MUNI
MED

Annual PhD Conference in Biomedical Sciences

Book of Abstracts

8-10TH NOVEMBER 2023

MASARYK UNIVERSITY CAMPUS, BRNO BOHUNICE

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FOREWORD

The Annual PhD conference in Biomedical Sciences aims to bring together PhD students from different fields offering a chance to present their research in front of a miscellaneous audience promoting communication and open discussions among participants. All students in the program take active part creating a team spirit and facilitating the exchange of ideas and experiences.

Since 2021, the PhD conference is held among students of all three specializations following the idea more people, more science & more fun! Building up on the success of this event, we keep this format that shall promote communication and exchange between various departments.

This year, the conference will last three days. Overall, 14 students will give talks as part of their doctoral state exams, 6 students will present their progress reports and 40 students will present posters during two poster sessions which create great environment for networking.

We believe that each student will receive valuable feedback on their research projects and get the most out of this annual gathering. On behalf of the organizing committee and doctoral board, I wish you a lot of success and fun.

Prof. Ondřej Slabý
Program Director

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8. Hašanová Klaudia
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11. Krishna Shwetha
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1. Andrejčinová Ivana
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5. Čimborová Katarína
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17. Kovačovicová Petra
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24. Musilová Zuzana
25. Niro Francesco
26. Nita Alexandru
27. Pereira de Sousa Daniel
28. Poovakulathu Abraham Sara
29. Portakal Yanik Turkan
30. Pospíšilová Michaela
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33. Smolková Karolína
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36. Torkashvand Marzie
37. Tuaima Haneen Riadh Ali
38. Ursarchi Vlad-Constanti
39. Vadovičová Natália
40. Vymazal Ondřej

1. The role of FSP1+ stromal cells in mammary gland development and lactation

Denisa Belisova[1], Ema Grofova[1], Jakub Sumbal[1], Zuzana Sumbalova Koledova[1]

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The production of milk is the essential function of the mammary gland. The development of morphologically and functionally mature mammary gland, able to produce and expulse milk, requires precise coordination of systemic and also microenvironmental regulatory systems.

To focus our research on the epithelial-stromal interactions, which are crucial for organ development and homeostasis, we employed a genetically engineered mouse model, *Fsp1-Cre* to constitutively target *Fsp1 (fibroblast specific protein 1*)-expressing stromal cells and investigate their role in mammary branching morphogenesis, alveologenesis and milk production.

Although the *Fsp1-Cre* mouse model was previously used in fibroblast-targeting studies, our results of lineage-tracing experiments using *Fsp1-Cre;mT/mG* model indicate that FSP1+ cells belong to a population of immune cells. Depletion of these cells using *Fsp1-Cre;DTA* mouse model leads to abrogated epithelial outgrowth during puberty, and to a lactation defect. The milk production is preserved but the milk is not delivered to the progeny despite normal maternal behavior, resulting in early mortality of the whole litter.

Taken together, we observed a strong lactation phenotype in mice with depleted FSP1+ stromal cells, indicating the important role of these cells in mammary gland development and function. Further experiments will be needed to evaluate if this defect results from systemic or tissue-specific effect of the FSP1+ cell ablation.

This work was funded by Internal Grant Agency of Masaryk University (MUNI/IGA/1311/2021), Grant Agency of Masaryk University (MUNI/G/1775/2020 and MUNI/A/1301/2022) and Brno city municipality (Brno Ph.D. Talent Scholarship).

2. Long non-coding RNAs in small extracellular vesicles in patients with colorectal cancer

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The prognosis of patients with colorectal cancer (CRC) depends on the extent of the disease at diagnosis; therefore, early detection is essential for successful treatment. To identify potential diagnostic biomarkers of CRC, we aimed to analyze and verify long non-coding RNAs (lncRNAs) isolated from small extracellular vesicles (EVs) in patient blood samples.

Small EVs were purified by size exclusion chromatography from 150 µl of blood serum and characterized by electron microscopy, DLS analysis and Western blot. After RNA isolation, cDNA libraries were prepared and sequenced using NextSeq 550. Differentially expressed lncRNA were validated by qPCR.

We successfully purified small EVs and prepared sequencing libraries despite a very low volume of starting material. Total RNA sequencing confirmed the presence of both protein-coding and non-coding RNA and data analysis revealed significantly altered levels of lncRNAs in CRC patients versus healthy controls. Selected lncRNAs were verified in a larger validation cohort, confirming their differential expression between the groups.

Our data showed that lncRNAs represent a fraction of RNA in small EVs and have the ability to distinguish CRC patients from healthy controls. Additional functional test will be performed to determine the role of selected lncRNA in CRC pathogenesis.

3. Investigating signaling dynamics in mammary epithelial morphogenesis

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3 Cell Biology and Biophysics Department, EMBL, Heidelberg, Germany (visiting scientist)

Mammary glands (MGs) are specialized organs responsible for the secretion of components crucial for offspring survival. The main functional component of the gland is the mammary epithelial tree. It develops under the influence of reproductive hormones and other signaling molecules such as growth factors (GF) in the process of branching morphogenesis. Various signaling pathways involved in this process are mediated by extracellular signal-regulated kinase (ERK), but the precise mechanism is unknown. We hypothesize that regulation occurs through dynamic ERK activity patterns. Here we employ transgenic ERK activity biosensor mouse strain EKAREV-NLS [1], with 3D organoid models and live imaging methods to follow dynamic changes during MG branching morphogenesis. We investigate effect of the biosensor construct on the MG development *in vivo* and perform long-term live imaging of acquired organoids to track ERK signaling dynamic in single cells.

Altogether, we found that biosensor expression has a significant impact on the MG development *in vivo*. After supplementation with reproductive hormones this effect is reduced, while the change of mouse genetic background completely rescues the phenotype. Further, we confirmed that biosensor expression and functionality are maintained. ERK dynamics can be detected in MG organoids after long-term light sheet imaging with different GF supplementation.

[1] Yuji Kamioka, Kenta Sumiyama, Rei Mizuno, Yoshiharu Sakai, Eishu Hirata, Etsuko Kiyokawa, Michiyuki Matsuda, Live Imaging of Protein Kinase Activities in Transgenic Mice Expressing FRET Biosensors, Cell Structure and Function, 2012, Volume 37, Issue 1, Pages 65-73

This project was funded by MUNI/G/1775/2020 and MUNI/A/13012022.

4. Shedding Light on Retinal Adaptation: Discovery of Light-Responsive microRNAs

Canan Celiker[1], Kamila Weissova[1,2], Katerina Amruz Cerna[1], Jan Oppelt[3], Birthe Dorgau[4], Francisco Molina Gambin[1], Jana Sebestikova[1], Majlinda Lako[4], Evelyne Sernagor[5], Petra Liskova[6], Tomas Barta[1*]

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The human retina comprises cells that swiftly adapt to a wide array of visual stimuli. This rapid adaptation to varying levels and wavelengths of light facilitates the regulation of circadian rhythms and enables the visual system to remain sensitive to higher levels of illumination. It has been shown that retinal microRNA (miRNA) molecules play a key role in regulating these processes. However, despite extensive research using various model organisms, light-regulated miRNAs in human retinal cells remain unknown.

In this study, we aim to characterize these miRNAs. We generated light-responsive human retinal organoids that express miRNA families and clusters typically found in the retina. Using a custom-designed photostimulation device we identified a subset of light-regulated miRNAs. Importantly, we found that these miRNAs are differentially regulated by distinct wavelengths of light and have a rapid turnover, highlighting the dynamic and adaptive nature of the human retina. In conclusion, our research suggests that retinal organoids are an effective model to study light-regulated miRNAs. These findings provide important insights into the mechanisms by which the human retina adapts to changes in the environment and could have significant implications for studying the molecular mechanisms of light perception in the retina.

This study was supported by the Czech Science Foundation (GA21-08182S).

5. Use of CRISPR/Cas9 screening to explore CD20 regulation

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CD20 is a B-cell surface antigen that has been used as a target of monoclonal antibodies (such as rituximab) in the therapy of B-cell malignancies for more than two decades. However, the resistance to the monoclonal antibodies is often observed, which leads to the therapy failure. Several mechanisms of the resistance have been described, with downregulation of CD20 being one of them.

To identify genes whose disruption may be able to restore CD20 expression on the surface of malignant B cells, we performed CRISPR/Cas9 screening in the rituximab-resistant $CD20^{low}$ cell line, which was previously generated in our laboratory to mimic the situation occurring in patients. The screening revealed several genes whose knockout upregulated CD20 surface expression. For three of these genes, CSK, SSR1 and CD37, we have validated results on single-cell clones generated using newly designed gRNA. We observed upregulation of CD20 using both flow cytometry and western blot. Additionally, the sensitivity to rituximab was restored. The thorough investigation of underlying mechanisms could provide a way to potentially enhance anti-CD20 monoclonal antibody therapy.

6. Monitoring the mechanosensing contribution to mouse incisor growth

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Rodents have developed throughout evolution a type of teeth able to constantly regrow. This continuously growing tooth serves as an attractive model widely used for studying the stem cell niche, differentiation, or tissue homeostasis [1]. Although, the exact mechanisms controlling the dynamics of incisor growth and its growth pace remain mostly not understood.

Here, we wanted to evaluate the specific role of mechanically gated ion channels (Piezo and Ryanodine family) to control incisor´s turnover. To address it, we developed a new protocol named BEE-ST that enables to quantify daily incisor growth [2]. The combination of BEE-ST with the use of genetically modified mice and different dental surgery procedures will help to target the mechanosensing receptors and uncover their function in incisor (re)growth. Furthermore, other state-of-the-art techniques e.g., X-ray tomography, immunohistochemistry, *in situ* RNA hybridization, will be performed to achieve a better understanding from the molecular and cellular perspective to its implication in the tooth as an organ.

Interestingly, this work will bring a new insight about the tooth mechanobiology regulation solving key questions of dental development and applications in regenerative dentistry.

[1] Krivanek, J., Soldatov, R.A., Kastriti, M.E. et al. Dental cell type atlas reveals stem and differentiated cell types in mouse and human teeth. Nat Commun 11, 4816 (2020). https://doi.org/10.1038/s41467-020-18512-7.
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7. Identifying the genome/epigenomic regulators to enhance CAR-T cell activity by preventing CD19 antigen escape in relapsed models

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Chimeric antigen receptor T cells are genetically engineered T lymphocytes widely being investigated for their ability to treat certain malignancies, especially leukaemia and lymphomas. They target, recognize and kill the malignant cells specifically and effectively. Despite their precisive mode of action, CAR-T cells still possess certain limitations, often leading to a relapsed disease state. Target antigen escape and downregulation of antigen is a prominent mechanism in relapsed patients treated with CAR-T cells. However, the mechanisms behind this antigen escape are poorly understood. In my project we aim to find the key genetic and epigenetic mechanisms that result in CD19 antigen escape and downregulation, thereby proposing novel combinational therapies to enhance CAR-T cell activity.

Support & Funding:

This work has received funding from the Czech Science Foundation, project no. 22-3527
This work was supported by the grant project MUNI/A/1224/2022

8. The reaction of the blood-cerebrospinal fluid barrier to nerve Injury

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There is convincing evidence indicating that peripheral nerve injury can induce inflammatory reactions in remote structures of the central nervous system (CNS). One possible pathway for the spread of neuroinflammatory reactions during neuropathic pain might be the blood-cerebrospinal fluid (BCSF) barrier localized in the choroid plexus (CP) of the brain ventricles. CP is composed of vascularized stroma and choroidal epithelial cells. Choroidal epithelial cells are connected by tight junctions (TJs). We assume that peripheral nerve injury could alter TJs of CP which might lead to a penetration of circulating monocytes into the epiplexus position.

In our study, we used Wistar male rats with a sterile chronic constriction injury (sCCI) and we used fluorescein-conjugated dextran FluoroEmerald (FE) to trace the permeability of the BCSF barrier. FE circulation time was 30 minutes and 5 hours. We also examined the number of ED1+ and ED2+ macrophages after sCCI.

Our findings revealed a significant increase in FE concentration within the cerebrospinal fluid (CSF) at 3 and 7 days post-nerve injury for both FE circulation durations. These outcomes suggest a modification of the BCSF barrier permeability. Subsequent to sCCI the numbers of both ED1+ and ED2+ macrophages gradually increased over time. To ascertain that there was no noticeable proliferation effect among the macrophages, we used Ki-67 immunostaining. Furthermore, Ki-67 was not increased we assume epiplexus macrophages both after the surgery itself and after nerve injury are derived from recruited monocytes.

1]M. Joukal, I. Klusáková, P. Solár, A. Kuklová, and P. Dubový, "Cellular reactions of the choroid plexus induced by peripheral nerve injury," Neurosci. Lett., vol. 628, pp. 73–77, Aug. 2016, doi: 10.1016/j.neulet.2016.06.019.

[2]P. Solár, A. Zamani, L. Kubíčková, P. Dubový, and M. Joukal, "Choroid plexus and the blood–cerebrospinal fluid barrier in disease," Fluids Barriers CNS, vol. 17, no. 1, p. 35, May 2020, doi: 10.1186/s12987-020-00196-2.

Supported by MUNI/A/1238/2022.

9. Comparison of genomes of the yaws bacterium, *Treponema pallidum* subsp. *pertenue*, of nonhuman primate origin

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Treponema pallidum subsp. *pertenue* (TPE) is the causative agent of human yaws. The disease was believed to have no animal reservoirs, but genetic evidence shows that TPE strains naturally infect and cause sexually transmitted infections in non-human primates (NHPs) in sub-Saharan Africa [1]. We have determined 21 new genomes of TPE isolates that originated from *Chlorocebus pygerythrus* (vervet monkey), *Papio anubis* (olive baboon), *Papio cynocephalus* (yellow baboon) and *Cercopithecus mitis* (blue monkey) and were collected in protected areas in Tanzania, including Lake Manyara National Park (NP), Ngorongoro Conservation Area, Serengeti NP, and Ruaha NP [2]. We performed genome-to-genome comparisons of all 21 genomes plus previously sequenced strain LMNP-1, and found an unexpectedly high degree of genetic similarity in 9 comparisons out of 231 combinations. While two of those cases were determined in samples taken from the same NHP species, in additional seven cases, samples were isolated from different NHP species, three times within the same national park and four times in different national parks. These findings suggest relatively frequent inter-species transmission of TPE among NHPs. Moreover, geographical separation of national parks appears not to limit this transmission.

[1] Knauf S, et al., Nonhuman primates across sub-Saharan Africa are infected with the yaws bacterium Treponema pallidum subsp. pertenue. Emerg. microbes & infect. 2018 Sep;7:1, 1-4, https://doi.org/10.1038/s41426-018-0156-4
[2] Chuma IS, et al., Widespread Treponema pallidum Infection in Nonhuman Primates, Tanzania. Emerg Infect Dis. 2018 Jun;24(6):1002-1009. https://doi.org/10.3201/eid2406.180037

10. Development of a microfluidic system for the study of lung development or disease

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Advances in material science and engineering have led to the development of microphysiological systems, also known as organs-on-chips. This biomimetic approach to cell cultivation has gained increasing interest in recent years as it allows the user to better recapitulate the microenvironment of *in vivo* tissues. Both the mechanical and the biochemical environment of the cell culture can be specifically tailored to mimic the desired tissue niche through the use of different cell types, hydrogels, and physical constraints.

In this work we show how a microfluidic system can be designed for cell cultivation and how the system can be adapted to allow for either suspension culture or cultivation of cells embedded in hydrogel. We further aim to recreate a variety of elements that contribute to the specific niche that enables lung development *in vivo*. To this aim, we show how a biochemical gradient can be created and maintained in a continually perfused system. Additionally, through a co-culture of endothelial cells and stromal cells, we managed to form an endothelial network within the microfluidic device. Lastly, we will speculate on how we plan to apply all these various elements in a single system to study the development of the lung.

11. The role of RNA adenosine methylation in cellular homeostasis

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RNA modifications have emerged as a widespread regulatory mechanism controlling various molecular and cellular events in higher eukaryotes. Among the numerous chemical modifications, the best characterised and most prevalent in mRNA is N6-methyladenosine (m6A) [1]. Their deposition is controlled by dedicated machineries whose perturbation leads to the disruption of cellular homeostasis and development of several clinical conditions. In addition to m6A, another closely related modification at the 5' terminus of mRNA, N6-2'-O-dimethyladenosine (m6Am) was also documented, although the precise functional relevance of this modification remain controversial [2,3].

Preliminary results from our lab suggested an involvement of m6A(m) demethylating enzyme FTO in events of DNA replication and repair. Several key proteins involved in these events such as RADX and XRCC4 were found to be proximally interacting with FTO. Our screening assays also show an increased synthetic lethality in FTO KO cell lines when targeting important DNA replication factors. Downregulation of FTO leads to a dramatic stalling of the replication machinery, possibly in an R-loop dependent manner. Moreover, proteins like RECQ5 and SETX were found to be proximal interactors of PCIF1, an m6Am methyltransferase. Collectively, our results highlight a significance of m6A(m) pathway in the maintenance of genome integrity.

[1] Dominissini, Dan, et al. "Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq." Nature 485.7397 (2012): 201-206.
[2] Akichika, Shinichiro, et al. "Cap-specific terminal N 6-methylation of RNA by an RNA polymerase II–associated methyltransferase." Science 363.6423 (2019): eaav0080.
[3] Boulias, Konstantinos, et al. "Identification of the m6Am methyltransferase PCIF1 reveals the location and functions of m6Am in the transcriptome." Molecular cell 75.3 (2019): 631-643.

12. Zygotic spindle orientation defines cleavage pattern and mitotic fidelity in human embryos

Volodymyr Porokh[1], Drahomíra Kyjovská[2], Martina Martonová[2], Tereza Klenková[2], Pavel Otevřel[2], Soňa Kloudová[1,2] and Zuzana Holubcová[1,2]

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The first embryonic division represents a starting point for the development of a new individual. In many species, tight control over the first embryonic division ensures its accuracy. However, the first division in humans is often erroneous and can impair embryo development. To delineate the spatiotemporal organisation of the first mitotic division typical for normal human embryo development, we systematically analyzed a timelapse dataset of 300 human embryos, which developed into healthy live births. We show that division at a right angle to juxtaposed pronuclei is preferential and favours proper chromosome segregation. Alternative configurations of the first division were more common in women of advanced reproductive age and were associated with reduced clustering of nucleoli and multinucleation at the 2-cell stage. Collectively, these data show that orientation of the first division predisposes human embryos to genetic (in)stability and may contribute to aneuploidy and age-related infertility.

13. Peri-TEB fibroblasts instruct 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. Branching morphogenesis of the mammary gland is led by terminal end buds (TEBs), specialized epithelial structures that contain proliferative cells and invade the surrounding fat pad. By single cell RNA sequencing of microdissected TEB-containing regions of mammary gland, we have demonstrated the existence of a subset of highly contractile peri-TEB fibroblasts. The peri-TEB fibroblasts express a specific set of extracellular matrix and paracrine factors. Moreover, by combining the single cell data with in vivo lineage tracing experiments, we mapped the hierarchy of fibroblasts population and its connection with other stromal lineages in the fat pad. Finally, we demonstrated in vitro and in vivo that experimental perturbation of WNT/CTNNB1 and YAP signaling, a signature of peri-TEB fibroblasts, affects fibroblasts contractility and their ability to instruct epithelial branching. 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.

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

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In eukaryotes gene expression is regulated at several levels, one of them being mRNA stability with RNA tailing marking transcripts for further processing or degradation. Mammalian cells encode several types of 3' to 5' exoribonucleases with specific targets and functions. Our lab has previously identified the DIS3L2 protein as a processive 3' to 5' exoribonuclease with specific affinity to 3' terminal oligoU extensions on different, even highly structured, RNAs in the cytoplasm [1][2][3]. We reasoned that its activity *in vivo* must be regulated to prevent unspecific disastrous degradation of cellular RNAs via posttranslational modifications or putative additional protein factors. To date, DIS3L2 was shown to interact with Xrn1 in an RNA-dependent manner [4]. To search for additional regulatory cofactors, we biotinylate proximal proteins of known TUT-DI3L2 pathway players using inducible expression of these proteins fused with promiscuous biotin ligase miniTurboID [5] in HEK293T cell line. The biotinylated proteins are then purified with streptavidin and identified by LC-MS/MS.

In collaborative project I also study another human 3' to 5' exoribonuclease ISG20L2. Previously ISG20L2 was only shown to be involved in the maturation of 5,8S rRNA [6], otherwise its role was rather enigmatic. In collaboration with Sánchez-Madrid group in Spain, we reveal that ISG20L2 has a role during activation of naïve T-cells targeting particular miRNAs and modulating cytokine secretion and formation of immunological synapse. It also negatively regulates immunoregulatory molecules. I contributed with the characterization of its nucleolytic activity by *in vitro* enzymatic assays. [7] We plan to further study ISG20L2 activity *in vitro* and *in vivo*.

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1. Cytokine response after COVID-19 correlates with changes in cardiovascular damage markers.

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Acute COVID-19 infection is associated with strong immune response leading to inflammation, subsequently affecting multiple organs including cardiovascular system. Dysregulated immune response can persist over time and put patients at higher risk of developing cardiovascular disorder (CVD). Here, we aimed to identify early immune markers associated with increased risk of future CVD.

We analyzed samples from 22 patients with severe COVID-19. Samples were obtained in three timepoints: acute phase during patients' hospitalization, 1 and 6 months after COVID-19 onset. We measured plasma levels of inflammatory cytokines together with proteins related to cardiovascular damage and subsequently investigated possible correlations between these markers. Using our established intracellular flow cytometry protocol, we measured NF-κB activity in monocytes and performed multi-parametric immunophenotyping of peripheral blood leukocytes by flow cytometry.

Levels of some cardiovascular damage markers decreased significantly after recovery from acute COVID-19. Correlation analysis between these cardiovascular damage markers and immune response markers revealed key cytokines with possible role in CVD development. We describe phenotypic changes in peripheral blood leukocytes. Analysis of monocyte subsets revealed significant changes in the frequency of $CD16^+$ monocytes in post-acute COVID-19. Further investigation aims to identify immune profiles that may be associated with increased risk of CVD development/exacerbation.

2. Early onset of cardiac progenitor maturation leads to their depletion recapitulating cardiac pathology development 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 skeletal muscle loss followed by cardiomyopathy. Current medical care for DMD is palliative, and a better understanding of DMD pathologies is needed for targeted interventions and improved therapies. In addition to the widely accepted role of dystrophin in myocytes, we have previously shown that DMD derived pluripotent stem cells present impaired self- renewal as well as elevated DNA damage. The damage was at least partially caused by deregulation of nitric oxide synthase (NOS) and subsequent production of reactive oxygen/nitrogen species.

Here we present that dystrophin deficient cells show impaired cardiac differentiation efficacy as illustrated by forming fewer spontaneously contracting organoids with higher rate of cardiomyocyte (CM) death, increased content of collagen and altered transcriptional program during the differentiation process. Characterization of the developing cardiovascular progenitor (CP) population shows both higher and earlier activation of DMD CP markers with subsequent attenuation of their transcription. Furthermore, earlier onset of transcription of genes associated with maturation compared to wild type coincides with a decrease in proliferation in the organoid. DMD CP population also presents higher levels of inflammation and DNA damage, thus recapitulating phenotype of mdx mouse and human DMD hearts. NOS inhibition attenuates DNA damage and improves beating organoid formation; however, it does not prevent CM death or significantly affect transcription of cardiac development related genes. Therefore, NOS inhibition may be tested as a complementary treatment to current medical care and as a target for further molecular-level investigation and modulation.

3. 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 a chronic inflammation of gastrointestinal tract and in general is caused by deregulated immune response targeting the gut microflora. MAIT cells, population of unconventional T cells, play a crucial role in the mucosal immune response. However, their specific role in IBD remains enigmatic. Activated MAIT cells express IL-26, a novel IL-10 family cytokine with a controversial role in IBD. Here, we investigated the role of MAIT cells and IL-26 in IBD patients using 3D state-of-art methods employing human intestinal organoids.

Within this study we analyzed MAIT cells in peripheral blood and intestinal tissue of Crohn's disease (CD) patients. Flow cytometry analysis was employed to describe MAIT cells' phenotype and IL-26 expression. 3D intestinal organoids as a complex in vitro model of human tissue were used to study the role of IL-26.

We showed a reduction of MAIT cells in peripheral blood and inflamed ileum of CD patients. Moreover, we showed dysregulation of IL-26 expression by MAIT cells in CD patients. IL-26 exhibited a protective role in healthy and proinflammatory conditions in the intestinal organoid model.

Our results show the crucial role of IL-26 and MAIT cells in the homeostasis of intestinal tissue and their involvement in the pathogenesis of IBD. They represent new potential therapeutic targets for CD patients.

4. By leaps and bounds: From *in vitro* lung tissue models to fostering collaboration in science

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The human respiratory system is constantly being exposed to potentially harmful exogenous substances. This can lead to various diseases or undesirable changes in the structure and function of lung tissue. The effect of the external substances can be studied using *in vitro* models based on scaffolds seeded with appropriate cellular elements.

In this study, we focused on preparation of *in vitro* models of the alveolar-capillary interface of human lungs. First, we prepared polymeric nanofibrous scaffolds made of poly-Ɛ-caprolactone. These scaffolds were designed to mimic the morphological structure of the extracellular component of the alveolar-capillary interface. We seeded the scaffolds with cells: mesenchymal stem cells (MSCs), early lung epithelial progenitors (ELEPs) [1] and human endothelial cells (ECs). To unravel detailed morphological interactions in the model, we mainly used advanced microscopic techniques.

The data we obtained have the potential for further use as OPEDAS (Other People's Data). Successful exploitation of this potential requires adherence to the FAIR (Findable, Accessible, Interoperable and Reusable) principles. A critical element here is an ecosystem based on the concept of data repositories: we participate in the development of National Repository Platform, which will contribute to the clarity and FAIRness of primary data.

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5. Development towards therapy of macular degeneration of retina by derivatives of human pluripotent stem cells

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Regenerative medicine in the field of ophthalmology was revolutionized in the 1990s through stem cell-based therapies focusing on treating corneal diseases. However, there still exist numerous incurable diseases associated with the perceptive part itself, the retina. The pathogenesis of the multiple forms of retinopathies is often associated with the dysfunction and loss of retinal cell types, including retinal pigment epithelium (RPE). In this respect, delivery of the laboratory-derived RPE cells has been considered an innovative tool for delaying, ceasing, or ultimately reversing the course of yet incurable retinal degenerative diseases, and possibly also retinal traumas.

Our objective is, thus, to develop an efficient and reproducible methodology for producing RPE cells derived from human pluripotent stem cells (hPSC), both human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC), and assure their compliance with current Good Manufacturing Practice (cGMP) requirements.

6. Flow cytometric analysis of normal and osteoarthritic chondrocytes

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Osteoarthritis (OA) is the most common degenerative joint disease characterized by increased degradation of cartilage tissue due to overexpression of proteolytic enzymes degrading extracellular matrix (ECM). Due to the inability of resident chondrocytes to regenerate the ECM with the same properties as it was formed during development, the probability of spontaneous regeneration is very low. The outcome of the disease tends to be a total replacement of the joint.

The main problem and challenge of advanced therapy medicinal product (ATMP) based on human articular chondrocytes (HACs) is that chondrocytes lose their properties and undergo dedifferentiation into fibroblast-like, spindle-shaped cells [1]. Our hypothesis was that modification of growth media can modify chondrocyte properties, mainly collagen type II (COL2) synthesis. We chose three surface markers (CD44, CD54, CD90) that should determine the state of the HACs and the differentiation status. Our results indicate that increased expression of CD54 is therefore associated with inflammatory stimulation. In healthy cartilage, the expression of this marker was not detected at all. The high level of CD44 and CD90 is directly connected with the good chondrogenic capacity of the HACs and the ability to produce a matrix with a high content of COL2 [2].

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7. The effect of amino acid deprivation on the growth signaling in malignant melanoma

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The mTORC1 signaling pathway is a crucial regulator of cancer cell growth, survival, and metabolism. Among its various features, it responds to the level of amino acids via specific sensors. Methionine, an essential amino acid, is sensed indirectly through its metabolite S-adenosylmethionine, which binds to the sensor SAMTOR and enables mTORC1 activation [1]. Its presence in the cell is vital for cell proliferation and growth of many cancer types [2].

We analyzed the impact of methionine deprivation on mTORC1 activity in *BRAF*-mutated melanoma cells. Based on the current knowledge, we expected mTORC1 inhibition after methionine restriction. Surprisingly, in *BRAF*-mutant cells, we observed an increase in mTORC1 activity. Moreover, this activation correlated with the increased activity of ERK and AMPK signaling pathways, which are closely connected to the mTOR signaling and growth regulation. Altogether our data indicate the involvement of another unknown regulatory loop affecting the mTORC1 signaling in *BRAF*-mutant melanoma cells after methionine restriction.

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8. Biased FGFR1 signalling in response to multiple FGF ligands

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Fibroblast growth factor receptors (FGFRs) belong to the family of receptor tyrosine kinases. Mammalian FGF family consists of 18 FGF ligands and eight FGFRs which makes this signalling pathway quite diverse and important to understand. Binding of FGF ligands to FGFRs, induce receptor dimerisation and triggers downstream signaling important for cellular migration, proliferation, and differentiation, skeletal growth and many more. FGFR1 interacts with FGF ligands such as FGF4, FGF8, and FGF9 which play a critical role in the skeletal system development. Each ligand has its own specific function, but the molecular mechanism behind the biased response of FGFR1 in response to FGF4, FGF8, and FGF9 is poorly understood.

Here, we use biochemical and biophysical tools to deduce differential structural and functional responses of FGFR1 signalling in response to three FGF ligands. We demonstrate that the three FGF ligands possess different affinities and efficacies for inducing signalling response in the cell. Furthermore, we find that in comparison to FGF4 and FGF9, FGF8 is a biased ligand. This FGF bias is caused by the structural differences in FGF-FGFR1 dimers resulting in the differential recruitment and activation of the downstream effector molecules, leading to differential FGFR1-mediated functional responses in cell growth and senescence. Thus, this study sheds an insight on the poorly understood molecular mechanism of FGF ligand bias.

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9. Chemotherapy alters the Kolmer cell of the choroid plexus

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Paclitaxel, an anti-cancer drug, stabilizes microtubules, inhibiting cell division. It also induces neuropathic pain with unclear mechanisms, possibly involving immune cell effects(Solár et al., 2020). This study focused on how paclitaxel affects choroid plexus (CP) macrophages, vital for the blood-cerebrospinal fluid barrier. Using an in vivo model, Wistar rats received paclitaxel or control, with brain samples collected at multiple time points (1, 7, 14, 21 days) for analysis. Immunohistochemistry examined activated and resident Kolmer cells and CP cell proliferation. ED1 marker staining revealed increased activated Kolmer cells in CP after both treatments, with no significant difference. Conversely, ED2 marker staining showed more resident Kolmer cells in CP after paclitaxel. Ki-67 staining indicated increased CP cell proliferation, likely Kolmer cell proliferation, after paclitaxel. This suggests paclitaxel's immunomodulatory impact on CP, potentially affecting the immune response and inflammation of the central nervous system. These findings offer insights for optimizing paclitaxel's anti-cancer benefits while minimizing immune-related side effects.

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10. Chromosomal Instability in Cancer Associated RAD51 S296L Mutant

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Homologous Recombination (HR) is important in the processing of stalled replication forks, and HR defects are common in cancer. RAD51, a critical component of HR, forms nucleoprotein filaments on single-stranded DNA (ssDNA) that enable pairing and strand exchange with template DNA. Defective strand exchange leads to defective double-strand break repair and processing of stalled forks, so it 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 Head and Neck Squamous Cell Carcinoma, leads to genomic instability.

We have shown that Mouse Embryonic Fibroblasts carrying the S296L mutation have increased chromosomal aberrations. Further experiments showed high levels of stalled forks, and defective pairing of ssDNA with template DNA. Detailed mechanistic characterization revealed alterations in the coordination of calcium ions which impacts the function of ATP hydrolysis, leading to defects in nucleoprotein filament formation and 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. Revealing axonal transport mechanisms of neurodegenerative disease-linked proteins: a close look to APP and TDP-43

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Neurons are highly polarized cells whose axons extend for up to 1 m within the human body [1]. In this context, axonal transport plays a crucial role in ensuring the delivery of a wide range of cargos from the cell body to the synaptic terminals and vice versa. [2]. Cargos are constantly moving along microtubules at speeds of 50-400 mm/day, thanks to motor proteins (kinesin and dynein), which are supported by numerous adaptor proteins [3]. Building on the central role of axonal transport, it is not surprising that this process becomes impaired in neurodegenerative diseases such as Alzheimer's disease (AD) or Amyotrophic Lateral Sclerosis (ALS) [4, 5].

In this project we investigate axonal transport of APP and TDP-43, two major proteins involved in AD and ALS pathology, respectively. First, we characterize the mechanism of movement of APP and TDP-43 in a human neurons model. Second, we investigate changes in either a pathological context due to a familial APP mutation (e.g., Swedish) or by determining a critical TDP-43 phenotype which can affect axonal transport in sporadic ALS. This study advances our comprehension of both the physiological and pathological aspects of APP and TDP-43 axonal transport and aims to shed light on the mechanisms triggering the pathological aspects of it during the progression of AD and ALS.

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12. Hypoxic stem cell niche in forming dental pulp

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Stem cells reside in a specific microenvironment called the niche, characterised by a low partial oxygen pressure. This physiological hypoxia helps stem cells to maintain their major characteristics such as multipotency or ability to differentiate. Within the mineralized tooth, the soft pulp contains a type of mesenchymal stem cells (MSC) called dental pulp stem cells (DPSCs).

From our recently published findings, the expression of the key trio of MSC markers in dental pulp is located mainly in differentiated odontoblasts and subodontoblastic layers.

To compare expression and localization of hypoxia inducible genes with MSC markers, qPCR along with immunohistochemistry analysis were performed at the stages of dental pulp formation. Additionally, hypoxia signalling pathways were examined by customised PCR Arrays.

HIFs were localised in the cytoplasm and translocated into the nucleus postnatally. The expression of investigated HIFs overlapped particularly in the sub-odontoblastic layer. Majority of hypoxia signalling pathway genes showed decreased expression during the development. The downregulating trend was identified among others e.g. in genes that encode glycolytic enzymes and glucose transporters. This finding suggests the shift from glycolysis to oxidative phosphorylation which corresponds to known metabolic flexibility of MSCs during their transition to more differentiated phenotype during development.

Supported by the Czech Science Foundation (GACR 21-21409S).
Holomkova K. is a PhD talent scholarship holder.

13. Extraintestinal pathogenic *Escherichia coli* from dead camel calves differ from fecal isolates of healthy camels

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Pathogenic *Escherichia coli* causes infections responsible for economic losses in animal herds worldwide. Although this bacterium is well studied in livestock and poultry, studies of camelid septicemic *E. coli* infections are limited.

In this study, the set of extraintestinal pathogenic camel *E. coli* (ExPEC; n = 207) and fecal *E. coli* from healthy camels (n = 139) were characterized with respect to 162 serotypes, phylogenetic groups, 35 virulence-associated genes and 36 bacteriocin determinants using PCR screening.

Serotypes O6, O78, O86, O118/O151, and phylogroups B2 and C were found to be significantly associated with ExPEC isolates. On average, ExPEC harbored significantly more virulence-associated genes compared to fecal isolates (5.8 and 1.6 per isolate, respectively, $p < 0.001$). Prevalence of 19 various virulence associated genes was significantly higher in the ExPEC set compared to fecal *E. coli* ($p < 0.001$). Moreover, ExPEC isolates frequently encoded bacteriocins; especially colicins B, Ia, M and microcins L, V, PDI. Based on identified characteristics, the corresponding analysis clearly distinguished ExPEC and fecal isolates.

These findings suggest an exogenous source of ExPEC infections in camel calves, likely wild birds and human keepers.

14. Building tissue models with synthetic and defined materials

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One of the approaches to fabrication of 3D tissue models is 3D bioprinting. It gives researchers control over the deposition of cell-laden material and thus over the spatial organization of the model, allowing mimicking of complex architecture of the tissue. Since one of the goals of tissue engineering is utilization of such models and techniques in regenerative medicine, there is always a need for suitable synthetic, xeno-free and defined materials and bioinks.

Presented results showcase three materials, with increasing level of definition. We utilize GelMA, a commonly used photocrosslinkable variant of gelatin, which allows formation of stable, three-dimensional endothelial networks. Next, we modified alginate with carefully selected peptide motif, and complemented it with increased porosity and printability. That resulted in an animal-free material surpassing even commercially available advanced alginate bioink. Lastly, we present fully synthetic and customizable poly amino acid hydrogel, with great printability and biocompatibility.

Thanks to these materials, we can fabricate highly organized 3D constructs to properly assess cell behavior in cocultures or increase the complexity of organoids.

This work was supported by following: Czech Science Foundation (CSF21-06524S), Grant Agency of Masaryk University (MUNI/A/1301/2022) and Ministry of Health of the CR (NU21-08-00561).

15. Uncovering mechanisms of Alzheimer's Disease development through iPSC- derived cerebral organoids

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Alzheimer's disease (AD) is a neurodegenerative disorder largely associated with amyloid plaques, neurofibrillary tangles, and neuronal degeneration [1]. Despite numerous therapeutic attempts, AD still remains an incurable disease. Currently, induced pluripotent stem cells (iPSCs) are widely used to study human neurodevelopment and disease, including AD. In the case of AD, iPSCs have been used to create 3D cerebral organoids (COs), which can adequately mimic AD in vitro [2], making them valuable for understanding the molecular mechanisms of the disease. This project aims to uncover mechanisms that lead to the development of AD using unique in vitro 3D stem cell models prepared in our laboratory. Importantly, preliminary data showed significant changes during the development of AD-COs compared to non-demented controls. Thus, our data provide further evidence that developmental disorders and altered neurogenesis could contribute significantly to the development of a familial form of Alzheimer's disease, which is the key pillar of this project.

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16. Modelling inflammation-driven fibrosis using human lung organoids

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The formation of fibrotic tissue is a crucial mechanism of physiological tissue repair. Pathologic fibrosis manifests as deregulated proliferation and activation of fibroblasts, excessive production of extracellular matrix, and stiffening of surrounding tissue. This can lead to tissue disruption, loss of function, and even organ failure. Fibrosis is associated with inflammation and immune response, with several cytokines such as TGF-β, TNFα, IL-6, and IL-1β playing a key role in its development. Yet, the precise molecular connection between the inflammatory response and the development of tissue fibrosis is currently not understood.

We intend to establish a new 3D human tissue model of inflammation-driven fibrosis using human iPSC-derived organoids. We aim to determine the most potent drivers of fibrosis, describe the involved mechanisms and their dynamics, and discern the key cell populations and molecular pathways involved in fibrosis initiation and progression. Our laboratory has previously shown that organoids are able to form an immunocompetent environment in response to pathogen stimulation, as seen by upregulation of inflammatory markers [1]. We show organoids' response to a panel of cytokines linked to fibrosis development assessed as structural changes, changes in fibroblast activation, proliferation/differentiation, function, and ECM composition.

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17. The application of AI in histology

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When artificial intelligence (AI) and machine learning methods came into research, there was a huge breakthrough in the automation of data processing. AI methods are mainly used in histopathology to make data evaluation faster, as there has been a significant increase in the amount of data that needs to be evaluated. They can be used, for example, for automatic classification in diagnosis, prediction of patient survival and response to treatment, segmentation, object detection, and analysis of microscope images [1]. They can learn and improve, and their accuracy is approaching, in some cases even surpassing, the performance of pathologists [2].

In my PhD project, I focus on development of AI tools for image and spectral data analysis in histology. So far, I participated in the application of machine learning methods to the discrimination of multiple myeloma and plasma cell leukemia patients and spectral data analysis. The result of this work is an article that is submitted for publication titled Improved screening of monoclonal gammopathy patients by MALDI TOF mass spectrometry. Next, I participated in the development of mass spectrometry-coupled AI tools for discrimination of single gene changes in cancer cells.

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18. LAMTOR1 is essential for the control of AMPK activity in *BRAF*-mutated melanoma cells

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Targeted therapy of malignant melanoma often aims at the components of the overactivated ERK pathway. However, patients treated with ERK pathway inhibitors usually develop resistance within several months. Our team recently identified new molecular mechanisms involving the metabolic sensor AMPK that 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.

In the current project, we focused on the LAMTOR1/p18 subunit of the Ragulator complex, a known AMPK and ERK regulator, and its role in AMPK activation in the cellular context of melanoma. We identified a partial disruption of the Ragulator complex in response to compounds promoting the LAMTOR1/p18 accumulation on the interfaces of enlarged endolysosomes in melanoma. Crucially, the AMPK activation by metabolic stress was disrupted under these circumstances in *BRAF*-mutated melanoma cells. The essential role of LAMTOR1/p18 for AMPK activation was verified using RNA interference targeting LAMTOR1 gene expression. Finally, we observed increased interactions between LAMTOR1/p18 and AMPK proteins after metabolic stress using proximity ligation assays and immunoprecipitations in *BRAF*-mutated melanoma.

Our results indicate the importance of the LAMTOR1/p18 for AMPK kinase activation in response to metabolic stress in *BRAF*-mutated melanoma cells.

19. Mapping NAK substrates

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Our objective is to gain an understanding of NAKs, a kinase family that has shown interactions with the endocytic machinery, yet remains relatively unexplored. Our primary focus is to identify novel substrates of NAKs using mass spectrometry techniques, aiming to unravel the cellular pathways they regulate.

Through *in silico* and *in vitro* methods, we have selected a list of promising substrates for validation in cell models. However, the detection and quantification of phosphopeptides pose challenges due to their low stoichiometry and often low abundance of the protein itself. Targeted mass spectrometry emerges as the viable option for this endeavour.

Combining cell line engineering to manipulate kinase activity, with parallel reaction monitoring (PRM) allowed us to observe the occurrences of target phosphopeptides in model cell lines. However, PRM alone did not provide sufficient sensitivity for quantification of phosphorylation. Therefore, we employed a combined PRM and immuno-affinity enrichment of pre-selected substrates.

We quantified the difference in abundance of the targeted substrates between cells with and without AAK1 perturbation. Our next objective is to repeat this experiment under endogenous conditions. Ultimately, the data obtained from this study could elucidate the involvement of AAK1, together with its substrates, in the formation of cell-substrate adhesions.

20. Impact of Diabetes Mellitus on Macrophages in the Choroid Plexus

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Diabetes mellitus (DM) is one of the most common and serious chronic diseases with a negative impact on the whole body. Apart from the best-known organ-target complications, there is another important chronic damage called diabetic encephalopathy (DE). DE is characterized by electrophysiological and neuroradiological changes, clinically expressed by cognitive impairment and psychomotor disorders [1].

We aimed to investigate whether DM induces cellular changes of activated ED1 (CD68+) and resident ED2 (CD163+) macrophages in the choroid plexus representing the blood-cerebrospinal fluid barrier. Rat model of DM was induced by intraperitoneal injection of streptozotocin (80 mg/kg), the control group received only vehicle. Animals were euthanized by CO2 inhalation three weeks after induction of DM (n=4) or application of vehicle (n=4) together with naive animals (n=2). The animals were perfused with Zamboni´s fixative solution and the brain was removed. The brain sections were immunostained with anti-CD68 and anti-CD163 antibodies and the number of positive cells in the choroid plexus per mm2 was examined. We found a significantly increased number of ED1 and ED2 macrophages in the choroid plexus in the DM group compared to naive and control groups. These findings correspond with the general pro-inflammatory state in poorly compensated diabetic subjects.

Supported by MUNI/A/1238/2022.

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21. 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. 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 these issues, 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.

22. Coinfection with two closely related *Treponema pallidum* subsp. *pertenue* strains in a yaws patient from Namatanai, Papua New Guinea

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Yaws, a disease that causes ulcerative lesions on the skin and hampers normal childhood growth and development, is caused by a spirochete called *Treponema pallidum* subsp. *pertenue* (TPE). In a 5-year-old yaws patient, closely related strains of TPE, known as JE11 and TE13, were found in an ulcer sample[1]. Cloning experiments confirmed the presence of two distinct but similar genotypes, T_E13 and J_E11, within the same individual. Although coinfection with closely related TPE strains has limited epidemiological and clinical significance, this is the first documented case of coinfection with genetically distinct TP strains in a single patient. Previous instances of similar coinfections may explain the presence of numerous recombinant loci in TP genomes, resulting from recombination events between different strains or subspecies[2].

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23. Modelling the Bardet-Biedl Syndrome using retinal organoid and cell models

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Bardet-Biedl Syndrome (BBS) is a rare autosomal-recessive ciliopathy that typically manifests with early onset of visual impairment. BBS patients experience a progressive retinal degeneration that is commonly diagnosed during early childhood. However, studying the retinal defects associated with BBS presents several significant challenges. These challenges include the limited availability of relevant tissue samples, the inability to study the human retina on a cellular and molecular level, the lack of a patient-specific drug testing platform, and the limitations of animal models in reproducing human pathophysiology. Addressing these challenges is crucial for advancing our understanding of BBS-related retinal degeneration and developing effective treatments for this condition.

We aimed to study genes *BBS2* and *BBS6* as they account for most of the mutations present in BBS with a severe retinal phenotype. We edited *BBS2/6* using CRISPR/CAS9 technology to introduce specific mutations underlying the BBS phenotype into two fully characterized hiPS cell lines from healthy individuals. We have differentiated these cells carrying BBS mutations into retinal organoids to define the photoreceptor phenotype and retinal pigmented epithelial cells to characterize the ciliary defects caused by the BBS genes mutations. Our work indicates that retinal organoid model represents a suitable tool to study BBS-related retinal defects.

24. 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.

25. An induced pluripotent stem cell-based model to study the role of EXTRACELLULAR MATRIX in myocardial fibrosis

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Cardiac fibrosis is the consequence of chronic insults to the myocardium, characterized by the abnormal accumulation of extracellular matrix (ECM). Differentiation of cardiac fibroblasts (cFbs) into myofibroblasts drives the pathological ECM remodelling, a process highlighted by biochemical and structural changes which compromise cardiomyocytes (CMs) contractile activity and eventually lead to heart failure [1].

Thus, we derived cFbs from induced pluripotent stem cells (iPSCs-cFbs), and optimized a protocol to induce their differentiation into myofibroblasts based on fine-tuning bFGF and TGF-beta signallings. Next, we obtained fibrotic ECM (dECM) by implementing a decellularization procedure of the activated iPSCs-cFbs and analyzed the pathological changes occurring during the deposition of diseased cardiac ECM. Then, we developed iPSCs-CMs and cultured them either on healthy or fibrotic dECM. Morphological and functional analysis were implemented to study how the biomechanical properties of pathological ECM affect CMs physiology and function. We further validated our model using cardiomyocytes differentiated from iPSCs derived from Duchenne muscular dystrophy (DMD) cardiopathic patients [2]. By capitalizing on this approach, we might be able to recapitulate the accumulation of fibrotic tissue during heart disease and investigate the contribution of pathological ECM to the progression of heart failure and many other cardiac pathologies.

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26. Fifteen novel receptor tyrosine kinases including Fgfr2 were found to function in the primary cilia

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The solitary primary cilium extends from the surface of most mammalian cells to orchestrate communication with the extracellular environment. This is highlighted by the presence and regulation of multiple signaling machineries within primary cilia. Numerous studies have shown the importance of cilia function in health and disease, by linking disrupted cilia architecture and signaling with developmental syndromes and homeostatic disorders including cancer.

The receptor tyrosine kinase (RTK) family comprises 58 receptors guiding cell face decision of virtually all vertebrate tissues. The relationship between RTKs and primary cilium has been revealed for only a few members of the family. In this study we reveal how cilia length is disturbed by increased activity of most RTKs through activation of the cilia disassembly pathways. Furthermore, we expand the pool of signaling receptors residing within the primary cilium with fifteen new RTKs. Finally, we expose the protein regulators and the intramolecular motif for cilia targeting of Fgfr2, and demonstrate it is critical for activation of Fgfr2 signaling and expression of the target morphogenic genes.

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

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Cardiac fibrosis is a pathological process mediated by cardiac fibroblasts (CFs) in which the maladaptive remodeling of the heart extracellular matrix (ECM) leads to heart failure. Yes-associated protein (YAP) has been shown to play a role in this process, providing a possible target for therapy.

Human pluripotent stem cells (PSCs) depleted or not from YAP were differentiated into CFs (YAP KO and WT CFs) and characterized by immunofluorescence (IF) and flow cytometry (FC). YAP KO PSCs showed an impaired capability to differentiate into CFs demonstrated by the lower expression of cardiac markers (NKX2.5 and GATA4). This was further confirmed by RNASeq which revealed more similar profiles between YAP WT CFs and HCF than YAP KO CFs. Their functionality was evaluated through a collagen gel contraction assay and staining of fibronectin and collagen in decellularized matrices. Furthermore, these CFs transdifferentiation into myofibroblasts was studied through the quantification of α-SMA and FAP expression by FC and SMAD2/3 nuclear localization by IF.

These results show that YAP KO CFs have impaired functionality shown by their hindered ability to contract collagen gels and deposit organized ECM, together with the disrupted capability to transdifferentiate as exposed by the erratic expression of α-SMA and FAP and lower number of nuclear $SMAD2/3^{+}$ cells.

Our findings reveal that YAP impacts CF differentiation and functionality by downregulating the expression of cardiac markers, impairing ECM remodeling and disrupting transdifferentiation.

28. Ciliogenesis associated kinase 1 (CILK1) functions in maintaining primary cilia and centrioles

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Primary cilia and centrioles represent two closely related structures of eukaryotic cells. Centrioles are essential for organization and function of the mitotic spindle, and transform into the basal body to induce ciliogenesis [1,2]. Proper function of cilia and centrioles is critical in development as well as homeostasis of the tissue [3,4].

Ciliogenesis associated kinase 1 (CILK1) is an evolutionarily conserved and ubiquitously expressed serine/threonine kinase, with significant functions in development of the skeletal, intestinal, renal, respiratory, auditory and neuronal tissues [5]. The so far reported mechanisms of CILK1 functions involve regulation of cilia architecture and intraflagellar transport, the Hedgehog signaling and mTORC1 signaling, and autophagy [5].

In this study, we show that CILK1 is involved in the centriole biology. Apart from the involvement in building primary cilia, CILK1 is also required for centriolar cohesion, and for blocking ectopic centriolar amplification. Moreover, loss of CILK1 caused aneuploidy, suggesting the role of CILK1 in chromosome segregation.

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29. Embryonic Stem Cell-Derived Expandable Lung-Like Epithelial (ELEP) Cells: A Versatile Model for Lung Research and Regenerative Medicine

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**equal contribution*

ELEP cells, derived from human embryonic stem cells (hESCs), are a valuable model for studying lung biology and regenerative medicine. These cells offer a unique opportunity for in-depth research into lung health and disease mechanisms.

Our hypothesis suggests that ER stress triggers significant changes, including the epithelial-mesenchymal transition (EMT), in ELEP cells. These changes involve increased expression of unfolded protein response (UPR) proteins and cadherins during ER stress.

Initial findings indicate the presence of TTF1 in ELEP cells, linking them to respiratory cell lineages originating from the foregut. ELEP cells exhibit impressive differentiation potential into both proximal and distal epithelial cells, including type 1 and type 2 pneumocytes. They also show heightened sensitivity to ER stress, leading to increased expression of critical UPR proteins like Bip and CHOP, resulting in morphological changes and altered adhesion molecule expression.

We are generating CRISPR-mediated TUSC3 and MAGT1 knockout clones of ELEP cells to explore their roles in ER stress response and lung differentiation pathways. These clones are essential for studying the impact of TUSC3 and MAGT1 on UPR signaling, cell adhesion molecules, and other relevant pathways in ELEP cells.

These studies aim to uncover the molecular basis for ELEP cells' increased sensitivity to ER stress, offering insights into potential therapies and lung-related disease mechanisms.

This research is supported by the Czech Science Foundation, project no. GA23-06675S, and Masaryk University, project no. MUNI/A/1301/2022.

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

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G-quadruplexes (G4s) are one of the alternative DNA secondary structures formed on guanine-rich regions in human cells. High-throughput sequencing identified more than 700,000 potential G4-forming sequences indicating the importance of G4s in controlling some biological processes [1]. They are generally believed to regulate replication, transcription and telomere maintenance. On the other hand, once formed, quadruplexes are highly stable and thus can pose an obstacle for replication fork progression [2]. Not only quadruplexes, but also other endo- and exogenous factors may obstruct replication. Therefore, cells developed several mechanisms to deal with it. The 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 the interaction of RAD51 with G4 and focused on the biochemical characterization of the formed complex and its biological role.

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31. Cellular distribution of the delta opioid receptor in the spinal dorsal horn in mouse neuropathic pain model

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The Delta opioid receptor (DOR) is distributed in the nervous system. We aimed to study exact cellular distribution of DOR in dorsal horn (DH) of lumbar and cervical segments in mouse neuropathic pain model.

We used mouse spared nerve injury model (SNIt) with spared tibial nerve (n=8). In sham-operated animals (n=8), the left sciatic nerve was only exposed. Both groups were left to survive for 7 and 21 days. Four mice without any operation were used as naive control. After Zamboni solution perfusion, spinal cord segments L3-L5, C4-C6 were dissected. Cryostat sections were immunostained with polyclonal DOR primary antibody. Double immunostaining with DOR and NeuN or OX42 or GFAP antibodies was to determine DOR distribution in neurons, microglia or activated astrocytes, respectively.

SNIt-operation resulted in increased intensities of DOR immunofluorescence (DOR-IF) in DH of both lumbar and cervical segments. Double immunostaining DOR/NeuN revealed perineuronal location of DOR predominantly in layer-I/II. SNIt-operation for 7 days induced activation of microglial cells in lumbar segments that displayed DOR-IF. Numerous reactive astrocytes in DH also showed DOR-IF after 7 days.

These results indicated that activated microglia and reactive astrocytes in the lumbar segments may contribute to opioid tolerance mainly 7 days from SNIt.

Supported by MUNI/A/1238/2022

32. Identification of mechanically responsive genes in ventricular cardiomyocytes

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Nuclear shape and size changes are essential in development, including cell division and migration (1). Differences in nuclear size can indicate conditions like cancer and aging (2). Mutations in the LMNA gene, encoding Lamin A and C proteins, crucial for nuclear integrity, are common in dilated cardiomyopathy (DCM), contributing to 6-8% of cases (3). Cardiac and skeletal muscles are most affected, with LMNA-related congenital muscular dystrophy (L-CMD) being the most severe form, marked by early-onset muscle wasting and premature cardiorespiratory failure (4). Recent research shows that mechanical stress, from the extracellular matrix or neighbouring cells, deforms the cell nucleus, altering chromatin condensation and gene expression (5). How this stress affects cardiomyocyte nuclei in the heart's hostile environment and its role in cardiac dysfunction remain unclear (6). Our work suggests that ECM remodelling-induced mechanical disruptions may involve the translocation of the Hippo pathway effector, Yes Associated Protein (YAP), into the nucleus (7). YAP overexpression affects nuclear heterochromatin in induced pluripotent stem cells and cardiomyocytes (8, 9). Preliminary findings hint that YAP's aberrant mechanosignalling may hinder nucleoskeletal component expression, altering nuclear shape (10). We aim to explore the interplay between cardiac ECM remodelling, nuclear morphology, and chromatin condensation, with a focus on YAP's role in ventricular cardiomyocytes.

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33. Validation of a novel reporter system for the identification of eIF4F inhibitors using high-throughput screening

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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 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. Using a promoter of one of the eIF4F-controlled genes, we built a reporter system, responding to eIF4F inhibition by changes in luciferase expression in a dose-dependent manner. The system was tested in a panel of cell lines, determining the impact of eIF4F inhibition on luciferase activity. The system validation in 384- and 1536-well format verified its applicability for HTS of bioactive compounds.

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

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This work was funded by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868), the European Union – Next Generation EU - the project National Institute for Cancer Research (Programme EXCELES, Project No. LX22NPO5102), Masaryk University (MUNI/11/ACC/2/2022), Brno city municipality (Brno Ph.D. Talent Scholarship), RVO: 68378050-KAV-NPUI and by the Ministry of Education, Youth and Sports of the Czech Republic (project number LM2023052).

34. Establishment of human embryonic stem cells for stem-cell therapies

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Human embryonic stem cells are able to differentiate into every cell type in the human body making them a tremendous cell source for regenerative medicine.

However, the production of human embryonic stem cell lines for clinical use is challenging as clean rooms, highly-qualified personnel, standard operating procedures for both manufacture and quality control are required.

Here we present the derivation of clinical-grade hESC lines in cooperation with the Centre of assisted reproduction – University Hospital Brno which provided 6-day old blastocysts. Laminin 521 in combination with Nutristem, Human serum albumin, and E-cadherin was used for the mechanical derivation. Human embryonic stem cells were cultured on laminin 521 in a Nutristem medium.

The derivation itself and subsequent culture are xeno-free, feeder-free, and fulfill standards of good manufacturing practices. In-depth quality control provides essential information about the safety, pluripotency, and differentiation potential of lines. Presented clinical-grade hESC lines were established according to state-of-the-art technology that makes them excellent cell source for stem cell-based therapies.

Supported by FNUSA-ICRC. Supported by the European Regional Development Fund – Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868). Supported by the European Regional Development Fund – Project MAGNET (No. CZ.02.1.01/0.0/0.0/15_003/0000492). This research was supported by the Ministry of Health of the Czech Republic, grant nr. NV18-08-00412 (all rights reserved). Funds by the Faculty of Medicine MU – MUNI/A/1398/2021. Tereza Souralova is Brno Ph.D. Talent Scholarship Holder – Funded by the Brno City Municipality.

35. The stability of meiotic spindle in vitrified-thawed oocytes

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Infertility has been recognized as a major global public health issue, which is growing in prominence worldwide. Cryopreservation of gametes represents an important advancement for assisted reproductive techniques allowing preserving fertility in patients undergoing intensive chemotherapy or getting a healthy pregnancy later in life. Despite that, the changes in delicate ultrastructure, mainly meiotic spindle stability, of oocytes remain understudied until now.

Chromosome segregation errors during female meiosis are a leading cause of pregnancy loss and human infertility. Proper segregation of chromosomes during meiosis is crucial for embryo development. As low temperatures induce depolymerization of microtubules, cryoinjury to fragile meiotic spindle may exacerbate genetic instability in female gametes. To evaluate the extent of cryoinjury on the microstructure of the meiotic spindle of oocytes, meiotic kinetochores fragmentation, possible loss of cohesins, and chromosome missegregation during the second meiotic division are contemplated. For this purpose, spare unfertilized human oocytes from young and healthy egg donors are subjected to live-cell imaging or observed with confocal fluorescence microscopy.

The proposed project contributes to gaining insight into how cryopreservation affects the stability of the meiotic spindle and thus developmental potential of female gametes.

Acknowledgments:

We thank the staff of Reprofit International for the recruitment of egg donors and the administration of informed consents.

36. 3D printed models of aneurysm according to patients' data

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Cardiovascular diseases including stroke are leading causes of death and disability worldwide. The onset of stroke can start with a brain aneurysm that ruptures. Aneurysms are characterized by weakening and ballooning of blood vessel walls. The treatment options for an aneurysm primarily involve clipping and coiling. Both of these methods require brain surgery, which can be associated with life threatening adverse effects[1]. Understanding the mechanisms and factors affecting aneurysm conditions is important in pursuing effective prevention and treatment strategies[2]. As Prof. Ludmila Nováková points out, it is crucial to comprehend the relationship between aneurysm rupture and its consequential impact on hemodynamics. To be able to understand these hemodynamic factors we employed 3D printing technology to create in vitro models. Consequently, 3D-printed models serve as invaluable tools for visualizing and simulating blood flow within an aneurysm.

In this study the *in vitro* models are printed from transparent resins on SLA 3D printer (Phrozen Sonic Mini 8K (China)) based on anonymized patients' data from CT scans obtained from Assoc. Prof. MUDr. Aleš Hejčl, to create 3D printed models representing both ruptured and unruptured aneurysms. The transparent resins should allow particle image velocimetry measurements.

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37. Molecular basis of accelerated tooth growth

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The mouse incisor is a continuously growing tooth, with the unique ability to grow and regenerate after injury[1]. Due to this property, it is considered a great model to study teeth development[2], [3]. However, not much is known about the adaptation of the stem cell niche after injury[4]. Our study investigates changes in gene expression of the mouse incisor mesenchyme in the healthy and injured conditions. The aim of this work is to identify various stem cell niches and their role in regeneration and after injury.

To address the dynamics of the stem cell populations and to better compare the changes between both the healthy and injured states, we took advantage of the single-cell RNA sequencing of healthy and injured mouse teeth. For this experiment mouse incisors were clipped, tissue was harvested at different time intervals and gene expression was analyzed. To validate changes in the gene expression suggested by single-cell analyses, we subsequently performed in situ hybridization.

Our results show how damaged tissue reacts to injury, and its effect on tooth growth. These results bring a better understanding of stem cell niche and could uncover the processes which stand behind the stem cell niche activation in more general perspective.

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38. The use of Phosphodiesterases to inhibit FGFR3 in Achondroplasia

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Fibroblast growth factor (FGF) signaling is crucial for proper development and homeostasis of bone and cartilage. It is very tightly regulated, as small change in the signaling pathway activity has serious impact on skeletal development and on the onset and progression of age-related conditions as osteoarthritis or osteoporosis. Aberrant FGF signaling is responsible for development of multiple skeletal dysplasia among which the most common is achondroplasia. Achondroplasia is characterized by short limbs, low stature and frontal asymmetry bossing. All these conditions are caused by gain-of-function mutations in the FGF receptor 3 (FGFR3) that leads to premature activation of Raf-Ras-ERK signaling pathway [1]. FGFR3 signaling is antagonized by CNP (C-Natriuretic peptide) signaling which uses cGMP as second messengers. Bone growth and homeostasis is also regulated by parathyroid hormone receptor signaling, which uses cAMP as secondary messenger. cAMP and cGMP levels are regulated by activity of enzymes called phosphodiesterases (PDEs), a huge family of enzymes. Currently, knowledge on the expression of PDEs in bone and cartilage is limited, but substantial body of evidence suggest that PDEs play important role in bone and cartilage biology. There are many diseases in which PDEs are involved and many PDE inhibitors are already approved in human medicine (e.g. Roflumilast, Milrinone, Cilostazol, Vardenafil) [2], but nothing is known about bone and cartilage.

Our results indicate that PDE 4B, 2A, 1B are expressed in bone and PDE 4B, 8A and 12 are expressed in the cartilage. Also, Roflumilast, Crisaborole and Cilostazol were able to improve the effect of CNP on cartilage cells i.e. to revert cartilage differentiation mediated by FGF. In addition, Cilostazol and Vardenafil were able to improve the calcification of bone cells. These results show that PDEs are expressed in bone and cartilage and PDE inhibitors could be used to improve the health of bone and cartilage.

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39. eIF4F controls AMPK activity in malignant melanoma

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The most effective drugs currently used in therapy for malignant melanoma are BRAF/MEK inhibitors, but resistance to treatment often emerges. The eukaryotic translation initiation complex (eIF4F) has been reported as the nexus of resistance to small molecule drugs targeting BRAF/MEK kinases [1]. Therefore, we performed a proteomic screen to characterize the crosstalk between the ERK and eIF4F pathways.

The analysis revealed an overlap of ERK and eIF4F targets in both melanoma subtypes. Apart from the cell cycle/DNA repair regulators, we found regulators of the primary cellular energy sensor, AMPK, that were downregulated - MO25, part of an AMPK-activating complex (LKB1-STRAD-MO25), or PP2Aα, an AMPK-inhibiting phosphatase.

Upon eIF4F inhibition, we observed ERK pathway activation and surprisingly, AMPK activation despite the downregulation of LKB1, the canonical activator of AMPK. This was also confirmed in LKB1-deficient $BRAF^{V600E}$-mutant melanoma cells.

Previous studies reported negative feedback regulation between the ERK pathway and LKB1 in $BRAF^{V600E}$-mutant melanoma cells. Active ERK and its target RSK phosphorylate LKB1, preventing it from activating AMPK, which would suggest mutually exclusive activity of AMPK and ERK [2]. However, in our experiments, eIF4F inhibition promoted non-canonical, LKB1-independent AMPK activation with simultaneous ERK activation. We describe a novel mechanism of AMPK activity control in $BRAF^{V600E}$-mutant melanoma cells.

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40. Neutrophils – immunity influencers influenced by NFAT

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Calcineurin (CN) - Nuclear factor of activated T-cells (NFAT) inhibitors are commonly used immunosuppressants inhibiting adaptive immunity but leaving patients vulnerable to opportunistic infections. CN-NFAT pathway is also active in myeloid cells, where inhibition of CN-NFAT affects anti-infectious responses, while the impact on neutrophils' function remains elusive [1-3].

We used neutrophils isolated from healthy donors and evaluated the effects of CN-NFAT inhibitors on response to heat-killed *Candida albicans* and *Aspergillus fumigatus*. We performed RNAseq to see global changes in expression and qPCR analysis of selected genes and used the ELISA method for analysis of released chemokines.

We showed that in response to pathogens neutrophils massively change gene expression patterns and CN-NFAT inhibitors inhibit the increase of expression of genes involved in inflammation regulation including expression of chemokines CCL2, CCL3, and CCL4. By chemotaxis assay using transwells, we showed the impaired neutrophil ability to chemoattract other immune cells in the presence of CN-NFAT inhibitors. NFAT inhibition in human neutrophils impairs their response to pathogens and dysregulates the inflammatory environment.

Presented negative effects of CN-NFAT inhibitors on human neutrophils complete our information about the impacts of NFAT inhibition in immune cells and can help explain the increased vulnerability of CN-NFAT inhibitors-treated patients.

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