) were analyzed and 5 growth temperatures, 5 pHs and 6 NaCl concentrations were tested. The results of these tests and of previous studies allowed us to select two strains, *Lactiplantibacillus (Lpb.) plantarum* UCLM-56 and *Levilactobacillus (L.) brevis* UCLM-47. Then, a study was carried out in order to determine the implantation capacity of strain *Lpb. plantarum* UCLM-56 in the Manchego cheese production process, using both thermised and raw milk. LAB strains that had been obtained from samples taken at different cheese ripening times were genetically characterized by RAPD-PCR. The results of this study showed differences between thermised and raw milk, which is to be expected, as raw milk cheeses have a rich and diverse spontaneous microbiota which our strain had to compete with. Nevertheless, the results in both cases were good and allowed us to conclude that this strain could be used as a biocontrol agent in the production of fermented dairy products.

Keywords: Lactic bacteria, biocontrol, biotechnology, food.

P162. Neuroscience, Psychiatry & Mental Health

Increased excitability of parvalbumin-positive interneurons in the premotor cortical area in a mouse model of obsessive-compulsive disorder

Santiago Reyes-León, Emilio Martínez-Márquez, Guadalupe Asensio-Gómez, Pablo García-Junco-Clemente, José Luis Nieto-González
Instituto de Biomedicina de Sevilla (IBiS)/HUVR/CSIC/ Universidad de Sevilla, Departamento de Fisiología Médica y Biofísica and CIBERNED, Seville, Spain

Obsessive Compulsive Disorder (OCD) is a severe, chronic, and ubiquitous neuropsychiatric disorder that affects 2–3% of the worldwide population. Corticostriatal dysfunction is considered a major factor in the pathogenesis of OCD. In *Sapap3* knockout (KO) mouse, a well-validated model of compulsive behavior, it has been reported that the striatal region receives increased levels of synaptic input from the secondary motor area (M2). M2 is thought to be homologous to the Supplementary Motor Area in humans, an area showing hyperactivity in OCD patients. Cortical

disinhibition in OCD patients due to GABAergic deficits has been proposed to be related to its pathogenesis. Therefore, we study, using a combination of *in vitro* and *in vivo* 2-photon experiments, the cortical GABAergic circuitry involving PV^+ interneurons in layer 2/3 of the premotor area (M2). We have developed a *Sapap3* null mouse line that expresses td-Tomato under the control of the PV promoter. The preliminary results show a decrease in the number of PV^+ interneurons in M2. Furthermore, using electrophysiological recording in brain slice preparation, PV^+ interneurons show increased input resistance, decreased rheobase and increased firing frequency gain. All together, the data indicate that PV^+ interneurons are hyperexcitable. *In vivo* calcium imaging experiments in awake animals are in progress to confirm the hyperexcitability of PV^+ neurons, and how the excitatory network is affected.

We are grateful to C. Cabrera Romero for excellent technical assistance. Supported by: RyC-2016-19,906, PRE2019-087729 (MCI/AEI/FSE, UE), VI PPIT-US and US-1264432 (US/JUNTA/FEDER, UE).

Keywords: Obsessive–compulsive-disorder, prefrontal cortex, interneuron, 2-photon electrophysiology.

P163. Cellular & Molecular Biology

Development of gapmers against MSI2, a new therapeutic target against muscle atrophy in myotonic dystrophy type 1

Natalia Riedel[1,2], Ariadna Bargiela Schönbrunn[3], Rubén Artero[1,2], Manuel Pérez Alonso[1,2]
[1]*BIOTECMED, University of Valencia, Valencia, Spain,* [2]*INCLIVA Biomedical Research Institute, Valencia, Spain,* [3]*Neuromuscular Research Unit, Neurology Department, Instituto de Investigación Sanitaria la Fe, Valencia, Spain*

The molecular causes of muscle atrophy in the rare neuromuscular disease myotonic dystrophy (DM1) are not yet fully understood. We recently discovered that *Musashi RNA-binding protein 2* (*MSI2*) is overexpressed in patient-derived myotubes and skeletal muscle biopsies. Importantly, silencing of *MSI2* raises the expression of miR-7, a critical regulator of excessive autophagy, and rescues atrophic phenotypes, while overexpression in a DM1 mouse model enhances them. Thus, *MSI2* is a new therapeutic target for muscle atrophy. To find the most effective gapmers against *MSI2* (antisense oligonucleotides that trigger RNase H-mediated decay of the target mRNA), we designed 27 versions and transfected each of them at eight concentrations in human-derived DM1 myoblasts with their respective controls, including untreated DM1 myoblasts, scramble version, and 2 gapmers previously used in proof-of-concept experiments. In addition, similar experiments were done to characterize cell toxicity using a cell viability assay. The results allow ranking the gapmers according to their therapeutic index to prioritize subsequent *in vitro* and *in vivo* validation work.

Keywords: Myotonic dystrophy type 1, gapmers, muscle atrophy, MSI2.

P164. Neuroscience, Psychiatry & Mental Health

Promoting brain regeneration through cellular reprogramming

Pablo Rodríguez Cumbreras[1,2], Fernando Cala Fernández[2], Francisco Manuel Vega Moreno[1,3], Benedikt Berninger[4], Ricardo Pardal Redondo[1,2], Aida Platero Luengo[1,2]
[1]*Departamento de Fisiología Médica y Biofísica, Universidad de Sevilla, Seville, Spain,* [2]*Instituto de Biomedicina de Sevilla (IBiS)/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain,* [3]*Departamento de Biología Celular, Universidad de Sevilla, Sevilla, Spain,* [4]*Centre for Developmental Neurobiology, King's College London, London, UK*

Although our knowledge about brain physiology has expanded significantly, most neurological conditions remain uncured. Aging is the major risk factor for many human diseases. In the lab, we take advantage of the reprogramming technology to address rejuvenation. Nuclear cell reprogramming has emerged as a method that allows the age of any cell to be reversed to a youthful stage by the action of the 4 Yamanaka Factors (4F). It has been shown that partial reprogramming (PR) by short-term cyclic expression of 4F ameliorates cellular and physiological hallmarks of aging. We propose to rejuvenate astroglia by PR in order to reduce age-related neuroinflammation to promote brain homeostasis and the maintenance of a healthy neuronal population.

Keywords: Aging, rejuvenation-by-reprogramming, partial-reprogramming.

P165. Cancer Biology & Oncology

Transcriptional regulation during acute myeloid leukemia resistance and plasticity

Alejo Rodríguez Fraticelli, Luis C. Rodríguez Escudero
IRB Barcelona – Institute for Research in Biomedicine, Barcelona, Spain

Despite decades of innovations in cancer therapy, survival rates for acute myeloid leukemia (AML) have barely improved. This dismal prognosis is a consequence of its high relapse frequency and the persistence of therapy-resistant cells. Targeting therapy-resistance remains a priority, but an understanding of the mechanisms involved in AML cellular plasticity and relapse are still lacking. Intriguingly, preliminary analyses show that TCF15 expression is a top predictor of AML-related mortality, and enriches for AML stemness and therapy resistance. Here, we propose a comprehensive experimental program, combining single-cell genomics, lineage tracing, mouse genetics and patient-derived xenograft studies to characterize TCF15 and its molecular targets as important AML biomarkers and potential druggable entities. The goals are: 1) to establish the role of TCF15 in AML initiation, maintenance and therapy resistance; 2) to dissect the molecular mechanisms of TCF15, including upstream regulators and downstream effectors; 3) to unbiasedly elucidate novel transcription-factor regulators of AML therapy resistance.

Keywords: Therapy resistance, relapse, cellular plasticity, acute myeloid leukemia, leukemic stem cells.

P166. Obesity, Diabetes & Other Diseases

Hypothalamic ER stress: the missing link between obesity and precocious puberty?

Elvira Rodríguez-Vázquez[1,2], Álvaro Aranda-Torrecillas[1,2], Violeta Heras[3], Cecilia Perdices-López[1,2], Francisco Ruiz-Pino[1,2], Ana Belén Rodríguez[1,2], María Jesús Sánchez-Tapia[1], María Jesús Vázquez[1,2], Francisco Gaytán[1,2], Miguel López[2,3], Juan Manuel Castellano[1,2], Manuel Tena-Sempere[1,2]

[1]Department of Cell Biology, Physiology and Immunology, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), University of Córdoba, and Hospital Universitario Reina Sofía, Cordoba, Spain, [2]CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Cordoba, Spain, [3]Department of Physiology, Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), University of Santiago de Compostela, and Instituto de Investigación Sanitaria, Santiago de Compostela, Spain

Puberty is a complex maturational process sensitive to the energy status of the organism. Indeed, early-onset obesity is frequently associated with precocious puberty, a phenomenon bound to long-term co-morbidities. Mounting evidence supports the idea that hypothalamic alterations in endoplasmic reticulum (ER) homeostasis, known as ER stress, are implicated in the pathophysiology of obesity. However, its contribution to the timing of puberty onset and its alterations remains unexplored. Our results showed that the hypothalamic expression of relevant ER stress markers changed throughout postnatal maturation in lean female rats. Specifically, the content of the phosphorylated PERK (p-PERK) and its downstream target, eukaryotic initiation factor 2 (p-eIF2α), decreased significantly at the prepubertal stage and remained low in the peripubertal period. Moreover, a significant reduction in p-IRE1 levels was detected at the peripubertal stage. Hypothalamic alterations of key ER stress factors were also observed in female rats with obesity-induced precocious puberty, including increased levels of p-PERK and p-eIF2α and reduced content of ATF6α. Chronic stimulation of hypothalamic ER stress with thapsigargin resulted in early puberty onset in lean female rats, as evidenced by advanced vaginal opening and first ovulation. Yet, this pubertal phenotype was not associated with significant changes in body weight, food intake, serum LH levels, or uterus weight. In contrast, central blockade of ER stress with tauroursodeoxycholic acid (TUDCA) partially normalized the timing of puberty in early overfed female rats. Collectively, our results document a novel role for hypothalamic ER stress in controlling puberty onset and its potential contribution to obesity-induced precocious puberty.

Keywords: Puberty, endoplasmic reticulum (ER) stress, obesity.

P167. Obesity, Diabetes & Other Diseases

Serum components of IL-6 signalling as predictors of severity and outcome in inflammatory diseases

M. Ángeles Rodríguez-Hernández[1], David Carneros[1], María Núñez-Núñez[2,3,4], Ramón Coca[5], Rosario Baena[6], Gema M López-Ruíz[1], María Elena Cano-Serrano[7], Alberto Martínez-Tellería[8], Ana Fuentes-López[9], Juan Manuel Praena-Fernández[10], Gemma Álvarez[5], Christoph Garbers[11], José Hernández-Quero[3], Federico García[1,4,9], Stefan Rose-John[13], Matilde Bustos[1]

[1]Area of Liver, Digestive and Inflammatory Diseases, Institute of Biomedicine of Seville (IBIS)/Virgen del Rocío University Hospital/Spanish National Research Council (CSIC)/University of Seville, Seville, Spain, [2]Department of Pharmacy, San Cecilio University Hospital, Granada, Spain, [3]Infectious Disease Unit, San Cecilio University Hospital, Granada, Spain, [4]Biosanitariy Research Institute of Granada (IBS), Granada, Spain, [5]Department of Clinical Analysis, Virgen de las Nieves University Hospital, Granada, Spain, [6]Department of Pharmaceutical Technology and Chemistry, University of Navarra, Pamplona, Spain, [7]Department of Anaesthesiology, San Cecilio University Hospital, Granada, Spain, [8]Department of Anaesthesiology, Virgen de las Nieves University Hospital, Granada, Spain, [9]Department of Microbiology, San Cecilio University Hospital, Granada, Spain, [10]Department of Statistics, Science Faculty, University of Granada, Spain, [11]Department of Pathology, Medical Faculty, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany, [12]CIBERINFEC, ISCIII, Madrid, Spain, [13]Institute of Biochemistry, Kiel University, Kiel, Germany

Background and objectives: (IL)-6 normalization strategies during maladapted inflammation have shown conflicting results in COVID-19 clinical trials. IL-6 can signal by classical mode with anti-inflammatory properties, or by trans-signaling with proinflammatory effects. The aim of this study was to investigate the ability of IL-6 signaling components (IL-6, sIL-6R and sgp130) to predict COVID-19 severity and outcome. *Methods*: Serum samples of hospitalized COVID-19 patients were collected and clinical and biochemical data were recorded. Levels of IL-6, sIL-6R and sgp130 were quantified. To estimate IL-6 trans-signaling, a ratio between the pro-inflammatory IL-6:sIL-6R complex and the inactive IL-6:sIL-6R:sgp130 complex, as well as the fold molar excess of sgp130 over sIL-6R (FME) were determined. *Results*: Our data demonstrated that high levels of IL-6, sIL-6R, sgp130 were independent predictors of COVID-19 severity in survivor patients (without death). Moreover, the combined analysis of IL-6 + sIL-6R + sgp130 improved the predictive capacity of their individual measurements, exhibiting the most robust specificity, accuracy and Odds Ratio. Notably, patients who needed a longer length of hospital stay were those with increased levels of the three variables. Conversely, in a subgroup of patients with a very poor prognosis, high levels of IL-6 and FME were early predictors of death. In this context, the combined analysis of IL-6 + FME + lymphopenia + creatinine had the most accurate predictive capacity. In conclusion, our study suggests that the screening of IL-6 signaling components at hospital admission can identify patients at risk of severe COVID-19, with clear implications for treatment and clinical decision-making.

Keywords: IL-6 signaling, COVID-19.

P169. Neuroscience, Psychiatry & Mental Health

An *in vivo* high throughput screening assay to test potential neuroprotective therapies for ischemic stroke

Marina Romero-Bernal[1], Carmen Del Río[1], Paloma Menéndez-Valladares[1], Joan Montaner[1,2]
[1]*Neurovascular Research Laboratory, Institute of Biomedicine of Seville (IBiS)/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain,* [2]*Department of Neurology, Hospital Universitario Virgen Macarena, Seville, Spain*

Stroke is the second cause of death worldwide and a leading cause of disability, with ischemic stroke accounting for 85% of all cases. Ischemic stroke is the result of the blockage of a cerebral artery by a clot and the only approved treatment for this condition is reperfusion therapy. However, these treatments are only applicable to a small percentage of patients and because of that there is an urgent need for neuroprotective therapies. Therefore, it is important to identify new therapeutic strategies and we thought to establish an *in vivo* screening model using *Drosophila melanogaster* where we can test multiple drug combinations already identified by *in silico* drug repositioning.
Flies from the Oregon R strain were exposed to anoxia or severe hypoxia (1% O_2) for different periods of time. We observed a sexually dimorphic response with females being more resistant than male flies to hypoxia. Then, we established the standard conditions to induce hypoxia in *D. melanogaster* males under defined environment using a hypoxia chamber. During post-hypoxic reoxygenation (21% O_2), we assessed daily mortality, fly locomotor activity and analyzed molecular characteristics (reactive oxygen species (ROS) production, caspase activation and protein carbonylation) at various time points. Most of the flies died within 24 h of exposure, and periods of severe hypoxia longer than 2 h affected flies′ survival and impaired locomotor ability. In conclusion, we established a reliable and reproducible high throughput screening assay to study the impact of different therapeutic approaches on hypoxia-reoxygenation injury using *D. melanogaster* as a model system.

Keywords: Ischemic stroke, neuroprotective therapies, *D. melanogaster*.

P170. Genetics & Epigenetics

Formation and repair of DNA topoisomerase 1 DNA double strand breaks associated with gene transcription

Diana Rubio-Contreras[1,2], Fernando Gómez-Herreros[1,2]
[1]*Instituto de Biomedicina de Sevilla (IBiS)/Hospital Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain,* [2]*Departamento de Genética, Universidad de Sevilla, Seville, Spain*

DNA double-strand breaks (DSB) are the most cytotoxic DNA lesions arising in cells. Their accurate repair is critical to prevent cell death and maintain genomic integrity. Some physiological DSBs have been associated with the activity of DNA topoisomerases, essential enzymes that transiently cut the DNA to release the torsional stress associated with DNA metabolism. Type 1 DNA topoisomerases (e.g., TOP1) cut one strand during their reaction cycle and remain covalently bound to the 3' DNA end before resealing it. However, these breaks can become abortive (irreversible) and trigger cellular repair pathways. Intriguingly, although abortive TOP1 cycles mainly generate DNA single-strand breaks (SSB), these can turn into DSBs by a poorly understood mechanism. More importantly, how these breaks are repaired and their effect on genome stability and cell death is still unclear. These TOP1-dependent DSBs can be induced with selective TOP1 poison camptothecin or its derivatives, commonly used as anticancer drugs. Additionally, physiological TOP1 DSBs might constitute a key cause of neuronal death in patients that carry mutations in the SSB repair machinery. In this work, we have characterized for the first time the effect of these breaks on genomic destabilization. Our results have important implications for the understanding of the molecular mechanism of DNA damage-associated neurodegenerative diseases and for the impact of gene transcription on genomic instability.

Keywords: Topoisomerases, double-strand-break, transcription, repair, instability.

P171. Neuroscience, Psychiatry & Mental Health

Acute genetic elimination of a synaptic co-chaperone to study and to revert presynaptic dysfunction and neurodegeneration

Fátima Rubio-Pastor, Santiago López-Begines, Pablo García-Junco-Clemente, Rafael Fernández-Chacón
[1]*Instituto de Biomedicina de Sevilla (IBiS)/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain,* [2]*Departamento de Fisiología Médica y Biofísica, Universidad de Sevilla, Seville, Spain,* [3]*CIBERNED, Seville, Spain*

Synapses operate throughout life, but synaptic proteins last only for several days or weeks. The mechanisms by which synaptic proteins are maintained are not well understood yet. We are interested in the role of the synaptic co-chaperone CSP-alpha/DNAJC5. Conventional KO mice lacking CSPα/DNAJC5 develop presynaptic degeneration and die soon after birth, preventing studies of synaptic protein maintenance in adulthood. We have bred mice bearing the *Dnajc5* floxed allele against UBC-Cre-ERT2 mice to target *Dnajc5* ubiquitously in a time-controlled manner. We demonstrated an efficient induction of Cre-recombinase by feeding the mice with tamoxifen which promotes a general decrease of CSPα/DNAJC5 expression in brain and a neurological and lethal phenotype in $UBC^{CreERT2}$:$Dnajc5^{flox/flox}$ mice. This approach has opened the opportunity to search for the time-window suitable to rescue the phenotype via the intravenous injection of recombinant adeno-associated viruses (rAVVs) that are permeable through the blood brain barrier. Using rAAVs encoding the fluorescent reporter tdTomato under the control of synapsin promoter we have found that the capsid of AAV-PHP.eB serotype is more efficient than the one from AAV-PHP.B4 serotype. Intravenous gene delivery of CSPα/DNAJC5 through rAAV-PHP.eB viruses turned out to be insufficient to rescue the neurological lethal phenotype of adult conditional CSPα/DNAJC5 KO mice but suitable, however, to investigate multiple features of phenotypic rescue of single neurons *in vivo*.
Support: Spanish AEI and MCIN (PID2019-105530GB-I00),

MICIU (FPU18/01700), Fundación Tatiana Pérez de Guzmán El Bueno, Andalusian CTEICU (P18-FR-2144), ISCIII (CIBERNED) and FEDER. Thanks to M.C. Rivero for previous technical assistance.

Keywords: CSP-alpha, presynaptic, co-chaperone, adeno-associated viruses.

P172. Cellular & Molecular Biology

Xylose-metabolized yeast *Ogataea* (Hansenula) *polymorpha* as a potential producer of second-generation ethanol

Justyna Ruchala[1], Roksolana Vasylyshyn[2], Marta Semkiv[2], Olena Dmytruk[1,2], Aksyniia Tsaruk[1], Kostyantyn Dmytruk[1,2], Andriy Sibirny[1]

[1]*College of Natural Sciences, Institute of Biology and Biotechnology, University of Rzeszow, Rzeszow, Poland,* [2]*Institute of Cell Biology, National Academy of Science of Ukraine, Lviv, Ukraine*

The development of viable processes for the production of lignocellulose biofuels is an important task in modern microbiology and biotechnology. Thermotolerant yeast *Ogataea polymorpha* is a promising organism for further development, since it robustly grows on glucose and xylose at 45–50 °C, which could be applicable for simultaneous saccharification and fermentation. Xylose is the main pentose released from lignocellulosic biomass and has a high potential as a renewable raw material for the production of bioethanol. The thermotolerant yeast *O. polymorpha*, unlike *Saccharomyces cerevisiae*, can metabolize and ferment not only glucose but also xylose. Using a combination of metabolic engineering and classical selection, mutant strains of *O. polymorpha* have been isolated which accumulated near 16 g/L of ethanol to form xylose at 45 °C. To overcome glucose inhibition of the use of xylose, the native Hxt1 carrier of *O. polymorpha* was designed and overexpressed. However, in non-conventional yeast, the regulation of glucose and glucose metabolism has not yet been well understood. Therefore, we characterized the role of transcriptional factors Mig1, Mig2, Tup1, and Hap4 in the natural yeast *O. polymorpha*. The deletion of *MIG1* and *MIG2* reduces the amount of ethanol produced on these sugars. Inversely, the overexpression of the *HAP4-A* and *TUP1* genes reduced ethanol production during xylose alcoholic fermentation. Therefore, *HAP4-A* and *TUP1* are involved in the suppression of xylose metabolism and fermentation in yeast *O. polymorpha* and their deletion could be a viable strategy to improve the production of ethanol from this pentose. *Research work supported by NSC no.: 2020/37/B/NZ1/0223.*

Keywords: Thermotolerant yeasts, lignocellulose, alcoholic fermentation, metabolic engineering.

P173. Genetics & Epigenetics

Whole exome sequencing as a tool for the genetic diagnosis of congenital sensorineural hearing loss

Juan Manuel Ruiz-Robles[1,2,3], Nerea Gestoso-Uzal[1,2,3], Carlos Gutiérrez-Cerrajero[1,2,3], Juan José Tellería[4,5], Luis Antonio Corchete[1,2], Fernando Benito-González[2,6], Jose Ignacio Benito-Orejas[7], Ana-Belén Herrero[1,2,3], Rogelio González-Sarmiento[1,2,3]

[1]*Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain,* [2]*Biomedical Research Institute of Salamanca (IBSAL), University Hospital of Salamanca-USAL-CSIC, Salamanca, Spain,* [3]*Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain,* [4]*Department of Cell Biology, Histology and Pharmacology, University of Valladolid, Valladolid, Spain,* [5]*Institute of Molecular Biology and Genetics (IBGM), University of Valladolid-CSIC, Valladolid, Spain,* [6]*Department of Otorhinolaryngology, University Hospital of Salamanca-IBSAL, Salamanca, Spain,* [7]*Service of Otorhinolaryngology, University Clinical Hospital of Valladolid, Valladolid, Spain*

Currently, the genetic diagnosis of congenital deafness is limited to the analysis of the mutational status of the genes with highest penetrance, such as GJB2, GJB5 and OTOF. Since hearing loss can be a consequence of a diversity of genetic alterations, and at least 600 genes associated with this disease are known, our group proposed a study of the whole exome (WES) of patients with congenital hearing loss negative for GJB2 analysis to identify new candidate genes. Interestingly, we have found that this methodology can also be useful at the clinical level, since in two different patients (2 of 12 in our cohort) we have found mutations that have been previously associated with syndromic forms of deafness. The mutations were the following: rs74315288 in patient 1, associated with Bartter syndrome; rs199472800 in patient 2, associated with Jervell and Lange-Nielsen syndrome and long QT syndrome. The detection of the mutations was prior to the knowledge of other severe clinical manifestations related to these syndromes, allowing early diagnosis, with the consequent improvement in the treatment and follow-up of these patients. Thus, we postulate WES as a biotechnological tool with biomedical interest, and propose its gradual incorporation into medical practice.

Keywords: Deafness, hearing loss, whole exome sequencing, genetic diagnosis.

P174. Cellular & Molecular Biology

Quality-controlled ceramide-based GPI-anchored protein sorting into selective ER exit sites

Susana Sabido Bozo[1], Sofía Rodriguez-Gallardo[1], Atsuko Ikeda[2], Misako Araki[2], Kouta Okazaki[2], Miyako Nakano[2], Auxiliadora Aguilera-Romero[1], Alejandro Cotes-Gómez[1], Sergio López[1], Miho Waga[3], Akihiko Nakano[3], Kazuo Kurokawa[3], Manuel Muñiz[1], Kouichi Funato[2]
[1]*Department of Cell Biology, University of Seville and Instituto de Biomedicina de Sevilla (IBiS)/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain,* [2]*Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan,* [3]*Live Cell Super-Resolution Imaging Research Team, RIKEN Center for Advanced Photonics, Wako, Saitama, Japan*

Glycosylphosphatidylinositol-anchored proteins (GPI-APs) exit the endoplasmic reticulum (ER) through a specialized export pathway in the yeast *Saccharomyces cerevisiae*. We have recently shown that a very-long acyl chain (C26) ceramide present in the ER membrane drives clustering and sorting of GPI-APs into selective ER exit sites (ERES). Now, we show that this lipid-based ER sorting also involves the C26 ceramide as a lipid moiety of GPI-APs, which is incorporated into the GPI anchor through a lipid-remodeling process after protein attachment in the ER. Moreover, we also show that a GPI-AP with a C26 ceramide moiety is monitored by the GPI-glycan remodelase Ted1, which, in turn, is required for receptor-mediated export of GPI-APs. Therefore, our study reveals a quality-control system that ensures lipid-based sorting of GPI-APs into selective ERESs for differential ER export, highlighting the physiological need for this specific export pathway.

Keywords: Endoplasmic reticulum, protein sorting, GPI-anchored proteins, quality control, ceramide.

P175. Pharmacology, Toxicology & Nutrition

Effects of miR-30c-5p modulation on the nucleolar stress response of dorsal root ganglia neurons after sciatic nerve injury

Raquel Francés[1], Jorge Mata-Garrido[2], Miguel Lafarga[3,4], María Teresa Berciano[3,4], Andrea Ysabel Saguillo Velásquez[4,5], Jessica Marques Llano[4,5], Mónica Tramullas[4,5], María Amor Hurlé[4,5]
[1]*Energy & Memory, Brain Plasticity Unit, CNRS, ESPCI Paris, PSL Research University, Paris, France,* [2]*Nuclear Organization and Oncology, Department of Cellular Biology and Infection, Institut Pasteur, Paris, France,* [3]*Instituto de Investigación Sanitaria Valdecilla (IDIVAL), Santander, Spain,* [4]*Departamento de Anatomía y Biología Celular, Facultad de Medicina, Universidad de Cantabria, Santander, Spain,* [5]*Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Cantabria, Santander, Spain*

Background and Aims: Neuropathic pain (NP) is a prevalent and debilitating chronic syndrome highly refractory to current analgesics. The development and maintenance of NP include long-term pathological plasticity in the nervous system. The nucleolar stress response is an important sensor of neuronal dysfunction in several neurodegenerative disorders. However, the impact of nucleolar dysfunction on NP development after nerve injury remains elusive. MicroRNAs (miRNAs) are small noncoding RNAs that post-transcriptionally modulate gene expression. Previous results of our group support a major role for miR-30c-5p in neuropathic pain development (Tramullas et al., 2018). Our study aims to assess nucleolar stress in neurons of dorsal root ganglia (DRG) in response to sciatic spared nerve injury (SNI) and the consequences of miR-30c-5p-gain and loss-of-function. *Methods*: NP was induced to rats by SNI. Mechanical allodynia was assessed with von Frey monofilaments. Lumbar dorsal root ganglia (DRG) were obtained and processed for immunofluorescence on days 5 and 10 post-SNI. *Results*: Our results indicate that SNI induces important structural alterations of the nucleolus in the DRG primary neurons, in association with NP development. The harmful effect of SNI was potentiated by the treatment with miR-30c-5p mimic, with pro-allodynic consequences. In contrast, SNI-induced DRG damage was prevented by the treatment with miR-30c-5p inhibitor with anti-allodynic consequences. *Conclusions*: the nucleolus is one of the cellular organelles affected by SNI, which is particularly vulnerable to alterations of miR-30c-5p expression. *[Supported by PID2019-104398RB-I00 and PDC2021-120878-I00].*

Keywords: Neuropathic pain, miRNAs, nucleolar stress, dorsal root ganglia.

P176. Cardiovascular Disease

Pulmonary embolism (PE), chronic thromboembolic pulmonary hypertension (CTEPH) and occult cancer: differences in their proteomic profiles

Verónica Sánchez-López[1,2], Julia Oto[3], Elena Arellano-Orden[1,2], Olga Tura[4,5], Carmen Font[4], María Eugenia Sáez[6], Pilar Medina[3], Isabel Blanco[4], Joan Albert Barberá[4], Remedios Otero-Candelera[1,2]
[1]*Institute of Biomedicine of Seville (IBiS)/Virgen del Rocio University Hospital/CSCI/US, Seville, Spain,* [2]*CIBERES, Madrid, Spain,* [3]*Hemostasis, Thrombosis, Arteriosclerosis and Vascular Biology Research Group, Medical Research Institute Hospital La Fe, Valencia, Spain,* [4]*Hospital Clínic-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain,* [5]*Biomedical Research Institute-IDIBGI, Girona, Spain,* [6]*CAEBI, Seville, Spain*

Introduction: In the follow-up of patients diagnosed with PE, either CTEPH or cancer can be identified. We hypothesized that cancer and CTEPH might share pathogenic pathways such as inflammation, cell proliferation, or apoptosis. *Aim*: Analyze the proteomic profile of patients with CTEPH, uncomplicated PE and PE patients with occult cancer to identify proteins dysregulated in PE group compared to those with occult cancer and CTEPH. *Methods*: Fourteen patients with CTEPH, 6 with venous thrombosis disease (VTD) who were subsequently diagnosed with cancer, and 7 with PE who did not present neither CTEPH nor occult cancer after 2 year follow-up were evaluated. Citrated plasma was obtained from all of them and used for protein quantification in a mass spectrometer by iTRAQ© labeling. After quality control and normalization, differential expression analysis was performed using the Kruskal-Wallis test with the Benjamini-Hochberg (BH) correction for multiple testing.

Results: A total of 382 proteins were determined in all groups, 27 were found to be differentially expressed in the 3 groups of patients. We selected Apolipoprotein M, Plasminogen, Lumican and Coagulation Factor XII-Mie based on their under-expression in the PE group and Zinc finger protein based on overexpression in the PE group compared to CTEPH and occult cancer. *Conclusion*: The circulating proteins identified could help to differentiate patients with PE who are at risk for presenting subsequent cancer or CTEPH.
Funding sources: Instituto de Salud Carlos III-FEDER (PI15/01085, PI15/00582).

Keywords: Thrombosis, occult cancer, pulmonary hypertension, proteomic, biomarkers.

P177. Cardiovascular Disease

Contribution of endothelial cell senescence to atherosclerosis

Katarzyna Sarad[1], Izabela Kraszewska[1], Urszula Jankowska[2], Bożena Skupień-Rabian[2], Józef Dulak[1], Agnieszka Jaźwa-Kusior[1]
[1]*Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland,* [2]*Proteomics and Mass Spectrometry Core Facility, Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland*

Aging is associated with increased oxidative stress and promotes cellular senescence in blood vessels. Despite the irreversible cell cycle arrest and inhibited cell turnover, the senescent cells produce an array of inflammatory and growth-promoting molecules that impact their environment. As such, cell senescence is a key biological process underlying aging-associated diseases including atherosclerosis. The study aimed to characterize the proteome of the human aortic endothelial cells (HAEC) subjected *in vitro* to oxidative stress-induced senescence and to validate the role of selected proteins in a mouse model of atherosclerosis. Senescence of HAEC was induced via exposure to hydrogen peroxide. Mass spectrometry analysis revealed 68 downregulated and 66 upregulated proteins involved in the regulation of the cell cycle, oxidative-stress response and cell–cell interaction. Among the top three-upregulated proteins were involved in antioxidant and anti-inflammatory response heme oxygenase 1 (HMOX1) and growth differentiation factor 15 (GDF-15) – known to be a part of the cellular response to activation of senescence-related pathways, but also to promote cellular senescence in nearby cells. In a mouse model of atherosclerosis based on overexpression of Pcsk9 protein, we observed the earlier formation of atherosclerotic plaques in old (18-month-old) vs. young (3-month-old) mice. This was associated with impaired anti-oxidant response, accumulation of senescent endothelial cells, and increased level of GDF-15 in serum. In summary, our results confirm the important role of age in susceptibility to atherosclerotic plaque formation in a new *Pcsk9* gene transfer-based model of atherosclerosis and indicate potential targets controlling cell senescence in the treatment of this vascular pathology.

Keywords: Senescence, atherosclerosis, endothelium, crosstalk, GDF-15.

P178. Cancer Biology & Oncology

From lost adhesion to tumor spreading – the upregulation of the RNA binding protein IGF2BP1 controls a targetable mesenchymal shift in ovarian cancer

Annekatrin Schott, Nadine Bley, Markus Glaß, Danny Misiak, Marcell Lederer, Tommy Fuchs, Stefan Hüttelmaier
Institute of Molecular Medicine, Division of Molecular Cell Biology, Martin-Luther-University Halle-Wittenberg, Halle, Germany

High-grade serous ovarian carcinomas (HGSCs), the most common histotype of epithelial ovarian cancers, represent a complex malignancy due to its heterogeneity, frequent metastatic spread at diagnosis and its occurring resistances to standard therapies. The upregulation within the mesenchymal C5 subtype of HGSC and the almost mutually exclusive expression within tumors, suggest a pivotal role of the post-translational regulator IGF2BP1 in HGSC. In this study, the IGF2 mRNA-binding protein 1 is shown to promote SRC activation by a previously unknown protein-ligand-induced, RNA-independent mechanism to disassemble adherens junctions. Concomitantly, IGF2BP1 enhances EMT-TF expression and affects AJ assembly through RAC1 to promote EMT and sustain a mesenchymal cell state. The IGF2BP1-driven loss of AJs creates the basis for invasive growth and tumor spreading. IGF2BP1 further regulates several key signaling molecules such as ERK2 and YAP1 RNA-dependently to control a plethora of cellular processes facilitating tumor progression and adaption. Together, this reveals IGF2BP1 as a post-translational regulator and biomarker of interconnected stimulation of SRC and ERK2 signaling in mesenchymal HGSC. Targeting IGF2BP1-positive tumors with a combination of SRC and MEK inhibitors was shown to overcome the IGF2BP1-driven invasive growth *in vitro* and *in vivo*. This provides a rationale for the therapeutic benefit of combinatorial SRC/MEK inhibition in mesenchymal HGSC.

Keywords: IGF2BP1, ovarian cancer, EMT, SRC, ERK.

P179. Immunology, Microbiology & Infectious Diseases

SARS-CoV-2 vaccine development based on the expression of viral proteins in *Komagataella phaffii*

Marta Semkiv[1], Anastasiya Zazulya[1], Roksolana Vasylyshyn[1], Lyubov Fayura[1], Kostyantyn Dmytruk[1], Magdalena Podlacha[2], Grzegorz Wĺgrzyn[2], Andriy Sibirny[1,3]
[1]*Department of Molecular Genetics and Biotechnology, Institute of Cell Biology NAS of Ukraine, Lviv, Ukraine,* [2]*Department of Molecular Biology, University of Gdansk, Gdansk, Poland,* [3]*Department of Microbiology and Molecular Genetics, University of Rzeszow, Rzeszow, Poland*

In 2019, a novel coronavirus emerged, named SARS-CoV-2, causing the pandemic disease COVID-19. Due to the worldwide spread of the disease, COVID-19 has become a serious concern, urging rapid development and worldwide administration of vaccines against SARS-CoV2. We aim to develop a vaccine against SARS-CoV-2 based on expression of the fragments of the spike S protein in the cells of humanized strain of yeast *Komagataella*

phaffii. A collection of transformants was obtained, containing cassettes for expression of fragments of subunits S1 and S2 of the spike glycoprotein of SARS-CoV-2 under the control of the methanol-inducible promoter of the gene *AOX1 K. phaffii*. Among the fragments of the spike protein are the following variants: RBD, NTD-RBD, FP-HR1-HR2 and NTD-RBD-FP-HR1-HR2. Each variant contains at the C-terminus a fibritin protein trimerization domain, an immunological adjuvant, a TEV protease site, and a 6-His sequence. Each of the variants was presented in two versions, with or without the N-terminal secretion signal – α-factor. It was found that fragments of spike protein with N-terminal α-factor are not secreted into the culture medium, though target proteins can be extracted from the insoluble fraction of cell-free extracts with guanidine hydrochloride. We also constructed recombinant strains that produce RBD or FP-HR1-HR2 with flanking linker regions rich in the amino acids glycine and serine. These variants are secreted into the culture medium and can be easily purified. Obtained preparations were tested on mice and Vero cell line and were shown to induce immune reaction and protect against SARS-CoV-2 infection.

Keywords: SARS-CoV-2, vaccine, yeast, *Komagataella phaffii*, recombinant proteins.

P180. Neuroscience, Psychiatry & Mental Health

Novel Ca^{2+}-modulated photoactivatable imaging reveals neuron-astrocyte glutamatergic circuitries within the nucleus accumbens

Irene Serra, Julio Esparza, Cristina Martín-Monteagudo, Laura Delgado, Marta Navarrete
Cajal Institute – CSIC, Madrid, Spain

Astrocytes have been traditionally studied as a homogeneous group; however, recent research has started to evidence their heterogeneity. Our hypothesis is that specialized astrocyte subsets are responsible for the modulation of specific neuronal circuits. We focused on the Nucleus Accumbens (NAc), an important integrator center in which converge different glutamatergic signals coming primarily from the medial prefrontal cortex (mPFC), basolateral amygdala (Amyg), and ventral hippocampus (vHip). In this work, we analyze whether astrocytes establish segregated populations in the NAc with intrinsic properties and functional consequences for the circuit. To this end, we have used optogenetic manipulations to afferent-specific synaptic stimulation to the NAc combined with a new adapted technique (calcium-modulated photoactivatable ratiometric integrator under GFAP promoter, $CaMPARI_{GFAP,}$) to specifically dissect the active astrocyte circuits with spatio-temporal precision. We demonstrate that NAc astrocytes show pathway-specific interactions with the glutamatergic afferents and that this activity does not correlate with the glutamatergic innervation patterns, suggesting unexpected astrocytic connectivity, i.e. activation of precise astrocytic populations in response to specific glutamatergic inputs. Moreover, the spatial activation of these astrocytic networks is not defined by alterations in astrocyte density or uneven expression of mGluR5. Finally, we show that different sub-populations of astrocytes in both NAc regions receive and integrate signals arising from all the excitatory afferents.

Our work reveals different neuron-astrocyte glutamatergic circuits in the NAc, showing pathway-specific astrocytic responses mediated by mGluR5. These results highlight the astrocytic contribution to NAc functionality, providing a potential explanation to comprehend how NAc integrates information from multiple glutamatergic inputs.

Keywords: Astrocyte-neuron interactions, circuits, nucleus accumbens, optogenetics.

P181. Cancer Biology & Oncology

Assessment of the synergistic effect of vitamin C and fasting-mimicking diet (FMD) on KRAS-positive carcinomas

Anastasiya Shmahala[1,2], Valter D. Longo[1]
[1]*IFOM ETS – The AIRC Institute of Molecular Oncology, Milan, Italy,* [2]*European School of Molecular Medicine (SEMM), Milan, Italy*

Pharmacological doses of Vitamin C (ascorbate) represent a potential anticancer therapy based on different pre-clinical and clinical studies. It exhibits toxicity towards specific cancer cell subtypes while having a protective effect on normal cell lines. The mechanism of action of ascorbate remains controversial, but most studies rely on the reactive oxygen species (ROS) and labile iron pool (LIP). Recent studies show that different carcinomas displaying mutations in KRAS oncogene are highly susceptible to ascorbate. For this reason, vitamin C might represent a potential weapon against several aggressive cancers, including undruggable KRAS mutated tumors. In recent years, our research group has demonstrated that fasting or fasting-mimicking diet (FMD) are able to delay tumor progression and sensitize a wide range of tumor types to the toxic effect of chemotherapy and other therapies. Based on these premises, the general aim of the current research project is the identification of highly effective low-toxicity treatments in the oncological field. Furthermore, given the already published *in vitro* and *in vivo* data on KRAS-positive colorectal carcinomas treated with FMD and ascorbate, we want to investigate the effect of this combined therapy on other KRAS-positive tumors, such as pancreatic and lung carcinomas. Moreover, FMD is able to activate specific starvation-induced escape mechanisms in triple-negative breast cancer (TNBC) cells that can be then targeted by specific drugs. Therefore, the ultimate purpose is to identify those escape mechanisms that allow cells resistant to FMD and ascorbate treatments to survive, establishing a low-toxicity or non-toxic targeted treatment.

Keywords: FMD, cancer, ascorbate, KRAS, ROS.

P182. Clinical Research, Translational Biomedicine & Personalised Medicine

Vitamin D increases CD38 expression in multiple myeloma cells and augments the efficacy of daratumumab

Belén Sierro-Martínez, Beatriz Guijarro-Albaladejo, Clara Beatriz García-Calderón, Javier Rojas-Martínez, Virginia Escamilla-Gómez, Laura Ortega-Pérez, Maribel Lara-Chica, Paola Hernández-Díaz, María Dolores De La Rosa-Garrido, Juan Luis Reguera-Ortega, Javier Delgado-Serrano, Estrella Carrillo-Cruz, Jose Antonio Pérez-Simón, Estefanía García-Guerrero

[1]*Instituto de Biomedicina de Sevilla (IBiS)/HUVR/CSIC/US, Seville, Spain,* [2]*UGC de Hematología, Hospital Universitario Virgen del Rocío, Seville, Spain,* [3]*Consejo Superior de Investigaciones Científicas (CSIC), Seville, Spain,* [4]*Centro de Investigación Biomédica en Red Cáncer (CIBERONC), Seville, Spain,* [5]*Universidad de Sevilla, Seville, Spain*

Introduction: Multiple myeloma (MM) consists of the abnormal proliferation of plasma cells. Although its treatment has improved in the last decade it is still considered an incurable disease. Daratumumab is an anti-CD38 monoclonal antibody used in the treatment of MM due to the high levels of expression of CD38 in plasma cells. In the present work, the effect of vitamin D in CD38 expression in multiple myeloma cells and its combination with Daratumumab was studied. *Methods*: Tumor cell lines and primary myeloma cells from 6 MM patients were treated with vitamin D (0.1 and 1 nM). Antibody-dependent cytotoxicity (ADCC) assays were performed against myeloma cells treated with vitamin D in the presence of mononuclear cells and Daratumumab (1, 10, 50 μg/mL) or isotype control. *Results*: A significant increase in CD38 expression was observed in three multiple myeloma cell lines (MM1.S, $n = 13$, OPM2, $n = 7$, H929, $n = 6$). Withdrawal of vitamin D produced a reduction in the expression of CD38 to its basal levels, which increased again after 48 h of re-exposure to treatment ($n = 3$). This increase was confirmed in primary myeloma cells from patients ($n = 6$). Activated T lymphocytes from peripheral blood of healthy donors were treated with vitamin D and an increase in CD38 levels was observed. A synergistic effect is observed in the combination of vitamin D and Daratumumab in myeloma cell lines (MM1.S and OPM2). *Conclusions*: Vitamin D increases CD38 expression levels in multiple myeloma and has a synergistic effect with Daratumumab in ADCC assays.

Keywords: Vitamin D, CD38, multiple myeloma, daratumumab.

P183. Pharmacology, Toxicology & Nutrition

Grape pomace upcycling for cosmeceutical application: green extraction and biological activity characterization in 3D human keratinocytes

Alessia Silla[1], Angela Punzo[2], Emanuele Porru[2], Rossana Comito[2], Paola Galletti[2,3], Emilio Tagliavini[2,3], Chiara Samorì[2,3], Patrizia Simoni[4], Aldo Roda[2,5], Cristiana Caliceti[5,6], Silvana Hrelia[1]

[1]*Department for Life Quality Studies, University of Bologna, Bologna, Italy,* [2]*Department of Chemistry "Giacomo Ciamician", University of Bologna, Bologna, Italy,* [3]*Interdepartmental Centre for Renewable Sources, Environment, Sea and Energy, University of Bologna, Bologna, Italy,* [4]*Department of Medical and Surgical Sciences, Alma Mater Studiorum – University of Bologna, Bologna, Italy,* [5]*INBB, Biostructures and Biosystems National Institute, Rome, Rome, Italy,* [6]*Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum – University of Bologna, Bologna, Italy*

Food waste is a critical global issue due to its environmental and economic impact, so the development of new functional applications for its upcycling is highly demanded. The winemaking process leads to an incomplete extraction of high-value compounds, leaving the pomace still rich in bioactive molecules, such as polyphenols. Therefore, this study aims at validating sustainable routes towards the extraction and further valorisation of these polyphenols, mainly for cosmeceutical applications. Phenolic compounds were extracted from red wine pomace using three different natural deep eutectic solvent (NaDES) mixtures: betaine-citric acid (BET-CA), betaine-urea (BET-U), and betaine-ethylene glycol (BET-EG). The polyphenol profile determined by HPLC-MS/MS analysis showed a similar malvidin concentration (51–56 μg mL-1) in each formulation, while BET-CA exhibited the highest skin permeation of malvidin in Franz cells, so it was further investigated to evaluate its biological effects in highly predictive human 3D keratinocytes (HaCat spheroids). Spectrophotometric bioassays ensured the safety of BET-CA treatment (malvidin range 0.05–1.1 μg mL^{-1}) for 24 h in HaCat. Antioxidant and anti-inflammatory activities were analyzed by a chemiluminescent bioassay for intracellular H_2O_2 detection and ELISA assay for interleukin (IL)-8 release, respectively. 24 h treatment of BET-CA showed a good antioxidant activity (IC_{50} 0.15 ± 0.02 μg mL^{-1} in malvidin) and significantly decreased ($P < 0.001$) the release of the pro-inflammatory cytokine IL-8, in HaCat injured with 25 μM menadione. Thus, the BET-CA formulation could be used as a cosmetic ingredient to reduce oxidative stress and inflammation, the main causes of skin aging.

Keywords: Red grape pomace, natural deep eutectic solvents, malvidin, skin permeation, human 3D keratinocytes, oxidative stress, inflammation.

P185. Cancer Biology & Oncology

Pigmentation level of melanoma cells and their proliferation

Justyna Sopel[1], Aleksandra Bienia[1], Anna Koziťska[1], Maŧgorzata Szczygieŧ[1], Michaŧ Sarna[2], Anna Markiewicz[3], Bożena Romanowska-Dixon[3], Martyna Elas[1]
[1]*Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology Jagiellonian University, Cracow, Poland,* [2]*Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology Jagiellonian University, Cracow, Poland,* [3]*Chair of Ophthalmology, Faculty of Medicine, Jagiellonian University Medical College and Ophthalmology and Ocular Oncology Clinic, University Hospital, Cracow, Poland*

Melanoma is an aggressive cancer metastasizing to various organs, including the liver and lungs. One of the characteristics of melanoma is the ability to synthesize melanin. In *in vitro* cultures, the melanin synthesis process by melanoma cells seems to be disrupted. Our aim is to present how melanoma cell pigmentation influences cell proliferation. In our research, we used amelanotic (non-pigmented) and melanotic (capable of pigmentation) cell lines. Melanoma cells were grown in a medium stimulating (addition of L-tyrosine) and not stimulating pigmentation. Under both conditions, a cell proliferation assay was performed. Spheroids were obtained by the hanging drop method from stimulated and unstimulated cells. The CAM (chick chorioallantoic membrane) model was used as the *in vivo* model. Melanoma cells were implanted on the chorioallantoic membrane. Tumor growth was observed over the next 6 days. In the models used, the markers of pigmentation and proliferation were assayed.
As expected, *in vitro* studies show that cells stimulated to pigmentation have inhibited proliferation. In the 3D model, a reduction in size was observed. The spheroids of various melanoma lines show different macroscopic appearance and degree of compaction. There are 3 patterns of cell aggregation: loose aggregate with clusters, spheroid with local areas of compaction, and fully compact spheroid. In contrast, in CAM models, we observe faster growth of tumor cells capable of pigmentation, along with a larger necrotic area and hemorrhagic necrosis. *This research was supported in part by CMUJ nr K/ZDS/007190 and NCN UMO-2020/37/B/NZ4/01313.*

Keywords: Melanoma, pigmentation, proliferation, cancer, spheroid.

P186. Neuroscience, Psychiatry & Mental Health

Organotypic hippocampal cultures (OHC) as a valuable tool to investigate the influence of cell-based therapies on injured brain tissue – optimisation and validation of the method

Martyna Strzelec, Jan Detka, Maŧgorzata Sobociťska, Patrycja Mieszczak, Marta Kot, Marcin Majka
Department of Transplantation, Institute of Pediatrics, Jagiellonian University Medical College, Cracow, Poland

Human Mesenchymal Stem Cell (hMSCs)-based therapies have attracted much attention in various aspects of regenerative medicine, showing high effectiveness in the treatment of patients with injured nervous tissue (e.g. in epilepsy, amyothopic lateral sclerosis, spinal cord injury). The influence of the hMSC secretome on damaged nervous tissue is among others assigned to their proneurogenic and anti-inflammatory properties. Organotypic Hippocampal Cultures (OHC) are a potentially useful tool to exhaustively investigate the mechanisms of hMSC secretome action, since they maintain tissue integrity, morphology and mutual cell interactions. In this study we provide optimisation of the OHC method as a tool to investigate cell-based therapies on injured brain tissue, as we also supply a strict selection of hMSC lines and a scheme of OHC medium hMSC conditioning. Experiments were performed by comparison of hMSC line secretomes under different conditions using the Luminex multiplex assay. Analysis pointed at optimal human donor and the most beneficial hMSC culture conditions. The optimisation of OHC was performed by the assessment of OHC morphology, followed by colorimetric cell viability tests and the measurement of chosen pro-inflammatory factors in the culture medium and OHC lysates. We achieved the most advantageous OHC medium composition and optimal scheme of OHC medium conditioning. Our data indicate that the OHC is a valuable tool to investigate the efficacy of stem cell-based therapies on injured mice brain tissue in preclinical studies as a method which maintains the native cytoarchitecture of studied tissue.
Project supported by National Scientific Center in Poland 2018/31/B/NZ3/01879.

Keywords: Organotypic hippocampal cultures, human mesenchymal stem cells, central nervous system, cell-based therapies, regenerative medicine.

P188. Computational Biology, Bioinformatics & Artificial Intelligence

Coupling cell proliferation and tissue organization in sea star embryos

Antonio Tagua[1], Vanessa Barone[2], Jesús A. Andrés San Román[1], Pedro Gómez Gálvez[1], Deirdre Lyons[2], Luis M. Escudero Cuadrado[1,3]
[1]*Instituto de Biomedicina de Sevilla (IBiS)/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla and Departamento de Biología Celular, Universidad de Sevilla, Seville, Spain,* [2]*Scripps Institution of Oceanography, La Jolla, CA, USA,* [3]*Biomedical Network Research Centre on Neurodegenerative Diseases (CIBERNED), Madrid, Spain*

The biophysical properties of epithelial cells determine how cell proliferation and cell packing coordinates drive morphogenesis. Improvements in microscopy techniques and the development of modeling and quantitative methods have enabled better interpretation of the bio-mechanical events controlling cell and tissue shape during animal development. In that regard, apico-basal cell intercalations (scutoids) have been described as essential dynamic changes to minimize line-tension energy in curved epithelia. Here, we will analyze how the presence of scutoids influence the embryonic development where the cells are in constant division while they change their shape. For that purpose, we have analyzed the early development of the bat star *Patiria miniata*, a suitable model system to study the dynamics of epithelial growth and organization. Sea star embryos present a holoblastic cleavage, which divide them into two halves along the animal-vegetal axis.

We have compared the differences between the two regions in terms of the connection between tissue organization and proliferation. We will show data about: 1) how cell–cell contacts and apico-basal intercalations correlate by analyzing high resolution live imaging (3D + time); 2) how physical constraints affect the packing of the tissue and the relation between scutoids and cell proliferation. In conclusion, our approach sheds light on the biophysical mechanisms that drive embryonic development by characterizing two fundamental processes of epithelial morphogenesis, such as cell shape remodeling (i.e. development of apico-basal intercalation) and cell proliferation.

Keywords: Proliferation, cell packing, scutoids, embryonic development.

P190. Cellular & Molecular Biology

Tyr48 phosphorylation of cytochrome *c* regulates its binding to nuclear histone chaperones

Joaquín Tamargo-Azpilicueta, Jonathan Martínez-Fábregas, Miguel Ángel De La Rosa, Irene Díaz-Moreno
Instituto de Investigaciones Químicas (IIQ), Centro de Investigaciones Científicas Isla de la Cartuja (cicCartuja), Universidad de Sevilla – CSIC, Seville, Spain

Post-translational modifications (PTMs) of proteins are ubiquitous processes present in all life kingdoms, involved in the regulation of protein stability, subcellular localization, and protein activity. Among them, phosphorylation consists of the reversible addition of a phosphate group via ester bonds to the lateral chains of tyrosine (Tyr), serine (Ser) or threonine (Thr) residues in proteins. In fact, phosphorylation at several Tyr residues at positions 48 and 97 of cytochrome *c* (C*c*) — a key component of the mitochondrial electron transport chain — downregulates C*c* electron transfer activity and hinders Cc-mediated apoptosis. Notwithstanding the efforts put into describing the role of Tyr-phosphorylated C*c* in the cytosolic and mitochondrial context, how phosphorylation of the hemeprotein works in the nucleus remains elusive. In this work, through isothermal titration calorimetry (ITC) experiments using Tyr-to-pCMF (p-carboxymethyl-l-phenylalanine) mutants, we *in vitro* characterize the modulation of complex formed between the hemeprotein and the histone chaperones SET/template-activating factor-Iβ (SET/TAF-Iβ) and nucleophosmin (NPM1), revealing that C*c* phosphorylation leads to: (i) a lower affinity of Tyr-phosphomimetic forms of C*c* towards SET/TAF-Iβ and NPM1 and (ii) a loss of binding cooperativity when Y48pCMF C*c* interacts with the chaperone. Based on these findings, we propose that C*c* Tyr phosphorylation constitutes a pivotal PTM in modulating the function of nuclear histone chaperones.

Keywords: Apoptosis, biophysics, cytochrome c, DNA damage response, histone chaperones, post-translational modifications, protein–protein interaction.

P191. Immunology, Microbiology & Infectious Diseases

Consumption of traditional plant-based diets and fermented banana beverages induced positive effects on the immune and metabolic systems in healthy adult men living in northern Tanzania

Godfrey Temba[1], Tal Pecht[2], Nadira Vadaq[3], Vesla Kullaya[4], Blandina Mmbaga[4], Leo Joosten[3], Reginald Kavishe[1], Andre Van Der Ven[3], Mihai Netea[3], Quirijn De Mast[3]
[1]Department of Medical Biochemistry and Molecular Biology, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania, [2]Department for Genomics and Immunoregulation, Life & Medical Sciences (LIMES) Institute, University of Bonn, Bonn, Germany, [3]Department of Internal Medicine, Radboud University Medical Center, Radboud Centre for Infectious Diseases (RCI) and Radboud Institute for Health Sciences (RIHS), Nijmegen, The Netherlands, [4]Kilimanjaro Clinical Research Institute, Kilimanjaro Christian Medical Center, Moshi, Tanzania

The effects of normal variation in the diet on the immune system remain inconclusive. Many communities in sub-Saharan Africa (SSA) are undergoing rapid urbanization with a transition in lifestyle and diet. We aimed to determine the immune effects of a short dietary switch from a traditional Tanzanian diet to a "globalized" diet and *vice versa*. A proof-of-concept intervention was performed on young healthy men in Northern Tanzania living in rural areas and consuming a predominantly traditional diet and or traditional fermented banana beverage or living in an urban area and consuming a "globalized" diet. Their diets were switched for 2 weeks. The study also included five controls who remained on their usual diet. Blood samples were collected at baseline and at weeks 2 and 6 for activation phenotype of leukocyte subsets, whole blood transcriptome, untargeted plasma metabolome, plasma proteome (inflammatory and cardiometabolic proteins), and whole blood cytokine responses. The fermented banana beverage had the most pronounced immunomodulatory effects. It reduced the activation state of circulating leukocytes, increased transcriptional pathways associated with humoral responses and complement activation, while decreasing the transcriptional pathways associated with cytokine production and increased IL-10 responses to pathogens. Targeted plasma proteomics (92 inflammation-related proteins and 92 cardiometabolic proteins) confirmed the anti-inflammatory effects of the fermented beverage and showed that a switch to a traditional diet decreased chemokines and metabolic regulators. In contrast, a switch to a high-fat, high-sugar 'globalized' diet increased pro-inflammatory and pro-atherogenic chemokines. This study shows how important it is to keep important parts of traditional African diets so that NCDs do not spread so quickly in SSA.

Keywords: Fermented banana beverage, plant-based diet, immunity, Tanzania.

P192. Cellular & Molecular Biology

Exploring DNA damage response in repair-defective neurons: effects on cell metabolism and role of nitric oxide

Federica Terraneo
IFOM ETS – The AIRC Institute of Molecular Oncology, Milan, Italy

DNA damage is a common hallmark of different pathological conditions, including cancer, neurodegeneration and genome maintenance syndromes, as well as natural aging. Persistent transcription stalling DNA damage causes metabolic redesign and altered redox balance in hepatocytes and fibroblasts, but the presence and the effect of this condition in other cell types have not yet been extensively investigated. Moreover, there is interesting evidence of crosstalk between DNA damage and inflammation. Nitric oxide (NO, an inflammatory signaling molecule) can form reactive species and damage biological molecules through oxidation and nitrosylation. Previous studies correlated NO to activation of the DNA damage response, but at the same time to inhibition of the repair pathways. The results, however, vary between cell type and dose and duration of the stimulus, and mechanisms responsible for these effects have not yet been fully clarified.
The aim of this project is to understand the interplay between DNA damage and repair, metabolism, and nitric oxide, specifically in neurons. Expression of shRNA against Ercc1 (a key enzyme in the nucleotide excision repair pathway, NER) was induced by IPTG in NGN cells, and repair capacity and metabolic output were deeply investigated in this NER-deficient context. In addition, the influence of NO donors on the DNA damage response (also in the presence of NO scavenging or ATM inhibition) was tested. This study has the potential to unravel the complex relationship between DNA damage and inflammation, and its prospective causative role in many related pathologies.

Keywords: Neurons, DNA damage and repair, cell metabolism, nitric oxide, iPSC.

P193. Pharmacology, Toxicology & Nutrition

4-Vinylcyclohexene diepoxide induced hepatotoxicity and nephrotoxicity in male and female Wistar rats: a mechanistic approach

Pere-Ebi Toloyai[1,2], Amos Abolaji[1], Titilope Odeleye[1], Susan Akinduro[1], Joao Batista Rocha[3], Ebeneezer Farombi[1]
[1]Molecular Drug Metabolism and Toxicology Laboratories, Department of Biochemistry, University of Ibadan, Ibadan, Nigeria, [2]Department of Medical Biochemistry, Delta State University, Abraka, Nigeria, [3]Departamento de Bioquimica e Biologia Molecular, Bioquímica Toxicológica, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, Brazil

4-Vinylcyclohexene diepoxide (VCD) is an occupational chemical that destroys ovarian and small pre-antral follicles in rodents. This study investigated the influence of VCD on selected hepatic and renal markers of oxidative stress and inflammation in both sexes of Wistar rats. Forty male and forty female rats were randomly distributed into four groups of ten rats per group, and orally administered VCD for 28 days. The control groups of both sexes of rats received corn oil only, while the remaining groups received VCD at 100, 250 and 500 mg/kg BW respectively. Thereafter, biomarkers of hepatic and renal oxidative damage, inflammation and immunohistochemical expressions of iNOS, COX-2, caspase-9 and caspase-3 were evaluated. The results revealed that VCD elevated markers of liver and kidney functions, oxidative damage and inflammation, and disrupted the antioxidant homeostasis of the rats ($P < 0.05$). Also, VCD enhanced the immunohistochemical expressions of iNOS, COX-2, caspase-9 and caspase-3 in the liver of the rats. Our data therefore implies that VCD induced toxicity in the liver and kidney of rats via the combined impacts of oxidative damage and inflammation.

Keywords: 4-Vinylcyclohexene diepoxide, inflammation, hepatic and renal oxidative damage, immunohistochemistry.

P194. Obesity, Diabetes & Other diseases

Role of Galectin-1 in adipose tissue development and adipocyte metabolism

Patricia Torres-Gérica[1], Ruth Álvarez[1], Johan A Fernø[2], Gunnar Mellgren[2], Simon N Dankel[2], Manuel Fresno[1]
[1]Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Madrid, Spain, [2]Hormone Laboratory, Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway

Galectins are a family of β-galactoside receptors, which have been described in many physiological and pathological processes. Among them, galectin 1 (GAL1) has been described in cancer or differentiation of the hematopoietic lineage. However, although recent studies show a link between GAL1 and adipogenesis, the results are contradictory, so its role has yet to be elucidated. We sought to clarify the role of GAL1 in adipose tissue at either the level of adipogenesis or the use and storage of fat, as well as its contribution to the development of obesity. To achieve this, the expression of *Lgals1* gene in adipose tissue, as well as the effect of *Lgals1* gene knockout in weight gain and adipocyte size were studied, together with the expression of different adipocyte markers. Furthermore, using cell lines for adipocyte differentiation and their derivative *Lgals1* knockdown cell lines, we studied the role of GAL1 during differentiation and adipocyte metabolism *in vitro*. We found that GAL1 is highly expressed in adipose tissue, and its expression in White Adipose Tissue increases with age and in response to nutritional challenge. Furthermore, the absence of GAL1 resulted in lower body weight and adiposity, as well as lower glucose levels. This was associated with impaired fatty acid uptake and an increase in brown adipose tissue markers in *Lgals1*$^{-/-}$ mice. This effect was also observed in *Lgals1* knockdown cells when differentiated *in vitro*. These results implicate GAL1 in adipocyte metabolism and energy homeostasis and, consequently, in the onset of obesity.

Keywords: Galectin-1, adipogenesis, fatty acid, lipid.

P196. Cancer Biology & Oncology

The significance of cancer specific PFK-II (PFKFB3 and PFKFB4) isoenzyme inhibition in overcoming resistance to BRAF inhibitors in malignant melanoma therapy

Sonia E. Trojan[1], Patrycja Kaczara[2], Justyna Totoń-Żurańska[3], Paulina Dudzik[1], Barbara Ostrowska[1], Ewelina Dratkiewicz[4,5], Piotr Laidler[1], Kinga A. Kocemba-Pilarczyk[1]
[1]Faculty of Medicine, Chair of Medical Biochemistry, Jagiellonian University Medical College, Krakow, Poland, [2]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland, [3]Jagiellonian University Medical College, Center for Medical Genomics-OMICRON, Krakow, Poland, [4]Department of Cell Pathology, Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland, [5]Science for Life Laboratory, Division of Genome Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

The fact that over 50% of melanomas harbor $BRAF^{V600E}$ activating mutation – the most common molecular disorder observed in melanoma patients – which promotes survival and proliferation and also accelerates glucose metabolism, deserves particular attention. Although treatment targeting mutated BRAF has been implemented for several years, the emerging resistance to applied inhibitors is the main obstacle to its effectiveness. Recent data show that glycolysis inhibition can induce cell death in BRAF inhibitor (BRAFi)-resistant cells. Moreover, it has been recently reported that the glycolytic pathway in cancer cells can be targeted by inhibition of cancer-specific isoenzymes (PFKFB3/PFKFB4) of phosphofructokinase II (PFK-II). Thus, the research hypothesis is that cancer-specific isoenzymes of PFK-II (PFKFB3/4), may be a novel target for anti-melanoma therapy affecting both growth and BRAFi resistance of melanoma cells. In the first step, the expression of PFKFB3/4 was confirmed in both wild type and resistant melanoma cell lines. Then, the cells were exposed to specific PFKFB3 and PFKFB4 inhibitors, and functional analysis of proliferation, survival, apoptosis and metabolic activity was performed. The significant response of chosen cell lines confirmed the specificity of the inhibitor applied in our study. The PFKFB3 and PFKFB4 inhibitors exhibit anti-tumor activity *in vitro*, regardless of the BRAF inhibitor resistance status. Of note, we observed a dose-dependent response for PFKFB3 inhibitor, whereas the cells exhibit slightly lower sensitivity to the PFKFB4 inhibitor. Our study suggests that currently available anti-melanoma therapeutic strategies may significantly benefit from agents targeting PFKFB3 and PFKFB4 activity.

Keywords: Melanoma, phosphofructokinase-II, BRAFi resistance, glycolysis.

P198. Pharmacology, Toxicology & Nutrition

Study of drug's effectiveness in NOMO1 knockout cell lines

Jorge Úbeda
Universidad de Salamanca, Salamanca, Spain

Colorectal cancer (CRC) is one of the tumors with the highest incidence and mortality in the world. Depending on the age of diagnosis, it can be classified into Early Onset Colorectal Cancer (EOCRC < 50 years old) and Late Onset Colorectal Cancer (LOCRC). Both present different clinic and molecular characteristics. For instance, the deletion of the gene *NOMO1* is more frequent in the first type than the second. One of the objectives for the *NOMO1* characterization project in EOCRC is to determine if its absence affects cell sensitivity to drugs used in clinical treatments, which could broaden the number of therapies available. In this work, we studied the effect of 5-Fluorouracil, Cisplatin, Irinotecan and Oxaliplatin on cell growth, viability and cell cycle of wild-type (WT) and knockout (KO) cell lines for *NOMO1*. Thus, we used proliferation and apoptosis assays as well as propidium iodide DNA staining. The results obtained show that the drugs inhibit cellular proliferation, as well as inducing cell death and affecting the cell cycle phases in the different lines studied (two CRC cell lines, HCT116 and HT29, and a mesenchymal one, HS5), with HT29 being the most resistant cell line to all the drugs used. We did not find significant differences in the sensitivity between WT and *NOMO1* KO clones.

Keywords: Drugs, cells, viability.

P199. Cancer Biology & Oncology

Calcineurin/NFAT signaling controls PDGF-activated Akt/mTOR pathway in MCF7 cells

Harun Un[1], Elif Cadirci[2], Zekai Halici[3], Yasin Bayir[4]
[1]Department of Biochemistry, Faculty of Pharmacy, Ağrı İbrahim Çeçen University, Ağrı, Turkey, [2]Department of Pharmacology, Faculty of Medicine, Atatürk University, Erzurum, Turkey, [3]Clinical Research, Development and Design Application and Research Center, Atatürk University, Erzurum, Turkey, [4]Department of Biochemistry, Faculty of Pharmacy, Atatürk University, Erzurum, Turkey

Cancer cells use many intracellular signaling pathways to progress the system. The Akt (protein kinase B)/mTOR (mammalian target of rapamycin) signaling pathway plays an important role in breast cancer. The Calcineurin/NFAT (nuclear factor of activated T cell) pathway also plays a role in cancer. The possible relations between the calcineurin/NFAT and Akt/mTOR pathways are not fully known. We found that calcineurin inhibition with FK506 suppressed the Akt/mTOR pathway and Akt inhibitor suppressed the calcineurin/NFAT pathway in PDGF (platelet derived growth factor)-activated MCF7 cells. This cross downregulation caused synergistic effects on cell proliferation and apoptosis. PDGF activated calcineurin/NFAT and Akt/mTOR pathways, and thus increased breast cancer cell proliferation and decreased apoptosis. Combined treatment with FK506 and Akt inhibitors decreased cell proliferation and increased apoptosis of cancer cells. Combined treatment also increased Bax, Caspase 9 and p53 gene expression, while NF-kappa beta, S6 ribosomal protein and Survivin levels were decreased. Importantly, decreased S6 protein and Survivin partially slowed down proliferation, thereby revealing these proteins as key factors downstream of Akt and calcineurin inhibition. We therefore propose that targeting the calcineurin/NFAT and Akt/mTOR pathways could have potential for breast cancer treatment.

Keywords: Calcineurin, NFAT, Akt, mTOR, MCF7.

P200. Cancer Biology & Oncology

A pharmacogenetic approach to identify novel combination treatments for AML therapy

Riccardo Valzelli, Fabio Santoro, Emanuel Soda, Elena Ceccacci, Saverio Minucci
European Institute of Oncology (IEO), Milan, Italy

Acute Myeloid Leukemia (AML) is consistently associated with epigenetic alterations. These variations play a crucial role in determination and maintenance of the leukemic phenotype. Numerous epigenetic drugs have been approved for clinical use, and others are in development. However, clinical responses have not yet been consistent, and therapies urgently need optimization based on innovative molecular findings. Interestingly, environmental changes such as nutrient availability represent significant drivers of epigenetic changes, tumor cell metabolism, and signaling pathways. Accordingly, we plan to identify, through the use of genetic dropout screens in the context of epigenetic and metabolic drug treatments, novel targets for further mechanistical and biological characterization. Hence, the overall aim of this project is to identify novel combination treatments for AML therapy. In particular, we plan to perform *in vitro* screening (initially using cell lines, and then, when possible, AML patient samples), by evaluating the depletion of cells infected with the pooled genetic library upon drug treatment. Specifically, we plan to inhibit the oxidative phosphorylation machinery, inhibit glycolysis, inhibit Lysine Demethylase 1A (LSD1) or other epigenetic targets, and utilize apoptosis-inducing drugs. Moreover, we plan to carry out *in vivo* screens using reduced complexity libraries by maintaining recipient mice in standard diet conditions, or subjecting them to intermittant fasting, in the presence or in the absence of the drugs considered *in vitro*. In conclusion, we expect this knowledge to generate novel combination treatments based on epigenetic and metabolic drugs/currently available therapies, which can be advanced to clinical validation.

Keywords: AML, synthetic lethality, screening, epigenetics, metabolism.

P201. Cellular & Molecular Biology

Unravelling the effect of DNA damage on aging-related cellular phenotypes

Jacinta van de Grint, Joris Pothof
Erasmus University Medical Center (Erasmus MC), Rotterdam, The Netherlands

Aging leads to aging-related cellular phenotypes within tissues, such as polyploidy, senescence and lower transcription levels. Using the newly developed FUNseq technique (FUNctional single-cell sequencing), it is possible to perform selection and isolation of cells with an aging-related phenotype for single-cell RNA sequencing. We isolated and cultured primary cells from young and old mice, with or without a genetic defect leading to accelerated aging. Using live-cell dyes, we visualized aging-related phenotypes and we will sequence mRNA of single, sorted cells. This will provide us with the expression profiles of specific aging-related phenotypes, which is superior to bulk single-cell sequencing, where phenotypes are undistinguished. Furthermore, this will allow us to pinpoint specific cellular pathways to aging phenotypes. In another project, we are analyzing the role of SNPs in DNA repair genes in human aging. A large GWAS for the age at natural menopause (ANM) in ~200.000 women discovered a list of SNPs that correlate with a young ANM. Interestingly, many of these SNPs are located in DNA repair genes, suggesting a link between DNA repair capacity and reproductive lifespan. We calculated polygenic risk scores based on these SNPs and analyzed DNA damage repair in PBMCs from donor blood with a high, average or low polygenic risk score. Together, these studies will further unravel the role of DNA damage in the aging process.

Keywords: Aging, DNA damage, DNA repair, cellular phenotypes, single-cell RNA sequencing.

P203. Cellular & Molecular Biology

L-lactic acid production from glucose and xylose with engineered *Ogataea polymorpha* yeast strains

Roksolana Vasylyshyn[1], Aksyniia Tsaruk[1], Andriy Sibirny[1,2]
[1]*Department of Molecular Genetics and Biotechnology, Institute of Cell Biology of the National Academy of Sciences of Ukraine, Lviv, Ukraine,* [2]*Department of Microbiology and Biotechnology, University of Rzeszow, Rzeszow, Poland*

L-lactic acid is a versatile industrial product widely used in the medical and pharmaceutical industries and also necessary for producing renewable bio-based plastics important in transplantology. To meet the growing demand, sustainable and environmentally-compatible lactic acid production from renewable resources, ideally from lignocellulosic waste streams, must be facilitated. This necessitates a fermentation organism, which is robust, thermotolerant, pH stable, and capable of efficient conversion of the two main lignocellulosic sugars: glucose and xylose. Because currently applied lactic acid-producing organisms (e.g., lactic acid bacteria) do not meet these requirements, our research has focused on engineering of the methylotrophic yeast *Ogataea polymorpha*. In this study, the alcohol fermentation pathway of wild-type strain and the best ethanol producer (BEP/*cat8*Δ) strain of the yeast *O. polymorpha* were successfully altered by the introduction of lactate dehydrogenase gene (LDH) derived from filamentous fungus *Rhizopus oryzae* was selected. The effect of carbon source, aeration and presence of neutralizing agent on the production of L-lactic acid was investigated. Our results have shown that cell aeration has a positive impact on lactic acid production, in that lactate production requires a lot of ATP because it is consumed largely to energize the efflux of lactic acid out of the cell. In addition, lactic acid accumulation causes cellular toxicity, making $CaCO_3$ neutralization of the medium during fermentation necessary for efficient L-lactic acid production. Engineered *O. polymorpha* strains demonstrated a significant increase in lactate production on a minimal medium with 10% glucose or with 10% xylose.

Keywords: L-lactic acid, yeast, *Ogataea polymorpha*.

P204. Cancer Biology & Oncology

Analysis of the target cell(s) in acute myeloid leukemia (AML)

Emanuela Villa, Elena Ceccacci, Saverio Minucci
European Institute of Oncology (IEO), Milan, Italy

PML-RAR is a leukemogenic protein responsible for the development of Acute Promyelocytic Leukemia (APL). We have investigated the target cell (TC) of APL in mice, triggering the expression of PML-RAR at different stages of hematopoietic differentiation. Hematopoietic stem cells, and progenitors devoid of self-renewal, can both be targeted by the fusion protein and give rise to identical leukemia. In the pre-leukemic phase preceding overt disease, transplantation of progenitor cells expressing PML-RAR in lethally-irradiated recipient mice led to the reconstitution of all normal hematopoietic lineages – including stem cells – demonstrating that they can functionally substitute for normal stem cells. Expression of PML-RAR leads, therefore, to re-programming of progenitor cells into a stem cell phenotype, accompanied by transcriptional induction of an adult stem cell signature, distinct from that observed in frankly established leukemic stem cells. To further investigate the effect of PML-RAR in leukemic cells, we performed single-cell RNA sequencing and a matched single-cell ATAC of normal and PML-RAR expressing cells, sorted into fractions enriched of HSC (Lin-Sca1 + cKit+), CMP (Lin-Sca1-cKit+) and LSK- (Lin-Sca1-cKit-) cells. The analysis revealed that the presence of PML-RAR induced changes in the chromatin state at less differentiated stages of the hematopoietic development, while also impacting the transcriptional landscape at later stages, resulting in a clonal expansion of myeloid progenitor lineage subpopulations. The subpopulations expanded due to PML-RAR expression in each target cell shared a great level of transcriptional similarity, hallmarked by cell cycle-related genes.

Keywords: PMLRAR, APL, scRNAseq, scATACseq.

P205. Cellular & Molecular Biology

Investigating a mechanism for eccDNA formation at telomeres and other repeats

Elia Zanella[1,2], Ylli Doksani[1]
[1]*IFOM ETS – The AIRC Institute of Molecular Oncology, Milan, Italy,* [2]*University of Milan, Milan, Italy*

Telomeres shorten due to the end replication problem, but telomere erosion is substantially affected by DNA damage. Our lab recently discovered that single-stranded DNA damage at telomeric repeats (i.e. nicks and gaps) induces the formation of intramolecular loops (i-loops) [1]. I-loops likely form due to the higher probability of homologous strand exchange when DNA damage occurs in the context of a repetitive sequence. Importantly, we found that damage-induced i-loops can be excised as extrachromosomal telomeric circles (t-circles). These results suggest a molecular mechanism that links telomeric damage with telomere shortening and generation of t- circles. We hypothesize that this mechanism is not unique to telomeres, but rather it represents a general mechanism of extrachromosomal circular DNA (eccDNA) formation throughout the genome, especially at tandem repeats. Furthermore, our results suggest that counteracting i-loop formation at sites of damage is necessary for telomere maintenance and stability of repetitive elements in general. The aims of my project are to investigate the probability of i-loop formation at other repetitive sequences and the contribution of candidate factors in i-loop formation and excision at telomeric repeats. To this end, I have developed an inducible Cas9-system to introduce strand-specific nicks at telomeres and other repetitive elements, which can induce the formation of i-loops. I'm using this system to monitor differences in i-loop formation by 2D-gel electrophoresis and eccDNA accumulation by an *in vitro* assay developed in our lab.
References: [1] Mazzucco, G. et al. Telomere damage induces internal loops that generate telomeric circles. Nat Commun 11, 5297, doi:10.1038/s41467-020-19,139-4 (2020).

Keywords: eccDNA, telomere repeats, DNA damage.