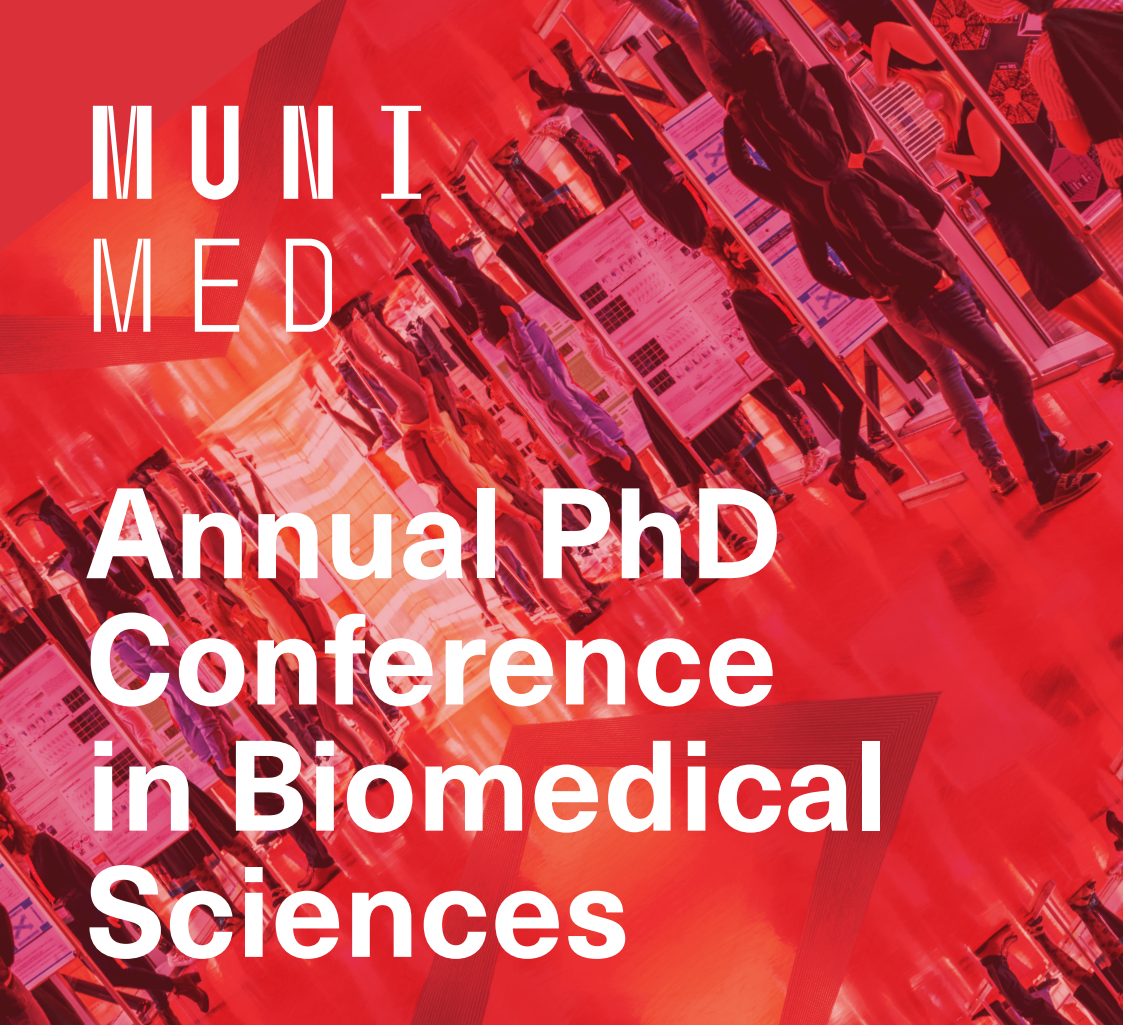

MUNI MED

# Annual PhD Conference in Biomedical Sciences

## Book of Abstracts

6–8TH NOVEMBER 2024

MASARYK UNIVERSITY CAMPUS, BRNO BOHUNICE

CEITEC

# 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 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, 7 students will give talks as part of their doctoral state exams, 8 students will present their progress reports and 45 students will present posters during three 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. RNDr. Ondřej Slabý, Ph.D.
Program Director

## LIST OF SPEAKERS - DOCTORAL STATE EXAMS

### Specialization Biochemistry and Molecular Biology

1. Smolková Karolína
2. Dudeja Pooja
3. Ursachi Vlad-Constanti

### Specialization Cell and Tissue Morphology

4. Čimborová Katarína
5. Holomková Kateřina
6. Cesnaríková Soňa
7. Kročianová Daniela

## LIST OF POSTERS

### Poster session A (1st and 2nd year students)

1. Cakmakci Riza Can
2. Emamiaref Parisa
3. Hlaváč Kryštof
4. Kafka Filip
5. Kaštánková Nikola
6. Koutná Jana
7. Kytková Lucia
8. Medaglia Alejandro
9. Molina Gambin Francisco
10. Papatheodorou Ioanna
11. Petra Orviská
12. Portakal Yanik Turkan
13. Satková Miriam
14. Stylianou Filio
15. Torkashvand Marzie
16. Tuaima Haneen Riadh Ali
17. Umar Mohammad
18. Vaňatková Kateřina
19. Vepřková Jana
20. Weselá Petra

Poster session B (3rd and 4th year students)

21. Brezak Matea
22. Celiker Canan
23. Dlugošová Slavomíra
24. Dorotíková Anna
25. Gonzáles López Marcos
26. Jongen Vincent
27. Koždoňová Kateřina
28. Kročka Erik
29. Porokh Volodymyr
30. Rábová Anna
31. Varma Gottmukkala Narendra

Poster session C (5th, 6th and 7th year students)

32. Beckerová Deborah
33. Belisová Denisa
34. Capandová Michaela
35. Dostálová Lenka
36. Chochola Václav
37. Janečková Klára
38. Lavický Josef
39. Morazzo Sofia
40. Nita Alexandru
41. Pereira de Sousa Daniel
42. Poovakulathu Abraham Sara
43. Pospíšilová Michaela
44. Souralová Tereza
45. Vadovičová Natália

# 1. A new reporter system for the identification of eIF4F inhibitors: validation of the compounds identified in the high-throughput screening

K. Smolkova[1], A. Janku[1], M. Lisova[2], P. Bartunek[2], S. Uldrijan[1]

*1 Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*2 CZ-OPENSCREEN, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic*

The eIF4F translation initiation complex plays a critical role in cancer. The complex's enhanced activity was identified as a nexus of therapy resistance and a promising target in melanoma [1,2]. However, the spectrum of available eIF4F inhibitors is limited, and none is in clinical use. One reason could lie in the relative complexity of techniques used to identify such inhibitors [3].

Thanks to our finding that eIF4F functions in the negative regulation of the RAS/RAF/MEK/ERK mitogen-activated protein kinase (MAPK) signaling pathway [4], we were able to build a unique cell-based reporter system suitable for the high-throughput identification of novel eIF4F inhibitors in small-molecule compound libraries. The system responds to eIF4F inhibition by changes in luciferase expression in a dose-dependent manner.

The reporter system was tested in a panel of cell lines, determining the impact of eIF4F inhibition on luciferase activity. Its applicability for the high-throughput screening (HTS) of bioactive compounds was verified in 384- and 1536-well format. Several highly diverse libraries comprising over 80 thousand compounds were assessed in the HTS. Surprisingly, apart from compounds potentially targeting eIF4F, the screening revealed possible new ways of ERK pathway regulation. We are currently validating the most potent compounds in orthogonal assays.

*[1] Pelletier J, et al. Targeting the eIF4F translation initiation complex: a critical nexus for cancer development. Cancer Res. 2015 Jan 15;75(2):250-63. doi: 10.1158/0008-5472.CAN-14-2789.*
*[2] Boussemart L, et al. eIF4F is a nexus of resistance to anti-BRAF and anti-MEK cancer therapies. Nature. 2014 Sep 4;513(7516):105-9. doi: 10.1038/nature13572.*
*[3] Shen S, et al. In situ detection of the eIF4F translation initiation complex in mammalian cells and tissues. STAR Protoc. 2021 Jun 23;2(3):100621. doi: 10.1016/j.xpro.2021.100621*
*[4] Valcikova B, et al., in press.*

This work was supported 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/LF-ACC/1335/2023), 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).

# 2. Biased signaling of fibroblast growth factor receptor 3

Pooja Dudeja[1] and Pavel Krejči[1]

*1 Department of Biology, Faculty of Medicine, Masaryk University*

Fibroblast growth factors (FGFs) play a crucial role in regulating various biological processes, including cell proliferation, differentiation, migration, cell lineage commitment and survival. The FGF family consists of 18 FGF ligands and four fibroblast growth factor receptors (FGFR1-4). Several FGFs can bind to the same FGFR, leading to different cellular responses. This phenomenon is known as ligand bias. Ligand bias in the FGF family remains poorly understood, particularly in relation to FGFR3, which plays a role in a number of skeletal dysplasias and cancers.

Here, we treated FGFR3-expressing cells with different FGF ligands and analyzed the FGF effect on the activation of downstream FGFR3 signaling pathways and on the cellular responses to the FGF stimulus, such as growth arrest, loss of extracellular matrix and induction of premature senescence.

Our results show distinct FGF-specific responses and provide clear evidence for ligand bias in FGFR3 signaling. Our results improve the understanding of FGFR3 signaling dynamics and open new avenues for the development of more selective FGFR3-targeted therapeutic strategies.

*Xie, Y., Su, N., Yang, J. et al. FGF/FGFR signaling in health and disease. Sig Transduct Target Ther **5**, 181 (2020). https://doi.org/10.1038/s41392-020-00222-7*

## 3. Modulation of fibroblast growth factor signaling for future therapies

Vlad-Constantin Ursachi[1], Pooja Dudeja1, Zuzana Feketova[1], Gustavo Rico-Llanos[1], Adolf Koudelka[1], Kalina Hristova[2], Lukas Trantirek[3], Bohumil Fafilek[1], Pavel Krejci[1]

*1 Department of Biology, Masaryk University, 62500 Brno, Czechia*
*2 Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, MA, USA*
*3 Central European Institute of Technology, 62500 Brno, Czechia*

Fibroblast growth factor receptors (FGFRs) are transmembrane receptors that regulate many cellular processes such as growth, differentiation, metabolism and survival. The main downstream signaling pathway activated by FGFRs is the ERK MAP kinase pathway, through which FGFRs control gene expression. The FGFR family consists of four receptors (FGFR1-4), which are present in most cells from the early stages of embryonic development. Impaired FGFR signaling, which is often caused by an activating mutation in the receptor itself, leads to numerous developmental disorders and also to cancer. Tyrosine kinase inhibitors (TKIs) have been considered as a potential therapy, but their low specificity at high concentrations is problematic for cancer treatment. In contrast, low dose TKIs may be used to treat FGFR-related developmental disorders such as achondroplasia. In this project, we are investigating different approaches to interfere with FGFR-ERK signaling, including TKIs, FGFR-specific DNA aptamers or the induction of stress in the endoplasmic reticulum. Ultimately, our experiments also lead to better understanding of the signaling mechanisms of individual FGFR variants, which in turn may contribute to the development of more targeted drugs.

# 4. From decellularization to proteomics: Exploring lung extracellular matrix across species

Katarína Čimborová[1], Vendula Pelková[1], Hana Kotasová[1, 2], Veronika Sedláková[1], Dáša Bohačiaková[1,2], Václav Pustka[3], Dávid Potěšil[3], Zbyněk Zdráhal[3], Aleš Hampl[1,2]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*2 St. Anne's University Hospital, International Clinical Research Center, Brno, Czech Republic*
*3 Central European Institute of Technology, Masaryk University, Brno, Czech Republic*

The extracellular matrix (ECM) is a fundamental, yet often overlooked, component of tissue architecture. Beyond serving as a passive scaffold, the ECM dynamically regulates cellular behavior and tissue homeostasis through complex biochemical and biomechanical signals. Its continuous remodeling enables adaptation to environmental changes, while dysregulation of ECM dynamics can profoundly impact organ function and contribute to a range of pathological conditions such as fibrosis, tumor growth, metastasis, and impaired wound healing.

While extensive studies have explored the ECM in individual species, there is a lack of comprehensive cross-species comparisons that can reveal both conserved and divergent aspects of ECM composition. Such comparisons are vital for understanding the evolutionary conservation of ECM components and for validating the relevance and translatability of animal models to human health and disease.

In this study, we performed a detailed proteomic analysis of lung ECM from mouse, pig, and human tissues. By employing the method of decellularization, we isolated the ECM from the lung tissues of each species, ensuring the removal of cellular components and genetic material while preserving the intricate ECM composition and structure as confirmed through histological, immunofluorescent, biochemical assays, and electron microscopy[1]. This approach allows for an accurate proteomic analysis of the ECM itself by eliminating predominant cellular proteins that might otherwise mask the detection of less abundant ECM components.

*[1] Čimborová, K., Kotasová, H., Pelková, V., Sedláková, V., Hampl, A. Decellularization of Pig Lung to Yield Three-Dimensional Scaffold for Lung Tissue Engineering. Methods Mol. Biol. Clifton NJ 2764, 21–33 (2024).*

This research was supported by Grant Agency of Masaryk University (MUNI/A/1598/2023).

# 5. Developmental aspects of vascularization, innervation and stem cells of the dental pulp

Holomkova K.[1], Svandova E.[1], Vesela B.[2], Lesot H.[3], Sharpe PT[4], Matalova E.[2]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*2 Department of Physiology, Veterinary University, Brno, Czech Republic*
*3 Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Brno, Czech Republic*
*4 Centre of Craniofacial and Regenerative Biology, King's College London, London, UK*

The dental pulp is a soft connective tissue that resides within the core of the tooth, surrounded by dentin and enamel. The dental pulp contains stem cells displaying potential for dental tissue regeneration. Stem cells dwell in a hypoxic microenvironment, which is crucial for their homeostasis and is involved in several mechanisms including enhancement of angiogenesis. The vascular system provides nutrient and oxygen supply for the tooth and together with nerve fibres, that are responsible for sensation of pain, play a vital role in pulp regeneration, responding to injury or infection and the dental pulp tissue maintenance. Despite increasing interest in dental pulp research, several aspects, such as developmental, remain unknown.

Therefore, stem cell markers and hypoxia-inducible factors expression dynamics and localisation have been investigated in postnatal development prior to tooth eruption. There are also on-going functional experiments associated with vascularization of the dental pulp. Additionally, the sensitive and sympathetic nerve fibres formation are evaluated at specific stages to identify the molecular background of their first appearance.

Understanding of stem cell physiology as well as vascularization and innervation of the dental pulp tissue is an interest for therapeutic strategies and represents a challenge for tooth tissue engineering techniques.

The research has been supported by the Grant Agency of the Czech Republic (GACR 23-06660S). KH is a Ph.D. Talent Scholarship Holder – Funded by the Brno City Municipality.

# 6. Investigating the processes of Alzheimer's Disease progression using iPSC-derived cerebral organoids

Sona Cesnarikova[1], Jiri Sedmik[1], Petr Fojtik[1, 2], Katerina Amruz Cerna[1], Veronika Pospisilova[1], Tereza Vanova[1, 2], Dasa Bohaciakova[1, 2]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University*
*2 International Clinical Research Center (ICRC), St. Anne's University Hospital*

Alzheimer's disease (AD) is a neurodegenerative disorder associated with amyloid plaques, neurofibrillary tangles, and neuronal degeneration [1]. AD remains incurable despite many therapeutic efforts. Induced pluripotent stem cells (iPSCs) are crucial for studying human neurodevelopment and diseases like AD. iPSCs have been used to create 3D cerebral organoids (COs) that mimic AD *in vitro* [2], helping to clarify the disease's molecular mechanisms. Our project uses these unique *in vitro* 3D stem cell models to investigate AD development. Our data provide further evidence that developmental disorders and altered neurogenesis could contribute significantly to the development of a familial form of AD (fAD).

Our preliminary data, from analyzing mature organoids (60, 85, 110, and 130 days old) revealed AD-COs' ability to mimic AD pathology. Single-cell sequencing of 60-day-old COs showed premature neural differentiation in AD-COs, suggesting altered early development. These changes manifest in COs' organization, structure, and different activity of key signaling pathways of younger COs (D4 – D25). Understanding these differences is crucial for following the early development of Alzheimer's disease. Examining these changes in AD organoids provides valuable insights into the mechanisms underlying the disease's progression.

*[1] Soria Lopez J.A., González H.M., Léger G.C.: Handb Clin Neurol. 167, 231-255 (2019).*
*[2] Barak M., Fedorová V., Pospíšilová V., Raška J., Vochyánová S., Sedmík J., Hříbková H., Klímová H., Váňová T., Bohačiaková D.: Stem Cell Rev and Rep 18, 792–820 (2022).*

## 7. Novel phosphorylation substrates reveal a spatially regulated role for AAK1 in cell migration

Daniela Kročianová[1], Alex Dagg[3], Rory Clayton[3], David Potěšil[2], Veronika Fedorová[1], Petra Martinková[2], Petr Gintar[2], Kateřina Hanáková[2], Adam Harmanec[5], Zbyněk Zdráhal[2], Zuzana Kadlecová[1,3]

*1 Masaryk University, Medical Faculty, Department of Histology and Embryology*
*2 Masaryk University, Central European Institute of Technology*
*3 University of Cambridge, Cambridge Institute for Medical Research*
*4 University of Cambridge, Department of Biochemistry*
*5 Czech Academy of Science, Institute of Information Theory and Automation*

AAK1 and its homolog BMP2K are serine/threonine kinases traditionally linked to clathrin-mediated endocytosis. AAK1 has also emerged as a drug target for antiviral therapies and neuropathic pain. Our research aims to identify novel substrates of AAK1 to unravel the cellular pathways it regulates. We have uncovered a new function for AAK1 in focal adhesion disassembly, identifying PDLIM5 as a novel substrate. PDLIM5 colocalizes with actin fibers and focal adhesions. Our data shows, that AAK1 itself also colocalizes with focal adhesions and enriches its interactome with focal adhesion components as well as proteins involved in actin stress fiber organization. Our findings suggest that phosphorylation by AAK1 regulates PDLIM5's colocalization with focal adhesions and actin stress fibers, playing a key role in focal adhesion disassembly and cell migration. This extends AAK1's role beyond endocytosis and highlights its potential as a regulator of focal adhesion dynamics. These insights are critical for the development of AAK1-targeted drugs and for understanding their potential side effects.

## 1. Investigation of the effects of endoplasmic reticulum stress and unfolded protein response in patient derived pancreatic adenocarcinoma cells

Riza Can Cakmakci[1], Ivana Acimovic[1], Lukáš Jan[1], Türkan Portakal[1], Michal Eid[2], Jakub Vlažný[3], Petr Moravčík[4], Zdeněk Kala[4], Petr Vaňhara[1,5]

*1 Department of Histology and Embryology, Faulty of Medicine, Masaryk University, Brno, Czech Republic*
*2 Internal Hematology and Oncology Clinic, University Hospital Brno, Brno, Czech Republic*
*3 Department of Pathology, University Hospital Brno, Brno, Czech Republic*
*4 Surgery Clinic, University Hospital Brno, Brno, Czech Republic*
*5 International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic*

Pancreatic adenocarcinoma (PDAC) is a deadly cancer, often diagnosed at advanced stages . Research into molecular signaling associated with the endoplasmic reticulum (ER) and the unfolded protein response (UPR) in PDAC cells can provide better understanding of PDAC etiopathogenesis and offer new treatment perspectives. Rapid cell proliferation leads to accumulation of misfolded proteins within ER and induction of cell stress. This study examines patient-specific expression changes of proteins coping with ER stress aiming to provide novel molecular markers for diagnostics, follow-up, and targeting. 3D culture model is employed to mimic PDAC cell heterogeneity *in vitro* for more accurate study and treatment.

Primary tumor cells from patient resections were cultured *in vitro*. ER stress biomarkers were analyzed using BIP, CHOP, TUSC3, TGF-β, E-cadherin, N-cadherin, and EGFR antibodies. Tunicamycin (TN) was used as an established ER stress inducer through inhibiting N-glycosylation. TUDCA and Salubrinal were used to inhibit ER stress. UPR activation was observed in TN groups with increased BIP and CHOP expressions. TUSC3 decreased in TN groups. EGFR expression increased in TN groups. Spheroid size and roundness varied between agarose scaffold and hanging drop method. This study highlights patient-specific differences in antibody expression in untreated PDAC cells, creating a path for unique treatment approaches.

This work was supported by Ministry of Health of The Czech Republic, grant no. NU23-08-00241 and by funds of Faculty of Medicine, Masaryk University, grant. no. MUNI/A/1598/2023.

# 2. The reaction of choroid plexus to chemotherapy

Parisa EmamiAref[1], Lucie Kubičková[1], Babak Bakhshinejad[2], Petr Dubový[1], Marek Joukal[1], Alemeh Zamani[1]

*1 Alemeh Zamani Research Group Department of Anatomy, Faculty of Medicine, Masaryk University*
*2 Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen*

Paclitaxel, a widely used anti-cancer drug, stabilizes microtubules to inhibit cell division but causes neuropathic pain, likely by affecting the immune system. This study investigates paclitaxel's impact on the choroid plexus (CP), which forms the blood-cerebrospinal fluid barrier and allows immune cell entry into the brain. The research focuses on macrophage-like Kolmer cells (KC), involved in CNS immune surveillance. Previous findings indicated paclitaxel increased activated and resident KC and proliferating cells. To explore the origins of these KC cells, male Wistar rats were administered paclitaxel (2 mg/kg) or a vehicle, followed by behavioral tests (thermal and mechanical sensitivity). Immunohistochemistry assessed colocalization of activated KC (ED1), resident KC (ED2), and proliferating cells (Ki67). Additionally, Z310 choroidal epithelial cells were treated with paclitaxel to evaluate changes in tight junction proteins (ZO1, Occludin) via Western blot. Behavioral tests confirmed neuropathic pain, with no colocalization of KC and proliferating markers, suggesting peripheral immune cell origins. In vitro, paclitaxel disrupted CP barrier integrity by altering Occludin and ZO1 levels. These results link paclitaxel-induced neuropathic pain to immune responses and CP barrier disruption, pointing to potential therapeutic targets to mitigate these side effects.

*[1] Xu H, Lotfy P, Gelb S, Pragana A, Hehnly C, Byer LIJ, et al. The choroid plexus synergizes with immune cells during neuroinflammation. Cell. 2024 Sep 5;187(18):4946-4963.e17.*

*[2] Thompson D, Brissette CA, Watt JA. The choroid plexus and its role in the pathogenesis of neurological infections. Fluids Barriers CNS. 2022 Sep 10;19(1):75.*

# 3. The role of FoxO1 transcription factor in response to targeted therapy in chronic lymphocytic leukemia

Krystof Hlavac[1,2], Laura Ondrisova[1,2], Vaclav Seda[1,2], Petra Pavelkova[1,2], Eva Hoferkova[1,2], Daniel Filip[1,2] and Marek Mraz[1,2]

*1 Central European Institute of Technology, Masaryk University, Brno, Czech Rep.*
*2 Dept. of Internal Medicine, Hematology and Oncology, University Hospital Brno and Faculty of Medicine, Brno, Czech Rep.*

FoxO1 transcription factor has an indispensable and intriguing role in B cell biology which is often dysregulated in related malignancies. Apart from its general functions in cell metabolism, DNA damage and others, FoxO1 has been found to promote the survival of chronic lymphocytic leukemia (CLL) cells in various ways which include homing capacity towards lymph node microenvironment or adaptation to B cell receptor (BCR) signaling inhibitor treatment [1,2]. We have discovered that FoxO1-regulated transcriptional network also affects pathways highly relevant to the microenvironment stimuli which were previously proven to have a major pro-proliferative and protective role in context of disease progression and targeted therapy resistance, respectively [3,4]. In our project, we investigate how FoxO1 activity impacts responsiveness to these stimuli through which CLL cells activate their proliferation programs and acquire resistance to targeted therapy. Our findings then serve as a base for preclinical testing of FoxO1 inhibition combined with approved small molecule inhibitors that are frequently used in CLL therapy.

*[1] Seda V, Vojackova E, Ondrisova L et al. FoxO1-GAB1 axis regulates homing capacity and tonic AKT activity in chronic lymphocytic leukemia. Blood. 2021; 138:758-772. doi: 10.1182/blood.2020008101.*
*[2] Ondrisova L, Seda V, Hoferkova E et al. S142: FoxO1-Rictor axis induces adaptive increase in Akt activity during BCR inhibitor therapy in CLL: Implications for combination therapy. 2023. HemaSphere 7, e8228434.doi: 10.1097/01.HS9.0000967480.82284.34.*
*[3] Hoferkova E, Seda V, Kadakova S et al. Stromal cells engineered to express T cell factors induce robust CLL cell proliferation in vitro and in PDX co-transplantations allowing the identification of RAF inhibitors as anti-proliferative drugs. 2024. Leukemia. doi: 10.1038/s41375-024-02284-w.*
*[4] Haselager MV, Thijssen R, Bax D et al. JAK–STAT signalling shapes the NF-KB response in CLL towards venetoclax sensitivity or resistance via BCL-XL. 2023. Mol Oncol, 1878–0261. doi: 10.1002/1878-0261.13364.*

This project was supported by the Ministry of Health of the Czech Republic, grant no. NW24-03-00369. This work was also supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU, and institutional support: MH CZ-DRO (FNBr, 65269705), MUNI/A/1558/2023.

# 4. Modelling inflammation-driven fibrosis using human lung organoids

Kafka F.[1,2], Bosáková V.[1,2], Lázničková P.[1], Hortová-Kohoutková M.[1], Frič J.[1,3]

*1 International Clinical Research Center, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*2 Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*3 Institute of Hematology and Blood Transfusion, Prague, Czech Republic*

Lung fibrosis is a serious condition characterized by an uncontrolled activation and proliferation of fibroblasts, resulting in excessive extracellular matrix deposition and impaired tissue function. It is strongly linked to inflammation and immune response, with cytokines including TGF-β, TNFα, IL-6, and IL-1β, as well as immune cells such as macrophages, shown to have a critical role in fibrosis development. It can also develop as a long-term consequence of lung damage and cytokine imbalance caused by sepsis.

We aim to establish a human tissue model of inflammation-driven fibrosis using iPSC-derived lung organoids and human fibroblast cell lines. Our team previously demonstrated that organoids can form an immunocompetent environment in response to inflammatory stimuli, evidenced by the upregulation of inflammatory markers [1]. Within this model, we aim to identify the drivers of fibrosis and assess organoids' response to a panel of fibrosis-related sepsis-born cytokines. By incorporating monocyte-derived macrophages into the organoid system, we aim to investigate macrophages' role in initiating and progressing fibrosis in human lungs. Comparing the organoids' responses to those of human fibroblast cell lines will underscore the significance of intercellular interactions within this complex 3D model for fibrosis research.

*[1] Jose S.S, et. al., Comparison of two human organoid models of lung and intestinal inflammation reveals Toll-like receptor signalling activation and monocyte recruitment, 2020*

# 5. Free circulating histones and their complexes in biofluids as a novel markers of paediatric brain tumours

Nikola Kaštánková[1,2], Jan Červený[3], Diana Búzová[3], Jan Fröhlich[2]

*1 Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czechia*
*2 International Clinical Research Centre, St. Anne's University Hospital, Brno, Czech Republic*
*3 Department of Adaptive Biotechnologies, Global Change Research Institute CAS, Brno, Czechia*

Paediatric brain tumours (PBT) are major contributors to cancer-related mortality in children. Traditional diagnostic methods, including imaging and tissue biopsies, face limitations due to their invasiveness and the difficulty of accessing tumour sites. This highlights the need for non-invasive alternatives like liquid biopsies, which analyse circulating tumour-derived components. Recent research indicates that circulating histones may serve as promising biomarkers for these tumours, providing insights into their state and progression.

This study aims to evaluate the diagnostic and prognostic value of circulating histones in PBT and to investigate the mechanisms underlying their release into the extracellular space. Our goal is to establish circulating histones as a non-invasive diagnostic tool, which could significantly improve early diagnosis, patient stratification, and treatment monitoring, thereby enhancing patient outcomes and reducing the risks associated with current diagnostic methods.

# 6. Analysis of monogenic and polygenic risk of hypercholesterolemia and development of atherosclerosis

Koutná J.[1], Freiberger T.[1, 2]

*1 Department of Clinical Immunology and Allergology, Faculty of Medicine, Masaryk University*
*2 Centre for Cardiovascular Surgery and Transplantation Brno*

Familial Hypercholesterolemia (FH) is an autosomal dominant genetic disorder which affects approximately 1 in 250 individuals [1,2]. It is defined by dyslipidemia that is characterized by lifelong exposure to elevated low-density lipoprotein cholesterol (LDLC) levels which leads to premature atherosclerosis and cardiovascular diseases. Early diagnosis and treatment can significantly reduce mortality. FH is traditionally considered a monogenic disorder caused mostly by pathogenic variants in *LDLR*, *APOB* or *PCSK9* genes, but rare cases of causal variants have also been identified in other genes involved in lipid metabolism. On the other hand, there is still a relatively high percentage of patients with a clinical diagnosis based on LDLC levels and other symptoms, but no causal variant detected by next generation sequencing (NGS). Recently, it has been suggested that the disease phenotype may also have a significant polygenic component contributed by the simultaneous occurrence of polymorphisms associated with increased levels of LDLC or increased risk of cardiovascular disease [3]. In my project, I will focus on analyzing biochemical markers and NGS data of the Czech newborn population and patients with an FH phenotype, but no causal variants identified, with aim to evaluate both monogenic and polygenic risks of FH. Especially studies of polygenic risks offer many possibilities to explore different approaches and risk scoring methods.

*[1] Sjouke B, Kusters DM, Kindt I, Besseling J, Defesche JC, Sijbrands EJ, Roeters van Lennep JE, Stalenhoef AF, Wiegman A, de Graaf J, Fouchier SW, Kastelein JJ, Hovingh GK. Homozygous autosomal dominant hypercholesterolaemia in the Netherlands: prevalence, genotype-phenotype relationship, and clinical outcome. Eur Heart J. 2015 Mar 1;36(9):560-5. doi: 10.1093/eurheartj/ehu058.*

*[2] Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard BG. Familial hypercholesterolemia in the danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication. J Clin Endocrinol Metab. 2012 Nov;97(11):3956-64. doi: 10.1210/jc.2012-1563.*

*[3] Talmud PJ, Shah S, Whittall R, Futema M, Howard P, Cooper JA, Harrison SC, Li K, Drenos F, Karpe F, Neil HA, Descamps OS, Langenberg C, Lench N, Kivimaki M, Whittaker J, Hingorani AD, Kumari M, Humphries SE. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study. Lancet. 2013 Apr 13;381(9874):1293-301. doi: 10.1016/S0140-6736(12)62127-8.*

# 7. TGFβ ligands in calva development and in calva-derived osteoblastic cells

Kratochvilova A[1], Kytkova L[1,2], Oralova V[1], Matalova E[1,3]

*1 Institute of Animal Physiology and Genetics, Academy of Sciences, Brno*
*2 Faculty of Medicine, Masaryk University, Brno*
*3 Department of Physiology, University of Veterinary Sciences, Brno*

The core members of the transforming growth factor beta (TGFβ) ligands, TGFβ1, TGFβ2, and TGFβ3, are multifunctional cytokines with a strong impact on osteoblastogenesis. Calva is a source of osteoblastic cells widely applied in bone research. This includes particularly mouse primary osteoblastic cells and the MC3T3-E1 cell line.

The aim of this investigation was to follow expression dynamics of Tgfb ligands during *in vivo* calva development and two calva-derived culture systems.

Calvarial samples were collected at the postnatal (P) days 0, 7, 14, 21 and 28. Calva-derived primary osteoblasts (isolated early postnatally) and MC3T3-E1 were also collected in week intervals during osteogenic differentiation. mRNA expression of Tgfb ligands was quantified by qPCR analysis.

*In vivo*, Tgfb1 and Tgfb3 peaked on days 7 and 28, while Tgfb2 demonstrated peaks on days 14 and 28. All ligands exhibited a significant drop on day 21 of postnatal calva development. In the *in vitro* setting, the peak was observed on day 14, with the exception of Tgfb2 in the MCT3-E1 cell line, which peaked on day 7, and Tgfb3 in primary cells, whose expression continued to increase until day 21.

The dynamics will be taken into account for the on-going experiments searching for mechanisms interconnecting cytokines during osteoblastogenesis as well the entire osteogenesis.

# 8. Construction of the interaction network of coding and non-coding RNAs in prostate cancer for potential personalized targeted treatments

Alejandro Medaglia-Mata[1,2], Vojtech Bystry[1]

*1 Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*
*2 Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

Prostate cancer (PCa) is one of the most prevalent malignancies affecting men, with an annual incidence of approximately 160,000 cases in the United States alone [1], [2]. It ranks as the third leading cause of cancer-related mortality in men. Although the 5-year overall survival rate is high at 98%, patients diagnosed with metastatic prostate cancer face a significantly reduced survival rate of about 30% [3]. These statistics highlight the critical need for a deeper understanding of PCa to improve diagnostic protocols and develop targeted therapies.

Non-coding RNA features have been used as a clinical biomarker for PCa. However, the interactions between coding and non-coding RNAs in PCa have been underexplored. Thus, the central objective of this project is to elucidate the complex interaction networks between coding and non-coding RNAs. By integrating bulk RNA-Seq and microRNA-Seq data into a Weighted Gene Co-expression Network Analysis (WGCNA), we aim to identify gene clusters with shared biological functions closely linked to PCa traits. This approach will lead to the generation of non-coding RNA-coding RNA and protein-protein interaction networks, which can be further investigated for their potential as targets for drug discovery and therapeutic intervention.

*[1] M. Liu et al., "LncRNA weighted gene co-expression network analysis reveals novel biomarkers related to prostate cancer metastasis," BMC Med Genomics, vol. 15, no. 1, Dec. 2022, doi: 10.1186/s12920-022-01410-w.*

*[2] Y. Wang, H. Su, Y. Lu, H. Li, and N. Ruan, "Regulatory Role of Fatty Acid Metabolism-Related Long Noncoding RNA in Prostate Cancer: A Computational Biology Study Analysis," J Oncol, vol. 2023, 2023, doi: 10.1155/2023/9736073.*

*[3] M. Liu et al., "LncRNA weighted gene co-expression network analysis reveals novel biomarkers related to prostate cancer metastasis," BMC Med Genomics, vol. 15, no. 1, Dec. 2022, doi: 10.1186/s12920-022-01410-w.*

# 9. Human Retinal Organoid and Cell Models for Studying Bardet-Biedl Syndrome Ciliopathy

Francisco Molina Gambin[1], Canan Celiker[1], Eva Hruba[1], Eman Elrefaay[2], Michaela Bosaková[2] and Tomas Barta[1]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Czech Republic*
*2 Department of Biology, Faculty of Medicine, Masaryk University, Czech Republic*

Bardet Biedl Syndrome (BBS) is a rare autosomal-recessive ciliopathy that results from mutations in a set of highly-conserved BBS genes. BBS patients experience retinal degeneration caused by a progressive loss of rods and cone photoreceptors, which 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.

Here we aimed to study gene *BBS6*, as it accounts for most of the mutations present in BBS with a severe retinal phenotype. We edited *BBS6* using CRISPR/Cas9 technology to introduce knockout 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 (RO) to define the photoreceptor phenotype and retinal pigmented epithelial (RPE) cells to characterize the ciliary defects caused by the BBS genes mutations. Our results show significant differences in cilia length and impaired phagocytosis function in RPE cells. In addition, photostimulation of ROs revealed differences in the expression of light-responsive genes after different light exposure time frames, proving that this model is a developmental biology tool with potential for the elucidation of BBS-related retinal anomalies.

This study was supported by the Ministry of Health of the Czech Republic, grant nr. NU22-07-00380

# 10. Interleukin 18 alters monocyte functionality without exacerbating inflammation

Ioanna Papatheodorou[1,2], Gabriela Blažková[1], Veronika Bosáková[1,2], Julie Štíchová[3], Petros Kolovos[4], Martin Helán[1,5], Marcela Vlková[3], Jan Frič[1,6], Marcela Hortová-Kohoutková[1]

*1 International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic*
*2 Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*3 Institute of Clinical Immunology and Allergology, St. Anne's University Hospital, Brno, Czech Republic*
*4 Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece*
*5 Department of Anesthesiology and Intensive Care, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*[6] Institute of Hematology and Blood Transfusion, Prague, Czech Republic*

Surgical intervention activates the innate immune response. To initiate recovery, innate immune cells as monocytes perform various tightly controlled functions, e.g. by production of cytokines. We aimed to investigate the determinants of successful recovery and how this affects monocytes. Blood samples were obtained from ORT patients before (T0), 24 hours (T1) and 3-5 days (T2) after surgery. We isolated plasma for cytokine profiling, and monocytes for immunophenotyping by flow cytometry and bulk RNAseq. We found that interleukin 18 (IL-18) was the only cytokine increased in T2 in comparison to other cytokines, mainly increased in T1. Transcriptomic analysis of ORT monocytes revealed upregulation of integrins, CXCL chemokines and hematopoiesis factor T-cell acute leukemia 1 (TAL1) in T2 but not of pro-inflammatory genes. Functional markers CD64, CD86 and CD11b were also increased in ORT patient monocytes in T2. We subsequently investigated the effect of IL-18 on monocytes *in vitro*. Healthy donor monocytes were stimulated with IL-18 and TNFα and analyzed by flow cytometry, western blot and PCR. IL-18 stimulation led to increased phosphorylation of Akt but not NFκB. Our findings support that IL-18 influences monocyte mobility and functional profile without exacerbating inflammation, suggesting a new role for this cytokine in monocytes.

# 11.The link between Alzheimer’s disease and diabetes mellitus in relation to neuroinflammation in the choroid plexus

Orviská, P., Emamiaref, P., Jeřábková, K., Baindur, S., Telenský, P., Ježek, K., & Zamani, A.

*Alemeh Zamani Research Group, Department of Anatomy, Faculty of Medicine, Masaryk University, Kamenice 3, CZ-62500 Brno, Czech Republic.*

Alzheimer's disease (AD) is a leading cause of neurodegeneration, with limited effective treatments. Emerging research points to a significant connection between AD and type 2 diabetes mellitus (DM), particularly through shared mechanisms of inflammation, oxidative stress, and insulin resistance. This project aims to investigate the role of the choroid plexus (CP) in AD and DM, focusing on its contribution to neuroinflammation. Understanding this connection could reveal new therapeutic targets for AD.

Using animal models of AD and hybrid AD/DM models (TgF-344-AD (an AD transgenic rat that overexpresses human APP and presenilin 1 genes associated with AD), and ZF AD+/DM+ (a hybrid between Tg F-344 AD, female and Zucker Diabetic Fatty, fa/fa male) and their controls, including ZF AD-/DM- (PS&APP1- Fa/Fa), ZF AD+/DM- (PS&APP1+ Fa/Fa), ZF AD- /DM+ (PS&APP1- fa/fa), ZF strain, Fisher F344, and diet-based diabetes model), the study will track molecular changes in the CP over time, analyzing both male and female subjects to identify sex-specific differences. Next-generation sequencing and mass spectrometry-based proteomics will be employed to monitor transcriptional and translational changes in CP tissue. Bioinformatics and artificial intelligence models will be used to integrate the data, identifying key molecular interactions and time points critical to disease progression.

Preliminary proteomics results on the rat CP epithelium model cell line Z310 suggest that neuroinflammation in the CP exacerbates insulin resistance and glucose metabolism, further promoting amyloid-beta production and inflammation. This study will advance knowledge of AD's pathophysiology and offer a comprehensive approach to understanding the complex interplay between AD and DM. Insights gained could support the development of novel therapeutic strategies targeting neuroinflammation and insulin resistance, ultimately contributing to more effective treatments for neurodegenerative diseases.

Funding: MUNI/11/JRG/1143/2023

# 12. ER Stress-Mediated EMT Induction by LPS in Expandable Lung-Like Epithelial Cells

Portakal T., Havlíček V., Herůdková J., Pelková V., Cakmakci R.C., Hampl A., Vaňhara P.,

*Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic*

Lipopolysaccharide (LPS) is an endotoxin in the cell wall of Gram-negative bacteria inducing inflammation. LPS has been studied for its effects on immune cells in the lung. However, its impact on epithelial cells is not well understood. This study aims to investigate the potential role of LPS in inducing epithelial to mesenchymal transition (EMT) via endoplasmic reticulum (ER) stress in expandable lung-like epithelial (ELEP) cells derived from human embryonic stem cells (hESCs).

During EMT, epithelial cells lose their characteristic properties and acquire mesenchymal features, which can lead to interstitial fibrosis and, ultimately, failure of lung functions. Severe ER stress activates the Unfolded Protein Response (UPR), and excessive UPR activation has been linked to EMT, abnormal produciton of extracellular matrix and/or cell death.

To explore the possible EMT effect of LPS via ER stress in ELEP cells, we performed functional and morphological analysys, migration assays, WB, PCR, and immunofluorescence. Additionally, studies were conducted using light microscopy, WB, and PCR in 3D conditions. The ER stress activator tunicamycin and inhibitor TUDCA were also used to investigate the role of ER stress in this process.

The results demonstrate that LPS triggers EMT in ELEP cells through increased expression of UPR-related molecules. Furthermore, LPS alters the expression of E-Cadherin and N-Cadherin, two key markers of EMT.

In conclusion, LPS can induce EMT in ELEP cells through the activation of ER stress and the UPR pathway. These findings contribute to our understanding of the effects of LPS on lung epithelial cells and the role of the mechanisms of LPS-induced EMT in lung pathologies.

*[1] Kotasová H, Capandová M, Pelková V, Dumková J, Koledová Z, Remšík J, Souček K, Garlíková Z, Sedláková V, Rabata A, Vaňhara P, Moráň L, Pečinka L, Porokh V, Kučírek M, Streit L, Havel J, Hampl A. Expandable Lung Epithelium Differentiated from Human Embryonic Stem Cells. Tissue Eng Regen Med. 2022 Oct;19(5):1033-1050. doi: 10.1007/s13770-022-00458-0. Epub 2022 Jun 7. PMID: 35670910; PMCID: PMC9478014*

This work was supported by Czech Science Foundation, grant no. GA23-06675S and by funds of Faculty of Medicine, Masaryk University, grant. no. MUNI/A/1598/2023.

# 13. Uncovering the role of *SORL1* mutations in Alzheimer's disease using iPSCs-derived glial models

Miriam Satkova[1], Jiri Sedmik[1], Jan Raska[1,2], Dasa Bohaciakova [1,2]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*2 International Clinical Research Center (ICRC), St. Anne's University Hospital, Brno, Czech Republic*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by the accumulation of amyloid-β plaques and the formation of Tau neurofibrillary tangles. Approximately 5% of AD cases are linked to mutations in genes such as *APP, PSEN1, PSEN2,* and *SORL1*. SORLA, encoded by the *SORL1* gene, plays a critical role in the intracellular trafficking of amyloid precursor protein (APP), and mutations in *SORL1* have been shown to affect APP processing in neurons. While these effects are well-documented in neurons, the impact of SORLA mutations on glial cells remains largely unexplored.

To investigate this, we optimized protocols to differentiate human induced pluripotent stem cells (iPSCs) into 2D astrocytes and 3D astrocyte-containing spheroids. These models, along with CRISPR/Cas9-edited iPSCs carrying *SORL1* mutations, will allow us to study the role of SORLA in glial biology and its contribution to AD pathology.

# 14. Analysis of alternative therapeutic options for Acute Myeloid Leukemia patients resistant to Venetoclax based on drug screening and expression profiling

Filio Stylianou[1], Adriana Ladungová[1], Jan Hýl[2], Daniel Buša[2], Martin Čulen[2], Barbora Weinbergerová[2], Jiří Mayer[2] and Michal Šmída[1,2]

*1 Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic*
*2 Department of Internal Medicine - Hematology and Oncology, Medical Faculty of Masaryk University and University Hospital Brno, Czech Republic*

Acute myeloid leukemia (AML) is one of the most challenging hematological cancers to treat due to its aggressive nature and heterogeneous genetic aberrations [1]. Although they are commonly used for disease stratification, they lack the ability to predict responses to therapy [2]. The BCL-2 inhibitor, Venetoclax (Ven), used in combination with the hypomethylating agent Azacitidine as a standard care for AML patients unfit for chemotherapy [3]. Despite promising responses, resistance to BCL-2 inhibitors emerges in many cases, emphasizing the need for exploring alternative therapies, identifying predictive response biomarkers and understanding the underlying mechanisms leading to resistance.

To explore the alternative therapies for Ven/Aza resistant patients, we assembled a drug library covering 300 FDA-approved drugs, including 23 drugs in combination with Ven. These drugs were screened on a collection of AML primary samples that failed on Ven/Aza therapy. The top effective compounds that affected the cell viability of these resistant samples were targeting proteasome, histone deacetylases and protein translation. In addition, we utilized qPCR to compare the expression profile of candidate genes at the time of diagnosis and relapse. This revealed a deregulation of multiple pro/anti-apoptotic genes in the resistant cells.

Dysregulated genes conferring resistance will be validated on Ven-resistant samples and the top compounds from the drug screening will be tested on patient-derived AML xenograft mouse models.

*[1] Daver, N. et al. (2020) 'New Directions for emerging therapies in acute myeloid leukemia: The next chapter', Blood Cancer Journal, 10(10). doi:10.1038/s41408-020-00376-1.*
*[2] Glytsou, C. et al. (2023a) 'Mitophagy promotes resistance to BH3 mimetics in acute myeloid leukemia', Cancer Discovery, 13(7), pp. 1656–1677. doi:10.1158/2159-8290.cd-22-0601.*
*[3] Saliba, A. N., John, A. J., & Kaufmann, S. H. (2021). Resistance to venetoclax and hypomethylating agents in acute myeloid leukemia. Cancer drug resistance, 4(1), 125–142. https://doi.org/10.20517/cdr.2020.95.*

# 15. 3D-printed models of aneurysms based on patients' data to study hemodynamic

Torkashvand M.[1], Nováková L.[2], Hejčl A.[3], Brunátová J.[4], Hron J.[4], Víteček J.[5], Vítečková Wünschová A.[1]

*1 Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic.*
*2 Faculty of Mechanical Engineering, J.E. Purkinje University, Prague, Czech Republic.*
*3 Department of Neurosurgery, J. E. Purkinje University, Masaryk Hospital, Ústí nad Labem, Czech Republic.*
*4 Faculty of Mathematics and Physics, Mathematical Institute, Charles University, Prague, Czech Republic.*
*5 Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic.*

Cerebrovascular diseases, including hemorrhagic strokes, are among the most fatal conditions globally, often triggered by ruptured brain aneurysms. Understanding the mechanisms and factors influencing brain aneurysms is crucial for developing effective prevention and treatment strategies. Notably, changes in hemodynamic conditions can lead to endothelial dysfunction, an aneurysm development, and rupture. CT scans of six cerebral aneurysms with known rupture sites were used to create virtual meshes. Computational Fluid Dynamics (CFD) simulations were conducted to analyze flow dynamics. Two aneurysms' meshes from this set were 3D printed to verify CFD simulations. These *in vitro* models were connected to a pump for Particle Image Velocimetry (PIV) measurements. Four of six aneurysms showed ruptures near a vortex with low wall shear stress (WSS) and a high oscillatory shear index (OSI). One ruptured in a high WSS flow jet, while another in a significant bleb without distinct hemodynamic parameters. The PIV measurements confirmed the CFD results. Our study confirmed that aneurysm rupture points are associated with at least three distinct hemodynamic conditions. These findings indicate a need for a larger study of aneurysms' hemodynamics to be able to predict their rupture.

# 16. Entangled niches: dynamics of stem cell niches in ever-growing incisors

Haneen Tuaima[1], Marina Struncova[1], Marcos Gonzales Lopez[1], Josef Lavicky[1], Michaela Kavkova[1], Adam Bogdanovic[1], Petr Taus[1], Jan Krivanek[1]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University*

A stem cell niche houses a complex microenvironment that regulates the behavior of stem cells, providing a supportive milieu to maintain physiological functioning of tissues and organs. Upon injury, temporal regulation of niche factors plays a pivotal role in orchestrating reparative processes and activating quiescence stem cells, while balancing self-renewal and lineage commitment. While the molecular mechanisms behind stem cells activation are well-studied, it is still unclear how the inter-communication within the stem cell niche contributes to accelerated regeneration post-injury. Our study investigates the changes in gene expression of mouse incisor mesenchyme in the physiological and injured conditions. The aim of this work is to further understand the interplay between distinct microenvironments as well as their role in regeneration and after injury.

To analyze the stem cell progeny and spatio-temporal gene expression patterns in the dental mesenchyme, we took advantage of methods such as single-cell RNA sequencing, followed by lineage tracing and *in situ* hybridization to validate and spatially map changes in gene expression suggested by single-cell analyses.

Investigating these dynamics provides insights into intricate modulation of stem cell niches *in vivo* and the regenerative potential and plasticity of stem cells post-injury, thus bringing a deeper understanding of tissue homeostasis.

# 17. *Caenorhabditis elegans* as a powerful model organism to study meiosis *in vivo*

Mohammad Umar, Jitka Blazickova and Nicola Silva

*Department of Biology, Faculty of Medicine, Masaryk University*

In sexually reproducing organisms, meiosis is a fundamental route to ensure the precise segregation of homologous chromosomes into the daughter cells in order to produce functional gametes bearing half the genetic material of the progenitor cells. Meiosis is necessary to produce genetic assortment, essential for progression and species survival, as well as maintaining genome stability. *Caenorhabditis elegans (C. elegans)* is an excellent model organism for studying meiosis because of its well-defined genetic makeup, rapid life cycle, and body transparency, which enable in-depth scrutiny of cellular activity. In addition, the *C. elegans* gonad contains a spatio-temporal organization of developing germ cells in all meiotic stages, allowing detailed analyses of premeiotic DNA replication, homologous recognition, establishment of the synaptonemal complex, as well as formation and repair of DNA double strand break. Moreover, the mechanisms governing meiosis are strongly conserved between worms and mammals, allowing the study of complex pathways in a simpler metazoan model and presenting as such as a tremendous tool to unravel novel details crucial also for human health.

Through a mass spectrometry-based approach we have identified several uncharacterized proteins that appear to play important roles in crossover formation and SC assembly, two fundamental processes to accomplish faithful chromosome segregation. I have recently undertaken the functional analysis of one of these factors in particular, and I will present preliminary data that show its involvement in preserving fertility.

# 18. The use of micro-CT and histological analysis in the diagnosis of Paget's disease

Vaňatková K.[1], Vargová L.[1], Horáčková L.[2], Němečková A.[3], Vymazalová K.[1], Macháček J.[4], Vopálenský M.[5], Adámková I.[5], Jilmová D.[6]

*1 Faculty of Medicine, Masaryk University, Kamenice 3, 625 00 Brno, Czech Republic*
*2 Department of Kinesiology, Faculty of Sports Studies, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*
*3 Department of Histology and Embryology, Faculty of Medicine, Charles University, Pilsen, Czech Republic*
*4 Department of Archaeology and Museology, Faculty of Philosophy, Masaryk University, Arna Nováka 1/1, 602 00 Brno, Czech Republic*
*5 Institute of Theoretical and Applied Mechanics, Czech Academy of Sciences, Centrum Telč, Batelovská 486, 588 56 Telč*
*6 City Museum and Gallery Břeclav, sídl. Dukelských hrdinů 2747/4a, 690 02 Břeclav, Czech Republic*

Paget's disease is a metabolic disease of unclear etiology, characterized by excessive bone resorption followed by the formation of structurally deficient bone. The most commonly affected bones are the vertebral column, pelvis, femur, skull, and sacrum. The disease manifests itself in the fifth or sixth decade of life. The current incidence in the Czech Republic is approximately 3 % and it is one of the most common metabolic diseases. However, findings on historical skeletal remains are still rare. During archaeological research in Líbivá near Břeclav, possible manifestations of Paget's disease were observed on one of the skeletons.

The skeletal remains of a male about 60 years old, subjected to anthropological and palaeopathological analyses, had significant pathological changes on the skull, vertebrae, long bones, and rib. On the postcranial skeleton, the spongiosis of the long bones was quite abundant, diploe of the skull was wide and showed a pumicelike structure. The histological examination of bone tissue sections revealed a mosaic pattern. Micro-CT of the cranial vault showed, that the bone has lost its homogeneous structure, with a clearing at the site of osteolytic foci, thickening at the site of new bone formation, and only sporadically there are fragments of normal bone tissue.

*Buikstra, J.E. (Ed.), 2019. Ortner's identification of pathological conditions in human skeletal remains. Academic Press, London.*

# 19. Establishment of cancer immunotherapy *in vitro* model

Jana Vepřková[1], Andrej Bešše[1, 2], Lenka Sedlaříková[1], Lenka Bešše[1, 2]

*1 Department of Biology, Faculty of Medicine, Masaryk University, 625 00 Brno, Czechia*
*2 Laboratory of Experimental Oncology, Department of Oncology and Hematology, Kantonsspital St. Gallen, 9007 St. Gallen, Switzerland*

T cells are key players in cancer immunotherapy, but a reliable and reproducible methodology for investigating the complex interactions between immune cells and cancer cells is rather limited. Although many *in vitro* models for immunotherapy have been published, there is variability in experimental setups, including cultivation conditions, immune and cancer cell types, co-culture methods and killing assays [1, 2].

Here, we present an optimized *in vitro* model that involve whole peripheral blood mononuclear cells (PBMCs) and commercially available cancer cell lines, to evaluate immunotherapy efficacy. Separated PBMCs from healthy donor buffy coats are stimulated and educated to achieve cytotoxic effects against cancer cells. Moreover, in this model, cancer cell lines are equipped with luciferase, which allows us to measure real-time, accurate and sensitive killing efficiency of educated and stimulated PBMCs (cancer cell viability).

We believe that this standardized approach can improve reproducibility of *in vitro* studies, can be used as a model with patients´ primary cells and PBMCs and thus may contribute to the development of more effective cancer immunotherapies.

*[1] CATTANEO, Chiara M., et al. Tumor organoid–T-cell coculture systems. Nature protocols, 2020, 15.1: 15-39.*
*[2] STAKHEEV, Dmitry, et al. LL-37 as a powerful molecular tool for boosting the performance of ex vivo-produced human dendritic cells for cancer immunotherapy. Pharmaceutics, 2022, 14.12: 2747.*

# 20. Classification of histological data using artificial intelligence

Petra Weselá[1,2], Petr Vaňhara[1,2]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University*
*2 International Clinical Research Centre, St. Anne's University Hospital*

Artificial intelligence has enabled significant progress in diagnostics. By using AI, patients can be diagnosed faster and more accurately. AI can also identify tumor types that would be undetectable in the early stages using standard diagnostic methods. An example of how this technology is used in practice is the Paige Prostate software, which helps detect areas that are suspicious for cancer, as a complement to routine detection methods of prostate cancer [1].

There are many ways how to develop artificial intelligence which will be able to help with diagnostics. Choosing the right model depends on data type, their size, whether it combines several different data sources or focuses only on, for instance, image analysis.

Here we present different types of machine learning models, the options for evaluating their reliability, as well as examples of applications in different fields of medicine. We also focus on limitations such as the problem of a sufficiently large dataset, proper annotation or generalizability to the whole population. This step is very important in the project because it will help us in determining how to select and build an AI model that will evaluate clinical, image or spectral data.

*[1] G. Campanella et al., "Clinical-grade computational pathology using weakly supervised deep learning on whole slide images," Nat Med, vol. 25, no. 8, pp. 1301–1309, Aug. 2019, doi: 10.1038/s41591-019-0508-1.*

# 21. Impact of ERK biosensor EKAREV-NLS on murine mammary gland development

Matea Brezak[1], Aleš Hampl[1], Zuzana Sumbalová Koledová[2]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University*
*2 Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, CZ*

Molecular biosensors are a highly effective tool for tracking and measuring protein activity within living systems. Still, their expression can disrupt endogenous signaling pathways, potentially resulting in developmental and physiological abnormalities. The EKAREV-NLS mouse model, that carries a FRET-based biosensor for monitoring extracellular signal-regulated kinase (ERK) activity, has been utilized across various organs and cell types. Here, we show a significant defect in mammary epithelial development in female EKAREV-NLS mice. Our findings show that these mice have strongly reduced mammary epithelial growth, connected with systemic defects like disrupted estrous cycling, reduced ovarian follicle maturation and lack of ovulation. Interestingly, estrogen supplementation was sufficient to stimulate mammary epithelial growth in the EKAREV-NLS females. We further confirmed the functionality of the biosensor in hormone-supplemented tissues by time-lapse imaging of primary mammary epithelial cells. Our results highlight the necessity for detailed characterization of biosensor models prior to their use in research. This work further highlights the influence of hormonal factors on mammary gland development and shows the importance of careful selection of biosensor strains for mammary gland related studies.

Acknowledgements: This project is funded by MUNI/G/1775/2020 and MUNI/A/1598/2023. We acknowledge the core facility CELLIM supported by MEYS CR (LM2023050 Czech-BioImaging).

# 22.Illuminating the path: light-induced mechanisms in retinal development

Canan Celiker[1], Eva Hruba[1], Tomas Barta[1*]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*

The human retina is a sophisticated, multilayered tissue essential for vision, capturing and processing light to create visual images. It comprises five main types of neurons: retinal ganglion, amacrine, horizontal, bipolar, and photoreceptor cells, all crucial for converting light into electrical signals. Retinal cell health, development, and maturation are vital for proper functioning and are regulated by active stimulation. Retinal development is influenced not only by morphogens through cell-to-cell communication and signaling pathways but also by light responses that uniquely shape this tissue. However, the signaling pathways involved in these light-induced responses are largely unknown.

In this study, we aim to use human pluripotent stem cells-derived retinal organoids as a model to closely investigate photo-induced effect on retinal differentiation by using a custom-designed photostimulation device. We assessed the effect of light on cell types in retinal organoids using single cell RNA sequencing and importantly found that the light stimulation improves maturation of cells in retinal organoids.

This study was supported by the GACR project 21-08182S. We acknowledge the CF Genomics supported by the NCMG research infrastructure (LM2023067 funded by MEYS CR) for their support with obtaining scientific data.

# 23.3D culture of human articular chondrocytes

Dlugošová Slavomíra[1], Koutná Irena[1,3], Dvořáková Jana[2], Proks Vladimír[2]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno*
*2 Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague*
*3 Cell and Tissue Engineering Facility, International Clinical Research Center, St. Anne's University Hospital, Brno*

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 challenge in developing advanced therapy medicinal products (ATMPs) based on human articular chondrocytes (HACs) is the tendency to lose their native properties and undergo dedifferentiation into fibroblast-like, spindle-shaped cells [1]. This process significantly impairs their ability to produce the extracellular matrix (ECM) essential for cartilage function. Our hypothesis posits that by modifying the culture conditions and transitioning from 2D to 3D cell culture environment, we can mitigate the dedifferentiation potential of HACs. We believe that this 3D culture approach will create a more favorable microenvironment, enabling chondrocytes to maintain their phenotype and thrive. Consequently, we anticipate that these conditions will facilitate the production of high-quality ECM rich in type II collagen, glycosaminoglycans, and proteoglycans, which are critical for cartilage integrity and functionality.

*[1] Portnoi PA, MacDonald A. Determination of the lactose and galactose content of cheese for use in the galactosaemia diet. J Hum Nutr Diet. 2009 Oct;22(5):400-8. doi: 10.1111/j.1365-277X.2009.00948.x.*

# 24. The DUSP6-ERK axis moderates the impact of methionine restriction on malignant melanoma

Anna Dorotíková[1], Natália Vadovičová[1], Stjepan Uldrijan[1]

*1 Masaryk University, Faculty of Medicine, Department of Biology, Brno, Czech Republic*

Methionine is an essential amino acid critical for various cellular functions. It is necessary for the initiation of translation, during protein synthesis, and serves as the only precursor of S-adenosylmethionine, a vital substrate for methylation reactions. Methionine also helps maintain redox homeostasis and influences the production of polyamines and nucleotides. A deficiency in methionine can induce metabolic stress in cancer cells and reduce tumor growth [1,2].

Interestingly, our studies using a BRAF mutant melanoma model showed that methionine restriction led to the upregulation of the ERK pathway, the primary driver of melanoma cell proliferation. The ERK activation also stimulated mTOR complex 1 (mTORC1), which responds to nutrient availability to regulate cellular metabolism protein synthesis. Notably, despite mTORC1 activation, overall translation levels decreased. After methionine restriction, we also observed a reduction in DUSP6, a negative regulator of the ERK pathway affected by translation intensity.

We propose that the upregulation of the ERK pathway following methionine restriction is a result of inhibited translation and a decrease in DUSP6 levels. This pathway activation may serve as a compensatory mechanism that enables cancer cells to adapt to nutrient limitations, mitigating the adverse effects of methionine deprivation on melanoma cells.

*[1] Sanderson SM, Gao X, Dai Z, Locasale JW. Methionine metabolism in health and cancer: a nexus of diet and precision medicine. Nat Rev Cancer. 2019 Sep;19:625–637. doi: 10.1038/s41568-019-0187-8.*
*[2] Wanders D, Hobson K, Ji X. Methionine Restriction and Cancer Biology. Nutrients. 2020 Mar;12(3):684. doi: 10.3390/nu12030684.*

This work is supported by the Czech Science Foundation (GA22-30397S), the European Union – Next Generation EU - the project National Institute for Cancer Research (Programme EXCELES, Project No. LX22NPO5102), and Brno city municipality (Brno Ph.D. Talent Scholarship).

# 25. Mechanosensing controlling continuously-growing incisor growth in mouse

Marcos Gonzalez Lopez[1], Maryam Azam[1], Michaela Kavkova[1], Margarita Gorbulina[1], Marina Struncova[1], Marcela Buchtova[2,3], Julian Petersen[4], Miroslav Vorechovsky[5], Jan Krivanek[1]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University*
*2 Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic*
*3 Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Brno, Czech Republic*
*4 Department of Orthodontics, University of Leipzig Medical Center, Leipzig, Germany.*
*5 Institute of Structural Mechanics, Faculty of Civil Engineering, Brno University of Technology, Czech Republic.*

Mechanosensing and mechanotransduction are pivotal mechanisms in wide variety of cells crucial for biological processes, such as development, regeneration or tissue repair. Mice, have adapted throughout evolution their dentition to their diet habits and lifestyle with specialized continuously growing teeth. This specific type of tooth renews completely several times during the mouse lifespan, thus serves as an attractive model to study tissue homeostasis *in vivo* and regeneration after injury.

However, this biological process is much dependent on the stem cell activity in the dental pulp the specific mechanisms triggering this regenerative process remains poorly understood. Our main goal is to understand how mechanical stimuli can change the normal incisor growth. In this poster, we address this question by using an injury tooth model where incisor gets clipped in a single or repeatedly way. Our results uncover phenotypical aspects such as changes in the rate of incisor regrowth, differences in the enamel and dentin thickening and microstructure of the incisor post-injury. Furthermore, we investigated if these adaptive changes in response to damage modifies the mechanical properties of the tooth and the surrounding alveolar bone. Finally, we focused on understanding at cellular and molecular level how the lack of pressure in shortened incisor affects the tooth regrowth by detecting mechanically-gated ion channels, mechanotransducers, immune cell distribution and cell proliferation rate in the incisor stem cell niche.

# 26. Translating the microenvironment from *in vivo* to *in vitro* using microfluidics

V.A. Jongen [1], J. Jaroš [1,2], A. Hampl [1,2]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech*
*2 International Clinical Research Center (ICRC) of St. Anne's University Hospital Brno, Brno, Czech Republic*

Organoids are increasingly used for both disease modelling and the study of healthy development. *In vivo*, the microenvironmental niche plays a crucial role in the development of tissues and organs. However, technical limitations hamper any effort to recapitulate this microenvironment in organoid cultures. *In vitro* organoid formation therefore heavily relies on the inherent ability of stem cells to self-organize into organ-like structures. The stochastic nature of this process leads to a high degree of variability between organoids. Moreover, the incorporation of, for example, biochemical gradients or mechanical forces may lead to a more physiologically relevant model of human development.

In an organ-on-chip model, microfluidic systems are specifically designed to take advantage of novel microfabrication techniques and microscale fluid dynamics in an effort to control the microenvironment of a cell culture. These devices thereby allow their user to mimic specific elements of the *in vivo* cellular microenvironment and translate it to their *in vitro* model in ways not possible in conventional cell culture vessels.

In this work a microfluidic device is designed to model the microenvironment of the fetal lung to support lung organoid cultivation, with special focus on the inclusion of concentration gradients of growth factors and small molecules.

This work was supported by Brno city municipality (Brno Ph.D. Talent Scholarship).

# 27. The essential role of LAMTOR1 in the control of AMPK activity in *BRAF*-mutated melanoma cells

Koždoňová K.[1], Vadovičová N.[1], Palušová V.[1], Uldrijan S.[1]

*1 Department of Biology, Faculty of Medicine, Masaryk University*

Oncogenic mutations hyperactivating ERK signaling are major drivers of melanoma growth. In previous research, we identified new molecular mechanisms by which the metabolic stress sensor AMPK could affect ERK signaling in melanoma, leading to the suppression of melanoma cell proliferation (1). In the current project, we focused on the LAMTOR1 subunit of the Ragulator complex, a known AMPK and ERK binding partner, and its role in AMPK and ERK signaling regulation in the cellular context of melanoma.

We identified a partial disruption of the Ragulator complex in response to compounds promoting the LAMTOR1 subunit accumulation on the interface of enlarged endolysosomes in melanoma. Crucially, the AMPK activation by metabolic stress was disrupted under these circumstances, indicating an essential function of LAMTOR1 in AMPK activation in *BRAF*-mutated melanoma. The data were subsequently verified by RNA interference targeting *LAMTOR1* gene expression. Furthermore, AMPK activation after metabolic stress was mediated by a non-canonical, LKB1-independent mechanism. Finally, we observed increased interactions between LAMTOR1 and AMPK proteins after metabolic stress using proximity ligation assays and immunoprecipitations in *BRAF*-mutated melanoma.

Our results indicate that the lysosomal LAMTOR1 protein plays an essential role in activating AMPK kinase in response to metabolic stress in *BRAF*-mutated melanoma cells.

*[1] Verlande et al. 2018. Metabolic stress regulates ERK activity by controlling KSR-RAF heterodimerization. EMBO Rep. 19: 320-336*

This work was supported by the Czech Science Foundation (No. GA22-30397S), the European Union – Next Generation EU - the project National Institute for Cancer Research (Programme EXCELES, Project No. LX22NPO5102).

# 28. Diabetes Mellitus and Cellular Changes in the Choroid Plexus

Erik Kročka[1,2], Lucie Kubíčková[1], Marek Joukal[1]

*1 Department of Anatomy, Faculty of Medicine, Masaryk University*
*2 Department of Internal Medicine, Hospital Nové Město na Moravě*

Diabetes mellitus (DM) is one of the most common chronic metabolic disorders worldwide. Apart from the best-known organ-target complications, there is another important chronic damage called diabetic encephalopathy (DE) [1]. One of the possible pathogenetic mechanisms of DE is lesion of the brain barriers.

We aimed to investigate whether DM induces changes of M1 (CCR7+) and M2 (CD206+) macrophages, and microglia OX-42 (CD11b/c) in the choroid plexus representing the blood-cerebrospinal fluid barrier (BCSFB). This study used Wistar rats (8–10-week-old males) divided into two groups: the diabetic group (n=3) and the control group (n=2). 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 or application of a vehicle. The animals were perfused by Zamboni´s fixative solution and the brain was harvested. The coronal brain sections were immunostained with anti-CCR7, anti-CD206 and anti-CD11b/c antibodies and the number of positive cells per $mm^2$ was examined and statistically evaluated. We found a significantly increased number of OX-42 and M1 cells in the DM group compared to control group, but no difference in M2 cells. Our results indicate inflammatory reaction of BCSFB in diabetic environment.

*[1] BIESSELS, G. J., A. C. KAPPELLE, B. BRAVENBOER, D. W. ERKELEN, et al. Cerebral function in diabetes mellitus. Diabetologia. 1994, **37**(7), 643-650. ISSN 0012-186X. DOI:10.1007/BF00417687*

Supported by MUNI/A/1563/2023.

# 29. Zygotic spindle orientation defines cleavage pattern and nuclear status of human embryos

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

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University*
*2 Reprofit International, Clinic of Reproductive Medicine*

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 organization of the first mitotic division typical for normal human embryo development, we systematically analyzed a unique timelapse dataset of 300 IVF embryos that developed into healthy newborns. The zygotic division pattern of these best-quality embryos was compared to their siblings that failed to implant or arrested during cleavage stage. We show that division at the right angle to the juxtaposed pronuclei is preferential and supports faithful zygotic division. Alternative configurations of the first mitosis are associated with reduced clustering of nucleoli and multinucleation at the 2-cell stage, which are more common in women of advanced age. Collectively, these data imply that orientation of the first division predisposes human embryos to genetic (in)stability and may contribute to aneuploidy and age-related infertility.

*[1] Porokh, V., Kyjovská, D., Martonová, M. et al. Zygotic spindle orientation defines cleavage pattern and nuclear status of human embryos. Nat Commun 15, 6369 (2024).*

# 30. Cellular distribution and semi-quantitative changes of delta opioid receptors in the spinal dorsal horn of a mouse spared nerve injury model

Rábová A., Dubový P., Kubíčková L., Joukal M.

*Department of Anatomy, Cellular and Molecular Research Group, Faculty of Medicine, Masaryk University, Kamenice 3, Brno, 62500, Czech Republic*

The primary sensory neurons (PSN) of the dorsal root ganglia (DRG) play a pivotal role in afferentation of peripheral nociception into the spinal cord. The majority of central branches of PSNs terminate on the neurons of the spinal dorsal horn (SDH). The aim of present experiments was to study semi-quantitative changes of DOR protein levels in lumbar (L-SDH) and cervical (C-SDH) of both sides (ipsilateral and contralateral) in a mouse model of neuropathic pain.

Unilateral spared nerve injury model (n=8) with spared tibial nerve (SNIt) was used. In sham-operated animals (n=8), the left sciatic nerve was only exposed. Both SNIt- and sham-operated animals were left to survive for 7 and 21 post-operation days (POD). Four mice were used as naive control. The spinal cord segments in the range of L3-L5, C4-C6 were dissected. A set of cryostat sections (12µm) was immunostained with polyclonal DOR primary antibody. Double immunostaining with IB4-FITC and DOR antibody was to distinguish DOR distribution in layers I and II of the SDH.

The results indicate that levels of DOR-IF were similar in both C-SDH and L-SDH of naive mice. Both sham or SNIt operations resulted in bilateral changes of DOR-IF levels in both C-SDH and L-SDH.

# 31. Serving or Stealing: Tunneling nanotube mediated transfer of mitochondria in B cell malignancies

Narendra Varma Gottumukkala[1,2], Jakub Pospisil[4], Michal Smida[1,3]

*1 CEITEC-Masaryk University, Brno, Czech Republic*
*2 Faculty of Medicine, Masaryk University, Brno, Czech Republic,*
*3 Masaryk University, Brno, Czech Republic*
*4 Cellular Imaging Core Facility CELLIM, CEITEC MU, Brno, Czech Republic*

Tunneling nanotubes (TNTs) are tiny, tube-like structures that allow cells to communicate directly with each other by transferring materials like mitochondria. This process has major implications in cancer biology. While originally observed in solid tumors, recent studies show that TNTs can also form in blood cancers like Acute Myeloid Leukemia (AML).

In our study, we explored this phenomenon in Chronic Lymphocytic Leukemia (CLL). We found that CLL cells, both from laboratory models and patient samples, consistently formed TNTs with T cells, which are crucial for the immune response. Through these TNTs, mitochondria were transferred from the T cells to the cancerous CLL cells. This “mitochondria theft” could be one reason why T cells become exhausted, meaning they lose their ability to fight effectively, which is a problem in CLL treatments like CAR-T cell therapy.

We believe that by blocking the formation of TNTs, we could potentially control T cell exhaustion, making treatments like CAR-T therapy more effective against CLL. This discovery opens new avenues for improving immune-based therapies for blood cancers.

This work was supported by a grant from the Czech Science Foundation (project no. 22-35273S) and the project MUNI/A/1558/2023.

# 32. Early onset of cardiac progenitor maturation leads to their depletion recapitulating cardiac pathology development in Duchenne Muscular Dystrophy

Deborah Beckerová[1,2], Martin Pešl[1,2,3], Hana Dobrovolná[4], Vladimír Rotrekl[1,2]

*1 Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*2 International Clinical Research Center ICRC, St. Anne's University Hospital, Brno, Czech Republic*
*3 First Department of Internal Medicine—Cardioangiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*4 Second Clinic of Internal Medicine, Masaryk University, Brno, Czech Republic*

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 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, DMD cells are affected already from the pluripotent stem cell (PSC) stage. Dystrophin deficient PSC present elevated DNA damage and mutagenesis, at least partially caused by deregulation of nitric oxide synthase (NOS) and subsequent production of reactive species, as we have previously shown.

Here we present impaired cardiac differentiation efficacy in dystrophin deficient cell lines as illustrated by forming fewer spontaneously contracting organoids with higher rate of cardiomyocyte (CM) death, increased content and earlier deposition of collagen and altered transcriptional program during differentiation. DMD cardiovascular progenitor (CP) population shows both higher and earlier activation of CP markers with subsequent attenuation of their transcription and an earlier onset of transcription of genes associated with maturation coinciding with a decrease in proliferation in the organoid. DMD CPs 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.

# 33. Depletion of FSP1+ stromal cells results in nursing failure caused by nipple underdevelopment

Denisa Belisova[1], Ema Grofova[1], Ales Hampl[1], Zuzana Sumbalova Koledova[2]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University;*
*2 Institute of Molecular Genetics of the Czech Academy of Sciences*

Stromal cells play an instructive role in the development of many organs, including the skin and its derivative, the mammary gland. Here we investigated the role of FSP1+ (fibroblast specific protein1 - positive) cells in mammary gland function using a constitutive cell depletion mouse model *Fsp1-Cre;DTA*.

We showed that the mammary branching morphogenesis is delayed in *Fsp1-Cre;DTA* mice, and lactating *Fsp1-Cre;DTA* dams show a severe nursing defect. Immunofluorescence analysis and oxytocin stimulation assay on lactating tissue revealed no defects in milk production or mammary alveolar contractility, suggesting no causative abnormality in the mammary epithelial function. Following examination of the nipple revealed its dramatic underdevelopment in the *Fsp1-Cre;DTA* females. Although the nipple sheath is formed normally during prenatal development, the nipple in the *Fsp1-Cre;DTA* females does not increase in size postnatally, making suckling impossible. Finally, to determine the identity of the FSP1+ cells, we analyzed available data from scRNAseq of the skin and mammary gland and performed immunofluorescence labeling. We found that FSP1+ cells are a heterogeneous stromal cell population, that includes both fibroblasts and immune cells, particularly macrophages, in both organs. Taken together, we show that FSP1+ stromal cells are crucial for postnatal nipple development to enable mammary gland function.

This work was funded by Internal Grant Agency of Masaryk University (project MUNI/IGA/1311/2021), Grant Agency of Masaryk University (MUNI/G/1775/2020, MUNI/A/1301/2022 and MUNI/A/1598/2023), Czech Science Foundation (GAČR 23-04974S), Ministry of Education, Youth and Sports (ERC CZ LL2323 FIBROFORCE) and Brno city municipality (Brno Ph.D. Talent Scholarship).

# 34. Alveolar-capillary cells, biomaterials; and infrastructure for research data

Michaela Capandova[1,2], Veronika Sedlakova[1], Zbynek Vorac[3], Hana Kotasova[1], Jana Dumkova[1], Lukas Moran[1], Josef Jaros[1,4], Matej Antol[2], Dasa Bohaciakova[1,4*], Ales Hampl[1,4]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Kamenice 3, 625 00 Brno, The Czech Republic*
*2 Institute of Computer Science, Masaryk University, Šumavská 416/15, 602 00 Brno*
*3 Department of Physical Electronics, Faculty of Science, Masaryk University, Kotlářská 267/2, 611 37 Brno, The Czech Republic*
*4 International Clinical Research Center, St. Anne's University Hospital Brno, Pekařská 53, 602 00 Brno*

Human respiratory system is constantly exposed to potentially harmful substances, leading to various diseases and changes in lung tissue. Studying the effects of these substances can be done using *in vitro* models with specific scaffolds seeded with appropriate cells. Our study explores using polycaprolactone nanofibers as a scaffold for cell cultures to model the alveolar-capillary interface of human lung, the most distal part of lung, where gas exchange takes place. We optimized nanofibers production, used UHELON net for the nanofibrous structures handling, and created 3D-printed inserts for specialized co-cultures of expandable lung epithelium (ELEP) [1] and human umbilical vein endothelial cells (HUVEC). Our findings show that nanofibrous scaffolds support cell attachment, proliferation, and differentiation effectively, offering a proof-of-concept model for the alveolar-capillary interface.

Further, we focused on adhering to the FAIR (Findable, Accessible, Interoperable, Reusable) principles in science. We participate in the development of the National Repository Platform, an integral part of National Data Infrastructure, to ensure research data life cycle in the FAIR way. Thus, we aim to promote the value of research data and to enhance the data reutilization across scientific disciplines [2].

*[1] Kotasová, H., **Capandová, M.,** Pelková, V. et al. Expandable Lung Epithelium Differentiated from Human Embryonic Stem Cells. Tissue Eng Regen Med 19, 1033–1050 (2022). https://doi.org/10.1007/s13770-022-00458-0*

*[2] Antol, M., Marek, J., **Capandová, M**., Juráček, J. a Matyska, L. EOSC CZ: Towards the development of Czech national ecosystem for FAIR research data. arXiv (2024). https://doi.org/10.48550/arXiv.2402.13343*

The author of this study is a recipient of Martina Roeselová Memorial Fellowship by IOCB Tech Foundation. We thank the core facility CELLIM, supported by the Czech-BioImaging large RI project (LM2023050 funded by MEYS CR).

# 35. The use of CRISPR/Cas9 knockout screen to study resistance to rituximab in B-cell malignancies

Lenka Dostalova[1,2], Aneta Ledererova[1], Helena Peschelova[1,4], Tomas Loja[1], Michal Smida[1,3]

*1 Central European Institute of Technology, Masaryk University, Brno, Czech Republic*
*2 Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*3 Department of Internal Medicine – Hematology and Oncology, Medical Faculty of Masaryk University and University Hospital Brno, Czech Republic*
*4 National Centre for Biomolecular Research, Masaryk University, Brno, Czech Republic*

Anti-CD20 monoclonal antibodies (mAb) such as rituximab (RTX) have been used as a first line treatment for several B-cell malignancies for more than two decades. However, therapy is often hampered by downregulation of CD20 antigen on the surface of malignant cells, resulting in mAb resistance and disease relapse. Therefore, it is crucial to study CD20 downregulation in B-cell malignancies to enhance the efficacy of anti-CD20 mAb. This project aims to identify genes whose disruption restores CD20 surface levels in RTX-resistant $CD20^{low}$ B-cell line using whole-genome CRISPR/Cas9 knockout screening.

CRISPR/Cas9 screening revealed several genes whose disruption increased CD20 surface expression. Based on their statistical significance and biological relevance, we selected several of these genes for further validations. Among selected there are genes involved in B-cell receptor pathway (CSK, PTEN) – an essential pathway in B cells, CD37, encoding immune cell specific protein, and genes associated with endoplasmic reticulum (SSR1-4, STT3A). Validation of these genes has been ongoing, and the mechanism of their function is being investigated. The understanding of underlying mechanisms could provide a way for a potential enhancement of anti-CD20 therapy in the clinic.

# 36. Advances in bioprinting and cell culture within highly-defined materials

V. Chochola[1,2], A. Golunova[3], V. A. Jongen[1], J. Dvořáková[3], V. Proks[3], J. Jaroš[1], and A. Hampl[1,2]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno*
*2 Cell and Tissue Regeneration, International Clinical Research Center, St. Anne's University Hospital Brno*
*3 Institute of Macromolecular Chemistry, AS CR, Prague*

Bioprinting has emerged as a revolutionary technique in tissue engineering, enabling the precise fabrication of complex biological structures. Selection of a proper material is a pivotal part, as it not only provides structural integrity, but may also mimic the physiological environment of native tissues.

Over the years, we explored three distinctive materials, all of which being utilized for bioprinting. Most used bioink would be GelMA, photocrosslinkable gelatin, which allowed us to print 3D constructs with living cells, including endothelial cells that form interconnected networks. Furthermore, we have optimized workflow, preparation and composition of alginate-based bioink to obtain ionically crosslinkable material that is otherwise unavailable on the market. Finally, our latest endeavor lies in completely synthetic polyaminoacid bioink. This represents defined, printable, biocompatible environment. We introduced several porogens into the bioink, ensuring increased survival and nutrient exchange, and analyzed the resulting porosity of the crosslinked hydrogel.

These advances will allow us to create complex tissue models and multicellular constructs, combining various cell types, organoids or even capillary networks, ultimately contributing to the development of advanced therapeutic solutions and personalized medicine strategies. This research expands the toolkit available for tissue engineering research as well as possibilities in regenerative medicine.

This work was supported by following: Czech Science Foundation (CSF21-06524S) and Grant Agency of Masaryk University (MUNI/A/1598/2023).

# 37. Genome-wide identification of genetic variability within infecting treponemal populations

Klára Janečková[1], Vojtěch Bartoň[2], Denisa Maděránková[2], Eliška Vrbová[1], Lenka Mikalová[1], Ivo Provazník[2], Sascha Knauf[3], and David Šmajs[2*]

*1 Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*2 Department of Biomedical Engineering, Brno University of Technology, Brno, Czech Republic*
*3 Institute of International Animal Health/One Health, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald – Insel Riems*

Human treponemal pathogens are divided into three subspecies; *Treponema pallidum* subsp. *pallidum* (TPA), the causative agent of syphilis, *Treponema pallidum* subsp. *pertenue* (TPE), the causative agent of yaws, and *Treponema pallidum* subsp. *endemicum* (TEN), the causative agent of bejel.

During infection of host, populations of infecting treponemes are not genetically identical but contain different subpopulations [1]. Previous studies revealed genetic heterogeneity on a genome-wide scale [2,3], but were limited in number of analyzed genomes. Other studies mapped the instrastrain heterogeneity in 37 genomes and found more than a hundred variable positions in more than 70 genes. However, these studies were limited by low depth coverage of the sequenced genomes [4], incomplete genome sequences [5], and limited number of available genomic sequences [6].

In this study, intrastrain heterogeneity including variability in homopolymeric tracts was determined in 13 genomes with available finished genome sequences where the average depth coverage of the sequenced genomes was higher than 100×. Moreover, unlike in previous studies, the complete genome sequences were analyzed in separate parts to allow analysis of paralogous genome regions.

*[1] Baseman JB, Nichols JC, Rumpp JW, Hayes NS. Purification of Treponema pallidum from Infected Rabbit Tissue: Resolution into Two Treponemal Populations. Infect Immun. 1974;10: 1062–1067. doi:10.1128/iai.10.5.1062-1067.1974*

*[2] Matějková P, Strouhal M, Šmajs D, Norris SJ, Palzkill T, Petrosino JF, et al. Complete genome sequence of Treponema pallidum ssp. pallidumstrain SS14 determined with oligonucleotide arrays. BMC Microbiol. 2008;8: 76. doi:10.1186/1471-2180-8-76*

*[3] Pětrošová H, Pospíšilová P, Strouhal M, Čejková D, Zobaníková M, Mikalová L, et al. Resequencing of Treponema pallidum ssp. pallidum Strains Nichols and SS14: Correction of Sequencing Errors Resulted in Increased Separation of Syphilis Treponeme Subclusters. PLOS ONE. 2013;8: e74319. doi:10.1371/journal.pone.0074319*

*[4] Čejková D, Strouhal M, Norris SJ, Weinstock GM, Šmajs D. A Retrospective Study on Genetic Heterogeneity within Treponema Strains: Subpopulations Are Genetically Distinct in a Limited Number of Positions. PLoS Negl Trop Dis. 2015;9: e0004110. doi:10.1371/journal.pntd.0004110*

*[5] Pinto M, Borges V, Antelo M, Pinheiro M, Nunes A, Azevedo J, et al. Genome-scale analysis of the non-cultivable Treponema pallidum reveals extensive within-patient genetic variation. Nat Microbiol. 2016;2: 1–11. doi:10.1038/nmicrobiol.2016.190*

*[6] Strouhal M, Mikalová L, Havlíčková P, Tenti P, Čejková D, Rychlík I, et al. Complete genome sequences of two strains of Treponema pallidum subsp. pertenue from Ghana, Africa: Identical genome sequences in samples isolated more than 7 years apart. PLoS Negl Trop Dis. 2017;11: e0005894. doi:10.1371/journal.pntd.0005894*

# 38. Unraveling transcription factor code involved in odontoblast differentiation

Josef Lavický[1], Marcos GonzalezLopez[1], Vladislav Rakultsev[1], Emma Wentworth Winchester[2], Haneen Tuaima[1], Lucie Pešková[1], Jan Verner[3], Eva Švandová[1], Marcela Buchtová[4,5], Justin Cotney[2], Tomáš Bárta[1], Jan Krivanek[1]

*1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*2 Department of Genetics and Genome Sciences, UCONN Health, Farmington, CT USA*
*3 Central European Institute of Technology, Masaryk University, Brno, Czech Republic*
*4 Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Brno, Czech Republic*
*5 Department of Experimental Biology, Faculty of Science, Masaryk University, Czech Republic*

Molecular mechanisms controlling the differentiation of stem cells into specialized cells and tissues are enormously complex. Deciphering the mechanisms behind this precisely regulated process is essential not only for understanding ontogenesis but also for interpretations of evolutionary dynamics. Recent emergence of multiomic approaches has overcome these barriers, providing unprecedented insight into the intricate workings of this molecular machinery. Based on single-cell RNA-seq analyses of continuously growing mouse teeth, four transcription factors were selected. Here, we provide evidence about their function in the differentiation of odontoblasts – dental cells responsible for the production of dentin. Our results revealed that controlled overexpression of specific transcription factors in pluripotent stem cells is sufficient to guide their differentiation into cells with an odontoblast-like phenotype. The differentiated cells exhibited expression of odontoblast-specific molecular markers, as well as production of collagenous and mineralized tissue. We demonstrate that deep insight into fundamental developmental events can provide powerful basis for innovative cell differentiation approaches. Taken together, this research might serve as a proof-of-concept for utilization of large -omics data in the generation of specific, differentiated cell types by controlled expression of specific transcription factor code. This may represent a turning point in both developmental biology and regenerative medicine fields.

# 39. ERK3 contributes to triple-negative breast cancer progression by modulation of EMT-related processes.

Sofia Morazzo[1,2], Soraia Fernandes[1], Marina Fortea[3], Helena Skálová[1], Marco Cassani[1], Kamila Vrzalová[1,4], Filip Kafka[1,2], Jan Vrbský[1], Daniel Pereira de Sousa[1,2], Veronika Bosáková[1,2], Jaeyoung Shin[5], Jan Fric[1,6,7], Kristina Haase[3] and Giancarlo Forte[1,8]

*1 International Clinical Research Center (ICRC), St Anne's University Hospital, Brno, Czech Republic*
*2 Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*3 European Molecular Biology Laboratory, Barcelona, Spain*
*4 Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic*
*5 AtoGen Co., Ltd., Daejeon, South Korea*
*6 Institute of Hematology and Blood Transfusion, Prague, Czech Republic*
*7 International Clinical Research Center (ICRC), Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*8 School of Cardiovascular and Metabolic Medicine and Sciences, King's College London, UK*

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer and is associated with high cell plasticity, recurrence, and metastatic rate [1]. During epithelial-to-mesenchymal transition (EMT), cancer cells display EMT plasticity, or partial-EMT features, which are required for breast cancer metastasis, such as collective migration [2]. ERK3 has been implicated in promoting migration and invasion of breast cancer, but the mechanisms remain elusive [3]. Here, we investigated ERK3 expression across patient-derived datasets of breast cancer and established its association with aggressive breast cancer phenotypes and poor clinical outcomes. Leveraging the hypothesis that ERK3 contributes to TNBC progression by supporting a partial-EMT state, we showed that ERK3 is essential in different steps of the metastatic process, especially by enabling collective migration but also by modulating cell-extracellular matrix adhesion, anchorage-independent growth, extravasation and colonization. In conclusion, our results demonstrate that ERK3 contributes to TNBC progression and potentially metastasis by promoting EMT plasticity and collective migration.

*[1] Carey L A, Dees E C, Sawyer L, Gatti L, Moore D T, Collichio F, Ollila D W, Sartor C I, Graham M L and Perou C M 2007 The Triple Negative Paradox: Primary Tumor Chemosensitivity of Breast Cancer Subtypes Clin. Cancer Res.* ***13*** *2329–34*

*[2] Lüönd F, Sugiyama N, Bill R, Bornes L, Hager C, Tang F, Santacroce N, Beisel C, Ivanek R, Bürglin T, Tiede S, Van Rheenen J and Christofori G 2021 Distinct contributions of partial and full EMT to breast cancer malignancy Dev. Cell* ***56*** *3203-3221.e11*

*[3] Al-Mahdi R, Babteen N, Thillai K, Holt M, Johansen B, Wetting H L, Seternes O-M and Wells C M 2015 A novel role for atypical MAPK kinase ERK3 in regulating breast cancer cell morphology and migration Cell Adhes. Migr.* ***9*** *483–94*

## 40. Partitioning of fibroblast growth factor receptor 2 (FGFR2) function to primary cilia

Alexandru Nita[1,2], Sara P. Abraham[1,2], Eman R. Elrefaay[1,2], Bohumil Fafilek[1], Eliska Cizkova[1], Vlad-Constantin Ursachi[1,3], Iva Gudernova[1,2], Adolf Koudelka[1], Pooja Dudeja[1,3], Tomas Gregor[1], Zuzana Feketova[1,3], Gustavo Rico[1,3], Katerina Svozilova[1,2], Canan Celiker[4], Aleksandra A. Czyrek[1,3], Tomas Barta[2,4], Lukas Trantirek[5], Antoni Wiedlocha[6], Pavel Krejci[1,2,3], Michaela Bosakova[1,2]

*1 Department of Biology, Faculty of Medicine, Masaryk University, 62500 Brno, Czech Republic*
*2 Institute of Animal Physiology and Genetics of the CAS, 60200 Brno, Czech Republic*
*3 International Clinical Research Center, St. Anne's University Hospital, 65691 Brno, Czech Republic*
*4 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, 62500 Brno, Czech Republic*
*5 CEITEC - Central European Institute of Technology, Masaryk University, 625 00 Brno, Czech Republic*
*6 Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Centre for Cancer Cell Reprograming, Faculty of Medicine, University of Oslo, Montebello, 0379 Oslo, Norway*

Primary cilium projects from cells to provide a communication platform with neighboring cells and the surrounding environment. This is ensured by the selective entry of membrane receptors and signaling molecules, producing fine-tuned and effective responses to the extracellular cues. Disruptions of the primary cilium structure, function or resident signaling pathways have severe developmental and pathological repercussions. In this study, we focused on one family of signaling receptors, the fibroblast growth factor receptors (FGFRs), their function in ciliogenesis, and their residence within cilia. For the fibroblast growth factor receptor FGFR2, we demonstrate its cilia expression in the developing mouse tissues and determine the molecular regulators including IFT144, BBS1, and the $T^{429}V^{430}$ motif within FGFR2. Finally, we demonstrate that FGFR2 cilia localization is necessary for its signaling and expression of target morphogenic genes.

# 41. YAP disrupts cardiac fibroblast monolayer and extracellular matrix organization

Daniel Pereira-Sousa[1,2], Francesco Niro[1,2,3], Vladimír Vinarský[1], Ece Ergir[1], Stefania Pagliari[1,3], Soraia Fernandes[1,4], Marco Cassani[1,4], Pau Guillamat[5], Xavier Trepat[5,6,7], Jorge Oliver-De La Cruz[1,5], Giancarlo Forte[1,3]

*1 Center for Translational Medicine (CTM), International Clinical Research Centre (ICRC), St. Anne´s Hospital.*
*2 Faculty of Medicine, Department of Biomedical Sciences, Masaryk University*
*3 School of Cardiovascular and Metabolic Medicine & Sciences, King's College London.*
*4 Department of Chemical Engineering, The University of Melbourne*
*5 Institute for Bioengineering of Catalonia, The Barcelona Institute for Science and Technology*
*6 Institució Catalana de Recerca i Estudis Avançats (ICREA)*
*7 Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN)*

Cardiac fibrosis is a pathological process mediated by cardiac fibroblasts (CFbs) 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 CFbs (YAP KO and WT CFbs) and characterized by immunofluorescence (IF) and flow cytometry (FC). YAP KO PSCs showed an impaired capability to differentiate into CFbs demonstrated by the lower expression of cardiac markers. This was further confirmed by RNA-seq which revealed more similar profiles between YAP WT CFbs and adult cardiac fibroblasts (HCF) than YAP KO CFbs. Then, YAP KO and WT CFbs were grown to confluence to assess their ability to organize in a monolayer. Here, YAP KO CFbs showed lower anisotropy and a higher percentage of disorganized regions. These trends were also observed in the ECM they deposited analyzed after decellularization, suggesting that YAP impacts both cell monolayer and ECM organization.

We next set at investigating whether YAP depletion had an effect on CFbs contractility. We adopted a collagen gel contraction assay, complemented by confocal imaging focal adhesions and Western Blot analysis of phosphorylated and total Myosin light chain 2 (MLC2). This set of experiments clarified that YAP KO CFbs showed impaired collagen contractility, very likely as a result of the lower number of focal adhesions and impaired phosphorylation of MLC2.

To validate these results, the YAP WT CFbs were treated with either an inhibitor of collagen crossilinking (β-Aminopropionitrile) or of MLC2 (blebbistatin) to assess their impact in CFbs monolayer and ECM organization. In this context, MLC2 inhibition phenocopied YAP KO behavior in terms of monolayer and ECM anisotropy, strongly suggesting that YAP impacts their organization by impairing the phosphorylation of MLC2.

# 42. Ciliogenesis associated kinase 1 (CILK1) functions in supporting centriolar cohesion and chromosomal segregation

Sara P. Abraham[1,2], Radek Fedr[3], Juraj Bosak[1], Bohumil Fafílek[1], Iva Gudernova[1,2], Tomáš Bárta[4], Miroslav Varecha[1], Karel Souček[3,5], Pavel Krejci[1,2,5], Michaela Bosakova[1,2]

*1 Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*2 Institute of Animal Physiology and Genetics of the CAS, Brno, Czech Republic*
*3 Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic*
*4 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*5 International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic*

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., which could be contributing to the cancer development as well.

*[1] Breslow DK, Holland AJ. Mechanism and Regulation of Centriole and Cilium Biogenesis. Annu Rev Biochem. 2019;88:691-724. doi:10.1146/annurev-biochem-013118-111153*

*[2] Kobayashi T, Dynlacht BD. Regulating the transition from centriole to basal body. J Cell Biol. 2011;193(3):435-444. doi:10.1083/jcb.201101005*

*[3] Nigg EA, Raff JW. Centrioles, centrosomes, and cilia in health and disease. Cell. 2009;139(4):663-678. doi:10.1016/j.cell.2009.10.036*

*[4] Basten SG, Giles RH. Functional aspects of primary cilia in signaling, cell cycle and tumorigenesis. Cilia. 2013;2(1):6. Published 2013 Apr 29. doi:10.1186/2046-2530-2-6*

*[5] Fu Z, Gailey CD, Wang EJ, Brautigan DL. Ciliogenesis associated kinase 1: targets and functions in various organ systems. FEBS Lett. 2019;593(21):2990-3002. doi:10.1002/1873-3468.13600*

# 43. RAD51 is capable of accommodating G4 within filamentous structure and promotes gap filling by template switching

Michaela Pospisilova[1,2], Karel Kubicek[1,3,4], Zbynek Prokop[5,6], Lucia Sommerova[7], Mark Skehel[8], Fedor Nikulenkov[1,2], Mario Spirek[1,2], Ross Chapman[7], Richard Stefl[1,3], Lumir Krejci[1,2]

*1 National Centre for Biomolecular Research, Faculty of Science, Masaryk University*
*2 Department of Biology, Faculty of Medicine, Masaryk University*
*3 CEITEC-Central European Institute of Technology, Masaryk University*
*4 Department of Condensed Matter Physics, Faculty of Science, Masaryk University*
*5 Department of Experimental Biology and RECETOX, Faculty of Science, Masaryk University*
*6 International Clinical Research Center, St Anne's University Hospital*
*7 MRC Weatherall Institute of Molecular Medicine, The University of Oxford*
*8 Proteomics STP, The Francis Crick Institute*

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.

*[1] Chambers VS., Marsico G., Boutell JM., Di Antonio M., Smith GP., Balasubramanian S. High-throughput sequencing of DNA G-quadruplex structures in the human genome. Nat Biotechnol. (2015) 33: 877.*
*[2] Kruisselbrink E., Guryev V., Brouwer K., Pontier DB., Cuppen E., Tijsterman M. Mutagenic capacity of endogenous G4 DNA underlies genome instability in FANCJ-defective C. elegans. Curr Biol. (2008) 12: 900-905*
*[3] Kolinjivadi AM., Sannino V., de Antoni A., Técher H., Baldi G., Costanzo V. Moonlighting at replication forks - a new life for homologous recombination proteins BRCA1, BRCA2 and RAD51. FEBS Lett. (2017) 591: 1083-1100.*

# 44. Truncated vitronectin with E-cadherin enables the xeno-free derivation of human embryonic stem cells

Tereza Souralova[1,2], Daniela Hulínová[2,3], Pavel Ventruba[4], Michal Jeseta[4], Ales Hampl[1,2], Irena Koutna[1,2]

*1 Masaryk University, Faculty of Medicine, Department of Histology and Embryology, Kamenice 3, 625 00 Brno, Czech Republic*
*2 St. Anne's University Hospital, ICRC, Cell and Tissue Engineering Facility, Pekarska 53, 602 00 Brno, Czech Republic*
*3 Masaryk University, Faculty of Science, Department of Experimental Biology, Kamenice 5, 625 00 Brno*
*4 Center of Assisted Reproduction, Department of Gynecology and Obstetrics, Faculty of Medicine, Masaryk University Brno and University Hospital, Obilni trh 11, 602 00 Brno, Czech Republic*

Human embryonic stem cells (hESCs) are able to differentiate into any cell type of a human body, have self-renewal abilities and very high proliferative activity [1]. These unique abilities enable their use in cell therapy, disease modelling, and drug development.

The derivation of hESCs is usually performed using an animal or human cell feeder layer, which is undefined and potentially contagious, and because of these difficulties, there is a tendency to replace feeders with xeno-free defined substrates in recent years [2].

We used truncated vitronectin with E-cadherin as a defined xeno-free substrate for the derivation of hESCs for the first time, derived three hESC lines, and confirmed their undifferentiated state, hESC morphology, and standard karyotypes together with their potential to differentiate into three germ layers (ectoderm, mesoderm, and endoderm) [3]. We confirmed for the first time that truncated vitronectin with E-cadherin is a defined xeno-free substrate that is suitable for the derivation of hESCs involved in cell therapies.

*[1] Thomson J. A., Itskovitz-Eldor J., Shapiro S. S., Waknitz M. A., Swiergiel J. J., Marshall V. S., Jones J. M. Sci. 282, 5391 (1998).*
*[2] Yamanaka, S. Cell Stem Cell 27, 523–531 (2020).*
*[3] Souralova, T., Hulinova, D., Jeseta, M., Ventruba, P., Hampl, A., Koutna, I. Sci Rep 13, 15062 (2023).*

This research was supported by funds from the Faculty of Medicine, Masaryk University—MUNI/IGA/1332/2021, MUNI/A/1301/2022 and MUNI/A/1598/2023. Supported by Ministry of Health of the Czech Republic, Grant No. NU22-08-00629. 

# 45. eIF4F controls ERK MAPK signaling in melanomas with *BRAF* and *NRAS* mutations

Natalia Vadovicova[1,2+], Barbora Valcikova[1,2+], Karolina Smolkova[1,2], Magdalena Zacpalova[1], Pavel Krejci[1,2], Shannon Lee[3], Jens Rauch[3,4], Walter Kolch[3,5], Alexander von Kriegsheim[6], Anna Dorotikova[1,2], Zdenek Andrysik[1,7], Rachel Vichova[8], Ondrej Vacek[2,8,9], Karel Soucek[2,8,9] & Stjepan Uldrijan[1,2]

*1 Department of Biology, Faculty of Medicine, Masaryk University*
*2 International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic*
*3 Systems Biology Ireland, School of Medicine, University College Dublin, Dublin, Ireland*
*4 School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland*
*5 Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Dublin, Ireland*
*6 Cancer Research UK Scotland Centre, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK*
*7 Department of Pharmacology, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
*8 Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic*
*9 Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic*

Oncogenic mutations in the RAS-RAF-MEK-ERK signaling pathway are common drivers of metastatic melanoma [1]. Patients usually respond to BRAF/MEK inhibitors, but resistance often rapidly emerges [2]. The eukaryotic translation initiation complex (eIF4F) plays a key role in melanoma resistance to small-molecule drugs targeting BRAF/MEK kinases [3]. In this study, we identify a novel function of eIF4F in the negative regulation of RAS/RAF/MEK/ERK pathway. eIF4F is necessary for maintaining balanced ERK signaling in melanoma cells with BRAF or NRAS mutations by supporting the production of DUSP6/MKP3, a negative feedback regulator of ERK. Inhibiting eIF4F disrupts this feedback, leading to ERK hyperactivation and increased EGR1 expression both in vitro and in vivo. Additionaly, our quantitative analysis suggests that eIF4F-dependent feedback maintains most ERK molecules in an inactive state, revealing a high spare signaling capacity of the ERK pathway. These findings underscore eIF4F's crucial role in controlling ERK signaling and suggest that eIF4F inhibitors can disrupt negative feedback mechanisms in melanoma cells with BRAF or NRAS mutations.

*[1] Fecher, L. A., Amaravadi, R. K. & Flaherty, K. T. The MAPK pathway in melanoma. Curr. Opin. Oncol. 2008; 20(2):183–189. doi: 10.1097/CCO.0b013e3282f5271c.*
*[2] Smalley, K. S. M. & Sondak, V. K. Inhibition of BRAF and MEK in BRAF-mutant melanoma. Lancet. 2015; 386(9992):410–412. doi: 10.1016/S0140-6736(15)60972-2.*
*[3] Boussemart, L. et al. eIF4F is a nexus of resistance to anti-BRAF and anti-MEK cancer therapies. Nature. 2014;513(7516):105–109.doi:10.1038/nature13572.*

This work was supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU; The Czech Science Foundation (GA22-30397S), and Masaryk University (MUNI/A/1393/2022).