ABSTRACT BOOK

MICROENVIRONMENTAL INTERACTIONS IN B CELL LEUKEMIAS AND LYMPHOMAS: IMPLICATIONS FOR TARGETED THERAPY

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The biology of multiple mature B cell leukemias and lymphomas largely depends on microenvironmental interactions that provide pro-survival and pro-proliferative signals. B Cell Receptor (BCR) signalling plays a pivotal role and this stems from its fundamental importance for the maturation, survival, and proliferation of B cells. B cell malignancies frequently harbor mutations in this pathway or complex non-genetic deregulation of BCR signalling. This is underscored by the remarkable clinical effect of inhibitors targeting BCR-associated kinases BTK and PI3K. Moreover, the differences in BCR signalling propensity contribute to variable clinical behavior of B cell lymphomas. Bedsides BCR signaling, the interactions with T cells seem to be essential in some B cell lymphomas, which is illustrated by the observation that in vitro malignant B cells will not spontaneously divide but can be induced into proliferation by factors such as CD40 produced by T cells. CD40 signaling synchronized with BCR signaling seems to be a key driver of malignant B cell proliferation, and this does not require any genetic aberrations in malignant cells, posing a question whether a large part of B cell tumor biology could be non-genetic making them a clear exception in the „universe" of cancer. The talk will focus on novel mechanisms tuning the propensity of BCR signalling together with T cell interactions during microenvironmental interactions of B cells, and also the related changes during therapy with BCR inhibitors or classical DNA-damaging drugs. The talk will include also novel data on "tonic" PI3K/pAKT activity and the possibility to use novel GAB1 inhibitors in the therapy of B cell neoplasms.

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HYPERBILIRUBINEMIA IS ASSOCIATED WITH CHANGES IN CHOLESTEROL METABOLISM AND FAT BREAKDOWN

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Hyperbilirubinemia in humans is associated with lower BMI. Animal studies also showed connection between high levels of unconjugated bilirubin and reduced adiposity. Recent report showed that hyperbilirubinemia is associated with reduced fat mass and increased hepatic mitochondrial biogenesis, specifically in female animals, suggesting a dual role of elevated bilirubin and reduced UGT1A1 function on adiposity and body composition.[1]

Our aim was to investigate the impact of unconjugated hyperbilirubinemia on metabolite and lipid composition in Gunn rats. Rat plasma of 14 weeks old animals of both genders was examined in metabolomics and lipidomics screening. Extracted plasma samples were measured on Orbitrap ID-X Tribrid using ZORBAX Eclipse Plus C18 column and on 6546 LC/Q-TOF using Accucore C30 column.

The most prominent differences in plasma metabolite composition were found in female cohort of Gunn rats. 5 subclasses of metabolites were found: bile acids, flavonoids, fatty acids, phenylalanine and, as expected, bilirubin and its metabolites. As opposed to male Gunn rats, lipidomic data analysis in female Gunn rats revealed substantial decrease of all measured lipid classes in comparison with control, with the main difference in compounds containing long unsaturated fatty acids.

This is the first study to comprehensively assess metabolomics and lipidomics in hyperbilirubinemic rats. Our findings show that hyperbilirubinemia, specifically in female animals, is associated with changes in cholesterol metabolism and breakdown of fat.

Reference: [1] Vidimce J., Pillay J., et al. *Front. Pharmacol.* **2021**, 12, 586715.

GOLDEN HAMSTER PIRNAS ARE NECESSARY FOR ZYGOTE DEVELOPMENT AND ESTABLISHMENT OF SPERMATOGONIA

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PIWI-associated RNAs (piRNAs) maintain the integrity of the germline genome by suppressing retrotransposon activity, and their functions in gene regulation have recently emerged. The understanding of the role of piRNA pathway in mammals was shaped by a mouse model in which piRNAs are essential for male but not female fertility. We report that the role of the piRNA pathway substantially differs in golden hamsters, whose piRNA pathway setup more closely resembles that of other mammals, including humans. We show that the loss of Mov10l1 helicase, an essential factor initiating piRNA biogenesis, generates novel phenotypes in hamsters. Unlike mice, female Mov10l1−/− hamsters are sterile. Mutant fully-grown oocytes show minor changes in the transcriptome and moderate to low increase in retrotransposon transcripts. Although Mov10l1−/− hamster oocytes appear largely normal and give rise to zygotes, they cannot support normal embryonic development. In another contrast to mice, Mov10l1−/− male hamsters have impaired establishment of spermatogonia accompanied by transcriptome dysregulation and a surge of expression of a specific young retrotransposon subfamily, which is the primary target of retrotransposon-derived piRNAs in early spermatogenesis. In addition, we examined groups of piRNAs during different stages of spermatogenesis and demonstrate that the regulation of specific retrotransposon subfamilies is stage-dependent. Our results not only refute the notion that in mammals the piRNA pathway is important only in males but also demonstrate the adaptive nature of the mammalian piRNA pathway, which allows to confront emerging genomic threats and acquire new critical roles in both sexes.

BBSome ASSEMBLE!

Avishek Prasai, Marketa Schmidt Cernohorska, Klara Ruppova, Veronika Niederlova, Monika Andelova, Ondrej Stepanek, Martina Huranova

Bardet-Biedl Syndrome (BBS) is a multiorgan genetic disorder caused by the dysfunction of the primary cilia, a microtubule-based sensory organelle. BBS is characterized by six major symptoms: retinopathy, polydactyly, genital and renal anomalies, obesity, and cognitive impairment. Twenty- four genes have been identified to lead to BBS, eight of which encode for a protein complex called the BBSome and three encode for chaperonin-like BBS proteins. The BBS chaperonins assist in the assembly of the BBSome, which traffics a wide range of signaling receptors into and out of the primary cilia. Mutations in the BBSome and the BBS chaperonins account for the majority of BBS patients with varying degree of disease severity. These mutations are thought to hamper the assembly of the BBSome resulting in its mis-localization from the cilia. However, it remains to be elucidated how the whole-body loss of this protein complex leads to such phenotypic variability. To investigate the role of individual BBSome subunits and the BBS chaperonins in the BBSome assembly pathway we generated a large library of genetically modified RPE1 cell lines deficient in these proteins. We uncovered that the BBSome assembly is spatially controlled by two of its subunits and is initiated by formation of an unstable pre-BBSome intermediate at the pericentriolar satellites. The next aim is to uncover which steps of this pathway are facilitated by the BBS chaperonins. Along with this, we will examine how the BBS linked mutations in the BBSome subunits and BBS chaperonins affect formation of the BBSome and its function in primary cilia. Overall, our results provide a framework for elucidating how BBS-causative mutations interfere with the biogenesis of the BBSome.

TEAM UP TO GO THE DISTANCE – CYTOSKELETAL CROSSTALK IN HCC CELLS

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Cytoskeleton consists of three distinct networks: intermediate filaments (IFs), actin fibers and microtubules. Recently, there has been mounting evidence indicating the importance of their interplay for proper cytoskeleton functioning. Physical linkage of cytoskeletal components is mediated by cytoskeletal linker proteins (cytolinkers) of the plakin protein family. Plectin, an ubiquitously expressed cytolinker, not only crosslinks IFs (and other networks) but also anchors them at junctional complexes. Over past years, plectin was found to be upregulated in various tumor types. Consistently, our preliminary data show plectin upregulation in hepatocellular carcinoma (HCC) and in HCC-derived cell lines, where its expression correlates with the cell motility and invasion. For this reason, we decided to employ plectin targeting to 1) study the role of plectin-mediated interplay in migratory and tumorigenic potential of HCC cells and to 2) validate plectin as potential target for HCC treatment.

First, using CRISPR/Cas9 approach, we generated plectin-defficient (KO) HCC cell lines to addres the role of cytoskeletal interplay in maintaining the proper cytoskeletal architecture. In micropattern-seeded HCC cells, we observed aberrant actin stress fibre architecture and collapsed vimentin IF network. To test whether the structural changes manifest on a functional level, we analyzed the contractility of HCC cells using traction force microscopy. In line with compromised actomyosin, lower contractile energy was determined in KO cells. Next, we studied the 2D and 3D migratory phenotype of plectin KO cells. In parallel, we addressed the effect of pharmacological targeting on the HCC cell movement using plecstatin, a high afinity plectin's ligand prepared by the lab of Dr. S. M. Meier (University of Vienna). Both genetic and pharmacological targeting effectively inhibit 2D and 3D migration of HCC cells. To explore the effect of plectin targeting *in vivo*, we studied the tumour growth of HCC-derived xenografts in immunocompromised NSG mice. Both targeting strategies resulted in reduced tumour growth. Together, these results demonstrate the importance of plectin-mediated cytoskeletal crosstalk in HCC cell migration, invasion and tumorigenicity. Our results also suggest plectin as potential pharmacological target for HCC theatment. In future research we plan to further address the anticancer potential of plecstatin in HCC cells extravasation using HD TVI assay.

REGULATORY T CELLS SUPPRESS THE FORMATION OF SUPER-EFFECTOR CD8 T CELLS BY LIMITING IL-2

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Regulatory T cells (Tregs) maintain self-tolerance by suppressing conventional T cells. On the other hand, Tregs promote tumor growth by inhibiting anti-cancer immunity. We identified that Tregs increase the threshold of self-reactive CD8+ T cells required for the induction of autoimmune diabetes. The major mechanism of the Treg-mediated suppression is limiting the availability of a key T-cell cytokine IL-2. Specifically, Tregs reduce IL-2 to prevent the formation of a previously uncharacterized subset of KLRK1 and IL-7R expressing CD8+ T cells, which are induced only by a combination of strong IL-2 signals and antigenic stimulation. Because these T cells produce high levels of effector molecules and show superior cytotoxic properties, we call them super-effector T cells. Accordingly, the administration of IL-2R agonists phenocopies the absence of Tregs and promotes autoimmune and anti-tumor CD8+ T cell responses.

COMPREHENSIVE RNA-SPLICING ANALYSIS OF MARINE DIPLONEMIDS USING COMPARATIVE GENOMICS APPROACH

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RNA splicing plays a critical role in regulating gene expression and transcriptome diversity in a variety of eukaryotes. Recent global surveys of marine biodiversity revealed that planktonic diplonemids are amongst the most abundant and diverse marine organisms. These so-far overlooked protists related to important kinetoplastid parasites (*Trypanosoma*, *Leishmania*) as well as ubiquitous prospective biofuel producers (*Euglena*) have unusual splice site and lack the canonical intron (GU-AG). This suggests the existence of an alternative mechanism of intron removal and implies diplonemids can be an excellent model for the study of intron evolution. We have therefore sequenced genomes and transcriptomes of five marine diplonemids. We have assembled the genome and transcriptome of all five diplonema. Further, We will be carrying out comprehensive splice-site analysis of these species. Firstly, we will be annotating the genomes of all sequenced marine diplonemids, identify non-canonical introns and determine common sequence and/or structural features. Finally, we will perform comparative intron/splice site analysis among diplonemids, reveal general mechanisms and pattern of RNA splicing and compare it to the canonical eukaryotic model.

A MODEL OF PREFERENTIAL PAIRING BETWEEN EPITHELIAL AND DENDRITIC CELLS IN THYMIC ANTIGEN TRANSFER

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Introduction: The presentation of self-antigens to developing T cells by medullary thymic epithelial cells (mTECs) and thymic dendritic cells (DCs) is essential for the establishment of central tolerance. While mTECs produce antigens in a self-autonomous manner, thymic DCs acquire them from mTECs by the process of Cooperative Antigen Transfer (CAT). Given the heterogeneity of mTEC and DC populations, unraveled recently by single-cell RNA sequencing (scRNAseq), the main objective of this study was to track the distribution of antigens generated by distinct subsets of mTECs among distinct subsets of thymic DCs.
Methods: Several Cre-based mouse models and our scRNAseq data analyses were employed to establish flow cytometry protocols to study the participation of DC subsets in the acquisition of mTEC-derived tdTomato antigen. Using a mouse model in which RFP or YFP were alternatively expressed in mTECs, we tested whether CAT occurs repetitively. We also analyzed whether antigens can be transferred between thymic DCs.
Results and Conclusion: We found that in regard to CAT: (i) each DC subset preferentially targets distinct mTEC subsets, (ii) the subset of XCR1$^+$ activated DCs is the most potent subset in CAT; (iii) single thymic DC can acquire antigen repetitively, (iv) the monocyte-derived DCs (moDCs) were the most efficient in repetitive CAT, and (v) moDCs represented also the most potent DC subset in the acquisition of antigen from other DCs. Thus, our findings outline some of the basic rules of the distribution of mTEC-derived antigens throughout the thymic medulla.

HIGH-RESOLUTION 3D IMAGING OF SINGLE SPERM CELLS USING CONFOCAL FLUORESCENCE AND FOCUSED ION BEAM SCANNING ELECTRON MICROSCOPY

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The specialized architecture of the spermatozoon plays a critical role in uniting gametes for fertilization. Teratozoospermia resulting from defects in spermatozoa structure(s) represent a spectrum of disorders that can compromise or abolish male fertility. Although the genetic basis for many male infertility disorders has been elucidated, to explain their mechanisms remains challenging, in part because we have an incomplete understanding of how sperm structure affects its function. To fill this gap, we applied cutting-edge imaging tools by combining confocal fluorescence and focused-ion beam scanning electron microscopy (FIB-SEM) to examine the 3D (ultra)structural organization of spermatozoa at the single-cell level. By comparing normal spermatozoa to teratospermic spermatozoa from subfertile mice, we noted differences in (sub)cellular structures in the healthy versus diseased state. Our results suggest that both imaging modalities are powerful tools to study the structure-function relationship of mammalian spermatozoa and can open new avenues for future investigation of the mechanisms and treatments of infertility.

DETERMINANTS OF FUSOGENICITY OF SYNCYTIN-1, CELLULAR GLYCOPROTEIN OF RETROVIRAL ORIGIN

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Syncytin-1 is an endogenous retroviral envelope glycoprotein specifically expressed in human placenta, where the protein was adopted for its novel physiological function. After interaction with specific receptor, transmembrane protein ASCT2, Syncytin-1 initiates cell-cell fusion leading to formation of multinucleated syncytiotrophoblast, which is essential for feto-maternal nutrients exchange. In my diploma thesis a new cell-cell fusion quantification assay was implemented for characterisation of Syncytin-1 fusion determinants. The assay uses Syncytin-1 and ASCT2 expressed separately with fragments of luciferase in heterologous cell-culture system. The assay enables to specifically quantify cell-cell fusions based on activity of reconstituted luciferase reporter and can be potentially used for quantitative high-throughput screening. After introducing several optimization steps, this method was validated for quantification of fusogenicity of Syncytin-1 and its mutants. Therefore, this method can serve as a powerful tool for describing main determinants of fusogenicity in the Syncytin protein structure and to further study the complex interactions between syncytins, their receptors and cell-cell fusion modulatory proteins.

THE PHYSIOLOGY AND/OR PATHOPHYSIOLOGY OF ADP-RIBOSE CHROMATIN SCARS AT SITE OF DNA DAMAGE

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DNA single strand breaks (SSBs) are amongst the most common DNA lesions arising in cells and if not repaired correctly can threaten genetic integrity and subsequently lead to cell death and embryonic lethality. Moreover, single-strand break repair (SSBR) defects are associated with various hereditary neurological disorders in humans. SSBs are detected by poly-ADP-ribose polymerases (PARPs), enzymes that subsequently synthesize mono-ADP-ribose and poly-ADP- ribose chains on themselves and other proteins including histones. ADP-ribosylation at the site of SSBs is important for recruitment of DNA repair proteins. Subsequently, ADP-ribose moieties are rapidly degraded by specific glycohydrolases.

Recently, mutations in the ARH3 glycohydrolase that catabolises ADP-ribose have been associated with episodic, stress-induced seizures, axonal neuropathy and cerebellar ataxia. Surprisingly, however, we find that neither poly-ADP-ribose metabolism nor the rate of DNA SSBR are affected in ARH3-mutated patient cells. Rather, we show that the elevated mono-ADP-ribose on histone residues in ARH3-defective cells is a chromatin 'scar' or 'memory' of endogenous sites of DNA repair. Moreover, we demonstrate that this mono-ADP-ribosylation is dependent on HPF1; a cofactor for serine ADP-ribosylation that confers serine specificity on PARPs.

To exploit our findings *in vivo* we have recently generated Arh3-deficient mouse model to identify location and rate at which endogenous SSBs and potentially pathological histone ADP-ribosylations arise in brain. Successfully, we detect substantially elevated levels of histone mono- ADP-ribosylation in highly proliferating organs such as spleen and thymus. This is consistent with our previous finding that ADP-ribosylation is predominantly associated with unligated Okazaki fragment intermediates of DNA replication. Excitingly, we also detect increased levels of histone mono-ADP-ribosylation in disease related organs, such as brain, especially in cerebellum; the region most commonly affected in patients with hereditary defects in SSBR.

We shall now extend our discoveries to identify and characterise the protein factors and pathways that couple aberrant ADP-ribose metabolism to neurodegenerative disease.

ROLE OF FAM83H IN IMMUNE SYSTEM HOMEOSTASIS

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Immune system homeostasis can be defined as the mechanisms that maintains an organism healthy and stable. Numerous genes could be important for this and a recent study suggests that Family with sequence similarity 83-member H (Fam83h) could be one of them. FAM83H was first characterized in *Autosomal-dominant hypocalcification amelogenesis imperfecta (AI)* which is a soft enamel disease. Fam83h has been suggested to be responsible for intracellular molecular transport, regulation of cytoskeletal networks, and enamel formation. Fam83h is quite ubiquitously expressed mainly in epithelial cells. Interestingly, two AI patients in one family in Czech Republic with confirmed mutation in FAM83H showed also symptoms of juvenile arthrosis. The roles of FAM83H in these diseases and in immune system homeostasis still remain elusive!

We show that Fam83h is ubiquitously expressed across different tissues with highest expression in ileum, colon and ameloblasts. In contrast to previous reports we show Fam83h to be expressed in immune organs (spleen, lymph nodes, thymus) and bone marrow, where the source of its expression are likely stromal cells. Fam83h deficient mice are subviable with the frequency 7% of KO animals in F2 litters, display growth retardation and have swollen joints and fingers. We have some images from uCT (microCT) of 3-week-old animals (KO and wt). It seems, that there is a bone neoplasia/ malformation on one of the fingers in the KO, also this animal seemed to be a bit developmentally late as compared with the wt littermate. Data from the IMPC screen show increased levels of potassium, ALT and urea in blood of KO mice, attesting for kidney and liver damage. Fam83 KO pups are smaller, neutrophilic, lymphopenic and show the highest degree of arthritis-like disease. Interestingly, weight and leukocyte counts return back to normal levels as animals age. The disease presents clearly an early onset and only few animals survive till week 16 of age.

The goal of the study is to reveal the mechanism of autoimmune disease in Fam83h KO mice. Our results will contribute to the understanding of the role of Fam83h in immune system homeostasis.

BIOLUMINESCENT ASSAYS FOR MEASURING CELLULAR METABOLISM

Vojtěch Ledvina, East Port Praha s.r.o.

Cellular metabolism comprises a dynamic network of pathways that undergo reprogramming in response to external and internal signals. This reprogramming has vital roles in proper cell function and also in many disease states, cancer metabolism, and immune metabolism. In this presentation, new plate-based bioluminescent assays for measuring the key metabolites glucose, lactate, glutamine, and glutamate will be presented. Their applicability will be shown on the study of glycolysis and glutaminolysis, two major fuel-metabolizing pathways.