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Book of Abstracts

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FOREWORD

The Annual PhD Conference in Biomedical Sciences brings together PhD students from different fields, offering a chance to present their research in front of their peers, supervisors, and the Doctoral Board/Committee. In 2021, the PhD conference was held for the first time among students of all three specializations following the idea "More people, more science & more fun!". Therefore, all students in the program actively created a team spirit, facilitating the exchange of ideas and promoting communication between different departments.

We believe this concept has proved to be very useful and enjoyable for the students and we would like to build on the success of the last conference this year.

In 2022, the conference will last almost four days, and all PhD students from our PhD school will actively participate. During the PhD conference, a total of 47 PhD students will present a poster. In addition to the poster, 33 students will give an oral presentation, 3rd year students have to pass the doctoral state exam, while 2nd, 4th and 5th year students present the progress of their PhD projects.

We believe that all participants will receive valuable feedback on their PhD projects, have a chance for professional networking, and, most importantly, enjoy their time at our campus with fellow PhD students, supervisors, and the Doctoral Board/Committee members.

On behalf of the Organizing Committee and Doctoral Board, I wish you a successful and mainly enjoyable meeting.

Ondřej Slabý
Chair of the Doctoral Board in Biomedical Sciences

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1. Andrejčinová Ivana
2. Beckerová Deborah
3. Belisová Denisa
4. Bosáková Veronika
5. Brezak Matea
6. Celiker Canan
7. Dostálová Lenka
8. Fedorová Veronika
9. Fedrová Pavla
10. Feiser Felicity Anne
11. Hašanová Klaudia
12. Hrala Matěj
13. Hrčková Anna
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27. Poovakulathu Abraham Sara
28. Pospíšilová Michaela
29. Souralová Tereza
30. Vadovičová Natália
31. Velezmoro Jauregi Gretsen
32. Vrbová Eliška
33. Vymazal Ondřej

ABSTRACTS OF POSTERS

34. Gonzalez Lopez Marcos
35. Gottumukkala Narendra Varma
36. Holomková Kateřina
37. Koždoňová Kateřina
38. Krishna Shwetha
39. Kročka Erik
40. Lodhi Yusuf
41. Moráň Lukáš
42. Okůnková Jana
43. Porokh Volodymyr
44. Pospíšil Jakub
45. Smolková Karolína
46. Sumbal Jakub
47. Tatíčková Martina

1. New roles of pattern recognition receptor-induced transcription factor signaling in monocyte responses

Andrejčinová I.[1,2], Bosáková V.[1,2],
Bendíčková K.[1], Hortová Kohoutková M.[1], Frič J.[1,3]

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Tight regulation of signaling cascades activated through pattern recognition receptors (PRRs) is crucial for innate immune cell functions. Efficient immune response followed by clearance of microbial pathogens requires strictly controlled cooperation of several transcription factors (TFs) including NF-kB and NFAT. Here I focus to define potential impact of treatment with calcineurin inhibitors on monocyte responses. Development of new tools for rapid single cell analysis of TF activation aims to improve diagnostics of acute inflammatory disorders such as sepsis.

We have analyzed the transcripts regulated by NF-kB and NFAT in stimulated monocytes. Based on the mRNA expression analysis in zymosan-stimulated monocytes, we hypothesized that NFAT co-regulates some immune response genes including CSF2, TNF and PTX3, but not IL6. Using commercial kit for flow cytometry, we have established intracellular flow cytometry (IC-FC) protocol as a tool for rapid evaluation of TF activity in monocytes. Preliminary results obtained from TF activity assessment indicate that this high-throughput method could be a valuable tool for studying TF activation in the cell subset of interest. Using this method, we identified non-classical monocytes as the major NFAT2-expressing subset. Further studies aim to identify NFAT2-regulated genes and interaction partners.

2. Modelling cardiovascular progenitor depletion and heart failure in Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a rare, X-linked neuromuscular disorder caused by mutations of the dystrophin gene resulting in progressive loss of skeletal muscle mass followed by cardiomyopathy. Current available medical care neither treats the cause nor improves muscle function. However, clinical trials and evaluations of patients' disease progress show that earlier intervention is more effective, and research shows that dystrophin deficiency affects cells from embryonic stage. DMD derived stem cells present higher DNA damage and impaired differentiation efficacy into beating embryoid bodies. The damage was previously shown to be caused at least partially by deregulation of nitric oxide synthase (NOS) and subsequent production of reactive oxygen/nitrogen species. Cardiovascular progenitors (CPs) are affected by increased DNA damage as well, possibly leading to a change of fate and their early depletion. DMD CPs are deployed and differentiate earlier, and the rate of cardiomyocyte death is higher. Initial experiments with NOS inhibitors show that some of the impairments connected to dystrophin deficiency can be rescued. Therefore, NOS inhibition could be a future target for pharmacological intervention preventing or delaying the onset of cardiomyopathy in DMD patients.

3. Investigation of the role of fibroblasts in the morphogenesis of branched organs

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Branching morphogenesis is a developmental process of many organs, which require large epithelial surfaces created within limited volume to fulfill their functions. Epithelial-mesenchymal interactions play a crucial role in this process. Fibroblasts, a mesenchymal cell type, provide crucial signals for epithelial morphogenesis, but their exact roles, both general and organ-specific, have not been fully elucidated.

In this study we investigate characteristics, distribution and function of Fsp1+ and Col1a2+ fibroblast populations in two branched organs: mammary gland and lacrimal gland.

We use genetically engineered mouse models for labeling (*Fsp1-Cre;R26-mT/mG* and *Col1a2Cre-ERT;R26-mT/mG*) and depletion (*Fsp1-Cre;R26-DTA* and *Col1a2Cre-ERT;R26-DTA*) of different fibroblast subpopulations. We analyze mammary and lacrimal gland tissue at specific developmental stages by histology and immunofluorescent staining techniques, tissue clearing techniques and state-of-the-art imaging approach.

We found that Fsp1+ and Col1a2+ cells are different cell populations and have distinct morphology and spatial distribution. While Fsp1+ cells are dispersed in stroma and have more rounded morphology, Col1a2+ cells are spindle-shaped and often localize in close contact with the epithelium. Depletion of Fsp1+ and Col1a2+ cells abrogated epithelial growth, suggesting important role of Fsp1+ and Col1a2+ cells in epithelial morphogenesis.

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4. Cellular and molecular interaction of MAIT cells in mucosal tissues and their role in inflammatory bowel disease

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Inflammatory bowel disease (IBD) manifests as chronic inflammation and is characterized by deregulated immune response. The identification of cellular immune players involved represents the major approach to unravelling the pathogenesis of IBD and may suggest new therapeutic strategies. Recently, MAIT cells have been identified as a possible key player in IBD. Activated MAIT cells produce cytokines including IL-26, a newly discovered cytokine involved in the pathology of IBD. IL-26 is not expressed in mice and therefore its effect on the course of inflammation has not been yet sufficiently investigated.

Our laboratory described a model of intestinal inflammation based on human iPSCs-derived intestinal organoids (IOs). Using this model, we aim to help to understand the role of MAIT cells and IL-26 in the pathology of IBD. We have described the relevancy of IOs to model IBD using various methods such as immunofluorescent labelling, flow cytometry and RNAseq. We have shown that MAIT cells can be isolated from human blood and intestinal tissue. Upon in vitro activation, they produce cytokines and effector molecules including IL-26 and their response differs depending on the origin of the stimuli. RNAseq and subsequent analysis have allowed us to describe the effect of IL-26 on healthy and inflamed tissue.

5. Optimisation of EKAREV-NLS mouse strain for investigation of ERK signaling patterns during mammary gland development

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An extracellular signal-regulated kinase (ERK) is a major signaling hub that mediates translation of signaling inputs into cellular responses. Its activity is regulated by a number of protein inhibitors and activators, resulting in distinct spatiotemporal ERK activation in the cells and tissues. These activation patterns have been linked to specific cell fate decisions, but the understanding of how these govern collective tissue morphology changes or cancerous transformation is not known.

Recent advancements in microscopy techniques and the enhancement of fluorescent protein characteristics have led to improvements in signaling research. Newly developed biosensor mouse strains enable tracking of kinase activity *in vitro* and *in vivo* in real-time, giving a more detailed insight into the complex nature of signaling pathways.

Here we utilise the EKAREV-NLS strain, that has endogenously expressed FRET-based reporter for ERK activity detection, to study processes during mammary gland development. The reporter construct contains potentially toxic fluorescent proteins that negatively affect mouse growth, life span and mammary gland development. This study investigates the effects of biosensor construct on EKAREV-NLS mouse mammary gland development and offers options for rescuing gland development. Further, we validate the biosensor activity under these conditions and describe ERK activation patterns occurring under growth factor treatments.

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6. Photo-stimulation of retinal organoids reveals light-regulated microRNA molecules in human retina

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MicroRNA molecules (miRNAs) act as post-transcriptional gene regulators that are expressed in a tissue-specific manner. In the retina miRNAs regulate many cellular functions including the adaptation of the retina to different light intensities, rapid turnover of the phototransduction cascade and circadian rhythms. Although light-regulated miRNAs have been recognized as important players, their functions in the retina is not fully understood, particularly in humans, as it is very challenging to study the miRNAs, because of the lack of human retinal tissues for experimental procedures.

Here we aimed to investigate light-regulated miRNAs in human retina using retinal organoid model. We developed a device that is capable to photo-stimulate retinal organoids using different intensities and different wavelengths of light. We photo-stimulated retinal organoids using this device and found 50 light-regulated miRNAs that are significantly up- or down-regulated. Detailed analysis of miRNA expression revealed that these miRNAs have a rapid turn-over and respond differently to different wavelengths of light. Taken together our data indicate that retinal organoids represent an elegant model to study light-regulated miRNAs and open up new avenues for the research on novel light-regulated miRNA targets, adaptation of human retina to light, and regulation of light-regulated circadian rhythms in humans.

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7. CRISPR/Cas9 knockout screening revealed genes involved in CD20 regulation

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CD20 is a surface antigen expressed almost exclusively on the surface of B cells. As such, it is the main target of monoclonal antibodies (mAb) like rituximab which are used as first-line treatment in the immunotherapy of B-cell malignancies. Unfortunately, malignant B cells often develop resistance to monoclonal antibodies, which leads to therapy failure and disease relapse. Several mechanisms responsible for the resistance development have been described, with the downregulation of CD20 on the surface of the malignant cells being one of them. Even though anti-CD20 mAbs have been used in the therapy of B-cell malignancies for more than two decades, the role and regulations of CD20 remain unclear.

We performed CRISPR/Cas9 screening on CD20-low Ramos cells resistant to rituximab, which were generated in our lab. The screening identified several genes whose disruption led to the upregulation of CD20 on the surface of cells, and we successfully validated the results in polyclonal knockout populations. Furthermore, we generated monoclonal knockout cell lines for identified genes and confirmed CD20 upregulation in these cell lines not only on the surface but also at the level of total expression in the cells. Experiments are currently ongoing to identify the molecular mechanisms responsible for the regulation of CD20 through our identified screening hits.

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8. The role of miRNA and protein-coding genes in human neural differentiation *in vitro*.

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Human neural differentiation is a critical and highly organized process that involves precise control of differentiation, self-renewal, and cell-fate commitment. When deregulated, it can lead to the development of severe congenital disorders as well as tumors of the nervous system. Here, we aimed to study neural differentiation *in vitro* to identify the key players in balancing differentiation versus self-renewal of stem cells. We first modeled early human neural differentiation using human pluripotent stem cells (hPSCs) via the formation of neural rosettes. Our data show the time-dependent activity of the major signaling pathways during the switch from pluripotency to neural cell fate and the mechanism by which they form [1]. We further studied miRNA regulation of neural differentiation *in vitro* since miRNAs are powerful regulators of gene expression during self-renewal and differentiation. For the first time, we present a comprehensive miRNA profile of human self-renewing NSCs and point to numerous miRNAs with yet undescribed roles during *in vitro* human neural development [2, submitted]. Lastly, we used our 3D neurodifferentiation cultures and optimized an *in vitro* Glioblastoma-Cerebral Organoid (GLICO) model. We show that exposure to the cerebral organoid microenvironment significantly changes the expression profile of the glioblastoma cell line [3, *under review*]. Altogether, our data provide new insights into the regulation of self-renewal of NSCs and neural differentiation *in vitro* as well as its pathological display in glioblastoma multiforme.

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[2] Fedorova, V., Amruz Cerna, K., Oppelt, J., Pospisilova, V., Barta, T., Mraz, M., Bohaciakova, D. MicroRNA profiling of self-renewing human neural stem cells reveals novel sets of differentially expressed miRNAs during neural differentiation in vitro, submitted.

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9. MinION as a tool for sequencing of variable genome sites of pathogenic treponemes

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Genus *Treponema* includes number of human and animal pathogenic species and subspecies, e.g., *Treponema pallidum* subspecies *pallidum* (TPA) causing syphilis and *Treponema pallidum* subspecies *pertenue* (TPE) causing yaws. Genome similarities among TPA genomes (99.95%) [1] and TPA and TPE subspecies (99.8%) [2] indicate that small differences in genome sequence play important role in pathogenicity. Regions that show high sequence variability between strains, genes containing sequence changes in rabbit syphilis, repetitive regions and paralogous genes are key for understanding pathogenicity of treponemes and host immune response. Those regions could be candidates for vaccine development [3].

Specific primers were designed to amplify regions of interest (ROI, n = 36) with unique 200 nt-long tags at the start and at the end of each region. All PCR products of each clinical sample were equimolarly pooled and barcoded, multiple samples were sequenced together using long-read sequencing technology (Oxford Nanopore, MinION). Unique 200 nt tags were mapped to masked reference genome to identify the read location. *De novo* assembly with filtered reads was performed followed by polishing of consensus for each ROI. Clinical samples of TPA and TPE with different sequence profiles were selected, sequenced and consensus sequences of ROIs were analyzed.

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[3] Šmajs D, Zobaníková M, Strouhal M, et al. Complete Genome Sequence of Treponema paraluiscuniculi, Strain Cuniculi A: The Loss of Infectivity to Humans Is Associated with Genome Decay. PLoS ONE. 2011;6(5). doi:10.1371/journal.pone.0020415.

10. RAD51 Filament Defects in Cancer-Associated S296L Mutant

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RAD51 is involved in double-strand break repair and processing of stalled replication forks. It forms ATP-dependent filaments on single-stranded DNA that protect it from nucleolytic degradation at replication forks. It also facilitates pairing with template DNA strands through the formation of a Displacement Loop (D-loop), which is a critical step in both homologous recombination and restart of stalled forks. D-Loop defects lead to fork collapse and defective DSBR, so is unsurprising that RAD51 mutations are found in many types of cancer. Here we aim to determine the mechanism by which a mutation at S296, which was found in HNSCC, leads to genomic instability and cancer.

Mouse Embryonic Fibroblasts (MEFs) carrying S296L have increased chromosomal aberrations. We evaluated fork protection and D-Loop formation as potential causes of these aberrations, but significant defects were found only in D-Loop activity. Detailed mechanistic characterization of this process revealed alterations in the nucleoprotein filament, leading to defects in subsequent strand exchange steps.

Our findings are consistent with defects caused by a high number of stalled replication forks which failed to restart. We hypothesize that these defects lead to fork collapse, which is repaired by error-prone pathways rather than HR, leading to the observed chromosomal aberrations.

11. Changes in blood-cerebrospinal fluid barrier integrity as a reaction on peripheral nerve injury

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Peripheral nerve injury can induce inflammatory reactions in remote structures of the central nervous system (CNS). One possible explanation is that products of Wallerian degeneration, damage-associated molecular patterns (DAMPs), from the distal part of the damaged nerve might migrate and spread the neuroinflammatory reactions into CNS through the blood-cerebrospinal fluid (BCSF) barrier. Changes in the barrier permeability might be caused by the alternation of tight junctions (TJs) of epithelial cells in the choroid plexus (CP), leading to disruption of the BCSF barrier. To test the tightness of the BCSF barrier following nerve injury (sterile chronic constriction injury - sCCI), we intravenously injected the fluorescent conjugated dextran FluoroEmerald (FE) at different times of circulation with subsequent analyzes in the CP.

Distribution of FE and simultaneous immunohistochemical detection ED2 and ED1 macrophages, antigen-presenting cells (MHC-II), T-cells (OX-52), and dendritic cells (OX-42) were analyzed using a fluorescence microscope. In samples from animals with sCCI, FE particles were found inside cuboidal cells, epiplexal Kolmer cells, and inside ventricular ependymal cells. A significant increase of the number of FE+ cells was found in CP after 3 and 7 days of CCI and FE circulation for 30min and 5h.

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12. Virulence and bacteriocinogeny among *Escherichia coli* causing sepses in dromedars

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Dromedar husbandry belongs to a rapidly developing part of food industry due to high demand for nutritious milk and meat. However, herd keeping leads to increased spread of infections among animals. Extraintestinal infections caused by *Escherichia coli* often cause deaths of dromedary camel calves. The aim of this study was to characterize these strains in terms of virulence and bacteriocinogeny.

Altogether, a set of 282 isolates from diseased and 139 isolates from healthy animals was isolated from dromedary farms in United Arab Emirates. These isolates were characterized using multiplex PCR for presence of determinants for 37 virulence factors and 38 bacteriocins.

Presence of virulence determinants was found higher in isolates from diseased animals. Among these isolates, determinants for siderophores, adhesins, hemolysins and genotoxins were mostly detected. Bacteriocinogeny was also higher in isolates from diseased animals. The most abundant encoded bacteriocins were colicins M, B, Ia and microcins L and V. *E. coli* isolated from dromedarswas also found to produce rarely detected bacteriocins such as colicin R and microcin PDI. Moreover, several strains are considered to produce a novel bacteriocin type, since no already known bacteriocin determinant was detected but these strains still inhibit indicator strain.

Obtained results may help to better understand pathogenesis of sepsis in dromedars and to better diagnose colisepticemia among dromedary camel calves.

13. Searching for new factors involved in RNA tailing and decay

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Gene expression in eukaryotes is regulated by diverse mechanisms. The key regulators include the production and stability of coding and noncoding RNAs. Every primary RNA transcript has to undergo further processing and modifications, often involving complex machineries. The second main parameter for RNA metabolism is its stability, which defines the time frame within which it performs its role.

Nontemplated RNA tailing plays critical roles in RNA processing, specificity as well as stability. Dysregulation of RNA tailing leads to disease. Here we focus on nontemplated addition of one or more uridines (oligo(U)) which is mediated by the activity of terminal uridyltransferases (TUTAses). The target specificity and function of oligoU is established by TUT associated cofactors. DIS3L2 is a 3' to 5' exoribonuclease recognising RNA molecules uridylated by TUT enzymes and degrading them, establishing the TUT-DIS3L2 surveillance (TDS) pathway. [1][2] However only scarce number of other factors employed in TDS is known to this date.

We use proximity labelling miniTurboID [3] to identify additional cofactors in this pathway. We use HEK293T cell line with inducible expression of proteins of interest fused with promiscuous biotin ligase to biotinylate proximal proteins. The biotinylated proteins are purified with streptavidin and identified by quantitative mass spectrometry.

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14. "Molecular" resection margins in squamous cell carcinoma of the oral cavity – report of the first results of the multidisciplinary view.

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The therapy of squamous cell carcinoma of the oral cavity has significantly intensified in the last decade. Here, we focused on sensitive mutation analysis of resection margins that could improve the prediction of relapse and/or sensitivity to specific drugs.

DNA was isolated from 26 patients (tumor, peripheral blood and margins from all patients, in total 3 samples/patient). We performed Illumina sequencing using a panel of 88 cancer genes. Only non-synonymous variants in tumor/a margin that were reported in the ClinVar database as “pathogenic”, “likely pathogenic” or “of uncertain significance”, and were simultaneously not present in the peripheral blood, were selected for further analysis.

In total, we found 21 mutated genes, among them mainly tumor suppressor genes involved in DNA repair. We detected mutations in DNA isolated from 22/26 tumors, and in 5/26 tumor margins. Gene TP53 was the most commonly mutated gene folloved by BRCA1/2 and CDKN2A. The median tumor load were 2 patogenic mutation per patient on average (range 0-9). This parameter did not correlate with the presence of histological markers like perineural invasion probably due to small cohort size. Similarly, we did not observe association of mutations in resection margins and probability of disease relapse.

The spectrum of the tumor mutations is similar as in other studies with the exception of mutations in the BRCA genes, which were not found frequently mutated in OSCC (12-19%). The data in the literature suggest BRCA mutation in OSCC examinated by imunohystochemistry technique range from 44% (139patients) to 63% (60patients). Variants identified in our dataset are often introducing stop codons leading to truncated and non-functional proteins. The potential application of BRCA(PARP) inhibitors for OSCC needs to be elucidated.

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15. RGDT alginate allows bioprinting and 3D culture of various cell types

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3D bioprinting allows control over spatial organization of cells in 3D cultures. The printed hydrogels must possess suitable viscoelastic properties necessary for the printing process, and moreover, properties specific for *in vitro* cultivation of individual cell types. Alginate, as natural polysaccharide hydrogel, is a common material of choice. Without further modifications, alginate however limits cell behavior such as migration of cells, their proliferation, and interaction of cells with the matrix and each other.

Modification by adhesive RGDT peptide motif [1] provided adhesion of different cell types (*e.g.* human pluripotent stem cells, mesenchymal cells and endothelial cells) and by optimization of bioink composition, concentration, and crosslinking method, we increased stability of bioink before and after the printing. We reached high proliferation rate of cells and allowed the printed spheroids to fuse into bigger structures. Via pre-aggregation of cells before the bioprinting, we were able to enhance dramatically cell survival in alginate.

In our modified alginate we could see increased growth of stem cells, spreading of stromal cells within the gel and ultimately, formation of three-dimensional endothelial networks. Thanks to 3D bioprinting, we are able to build pre-vascularized tissue models, which include combination of different cell types and hydrogels.

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16. Whole genome sequencing and analysis of *Treponema pallidum* subsp. *pertenue* of non-human primate origin

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Treponema pallidum subsp. *pertenue* (TPE) is the causative agent of yaws disease, endemic in tropical regions of Africa, Asia and Pacific. While this disease mainly affects human children, this bacterium also causes infection in wild non-human primate populations in Africa [1]. Previously, there were only two TPE whole genome sequences from samples isolated from non-human primates [1]. One of the strains was found to be highly similar to the human infecting strains [2]. To analyze the similarity between the strains of human and non-human primate origin, we determined whole genome sequences of eight different samples and 13 draft genomes, obtained from four different species from four different areas in Tanzania [3]. These samples were selected from available set of samples based on genetic diversity. Non-human strains were compared to the strains of human origin and no consistent genome differences were found, suggesting that the non-human primate populations serve as a reservoir of TPE. We also compared all 22 Tanzanian genomes among themselves. Out of 231 possible combinations of genome-to-genome comparisons, 4 comparisons revealed an unexpectedly high genetic degree of genetic similarity between different NHP species supporting the inter-species transmission of TPE among NHPs.

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17. One-stop microfluidic device for retinal organoid formation and long-term cultivation

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Organoids are self-organizing stem-cell derived 3D constructs that mimic in vivo structure and function. While organoids make for excellent models to study organ development and disease, a variety of challenges remain. Most, if not all, organoids suffer for labor-intensive cultivation protocols as well as heterogeneity between organoids. Most organoids also suffer from tissue specific drawbacks, for example, retinal organoids poor photoreceptor maturation and retinal ganglion cell degradation in long term culture [1].

Microfluidic platforms have the potential to help overcome some of these challenges by allowing for automation and precise control of the organoid microenvironment. Previous work has shown the clear advantages of utilizing microfluidic devices for the maturation of retinal organoids, by decreasing oxidative stress as well as workload [2]. Nonetheless, this approach still requires the manual transfer of previously formed organoids to the microfluidic device. In this work we therefore aimed to create a microfluidic device that is capable of both organoid formation as well as maturation without requiring direct handling of the organoids. We formed retinal organoids over 120 days within continuous medium perfusion and compared them to organoids grown in static conditions by analysis of morphology, proliferation and cell differentiation.

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18. Microfluidic systems for easy and uniform generation of cerebral organoids

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Cerebral organoids are three-dimensionally formed by pluripotent stem cells with multi-layered organization that partially recapitulate brain development. Despite of potential of organoid models, the recent protocols for generation of organoids are limited mainly in initial formation of organoids, their non-uniform size, labor-intensive manipulation, and continuity of medium exchange leading to final divergence of individual organoids. Microfluidic system is promising platform to overcome these disadvantages. Due to automation, microfluidic is capable of continual medium flow, which allows to create fully controllable microenvironments.

In this work, we present microfluidic system including microwell-based chips allowing formation of hPSCs and further differentiation towards cerebral organoids. Upon in silico simulations, we optimized several chip designs supporting effective nutrient exchange around organoids. We also simulated organoid production of lactate and diffusion in microfluidic chip, where dynamic exchange of medium provides decrease concentration of lactate around organoids. Our designs of multilayer chip enable to create uniform aggregates, differentiate them to cerebral organoids and influence their quality by flow control. Overall, we create a device which allows to produce uniform population of 3D organoids in closed system and simplify manipulation for 3D cultivation.

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19. Differentiation of pluripotent stem cells using odontoblasts-specific transcription factors

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Tooth morphology and function are mainly determined by dentin – the most abundant hard dental tissue. Dentin is produced by odontoblasts which are also responsible for its maintenance and make this hard tissue sensitive to external stimuli (e.g., carries, changes in temperature). While indispensable for the function of teeth, dentin has only limited reparative capacity. Currently, there are no widely used dental treatments for facilitating dentin regeneration or reparation that use innate biological mechanisms. Furthermore, attempts to obtain odontoblasts in vitro remain generally unsuccessful, likely due to their highly specialized, postmitotic phenotype.

To address this issue, we have designed a novel approach to enable direct differentiation of pluripotent stem cells into odontoblasts by harnessing the knowledge about their developmental trajectories constructed using single-cell RNA-seq data from the continuously growing mouse incisor.

Here we show a controlled differentiation of pluripotent stem cells into odontoblast-like cells utilizing overexpression of selected, odontoblast-specific transcription factors. Our results show upregulated expression of late odontoblast markers (DSPP, DMP1) associated with the overexpression of the selected genes. This suggests that the chosen transcription factors are important regulators of the odontoblast-like phenotype.

We anticipate our results to highlight the role of the selected transcription factors in odontoblast development (differentiation) and introduce their possible utilization in regenerative dentistry.

20. Sequencing of long non-coding RNAs in exosomes of colorectal cancer patients

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Current research shows that exosomal long non-coding RNAs (lncRNAs) are associated with cancer development. As lncRNAs are often tissue specific, their quantification in exosomes is proposed as a non-invasive method for early detection of colorectal cancer (CRC) [1,2].

Exosomes were isolated by gel chromatography from 150 µl of serum of CRC patients and healthy donors. Their quality and quantity were confirmed by electron microscopy and DLS analysis; protein markers were detected by Western blot. After RNA isolation, cDNA libraries were prepared and sequenced using NextSeq 550.

Sequencing data confirmed the presence of both protein-coding (50%) and non-coding RNAs, which consisted mainly of lncRNAs (28.2%), pseudogenes (15.2%) and other RNA types (6.5%). Results also showed significantly altered levels of some lncRNAs that could distinguish samples from CRC patients and healthy controls. Using GSEA analysis, we observed significantly enriched classes of genes related to DNA repair or cell cycle regulation.

Our preliminary data suggest that lncRNAs represent a significant fraction of the RNA present in exosomes and their distinct levels can separate CRC patients from healthy controls. The analysis of enriched genes also showed a significant representation of lncRNAs involved in cell cycle regulation and DNA repair, suggesting their possible involvement in cancerogenesis.

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21. Multiple *Haemophilius ducreyi* strains detected in samples of yaws suspected cases from Papua New Guinea

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Yaws is a neglected tropical diseases (NTD) being the focus of WHO's current NTD roadmap aimed at eradication of the disease by 2030. The etiological agent of yaws, *Treponema pallidum* subsp. *pertenue* (TPE) causes infections that are manifested as skin lesions in the lower limbs before disseminating into the blood[1]. During the TPE analyses of 1080 suspected yaws samples acquired from Papua New Guinea (PNG), it was observed that 33.8% (n=365) of the skin ulcers were positive for *Haemophilus ducreyi* (HD) and 23.5% (n=255) were positive for TPE. In addition, TPE and HD coinfections were detected in 7.6% (n=83) of the total samples analysed. Since HD causes similar clinical manifestations as that of yaws, it is commonly misdiagnosed as yaws ulcers. This misdiagnosis can potentially hinder the monitoring and control of yaws eradication program; thus, molecular detection of TPE appears to be required.

Subtype analyses of HD were performed to ascertain if the circulating strains in Namatanai, PNG, corroborate with the HD results from a previous study conducted in Lihir, PNG [2]. The suspected yaws samples showed the presence of seven genotypes circulating in Namatanai. From the seven genotypes, four genotypes were identified in previous studies, namely, I.3, I.4, I.5, and II.3. In this study we detected three new genotypes exclusive to Namatanai, namely, I.9, I.10, and I.11.

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22. ERK3 regulates key mechanisms of Breast Cancer progression.

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Extracellular-regulated kinase 3 (ERK3) is overexpressed in breast cancer (BC) and strongly correlates with poor patient survival [1]. However, the mechanisms by which ERK3 contributes to BC remain unknown. Therefore, this study aims to elucidate the role of ERK3 in breast cancer progression. We used BT549 and MDA-MB231 cell lines transfected with siRNA to knockdown ERK3 expression, followed by a variety of biochemical and functional assays. Our results show that ERK3 promotes migration in the wound healing assay while it has no effect in the transwell migration assay. Additionally, ERK3 reduces cell adhesion to different extracellular matrix (ECM) substrates, such as collagen IV, a key basal membrane protein; and reduces the expression of key transcription factors involved in epithelial-to-mesenchymal transition (EMT), i.e. SNAIL and SLUG. These findings suggest that ERK3 exclusively regulates collective cell migration, which is the main form of BC migration and invasion [2]. Finally, the downregulation of EMT suggests that the cells undergo mesenchymal-to-epithelial (MET) transition, a process necessary for secondary organ colonization [2,3]. In conclusion, our model shows that ERK3 plays a role in collective BC migration and metastasis by regulating cell motion, adhesion to the basal membrane and MET.

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23. Vascularization of the wrist and dorsal capsulotomy Pronator quadratus muscle free flap – vascular pedicles of the radial artery

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The radial-based dorsal capsulotomy is commonly used surgical approach to the radiocarpal joint when the incision is made along the radiocarpal (RCL) and the intercarpal ligaments (ICL). The radial forearm flap is used for the reconstruction of the wide variety soft-tissue surgical defects and can include pronator quadratus muscle (PQM) flap and its vascular pedicles of the radial artery. The aim of the study was to provide detail description of arteries supplying dorsal portion of the radiocarpal joint capsule in relation to incisions and anatomical location of arterial branches of the radial artery perfusing PQM.

The anatomical study was based upon analysis of fresh cadaver upper extremities. The radial, anterior interosseous and ulnar arteries were cannulated and injected by colored silicon rubber followed by macroscopical and microscopical dissection.

The dorsal portion of the wrist joint capsule is vascularized from the radial dorsal carpal branch, supplying branches run upwards and across the ICL. Based on these results we suggest the capsulotomy with incisions respecting the position of the radial branches crossing the ICL. The most constant and widest radial vascular branches of the PQM are mostly localized in distal radial quarter of the muscle. The PQM flap should be harvested and raised from the distal radial pedicels.

24. An induced pluripotent stem cell-based model to study the mechanobiology of myocardial fibrosis

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Cardiac fibrosis onsets following chronic insults applied to the myocardium, and it is characterized by the abnormal accumulation of extracellular matrix (ECM). The differentiation of cardiac fibroblasts (cFbs) into myofibroblasts drives pathological ECM remodelling, which compromises cardiomyocytes (CMs) homeostasis and eventually leads to heart failure [1]. Here, we adopted bioengineering tools and induced pluripotent stem cells (iPSCs) to investigate how fibrotic ECM affects CMs properties.

We derived cFbs from iPSCs (iPSCs-cFbs) and optimized a protocol to induce their differentiation into myofibroblasts based on the fine tuning of TGF-beta signalling. Next, we established a decellularization procedure which allowed us to obtain fibrotic ECM (dECM). Through this strategy, we succeeded in analyzing the pathological changes occurring during the deposition of diseased ECM by activated cFbs. Then, we generated iPSCs-CMs and cultured them either on healthy or fibrotic dECM and further validated our model by using cardiomyocytes differentiated from iPSCs derived from Duchenne muscular dystrophy (DMD) cardiopathic patients [2].

We finally established a 3D *in vitro* culture system, which entails the co-culture of isogenic iPSCs-CMs and -cFbs and better reproduces the cellular complexity and functionality of the human heart. This system represents a powerful tool for personalized medicine applications [3].

By capitalizing on this approach, we might be able to recapitulate the accumulation of fibrotic tissue occurring during heart disease and investigate the contribution of pathological ECM to the progression of heart failure.

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25. Receptor tyrosine kinases signaling involves the primary cilium

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The primary cilium acts as a signaling center of most mammalian cells. It's involved in key regulatory functions in many cellular processes, either by spatial compartmentalization of the signaling molecules, or precise fine-tuning of their action. This is highlighted by the growing list of pathologies that are now being associated with the primary cilia. These diseases commonly present symptoms that include developmental disorders of the bone, congenital heart defects, renal malformations, cognitive disorders, obesity, and multiple types of cancer. Receptor tyrosine kinases (RTKs) are key regulators of critical cellular processes, such as proliferation and differentiation, cell survival, cell migration and cell-cycle control. Numerous diseases result from genetic changes or abnormalities in the RTKs' regulation, activity or distribution. A handful of these mutations are linked to cancers, severe bone, metabolic and developmental disorders. Out of 58 existing members of the RTK family, only a small number of receptors has been associated with the primary cilium. The evidence connecting other RTKs with primary cilium is lacking, despite its high potential in addressing many human pathologies. In the current study, we set to address and expand the current understanding on the relationship between RTKs and primary cilium. We identified a considerable number of RTKs localize to the ciliary compartment, while noting differences between wild-type and disease-associated variants. Our data highlights the importance of ciliary RTK localization for a precise regulation of its signaling. These findings expand our understanding of the RTK signaling and the primary cilium compartment in development and disease.

26. Unveiling the function of YAP in the activation of cardiac fibroblasts

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Cardiac fibrosis, one of the primary reasons of end-stage heart failure, is a pathological process mediated by cardiac fibroblasts (cFbs). It is determined by the maladaptive remodeling of the heart extracellular matrix (ECM), resulting in its excessive deposition and stiffening. Despite its role in most cardiac dysfunctions, effective therapies against fibrosis are currently not available. Yes-associated protein (YAP), a mechanosensitive protein, mediates cardiac fibroblast activation and consequently the resulting cardiac fibrosis, providing a possible target for therapy.

To study YAP involvement in cardiac fibrosis progression, CRISPR YAP WT and KO pluripotent stem cells (PSCs) were differentiated into cFbs. YAP KO PSCs showed an impaired capability to differentiate into cFbs demonstrated by the lower expression of cardiac markers (NKX2.5 and GATA4). This was further confirmed by RNASeq which revealed more similar profiles between WT cFbs and primary cFbs than the YAP KO cFbs. Also, the latter can be activated by expressing typical myofibroblast markers (α-SMA and FAP) but show impaired functionality demonstrated by the hindered capability of YAP KO cFbs to contract collagen gels compared to the WT counterpart.

These observations show that YAP has a role in cFbs differentiation and activation, but further mechanistic dissection is needed to fully understand its involvement.

27. Regulation of Primary Cilia by Ciliogenesis associated kinase 1 (CILK1)

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Primary cilium is a microtubule-based organelle protruding outside of the cell, transducing extracellular cues and regulate cell signalling. Proper function of cilia is important in development as well as homeostasis of the tissues. Ciliogenesis and maintenance of the cilia is a dynamic and complex processes, and loss-of-function of the critical regulators produces signalling defects that manifest in a pleotropic group of disorders collectively termed as ciliopathies[1].

CILK1 (Ciliogenesis associated kinase 1), an RCK (ros cross-hybridizing kinases) family member, is an evolutionarily conserved and ubiquitously expressed serine/threonine kinase that functions in multiple organ development. CILK1 has been shown to regulate cilia formation, architecture and signalling. Loss of CILK1 activity leads to ciliopathies such as short rib-polydactyly syndrome and endocrine-cerebro-osteodysplasia due to deregulation of ciliary structure and signaling[2-4]. In this study, we used microinjection of small amounts of active CILK1 to challenge primary cilia at the physiological level of CILK1 expression. We also implemented a conditional knockdown system to determine the effects of endogenous CILK1 downregulation, in order to identify the molecular mechanism of CILK1 action. We also identified IFT-B complex proteins being phosphorylated by CILK1 in vitro, significance of which is to be determined in the future. Understanding these mechanisms would allow for better therapeutic options for the ciliopathy patients.

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28. Role of RAD51 in the metabolism of G-quadruplex structures

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G-quadruplexes (G4Q) are one of the alternative DNA secondary structures formed in human cells on guanine-rich regions. High-throughput sequencing identified more than 700,000 potential G4Q forming sequences indicating the importance of G4Q in controlling some biological processes [1]. They are generally believed to regulate replication, transcription and telomere maintenance. On the other hand, once formed, quadruplex is highly stable and thus can pose an obstacle for replication fork progression [2]. Not only quadruplexes but other endo- and exogenous factors may also obstruct replication. Therefore, cells developed several mechanisms to deal with it. RAD51 protein is well described as a key protein of homologous recombination, one of the pathways used to repair DNA double-strand breaks. However, it has recently been shown to participate in the protection and restart of stalled replication forks [3]. This work identified RAD51 interaction with G4 and focused on the biochemical characterization of the formed complex and its biological role.

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29. Xeno- and Feeder-Free Clinical-Grade Human Embryonic Stem Cell Lines for Cell Therapy

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Human embryonic stem cells (hESCs) are increasingly involved in clinical trials as they can be a gamechanger for treatment of many human diseases. To achieve the therapeutic effect, the quality of hESCs has to be taken into account, therefore current good manufacturing practice (cGMP) has to be implemented in the transport of embryos, derivation of inner cell mass to xeno-free, feeder-free and defined hESC culture and freezing. In-depth characterization of hESC lines focused on safety, pluripotency, differentiation potential and genetic background has to complement this process.

We established cGMP defined xeno-free and feeder-free system for the derivation, culture, and banking of clinical-grade hESC lines with use of laminin 521 and cGMP defined xeno-free Nutristem medium. We developed quality control testing with criteria concerning sterility, safety, and characterization that ensures the clinical-grade quality of hESC lines. Characterization of hESC involves the evaluation of pluripotency and differentiation potential. Sterility testing is accompanied by endotoxin level establishment, mycoplasma detection, and environmental screening. Safety testing involves, apart from karyotype establishment, the sequencing panel CZECANCA for the cancer predisposition evaluation that provides important information for future use of hESC lines in cell therapies. We successfully validated established procedures on three clinical-grade hESC lines MUCG01, MUCG02 and MUCG03 that are suitable for cell therapy according to current good manufacturing practice.

This project was evaluated as excellent in terms of the results and meeting the project objectives by the Ministry of Health of the Czech Republic.

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30. eIF4F controls AMPK activity in BRAF^{V600E}-mutant melanoma cells

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Oncogenic mutations in the RAS-RAF-MEK-ERK signaling pathway are common drivers of metastatic melanoma. Patients usually respond to BRAF/MEK inhibitors, but resistance to treatment often emerges.

The eIF4F eukaryotic translation initiation complex plays an important role in melanoma resistance to drugs targeting BRAF and MEK kinases [1]. Therefore, we decided to characterize the crosstalk between the ERK and eIF4F signaling pathways in melanoma in a proteomic screen. It revealed several AMP-dependent protein kinase (AMPK) regulators as potential common targets of ERK and eIF4F. In the subsequent experiments, we concentrated on MO25, part of an AMPK-activating complex (LKB1-STRAD-MO25), and PP2Aα, an AMPK-inhibiting phosphatase.

RNA interference revealed LKB1-independent AMPK activation in melanoma cells upon eIF4F inhibition. This was later confirmed in LKB1-deficient BRAF^{V600E}-mutant melanoma cells. Furthermore, PP2Aα downregulation seems to play an essential role in AMPK activation, as RNAi-mediated knockdown of PP2Aα and a small-molecule PP2A inhibitor, okadaic acid, both potently promoted AMPK activity.

Previous studies reported negative feedback between ERK and LKB1 in BRAF^{V600E}-mutant melanoma cells. Both ERK and its target RSK phosphorylated LKB1, compromising its ability to activate AMPK [2]. However, our findings show the existence of a non-canonical, LKB1-independent control of AMPK activity in BRAF^{V600E}-mutant melanoma cells, mediated by eIF4F and PP2A.

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31. Role of Amyloid Precursor Protein in Astrocytes in Alzheimer´s Disease

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Astrocytes are specialized glial cells that when exposed to inflammatory factors go through a process known as reactive astrogliosis. Reactive astrocytes are a neuropathological feature of Alzheimer's disease (AD) [1]. The etiology of AD is often explained by the accumulation of β-amyloid peptides, a proteolytic product of the amyloid precursor protein (APP) [2]. However, inflammation also plays a prominent role in the development of AD [3]. How reactive astrocytes participate in the pathogenesis of AD remains poorly understood. To elucidate the role of astrocytes in the pathogenesis of AD we exploited different *in vitro* and *in vivo* models to investigate the role of Amyloid Precursor protein in setting the immune response of astrocytes. Our results show that overexpression of APP in human cortical astrocytes induces reactive phenotype, similar to astrocytes found in brains of AD patients. These reactive astrocytes produce significantly increased levels of INF-γ in comparison with control astrocytes. Similar positive correlation between APP levels and INF-γ is found also in astrocytes from AD transgenic and traumatic brain injury mice models. Our data indicate that astrocytes are activated by high levels of APP, which promotes production and secretion of interferons and recapitulates salient features of astrocytes in AD.

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32. Genotyping of the causative agent of syphilis: updated MLST results for samples collected in the Czech Republic between 2004-2021

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Syphilis is a multistage venereal disease caused by *Treponema pallidum* subsp. *pallidum*. There are about 5,6 million new cases worldwide annually[1], with a few hundred of them in the Czech Republic (e. g., 612 in 2019, and 870 in 2020, respectively)[2].

The aim of this study was to map circulating strains in the Czech population and how they differ over time. Besides determining allelic profile of TPA strain, macrolide resistance in the samples was examined. Clinical samples were collected from Brno and Prague by collaborating laboratories during 18-year period. DNA isolated from this material was used for nested PCR of 4 loci, 3 typing loci (TP0136, TP0548, TP0705) and macrolide resistance locus 23S. Loci were sequenced by Sanger sequencing.

In total, there were 1186 samples examined from 1015 patients. From all samples, 582 were PCR positive (437 swabs, 138 whole blood, and 4 others), and 604 were PCR negative (262 swabs, 330 whole blood, and 12 others). Out of 582 PCR-positive samples, 454 were typeable, with more than 50% fully-typed samples containing over 25 allelic profiles, while previous report[3] from 2018 showed 16 different fully-typed allelic profiles. From 360 samples positives for 23S rDNA, 260 had mutation encoding macrolide resistance.

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33. Neutrophils – immunity influencers influenced by NFAT

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Calcineurin (CN) - Nuclear factor of activated T-cells (NFAT) inhibitors are commonly used immunosuppressants to inhibit adaptive immunity, but they frequently leave patients vulnerable to opportunistic infections. CN-NFAT signalling is not exclusively used by T-cells, but surprisingly, many other immune cells are undesirably affected. This fact is highly important for understanding the adverse effects of CN-NFAT inhibitors therapy. The exact impacts on human neutrophils' function after NFAT inhibition remain elusive.

We evaluated the effects of CN-NFAT inhibitors on neutrophil response induced by heat-killed *Candida albicans, Aspergillus fumigatus*, and other pattern recognition receptors (PRRs) ligands. We performed RNA sequencing to see global changes in gene expression. We also used qPCR analysis of selected genes and ELISA to understand the kinetics of NFAT-controlled functions.

We showed that after PRRs activation, neutrophils significantly increase the expression of molecules involved in inflammation control. Their expression is inhibited using CN-NFAT inhibitors, as specifically shown in the expression of the inflammation-mediating molecule Cyclooxygenase-2 and in the production of chemokines CLL-2 and CCL-3. We will monitor the possible inhibition of neutrophils' chemotactic ability by migration assay with monocytes.

These results show neutrophils as crucial influencers of immune response to pathogens and reveal that their ability to express modulatory molecules is highly influenced by NFAT inhibitors.

34. Spatiotemporal visualization of tooth mineralization uncovered new features in mouse dental development

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Teeth are one of the major mineralized tissues in vertebrates, they are formed from specialized cell types – odontoblasts - able to produce a hard calcified matrix rich in phosphate and carbonate minerals [1]. Rodents, such as mouse, have evolved a continuously growing incisor able to regenerate as an adaptation to their highly specific dietary habit [2]. In contrast, their molars exhibit limited regenerative properties in the same way as human dentition. For this reason, mouse model is an attractive model in regenerative dentistry to identify possible pathways leading to a natural restore of the tooth. Although these are actively investigated topics, the exact mechanisms controlling the dynamics of dental development and pace of growth remain mostly not understood [3].

Here, we characterized how mandibular and maxillary incisors grow in natural conditions according to the age and sex of the animal. Moreover, we studied this model in the damage context connecting this regenerative effect with the potential role of mechanosensing *in vivo* and *in silico*. Finally, we also explored the dynamics of calcification and growth in the molars, focusing on the crown shaping and elongation of the roots into the jawbone during early development.

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35. Chasing the epigenetic landscape to enhance CAR-T cell activity by preventing CD19 antigen escape in relapsed models

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Chimeric antigen receptor T cells (CAR-T cells) are genetically engineered T lymphocytes that will target, recognize and kill the malignant cells in a specific and effective manner. Despite their precisive mode of action, CAR-T cells still possess certain limitations, often leading to a relapsed disease state.

Our mouse model demonstrated a certain fraction of recurring tumors. Their closer analysis revealed that these relapsed cells had lost CD19 expression. With the help of epigenetic library screening, we identified two main groups of drugs that have a role in CD19 downregulation in relapsed state. These act as DNA methylation&Aurora kinase inhibitors. Bisulfate sequencing results further confirmed the hypermethylation at the CD19 promoter. However, we also showed that the change in the methylation at the CD19 promoter is a site specific but not global. Furthermore, using DNA demethylation agent azacytidine we managed to restore CD19 levels in vitro and also to prevent its loss in recurrent mouse model in vivo.

Our findings showed how the epigenetic landscape down-regulates the CD19 expression, which often leads to antigen escape in relapsed conditions. It also opened a possibility to use a combinational approach (by targeting epigenetic regulators) to enhance CART-cell activity by preventing/restoring CD19 antigen escape.

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36. Evaluation of mesenchymal stem cells markers in dental pulp *in vivo*

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The dental pulp is a highly vascularized and innervated connective tissue hosting cells with capacity for regeneration. Dental pulp derived stem cells (DPSCs) are able to differentiate into several types of dental but also non-dental cells. As such, they provide a promising source for treatment strategies and offer an accessible system to investigate mesenchymal stem cells (MSCs). So far, the majority of DPSCs research has been performed *in vitro* and based on the data a set of positive and negative DPSCs markers was established.

To follow expression of these molecules in the developmental context of the dental pulp *in vivo,* postnatal stages of the dental pulp formation were examined by customized PCR Array along with *in situ* analysis based on immunohistochemistry. The key trio of markers coded by *Nt5e, Thy1, Eng* genes was in focus in the mouse first molar tooth.

The expression of these markers was divergent during the investigated period. Markers displayed specific patterns of expression overlapping particularly in the sub-odontoblastic where the vascular and neural network is located. Additionally, the results show dynamics in the expression of DPSCs/MSCs markers *in vivo*, which reflects their natural tissue environment and supplements the *in vitro* based knowledge.

37. The essential role of lysosomal complex Ragulator in the control of AMPK activity in melanoma cells

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The therapy of malignant melanoma usually targets the hyperactivated ERK signaling pathway, especially BRAF and MEK kinases. However, patients treated with ERK pathway inhibitors commonly develop resistance to these compounds within several months. Therefore, it is necessary to identify new ways to target the ERK signaling in melanoma therapeutically.

Our team recently identified new molecular mechanisms by which the metabolic stress sensor AMPK could significantly affect ERK signaling pathway activity in melanoma despite the presence of *NRAS* and *BRAF* oncogenic mutations, leading to the suppression of melanoma cell growth (1).

In melanoma cells, we also found conditions leading to partial disruption of the lysosomal complex Ragulator, whose subunit LAMTOR1/p18 can participate in AMPK activation. Partial delocalization of LAMTOR1/p18 from the Ragulator complex diminishes AMPK activation in response to metabolic stress. The essential role of this Ragulator subunit for AMPK activation in melanoma was also verified using RNA interference targeting *LAMTOR1* gene expression.

Our results indicate the importance of the lysosomal Ragulator complex for AMPK kinase activation in response to metabolic stress and suggest another potential therapeutic target in melanoma cells. We further plan to study dynamic interactions between LAMTOR1/p18 and AMPK under metabolic stress using proximity ligation assay and immunoprecipitations.

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38. Involvement of adenosine methylation in cellular differentiation

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Reversible RNA modifications have emerged as key players in the complex transcriptional changes that underlie timely transitions in cellular states during early embryonic development. One of the prevalent internal mRNA modifications, N6-methyladenosine (m6A), is known to crucially drive this transition by degrading pluripotency-associated transcripts and stabilizing lineage-specific transcripts [1]. However, the functions of another widely reported and a closely related modification, N6-2'-O-dimethyladenosine (m6Am), remain unclear.

Preliminary results from our lab indicated this abundant mRNA mark m6Am is regulated during the differentiation of induced pluripotent stem cells (iPSCs) towards ectodermal lineage. This is for instance represented by increased expression on the responsible methyltransferase PCIF1 (writer). Furthermore, depletion of the m6Am demethylase FTO (eraser) leads to increased levels of m6Am. The preliminary results also indicated that depletion of either the m6Am writer or eraser display aberrations in the pluripotency and differentiation. Collectively, our results suggest a relevance of m6Am pathway in neuronal differentiation.

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39. Changes of the Brain Barriers induced by Diabetes Mellitus

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Diabetes mellitus (DM) is one of the most common and serious chronic disease, causing life threatening, disabling and costly complications, and reducing life expectancy. The prevalence of DM in the last year is estimated to 573 million adults worldwide. The total number of DM is predicted to rise to 783 million by 2045. Over 90% of DM is classified as type 2 DM [1]. Chronic hyperglycaemia seen in badly compensated patients causes tissue damage and vascular injury called macro- and microangiopathy. The best-known organ-target complications are ischemic heart disease, angiopathy with "diabetic foot syndrome", diabetic kidney disease, neuropathy and retinopathy. Another important, but not so well known complication, is diabetic encephalopathy (DE). DE is characterized by electrophysiological and neuroradiological changes, clinically expressed by cognitive impairment, psychiatric and motor disorders [2].

Our research is focused on morphological and functional changes in blood-brain and blood-cerebrospinal fluid barriers in animal model of DM. Especially, major interests of investigation are expression of selected pro-inflammatory and anti-inflammatory cytokines, their cellular distribution and description of changes in vessels supplying the choroid plexus.

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40. Genome-wide Screening Optimizations and High-throughput Compound Screening to Identify Novel Treatment Options in Venetoclax-resistant AML

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Recent advances in Venetoclax-based regimens have proven useful in the treatment of acute myeloid leukemia (AML) for patients deemed unfit for standard chemotherapy regimens. However, drug resistance remains a critical issue highlighting the need to find alternative compounds which may be able to overcome this resistance.

As a model of resistance, we are in the process of generating venetoclax-resistant AML cell lines for HL-60, OCI-AML3, MV4-11 and MOLM-13 which has been completed. Using a library consisting of 859 drugs approved by FDA or EMA for various indications, we performed a primary drug screen on our MOLM-13 venetoclax-resistant cell line which revealed some top hits validated through 10-point dose-response curves. We plan to expand this to our other Venetoclax-resistant AML cell lines. To understand the biological mechanisms responsible for Venetoclax-associated resistance, we intend on performing a genome-wide knockout screen. This is to identify depleted genes using the Brunello CRISPR library in these resistant AML cell lines and primary AML cells. We optimized the transduction conditions in primary AML cells. We tested a variety of different viral titres and also, the addition of BX795, reported to increase transduction efficiency. Using lentivirus expressing a GFP reporter, we were able to successfully determine via FACs optimal conditions for our infection of primary cells.

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41. Endoplasmic reticulum stress alters the morphology of ovarian surface epithelium

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Ovarian surface epithelium (OSE) represents a superficial layer of cubic cells covering the ovaries. OSE is constantly exposed to numerous stress factors, actively participating in ovulatory cycle and regularly undergoing wound and repair cycles. Understanding the background of morphological changes of OSE cells and their response to stress induced during aging or incessant ovulation may clarify histopathological background of ovarian dysfunction, infertility, and cancer.

The endoplasmic reticulum (ER) is an essential cellular organelle responsible for protein synthesis, posttranslational modifications, membrane biosynthesis, and Ca^{2+} management. ER is also an important signaling hub integrating various forms of stress and organizing the signaling response. ER stress can be triggered by a variety of factors and has been demonstrated to play a role in many pathologies.

Here we study the morphological changes of OSE evoked by ER stress. Our findings can help to clarify key aspects of carcinogenesis, regeneration, or functions. In addition, the identification of biologically relevant links between the ER molecular machinery and ovarian biology can extend the portfolio of molecular targets for the treatment in the female reproductive system.

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42. Metabolic regulation of stem cell fate determination

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Human pluripotent stem cells are an important research tool and show great promise in drug screening and regenerative medicine. Maintaining their pluripotency and differentiating them into somatic cell types in vitro are essential elements for successful research work. Increasing evidence implicates metabolic pathways as key regulators of cell fate and function. Stem cells rely heavily on aerobic glycolysis as their energy source, but throughout differentiation the balance shifts more towards Krebs cycle and oxidative phospholyration. This metabolic switch seems to be one of key elements in differentiation priming, yet the exact mechanism how it drives specification of all three germ layers (endoderm, mesoderm, ectoderm) remains unclear. The aim of my PhD project is to dissect the metabolic pathways that drive stem cell fate determination.

To achieve this, various regulators of metabolic pathways will be administered to stem cells spontaneously differentiating in the form of embryoid bodies. Suitable working concentrations as well as time points for sample collection are being optimised. To assess the effect of the regulators on cell fate determination, we will analyse expression of specific markers for each germ layer. Ultimately, our results will contribute to better understanding of molecular mechanisms behind stem cell fate decision.

43. Illuminating life: visualizing the egg to embryo transition in non-rodent mammals

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After fertilisation, the single cell (zygote) undergoes successive rounds of cell divisions, which are highly prone to errors. The ability to correctly handle genetic information is thus pivotal for the early development and establishment of a healthy pregnancy. Intriguingly, mitotic errors are common in human embryos but are rarely seen in mice. Unlike in mice, human sperm not only transmit genetic information from the father but also carries the non-membranous organelle – centrosome. We hypothesize that successful embryo development in non-rodent mammals is dependent on the ability to integrate the sperm-derived centrosome.

Here we developed the live-cell imaging setup for the visualization of centrosomes in bovine fertilized eggs. By injecting mRNA coding fluorescently labelled centrosomal protein 152 (CEP152) we investigated the temporal and spatial organization of sperm-derived centrosome. CEP152 became localized to the base of the sperm tail approximately 7 hours after fertilization. This is in line with the recently reported onset of S-phase in the one-cell embryo and suggests that the same molecular machinery could regulate centrosome duplication in embryonic and somatic cells.

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44. Nanopatterned Surface for Super-resolution Microscopy localization of Cell Interactions

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Cellular behavior and cell fate are controlled not only by intrinsic processes but can be modulated also by extracellular environment. Interactions with environment usually occur at nanoscale level and therefore it is complicated to evaluate their impact. To elucidate in more details, influence of surrounding environment *in vitro,* we have to be able to prepare suitable biomaterials with controlled nano-distribution of selected molecules in precise and reproducible manner. The main challenges are obtaining the nanoscopic features, high demand on biocompatibility, and finally, the necessity for transparent properties of prepared biomaterial, allowing subsequent super-resolution microscopy analysis. Recent progress in nanotechnology allows for mimicking of the microenvironment by the patterned distribution of biomolecules at the nanoscale level, particularly by using highly promising electron-beam lithography (EBL). Although this nanopatterning technique can generate nanostructures of good quality and resolution, it has been used, thus far, for the preparation of nanomaterials without a biological agent, and mostly non-transparent, leaving its potential in biological applications unfulfilled. Here, we performed the EBL on conventional cell culture material coated with a transparent electron-conductive indium tin oxide layer, resulting in complex nanopatterns, further biofunctionalized via novel HaloTag recombinant protein. This biocompatible system allowed us to nanoprobe the cell interactions toward a specific region. We prove successful cellular localization by scanning electron microscopy and by super-resolution (SIM) microscopy. Furthermore, we suggest, that such a universal system can be effectively used for probing biological samples for the corelative-microscopy technique and may provide an understanding of cellular signaling mechanisms at a single-molecule level.

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45. A new reporter system for high-throughput identification and validation of eIF4F inhibitors

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The eIF4F translation initiation complex has a critical role in cancer. The complex enhanced activity was identified as a nexus of drug resistance and a promising therapeutic target in melanoma [1,2]. However, the spectrum of available eIF4F inhibitors is limited, and none of them is in clinical use [3]. One of the reasons could lie in the relative complexity of techniques used to identify such inhibitors [4]. Here we report a unique cell-based reporter system suitable for the high-throughput identification of novel eIF4F inhibitors in small-molecule compound libraries.

We identified several eIF4F-regulated pathways controlling melanoma cell proliferation in a proteomic screen. Then we used a promoter of one of the eIF4F-controlled genes to build a reporter system, responding to eIF4F inhibition by changes in luciferase expression in a dose-dependent manner. Subsequently, we validated the system in a panel of cancer and non-cancer cell lines, determining the impact of eIF4F inhibition on luciferase activity.

Our technique is not only highly specific and suitable for high-throughput screening; it also overcomes several limitations of the current state-of-the-art eIF4F inhibitor screening assays, e.g., the proximity ligation assay. It is sensitive, less cost-intensive, significantly faster, and does not require expensive fluorescent microscopy/image analysis equipment.

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46. Spatial mapping of stromal microenvironment during mammary branching morphogenesis

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Branching morphogenesis is a conserved set of cellular processes that ensures high relative surface-to-volume ratio. In several organs, including mammary gland, the pivotal role of stromal microenvironment for epithelial morphogenesis has been demonstrated. In my project I started with the hypothesis, that actively proliferating part of developing mammary gland, the terminal end bud (TEB) is surrounded by specialized set of stromal fibroblasts, that support its growth and invasion through surrounding matrix. To entertain this hypothesis, we performed microdissection of "active" part of mammary gland, containing TEBs and "quiescent" part of mammary gland, containing ducts, followed by single cell RNA sequencing. By unsupervised cluster analysis and integration with published datasets, I have demonstrated existence of specialized peri-TEB fibroblasts, specified in both time and space. Furthermore, I spatially mapped the cluster identified by sequencing by combination of immunohistochemistry and in situ mRNA hybridization. Finally I present preliminary results on lineage tracing of the peri-TEB fibroblasts and in vitro insight into cell signaling pathways that specify the peri-TEB phenotype. Our data shed light on fibroblasts heterogeneity during morphogenesis and homeostasis that will help to understand involvement of these cells in both developmental and pathological processes, such as cancer or fibrosis.

47. Volumetric imaging provides insight into the 3D ultrastructural organization of maturing human oocytes

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Egg quality is a limiting factor of female fertility. Complex structural and biochemical changes in the cytoplasmic compartment are necessary to confer the female gamete the capacity to undergo successful fertilization and sustain embryonic development.

Transmission electron microscopy is traditionally used to inspect the ultrastructure of female gametes. However, two-dimensional micrographs contain only fragmentary information about the spatial organization of the complex oocyte cytoplasm. We employed Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) which provided an unprecedented view of ooplasmic architecture. By comparing samples fixed at three developmental stages, we mapped out how human oocyte cytoplasm reorganizes during meiotic maturation in vitro. The image data obtained showed that the major reorganization of cytoplasm occurs before the first polar body extrusion. The organelles initially concentrated around the prophase nucleus were repositioned toward the periphery and evenly distributed throughout the ooplasm. The most prominent feature was the formation of heterologous complexes composed of the endoplasmic reticulum and multiple mitochondria with primitive morphology. The quantitative analysis of reconstructed 3D images revealed that the most abundant organelles, the mitochondria, occupy ~ 4.26 % of the maturing oocyte cytoplasm. In conclusion, this work enhances our knowledge of human oocyte morphology and cytoplasmic maturation.

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