10th
SCRM PhD Students Retreat

Gurten Park

1st September 2023

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Bern Stem Cell Research
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Gurten Park, Bern
1st September 2023

08:30	08:35	Funicular from Wabern to Gurten
08:45	09:00	Welcome Coffee
09:00	09:10	Welcome by Organizing Committee
Morning Session Chair: Francesco Bonollo		
09:10	09:30	Paola Bermudez-Lekerika
09:30	09:50	Daniel Batora
09:50	10:10	Tuo Zhang
10:10	10:30	Vera Tscherrig
10:30	11:00	**Coffee Break**
11:00	11:20	Roxana Manaila
11:20	11:40	Cong Wang
11:40	12:00	Amal Fahmi
12:00	13:00	**Mentor Talk: Dr. Lorenzo Leoni**
13:00	14:00	**Lunch Break**
Afternoon Session Chair: Ainhoa Asensio Aldave		
14:00	14:20	Siavash Rahimi
14:20	14:40	Anna Matveeva
14:40	15:00	Wenjuan Ning
15:00	15:30	**Coffee Break**
15:30	15:50	Jiaqi Li
15:50	16:10	Isabel Schlutz-Pernice
16:10	16:30	Vedat Burak Ozan
16:50	18:00	**Mentor Talk: Prof. Anna Cereseto**
18:00	18:10	Conclusive Remarks and Thanks from the SCRM Steering Committee
18:15		**Apéro**

This event was made possible with the generous support of:

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We want to thank you for your support,
which made this event possible!

7th Retreat, Gurten Park, 4th September 2020

8th Retreat, Gurten Park, 3rd September 2021

9th Retreat, Gurten Park, 2nd September 2022

Dear participants,

Welcome to the 10th SCRM PhD Students Retreat! We are happy to continue this successful history of PhD students retreats, which started in 2014 and was initiated by our colleague Dr. Luca Tamò, with you.

The day starts with a coffee and a short welcome from the organizing committee. The program will continue with a morning and an afternoon session of PhD project presentations and two coffee breaks for networking and discussions.

We are also excited to attend two interesting keynote lectures, which will be given by this year's mentors **Dr. Lorenzo Leoni**, manager partner at Ti-Ventures, entrepreneur, and founder of several biomedical companies; and **Prof. Anna Cereseto** from the University of Trento, Italy. We are very grateful to them for being our mentors today.

The retreat will be concluded with brief remarks and thanks by the SCRM Steering Committee, followed by the Apéro.

We are looking forward to meeting you and we wish you a fruitful and pleasant time during the retreat.

Sincerely yours,

The organizing committee

Ainhoa Asensio Aldave
Francesco Bonollo
Siavash Rahimi
Vedat Burak Ozan

Paola Bermudez-Lekerika

The metabolic role of IL-4 and IL-10 in Intervertebral Disc Degeneration

Paola Bermudez-Lekerika, Sofia Tseranidou, Exarchos Kanelis, Katherine B. Crump, Andrea Nuesch, Christine Le Maitre, Karin Wuertz-Kozak, Leonidas G. Alexopoulos, Jerome Noailly and Benjamin Gantenbein

Introduction: Intervertebral disc (IVD) degeneration is a pathological process often associated with chronic back pain and considered a leading cause of disability worldwide[1]. During degeneration, progressive structural and biochemical changes occur, leading to blood vessel and nerve ingrowth and promoting discogenic pain[2]. In the last decades, several cytokines have been applied to IVD cells in vitro to investigate the degenerative cascade. Particularly, IL-10 and IL-4 have been predicted as important anabolic factors in the IVD according to a regulatory network model based in silico approach[3]. Thus, we aim to investigate the potential presence and anabolic effect of IL-10 and IL-4 in human NP cells (in vitro) and explants (ex vivo) under hypoxia (5% O2) after a catabolic induction.

Methods: Primary human NP cells were expanded, encapsulated in 1.2% alginate beads (4 x 106 cells/ml) and cultured for two weeks in 3D for phenotype recovery while human NPexplants were cultured for five days. Afterwards, both alginate and explant cultures were i) cultured for two days and subsequently treated with 10 ng/ml IL-10 or IL-4 (single treatments) or ii) stimulated with 0.1 ng/ml IL-1β for two days and subsequently treated with 10 ng/ml IL-10 or IL-4 (combined treatments).

Results: The presence of IL-4 receptor, IL-4 and IL-10 was confirmed in human intact NP tissue (Fig 1). Additionally, IL-4 single and combined treatments induced a significant increase of proinflammatory protein secretion in vitro (Fig. 2A-C) and ex vivo (Fig. 2D and E). In contrast, no significant differences were observed in the secretome between IL-10 single and combined treatments compared to control group.

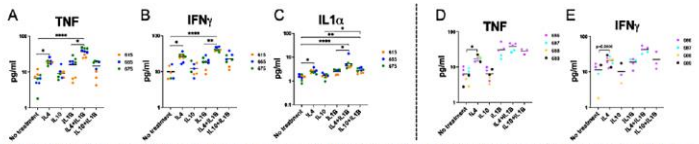

*Figure 1: In vitro(A-C) and ex vivo (D and E) secretome analysis of proinflammatory and catabolic proteins. Shown are medians, N = 3, p-value: * <0.05, ** <0.01, *** <0.005 and **** <0.001.*

Conclusion: Overall, IL-4 containing treatments promote human NP cell and explant catabolism in contrast to previously reported IL-4 anti-inflammatory performance[4]. Thus, a possible pleiotropic effect of IL-4 could occur depending on the IVD culture and environmental condition.

References: [1]J. Hartvigsen et al (2018) The Lancet 391:2356-67, 2PPA. [2]Vergroesen et al (2015) Osteoarthritis and Cartilage 23:1165-77, [3]S. Tseranidou et al, EBS 2023 28th Congress, July 9-12, 2023, Maastricht, The Netherlands, [4]H. Kedong et al (2020) The Spine Journal 20:60–68

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- Prof. Benjamin Gantenbein
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The **Stem Cell Research and Regenerative Medicine** (SCRM) Platform is an inter-faculty and inter-institutional research cluster of the University of Bern and the Inselspital, University Hospital Bern. The platform was founded in November 2012 with the aim of facilitating new ideas and skills in translational stem cell research and cell-based therapies to flow seamlessly through the member groups.

The SCRM Platform comprises **over 30 member groups** affiliated with the Medical, Vetsuisse and Phil.-nat. Faculty of the University of Bern and the Inselspital, University Hospital Bern.

The SCRM Platform organizes a number of events every year including a monthly lunch seminar, an annual PhD Students Retreat and an Annual Meeting. To learn more follow us in our official website!

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Vedat Burak Ozan

Induced Pluripotent Stem Cell Derived Multicellular Alveolar Lung Organoids with Endothelial and Immune Cells

Vedat Burak Ozan[1,2,3], Anna-Barbara Tschirren[1,2], Amiq Gazdhar[1,2], Thomas Geiser[1,2]
[1]Department of Pulmonary Medicine, University Hospital Bern; [2]Department of Biomedical Research, University of Bern; [3]Graduate School for Cellular and Biomedical Sciences, University of Bern

Introduction: Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease with high morbidity and mortality for which novel therapeutic options are urgently needed. Lack of representative *in vitro* and *in vivo* models and shortage of patient material, specifically alveolar epithelial cells, are the major limitations in understanding disease pathophysiology and identifying new drug candidates. Induced pluripotent stem cells (iPSCs) are a promising technology that can be used to generate patient and disease-specific cells. By differentiating iPSCs into alveolar lung organoids, endothelial cells, and macrophages, and culturing them in 3D, we aim to recapitulate the complex alveolar microenvironment *in vitro* for use in basic research, disease modelling, and drug screening.

Methods: iPSCs were grown in defined media and sequentially differentiated into Definitive Endoderm, Anterior Foregut Endoderm, and into Alveolar Lung Organoids (ALOs), with mature organoids acquired around day 35. iPSCs were differentiated into endothelial cells in a two-step protocol and were then selected by cell sorting and further underwent a round of selective passaging. iPSC to macrophage differentiation was a three-stage process that started with embryoid body formation, followed by progenitor cell generation and then maturation into macrophage cells by growing in media supplemented with macrophage specific cytokines. Each differentiation was validated through qPCR, flow cytometry, and microscopy using specific markers.

Results: iPSCs were successfully differentiated into ALOs, displaying markers for alveolar epithelial type I cells (HTI-56, AQ5), alveolar epithelial type II cells (HTII-280, SPC, SPB) and of basal cells (p63). iPSCs were successfully differentiated with high efficiency into endothelial cells yielding CD31 and von Willebrand Factor positive cells. Tube formation assays further demonstrated the functionality of these endothelial cells. iPSC-derived macrophages were generated with preliminary characterization by flow cytometry of relevant macrophage markers CD68 and CD206, followed by phagocytosis assays to demonstrate functionality.

Conclusion: iPSCs can differentiate into mature ALOs, endothelial cells and macrophages; different factors such as cell-line specific growth rates affect the efficiency of differentiation of iPSCs into the targeted cell types. Further complexity in this model will be achieved by establishing a 3D co-culture of ALOs with endothelial cells and macrophages. This will pave the way for a complex, patient-specific in vitro model of IPF.

Daniel Batora

Targeted metabolomics versus random numbers: which are the better predictors for disease symptoms?

Introduction: The application of state of the art bioanalytical and omics methods is critical for the discovery of novel predictors of disease parameters, however the translational impact of biomarkers is challenged by the generalizability of the findings. Preanalytics, the sample size and heterogeneity are inherent constrains in clinical bioanalytics which increases the stochasticity in the generated matrices and thereby the likelihood of false discovery. To overcome these general limitations, we first developed a methodological framework based on Random Matrix Theory (RMT) to validate bioanalytical datasets and eventually optimize resource allocation. Next, using an activity-based proteomics (ABP) approach we introduce a previously unexplored class of analytes, enzyme-activity fingerprints.

Methods: In our clinical study, we collected plasma from 90 enrolled hypercalcemia patients over the curation of the disease who underwent parathyroidectomy and the resected parathyroid adenomas from the surgical intervention. As molecular mechanisms that drive the manifestation of key clinical symptoms remain unknown, we employed targeted metabolomics to quantify inflammatory lipids and amino acids in plasma and the surgically resected adenomas. Furthermore, we generated enzyme-activity fingerprints using an activity-based proteomics approach on the resected adenomas.

Results: Our results unveil strong alterations in the level of specific inflammatory lipids and amino acids that may contribute to the development of the major symptoms associated with the disease. We found several significant correlations between the severity of the symptoms and the measured analytes. By applying the recently developed RMT approach, we could test the robustness of our empirical results by filtering out the random component.

Discussion: In summary, our study introduces novel model- and data-associated paradigms for bioanalytics. Our clinical study reveal novel correlates with the key symptoms of hypercalcemia whose mechanistical origins largely remained unknown.

Tuo Zhang

Ribosome biogenesis and ferroptosis resistance defines cancer stem cells in mesothelioma

Introduction: Malignant pleural mesothelioma (MPM) is an aggressive tumor arising from the serosal outer linings of the thorax. 90% of MPM cases present late in the course of the disease, with the 5-year survival rate continuing to languish at 5 to 10 %. Clinical and experimental evidence reveals that tumour initiation and propagation is driven by a subpopulation with self-renewal and enhanced tumourigenic capacity, termed cancer stem cells (CSCs). They have emerged as key contributors to drug resistance.

Results: It has been shown that high levels of ribosome biogenesis (RiBi) and ferroptosis resistance are necessary for the survival of hematopoietic stem cells. This implies that by inhibiting ribosome biogenesis together with inducing ferroptosis, we could precisely eliminate MPM CSCs. Accordingly, we demonstrated that the ribosome biogenesis inhibitor and ferroptosis inducer have synergistic inhibitory effect on the MPM cell lines MESO1 and H2452 in vitro. We also performed single-cell RNA sequencing (sc-RNAseq) to illustrates the hierarchy of MPM cells in clinical samples and found that typical CSC markers, e.g., ALDH1 and SOX2, are ubiquitously expressed across all cell populations in MPM, suggesting that alternative approaches are needed to identify MPM CSCs. In light of recent evidence that protein translation and RiBi oscillate during stem cells differentiation, with less-differentiated, higher-grade tumours characterized by decreased translation rate but increased RiBi, our scRNA-seq data revealed an MPM subset associated with high RiBi, suppressed translation, and an enriched gene signature of ferroptosis resistance.

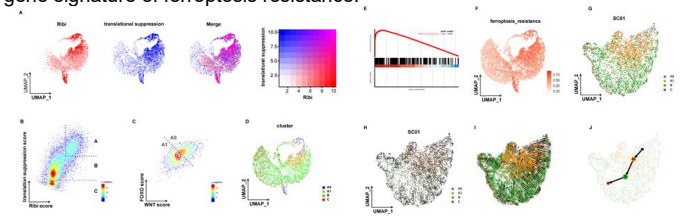

Figure 1 A. UMAP plots showing single sample gene set enrichment (ssGSEA) score of Ribi and translational suppression in the untreated patient (SC01, SC09, SC12) derived MPM cells (n=7335). **B.** In-silico FACS for MPM cells in A. The x axis and the y axis represents the normalized ssGSEA score of Ribi and translational suppression, respectively. The MPM cells is separated into A, B, and C clusters according to these ssGSEA score (cluster A: Ribi>0.48 and translational suppression>0.54; cluster B: cells not belong to cluster A and cluster C; cluster C: translational suppression<0.20), as shown in the B. **C.** In-silico FACS for cluster A MPM cells from B. The x axis and the y axis represents the normalized ssGSEA score of WNT pathway and FOXO pathway, respectively. The MPM cells is separated into A0 and A1 clusters according to these ssGSEA score (cluster A0: WNT + FOXO>1.25; cluster A1: WNT + FOXO<1.25), as shown in the picture. **D.**UMAP plot of MPM cells in A, showing the formation of 4 main cluster (A0, A1, B, C) from B and C. **E.** Enrichment plot for the high-expressed genes in A0 cells for previous reported signatures of ferroptosis resistance(n=65). **F.** UMAP plots showing the ssGSEA score of ferroptosis resistance in the MPM cells of A. **G.** UMAP plot of A0 MPM cells, colour coded by 4 main clusters (A0, A1, B, C) from B and C. **H.** RNA velocity visualized on diffusion map of MPM cells from SC01, coloured by clusters. **I.**RNA velocity visualized on stream map of MPM cells from SC01, coloured by clusters. **J.**Partition-based graph abstraction (PAGA) plots showing the development trajectory of MPM cells from SC01, coloured by clusters.

Discussion: These results demonstrate that MPM cells with high levels of RiBi but elevated resistance to ferroptosis are putative CSCs in MPM, suggesting a novel approach to target and eliminate MPM CSCs.

Isabel Schultz-Pernice

Mpox virus infection leads to neuronal injury in human neural organoids

Isabel Schultz-Pernice, Amal Fahmi, Chiu Yen-Chi, Blandina I. Oliveira Esteves, Teodora David, Antoinette Golomingi, Beatrice Zumkehr, Damian Jandrasits, Roland Züst, Selina Steiner, Carlos Wotzkow, Fabian Blank, Olivier B. Engler, David Baud, and Marco P. Alves

Introduction: On May 6th 2022 the first case of mpox virus (MPXV) infection without traceable contact to African population or fauna was reported in UK. Since then, 110 countries reported cases of infection, marking the largest recorded outbreak outside of endemic regions. First manifestations of MPXV infection include fever, lymphadenopathy and muscle aches, followed by characteristic vesiculopapular rash development. Recently, MPXV DNA was detected in the cerebrospinal fluid of a young, previously healthy women affected by encephalitis, suggesting neuroinvasive potential of MPXV. Despite reports of deadly encephalitis cases date back to 1987, mechanisms driving acute neurological manifestations during MPXV infections have been poorly investigated.

Methods: We used Human neural organoids (hNOs) to explore the susceptibility of neural tissue to MPXV infection. MPXV efficiently replicates in hNOs as indicated by an elevated frequency of MPXV-positive cells over time as well as the exponential increase of viral loads upon infection as detected with a qPCR assay.

Results: When comparing extra- and intra-cellular MPXV titers, we observed up to 100 times more cell-associated infectious virus, suggesting MPXV spread through cell-to-cell contact. In line with this, we detected the presence of viral antigen in neurites and in foci of grouped cells distributed throughout the tissue. Furthermore, while no evident morphological changes were noticed, we microscopically observed spheroid formation on neurites, a phenomenon termed axonal/dendritic beading, indicative of neuronal damage.

Discussion: Our findings underline the value of sophisticated in *vitro* systems to model neuropathogenesis in the human host and underline the urgent need to shed light on the mechanisms driving severe complications following infection by this previously neglected pathogen.

Jiaqi Li

The effect of oxidative stress on evolutionary dynamics within intestinal microbiota of inflammatory bowel disease patients

Jiaqi Li[1,2], Sebastian Jordi[1,2], Dmitrij Marchukov[2], Isabel Bärtschi[1,2], Benjamin Misselwitz[1,2], Andrew Macpherson[1,2], and Bahtiyar Yilmaz[1,2]
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Introduction: An initially mutualistic relationship between the host and its microbiota can lead to alterations in microbial consortia and their metabolic functions, accompanied by a loss of fitness of the host — this may manifest a disease such as inflammatory bowel disease (IBD). The gut microbiota in IBD is relatively different from non-IBD, mainly with an increase of enteropathogenic strains due to the generated reactive oxygen species in an altered metabolic niche. However, the effect of oxidative stress on gut residents in a long-term adaptive manner is unknown. Knowing these led us to ask how IBD microbiota under oxidative stress genetically adapts over time within individuals and how this relates to the trajectory of activity and treatment of IBD.

Results: Using mucosa-associated samples from IBD patients with the active and nonactive disease, we found numerous altered taxa in IBD, including *Bacteroides*, *Faecalibacterium*, *Lachnospiraceae*, *Muribaculaceae*, and *Akkermansia,* are altered and is directly associated with lack of oxidative stress responsiveness in the host. We are currently processing the biopsies longitudinally collected from the Swiss IBD cohort to characterize the variants in gut microbiota and to understand how disease activity contributes to this. Lastly, we aim to characterize gut microbial strains for their contribution to oxidative stress and gut homeostasis using mouse-model. We have been isolating over 150 strains from subjects with a stoma using different selected media and pre-treatments. We aim to address whether these strains are intrinsically capable of exacerbating or resisting oxidative stress and potentially make the host more susceptible to further inflammation with ongoing oxidative stress.

Discussion: Overall, understanding how oxidative stress affects genomic variation in IBD gut microbiota and whether this is beneficial to the aetiopathogenesis of pathobionts in an altered metabolic niche will uncover the molecular biomarkers, including particularly relevant variants to human health and well-being

Vera Tscherrig

MicroRNA in small extracellular vesicles from the umbilical cord have the potential to prevent and treat brain damage upon preterm birth

Vera Tscherrig[1,2], Valérie Haesler[1], Marel Steinfort[1,2], Amanda Brosius-Lutz[1], Daniel Surbek[1], Andreina Schoeberlein[1], Marianne Jörger-Messerli[1]
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[2]Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern, Switzerland

Introduction: Preterm birth is still the leading cause of childhood morbidity and mortality. It often results in white matter injury (WMI), leading to long-term neurobehavioral and neurodevelopmental disabilities. Intranasally administered mesenchymal stromal cells (MSC), and importantly also their secreted factors such as MSC-derived small extracellular vesicles (MSC-sEV), have a therapeutic potential for preterm WMI. We aim to investigate the beneficial mechanisms of MSC-sEV in preterm WMI. They contain small non-coding RNAs, such as microRNAs (miRNAs), being able to repress genes involved in preterm WMI. We hypothesize that miRNAs released by sEV upon uptake in their target cells are crucial for the beneficial effects of MSC-sEV in preterm WMI.

Methods: MSC were isolated from the connective tissue of human umbilical cords, the Wharton's jelly. sEV were purified from the conditioned medium of the MSC by serial ultracentrifugation and size exclusion chromatography (SEC). The sEV-containing SEC fractions were collected and analysed for protein and miRNA expression.
The MSC were knocked-down for DROSHA, an enzyme important for miRNA maturation, to evaluate the regulatory activity of sEV miRNA. The miRNA-mediated repression of target genes was analysed using a luciferase assay.

Results: The sEV-containing SEC fractions expressed characteristic markers for sEV (CD81, CD63, CD9) and MSC (CD73, CD90, and CD105). They contained high amounts of miRNAs, such as miR-21-5p, miR-22-5p, miR-27b-3p, and let-7 family members. sEV significantly reduced the luciferase signal for TP53 and TAOK1, genes involved in WMI, indicating miRNA-mediated repression. sEV enhanced the differentiation of the oligodendrocyte lineage. After oxygen-glucose deprivation, sEV reduced apoptotic markers. sEV from DROSHA knock-down MSC had fewer miRNA and no significant effects on the in vitro assays, in contrast to naïve sEV.

Discussion: Thus, miRNAs are one of the main players in the therapeutic potential of MSC-sEV in preterm WMI. Intranasal administration of MSC-sEV holds strong potential for the future treatment of neonatal brain injuries.

Roxana Manaila

Loss of S1P Lyase Expression in Human Podocytes Causes a Reduction in Nephrin Expression That Involves PKCδ Activation

Group of Prof. Dr. Andrea Huwiler, Institute of Pharmacology, University of Bern

Introduction: Sphingosine 1-phosphate (S1P) lyase (SPL, *Sgpl1*) is an ER-associated enzyme that irreversibly degrades the bioactive lipid, S1P, and thereby regulates multiple cellular functions attributed to S1P. Biallelic mutations in the human *Sglp1* gene lead to a severe form of a particular steroid-resistant nephrotic syndrome. Based on these observations, we therefore hypothesized that the S1P metabolism plays a key role in podocyte physiology and that the molecular mechanism that leads to disrupts the normal architecture of the slit diaphragm and development of the nephrotic syndrome by SPL loss-of-function mutations or depletion is interference with the production of core protein of the SD, nephrin.

Methods: In this study, we have investigated the molecular effects of SPL knockdown (kd) in immortalized human podocytes to better understand the mechanism underlying nephrotic syndrome in patients. A stable SPL-kd was generated by the lentiviral shRNA transduction method and was characterized for reduced SPL mRNA and protein levels and increased S1P levels with LCMS.

Results: We show here that SPL-kd leads to the downregulation of the nephrin protein and mRNA expression, as well as the Wilms tumor suppressor gene 1 (WT1), which is a key transcription factor regulating nephrin expression. Mechanistically, SPL-kd resulted in increased total cellular protein kinase C (PKC) activity, while the stable downregulation of PKCδ revealed increased nephrin expression. Furthermore, the pro-inflammatory cytokine, interleukin 6 (IL-6), also reduced WT1 and nephrin expression. In addition, IL-6 caused increased PKCδ Thr^{505} phosphorylation, suggesting enzyme activation.

Discussion: Our data demonstrate that nephrin is a critical factor downregulated by the loss of SPL, which may directly cause podocyte foot process effacement as observed in mice and humans, leading to albuminuria, a hallmark of nephrotic syndrome. Furthermore, our in vitro data suggest that PKCδ could represent a new possible pharmacological target for the treatment of a nephrotic syndrome induced by SPL mutations.

Wenjuan Ning

Combined inhibition of $KRAS^{G12C}$ and DNA damage repairs induces enhanced apoptosis in $KRAS^{G12C}$ mutant cancer.

Introduction: KRAS is one of the most common and notorious oncogenes as it was previously deemed as 'undruggable'. The advent of $KRAS^{G12C}$ inhibitors partly conquered this issue. However, to maximize the efficacy of $KRAS^{G12C}$ inhibitor therapy, drug resistance must be addressed and overcome.

Method: We performed gene set enrichment analysis (GSEA) of the published RNA sequencing (RNAseq) data from $KRAS^{G12C}$ inhibitor-treated $KRAS^{G12C}$ mutant cell lines to identify the potential mechanisms underlying resistance to of $KRAS^{G12C}$ inhibitors. To confirm enhanced anti-tumor efficacy of combined inhibition, cell viability assays, western blotting, colony formation, and flow cytometry were performed. As well, 2 xenograft murine models were established with the H2122 and PF139 cell lines.

Result: The synergistic effect of multiple combined treatments of $KRAS^{G12C}$ and double strand break repair inhibitors was confirmed by in vitro cell viability and apoptosis assays as well as *in vivo* xenograft tumor models. We also observed that the synergy of combined inhibition of $KRAS^{G12C}$ and DNA damage repair is accompanied by the enhanced inhibition of KRAS downstream pathways, but the increase of DNA damage and pre-apoptotic makers.

Conclusion: Inhibition of DNA double strand breaks enhanced the anti-tumor activity of $KRAS^{G12C}$ inhibitors in $KRAS^{G12C}$ mutant cancer both *in vitro* and *in vivo*, and the synergy was achieved by eliciting apoptotic cell death.

Anna Matveeva

A research proposal: Potential of hiPSC-derived POR*28 hepatocytes for drug screening and CYP450 activity assessment

Department of Biomedical Research, Pediatric Endocrinology, Diabetology, and Metabolism, University Children's Hospital, Bern, Switzerland

Introduction: Hepatic cytochromes P450 (CYPs P450) are crucial in phase I drug metabolism, accounting for the oxidation, reduction, and hydrolysis of around 70-80% of clinically utilized drugs. P450 oxidoreductase (POR) is vital for the activity of cytochromes P450, facilitating electron transfer from NADPH to CYPs P450. The specific variant of POR named POR*28 or variant A503V was found in 30% of sequenced alleles according to Genome Aggregation Database, mostly in European population. The variant is considered benign since it doesn't cause POR deficiency manifestations as to skeletal deformities and disordered steroidogenesis. It allows to assume that functionalities of cytochromes involved in steroidogenesis as to 17A1, 19A1, and 21A2 are not affected. However, several studies showed aberrant drug metabolism in A503V carriers. Thus, POR*28 patients showed 45% lower metabolic ratio of midazolam – a widely used sedative for anxiety reduction, pre-surgery sedation, and seizure emergency treatment. Immunosuppressor tacrolimus is applied in the post-transplantation period to prevent organ rejection. In POR*28 carriers, who underwent heart transplantation, more intensive tacrolimus metabolism was observed. The findings highlight the importance of dose adjustment when treating these patients. Apparently, the dysmetabolism of both drugs is attributed to increased activity of POR A503V towards drug-metabolizing cytochromes, notably CYP3A5, which is among the most efficient enzymes in drug oxidation.

Hypothesis & Aims: Our study aims to obtain hiPSC-derived hepatocytes carrying POR A503V and evaluate their potential for drug screening and drug-metabolizing cytochromes activities. hiPSC – derived hepatocytes provide a more physiologically relevant model for studying molecular mechanisms of drug metabolism than animal models or immortalized cell lines. hiPSCs will be generated by adult human POR*28 fibroblasts reprogramming followed by differentiation to hepatocytes. In the next approach we aim to evaluate tacrolimus and midazolam metabolic ratios in A503V hiPSCs utilizing HPLC. For cytochromes activity measurements, enzymatic reactions with fluorescent CYPs substrates will be performed.
A novel iPSC-based model will be developed for studying the POR A503V phenotype. This model will yield valuable data that can be compared to existing approaches, including purified systems, animal, and cellular models, as well as clinical data.

Cong Wang

Novel insights on liver endothelial mechanobiology in cirrhosis: role of calcium integrin-binding protein 1.

Cong Wang[1*], Eric Felli[1*], Sonia Selicean[1*], Yeldos Nulan[1], Sergi Guixé-Muntet[3], Juan José Lozano[3], Jaume Bosch[1,2,3], Annalisa Berzigotti[1,2], Jordi Gracia-Sancho[1,2,3]
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Introduction: Liver sinusoidal endothelial cells (LSECs) are central players in liver microcirculatory malfunction. This study aimed at investigating the role of calcium integrin-binding protein 1 (CIB1) in LSECs stiffness-mediated dysfunction in chronic liver disease.

Methods: We investigated CIB1 expression in human liver. Rat LSECs were cultured 24h on tunable stiffness polyacrylamide gels. The effects of depleting CIB1 using siRNA were investigated by RNAseq.

Results: Immunofluorescence on human cirrhotic liver showed that CIB1 was upregulated and translocated to the cytoplasm (+46.7% and +93.3% respectively). In healthy rat LSECs pathologic stiffness (30kPa) induced significant upregulation (+57.0%) and translocation to cytoplasm of CIB1 (+46.7%), which was prevented using cytoskeleton disruptors(-25.8% and -212.2%) (all $p<0.05$). Importantly, CIB1 knock-down reversed LSECs nuclear morphology to a healthy spherical shape on 30kPa, which was associated with improved LSECs phenotype as demonstrated by the amelioration of pathways related to inflammatory response, reactive oxygen species production and LSECs differentiation.

Conclusions: Our results demonstrate that CIB1 modulates LSECs mechanotransduction and dysfunction in liver cirrhosis. The reversibility of the effects of CIB1 or its downstream molecular pathways, may be potential novel therapeutic targets for chronic liver disease and portal hypertension.

Amal Fahmi

West Nile virus infection impairs human neural organoid development

Amal Fahmi1,2,3, Isabel Schultz Pernice1,2,3, Beatrice Zumkehr1,2, Antoinette Golomingi Mujyigna1,2, Blandina I. Oliveira Esteves1,2, Melanie Brügger1,2, Thomas Démoulins1,2,4, and Marco P. Alves1,2,5
1Institute of Virology and Immunology, Bern, Switzerland 2Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland 3Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland 4Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland
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Introduction: The incidence of flavivirus infection in humans is rising and represents a significant threat to global health. West Nile virus (WNV) is a neurotropic flavivirus of particular concern due to its worldwide spread and high potential to cause outbreaks in humans. WNV is mainly transmitted by mosquitoes, although other routes of transmission have been described, including vertical transmission. Due to poor monitoring of pregnant women during outbreaks and scarce experimental investigation available, the impact of WNV infection on human fetal brain remains poorly described.

Methods: To explore the potential neuroteratogenic effects of WNV and its interference with brain organogenesis, we employ human neural organoids (hNO), an advanced *in vitro* system commonly used to study human brain development.

Results: Our findings show hNO to be highly permissive to WNV infection as demonstrated by a rapid exponential increase of infectious virus release over time. In terms of cell tropism, WNV preferably targets neurons; however, WNV envelope protein is also detected in neural progenitors. Notably, WNV infection results in compromised hNO morphology and growth as demonstrated by atrophy and decrease in cell number. In line with this, WNV induces massive apoptosis in both neurons and neural progenitors, as assessed with a cleaved-caspase 3 assay. Finally, following WNV infection at low multiplicity of infection, we observed robust induction of antiviral and pro-inflammatory mediators including interferons, IL-6, and IL-8 potentially contributing to neurological damage.

Discussion: Our results stress the requirement to perform further work to evaluate better the risks represented by WNV infection during pregnancy.

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Role of proliferation and mechanostructural signaling in Pemphigus vulgaris

Introduction: Previous studies have shown that mechanostructural properties of keratinocytes alter upon anti-Dsg3 antibody treatment. However, the involvement of these changes in the pathogenesis of Pemphigus vulgaris (PV) has not been fully elucidated.

Methods: In this study, we first utilized a keratinocyte dissociation assay (KDA) to find, via pharmacological modulators and knock-out cells, whether transcription, actomyosin contractility, E-cadherin, and cell proliferation are required for loss of keratinocyte cohesion. Then we performed flow cytometric assays on EdU incorporation into keratinocytes treated with AK23 (anti-Dsg3) antibody to uncover the effect of AK23 on proliferation. Bulk RNA-sequencing was done on 2D cultures of primary human epidermal keratinocytes treated longitudinally with experimental antibodies, AK23 or PX43 (anti-Dsg1/3), to elucidate the transcriptional changes involved in disease-induced cell contraction and proliferation.

Results: KDA results suggest that proliferative mechanisms, actomyosin contraction, and transcriptional regulation are required for AK23-induced fragmentation, while E-cadherin was not involved. Flow cytometric analysis of EdU incorporation at 24 hours confirmed that AK23-treated keratinocytes were more proliferative compared to control antibody-treated cells. Consistent with the abovementioned findings, RNA-seq revealed modulation of replication, cell cycle, WNT, Rho GTPases, and apoptosis-related genes through time.