

OR Vývojové a buněčné biologie

Vypsané doktorské práce pro akademický rok 2022/2023- II. kolo přijímacího řízení

Characterization, regulation, and enhancement of mitochondria-targeted compounds-induced cell death.

RNDr. Ladislav Anděra, CSc

ID 248045

Despite significant advances in recent anti-cancer therapies (targeted chemotherapeutics, focused irradiation, or boosting anti-tumor immune response), malignant diseases are still one of the major causes of human fatalities and thus there is a need for novel preferentially combined therapies leading to a synergistic elimination of cancer cells. One of the main features of cancer cells is their acquired resistance to cell death and thus to regulated cell death (RCD) triggering anti-cancer drugs. The major goal of this PhD Thesis project is to pave the way for overcoming these resistances and triggering efficient RCD activation in cancer cells, which would lead to their effective elimination. Mitochondria represent a hub for a number of both cellular metabolism and energy-producing pathways (MEP) and several RCD modalities. Thus we aim to simultaneously target rationally selected signaling pathways in the mitochondria of cancer cells using specific compounds - mitocans (RCD activators such as BH3 mimetics, triphenylphosphonium (TPP)-modified compounds such as MitoTam and others) and MEP inhibitors such as glucose transporter 1 inhibitor BAY-876, lipoic acid antagonist CPI-613 and others) and their combinations in model cancer cells. The initial screenings will be performed in vitro using semi-high throughput time-lapse assays performed in Incucyte SX3 and similar analyzers (e.g. Lumascope L720) and relevant cell death/proliferation assays (e.g. staining dead cells using Sytox Green). The obtained data will be confirmed by alternative cell death (e.g. Annexin V-FITC/PI staining and flow cytometry) and survival assays (e.g. colony formation test). Activation of relevant RCD signaling pathways will be assessed by chemical (pathway inhibitors – e.g. blocking apoptosis/pyroptosis by pan-caspase inhibitor Q-VD-Oph) and then also by genetic means (CRISPR-mediated knockout of RCD pathways essential genes – e.g. MLKL for suppressing necroptosis). The best-performing pairs of mitocans and MEP inhibitors will be then examined for their effectiveness in suppressing the tumorigenic capacity of relevant human and mouse cancer cells in vivo (human cancer cells in immunodeficient NSG mice and murine cancer cells in a corresponding syngeneic mouse strain). In its outcome, this PhD project will provide not just detailed knowledge of RCD modalities activated by these anti-cancer compounds and their binary combinations but might also significantly contribute to future improvements in cancer therapy.

Screening for proteins involved G4-structure metabolism and their role in prevention of genotoxic stress

RNDr. Jana Dobrovolná, Ph.D.

ID 246713

DNA replication is an essential and one of the most complex processes in the cell that ensures faithful, complete and timely duplication of genome. Not only exogenous DNA damage but also intrinsic DNA structures including G-quadruplexes (G4) and R-loops, their stabilization or unscheduled formation represent major replication obstacles with possible detrimental effects on genome integrity. Not surprisingly, those processes are pharmacologically targeted in anticancer therapy, despite the fact that only little is known about the underlying molecular mechanisms. It becomes apparent that maintenance of processive DNA replication requires sophisticated protein networks beyond the core replisome. Whether there is a direct crosstalk between G4 and R-loops, what proteins are involved in their homeostasis and what are the factors diversifying between their beneficial and pathological roles is not well understood. The goals of our research are to identify proteins associated with G4 and R-loop structures and understand their roles in G4/R-loop formation and resolution as well as relationship to replication fork progression and associated repair. PhD student will be involved in preparation of tools for study of G4 structures (establishment of various cell lines) and in identification of proteins involved in metabolism of these structures by mass spectrometry-based proteomics approaches, including APEX-based proximity labeling and chromatin affinity precipitation methods, and by functional siRNA screen. Proteins identified in these screens will be selected for further validation and characterization based on their relevance to G4/R-loop and replication fork metabolism, and role in maintenance of genome integrity.

Development of scaffolds for a regeneration of bones and osteochondral defects

Mgr. Eva Filová, Ph.D.

ID 254213

The aim is to develop biodegradable scaffolds which are able to release bioactive substances supporting formation of new bone or cartilage tissues (e.g. growth factors, peptides, exosomes from stem cells or plant cells), chemicals supporting angiogenesis or drugs (e.g. antibiotics).

Foam scaffolds based on collagen, tricalcium phosphates (alpha-TCP, beta-TCP, hydroxyapatite (HA)), ceramic scaffolds based on TCP and HA with different nanostructure or microstructure. Moreover, composites with nano/microparticles will be prepared for a drug delivery.

Scaffolds will be characterized with physico-chemical methods, SEM etc., seeded with cells - cell lines, stem cells, coculture with other cell types, and tested from cell adhesion, proliferation and differentiation. Selected scaffolds will be tested in vivo (on rats, rabbits, pigs) and evaluated histologically.

In vitro evolution and engineering of XNA polymerases

prof. Ing. Michal Hocek, CSc., DSc.

ID 246675

XNA (xenobiotic nucleic acids) are modified artificial analogues of biopolymers orthogonal to natural nucleic acids (DNA or RNA). They mostly contain modified sugar part that makes them resistant to cleavage by nucleases and enables unique secondary folds. Natural DNA or RNA polymerases typically do not tolerate dual base- and sugar-modified nucleoside triphosphates (XTPs) as substrates which seriously limits the application of the XNA methodology. To this end, we propose a project that aims at the in vitro evolution of engineered XNA polymerases to enable efficient enzymatic synthesis of base-modified XNAs from modified XTPs bearing a portfolio of useful modifications. We will use best performing exonuclease-deficient *Thermococcus gorgonarius* (Tgo) polymerase variants as a platform for mutant library generation using transposon-directed mutagenesis and targeted mutagenesis. Having the library in hands, we will adopt established methods for directed evolution, e.g. phage display or emulsion-based selections. The ultimate and groundbreaking goal would be to develop a polymerase capable of robust synthesis of longer sequences of dual base- and sugar-modified XNA using full set of all-four-base-modified XTP building blocks. However, even a polymerase capable of incorporation of several base-modified nucleotides would be very desirable.

Molecular mechanisms of spatiotemporal regulation of cancer cell quiescence

RNDr. Radoslav Janoštiak, Ph.D.

ID 246621

Tumors maintain balance between proliferative and dormant cells and the ratio is altered in response to therapy or foreign site colonization. This can be explained by continuous paracrine or cell-to-cell communication between proliferative and quiescent cells. Recent evidence also suggests that individual quiescent cells have different reawakening potential implying the existence of subpopulations of quiescent cells. Therefore the aim of the project is to investigate the hypothesis that cell quiescence is actively maintained state, not a passive one that is caused by the lack of pro-proliferative signaling. To this end, we plan to analyze the complex network of communications (cell-to-cell, paracrine, cell-to- ECM) to identify the crucial signaling nodes regulating interaction between proliferative and quiescent cells. Moreover, we plan to analyze the population of dormant cancer cells and characterize signaling pathways regulating transition between individual phases of dormancy until the entry into the cell cycle. Besides basic cell and molecular biology techniques, we will use a wide range of approaches such as large scale proteomics, single cell analysis, advanced fluorescent microscopy or 3D organoid cultures.

Mechanistic characterization of LACTB-induced tumor suppression

Mgr. Zuzana Kečkéšová, Ph.D.

ID 246275

Lactamase-B-like (LACTB) protein is a recently discovered mitochondrial tumor suppressor that acts through reprogramming of cancer metabolism and induction of cancer stem cell differentiation. The detailed mechanisms of this tumor suppression is currently unknown. This project, through utilization of many molecular biology and biochemistry approaches, will intend to reveal the binding partners of LACTB and their role in regulation of LACTB. Furthermore, it will provide an insight into requirements for correct LACTB's conformation and structure and its effect on LACTB-induced tumor suppression. This PhD study will uncover novel aspects of this tumor suppressor pathway; the knowledge of which can help us design new approaches for therapeutic differentiation of cancer stem cells.

Molecular mechanisms of apoptosis induction by saturated fatty acids in pancreatic beta-cells

prof. RNDr. Jan Kovář, DrSc.

ID 246990

As experimental model, we employ pancreatic beta-cell lines of human as well as of animal origin. We also use, as to pancreatic beta-cells, animal in vivo models. The main aim of the project is to describe molecular mechanisms how saturated fatty acids induce apoptosis in pancreatic beta-cells.

Furthermore, we also want to describe the mechanism how unsaturated fatty acids inhibit apoptosis induction by saturated fatty acids. There are several other players involved in apoptosis induction/inhibition by fatty acids, i.e. ER stress, hypoxia (HIFs) and several signaling pathways such as p38 MAPK pathway, PKB/Akt pathway and ERK pathway. Studies should help to propose new strategies how to protect efficiently against the development of type 2 diabetes mellitus.

Molecular mechanisms of the resistance of cancer cells to chemotherapy

prof. RNDr. Jan Kovář, DrSc.

ID 246989

We mainly use breast cancer cells and ovarian cancer cells as experimental model. We employ cancer cells lines in vitro and in collaborations also patient's tumor samples as well as tumor xenografts in mice. The main aim of the project is to describe the mechanism how selected molecule(s) produce acquired resistance to tested chemotherapeutics (taxanes, mitocans etc.). Studied molecules are both well known molecules with the role in resistance (ABC transporters) and newly revealed molecules with potential role in resistance (TRIP6). These studies should result in new strategies how to overcome the resistance of cancer cells.

Evolution of vertebrate eyes: insight from photoreceptors of a basal chordate

RNDr. Zbyněk Kozmik, CSc.

ID 246676

Project aims to provide new insight into evolution of vertebrate eyes using invertebrate chordate amphioxus as a laboratory model. Project will specifically focus on amphioxus photoreceptors that are not associated with pigment cells: Joseph cells, bearing similarity to intrinsically photosensitive retinal ganglion cells of vertebrates, and lamellar body that is likely homologous to vertebrate pineal organ. The methods used will include basic bioinformatics analysis, gene isolation, gene expression studies by transcriptomics, whole-mount in situ hybridization and immunohistochemistry, biochemical characterization of photosensitive proteins (opsins) in vitro, characterization of selected gene knockouts produced in the lab by CRISPR/Cas9 system, and behavior visual tests (in collaboration with laboratory in Vienna).

Isoforms of microtubule-associated proteins and their regulation of microtubule envelopes

RNDr. Zdeněk Lánský, Ph.D

ID 254003

Microtubule-based processes are essential, e.g. for intracellular transport. Microtubule-associated molecular motors, which drive the transport, are regulated by many factors, including microtubule-associated proteins (MAPs). We have found that some MAPs (e.g. Alzheimer's disease-associated protein tau) cooperatively form protective layers on microtubule surface termed envelopes(1,2), differentially affecting motility of molecular motors(3,4). In human brain these MAPs are present in various isoforms. How do these isoforms affect the formation and functionality of envelopes is unclear. The student will generate isoforms of selected neuronal MAPs, such as tau and will use them to probe the self-assembly and functionality of the envelopes. To do this, the student will use cutting edge microscopy techniques, including imaging with single molecule resolution and custom written image analysis tools. The project will uncover the role of MAP isoforms in regulating the functionality of microtubule envelopes.

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Regulation of tau envelope by tau post-translational modifications

RNDr. Zdeněk Lánský, Ph.D.

ID 246454

Lab profile: Cytoskeletal networks form the internal dynamic scaffold of living cells essential for key cellular processes, such as cell division, cell motility or morphogenesis. Our aim is to understand how the individual structural elements of the cytoskeleton mechanically cooperate to drive these cellular processes. We use reconstituted cytoskeletal systems to study the system's self-assembly and dynamics. Central to our approach are imaging, manipulation and force measurement techniques with single molecule resolution.

Project description: Modulating the accessibility of the cytoskeletal filaments for the filament-associated proteins is one of the fundamental regulatory mechanisms in the cytoskeleton. Unstructured microtubule-associated proteins, such as the Alzheimer's disease-associated protein tau, can form cohesive envelopes around microtubules, selectively modulating the microtubule accessibility by locally excluding specific proteins from the microtubule surface while recruiting others. The aim of the project is to explain the role of tau post-translational modifications in envelope formation and function.

Candidate profile: We are looking for an enthusiastic PhD student motivated to work on cross-disciplinary projects. The candidate should hold a master's degree in (bio)chemistry, (bio)physics, molecular/cellular biology or an equivalent field.

Regulatory roles of the microtubule envelope

RNDr. Zdeněk Lánský, Ph.D.

ID 222732

Lab profile: Cytoskeletal networks form the internal dynamic scaffold of living cells essential for key cellular processes, such as cell division, cell motility or morphogenesis. Our aim is to understand how the individual structural elements of the cytoskeleton mechanically cooperate to drive these cellular processes.

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Project description: Modulating the accessibility of the cytoskeletal filaments for the filament-associated proteins is one of the fundamental regulatory mechanisms in the cytoskeleton. Unstructured microtubule-associated proteins, such as the Alzheimer's disease-associated protein tau, can form cohesive envelopes around microtubules, selectively modulating the microtubule accessibility by locally excluding specific proteins from the microtubule surface while recruiting others. The aim of the project is to explain the envelope formation and its regulatory and (patho)physiological roles.

Candidate profile: We are looking for an enthusiastic PhD student motivated to work on cross-disciplinary projects. The candidate should hold a master's degree in (bio)chemistry, (bio)physics, molecular/cellular biology or an equivalent field.

The role of non-canonical RNA caps in eukaryotic cells

Ing. Hana Macíčková Cahová, Ph.D.

ID 246163

Recent discovery of RNA caps such as NAD and CoA leads to reassessment of RNA structure in all types of cells. In our search for new RNA modifications, we have discovered brand new class of 5'caps - dinucleoside polyphosphates (NpnN) in bacteria as well as in eukaryotes. The project will focus on elucidating the role of newly identified non-canonical RNA caps in RNA stability, cellular localization or in RNA splicing. In addition, for RNA caps identified on mRNA molecules, their potential role in mRNA translation in eukaryotic cells will be investigated.

The role of SEL-5/AAK1 kinase in cell migration and cell growth

Mgr. Marie Macůrková, Ph.D.

ID 246671

Endocytóza závislá na klatrinu patří k důležitým mechanismům, kterými buňka reguluje vstup molekul dovnitř buňky, přenos signálu nebo homeostázu membrán. Proces je striktně regulován na několika úrovních. Sestavení klatrinového váčku na plasmatické membráně napomáhá řada adaptorových proteinů, mezi nimi například adaptorový proteinový komplex 2 (AP2). V savčích buňkách je aktivita AP2 komplexu regulována fosforylací prostřednictvím kinázy AAK1. V modelovém organismu *Caenorhabditis elegans* studujeme funkci proteinu SEL-5, ortologu kinázy AAK1. Z našich výsledků vyplývá, že funkce SEL-5 není u *C. elegans* nezbytná pro běžnou regulaci endocytózy, nicméně ztráta funkce SEL-5 vede k ovlivnění migrace neuronálních prekursorů a také k poruše růstu konkrétního typu buněk. Je zajímavé, že poruchu buněčné migrace je možné pozorovat i po ztrátě exprese AAK1 u savčích buněk v kultuře. Zdá se tedy, že SEL-5 a AAK1 mají jak společné, tak unikátní role. Cílem projektu je objasnit mechanismus zapojení SEL-5 do regulace buněčné migrace a buněčného růstu a v případě buněčné migrace porovnat mechanismy funkce SEL-5 a AAK1 u savčího a *C. elegans* modelu. V projektu bude využito pokročilých genetických manipulací u *C. elegans*, jako je například cílená mutagenese pomocí CRISPR/Cas9 nebo in vivo značení endogenních proteinů, a biochemických a mikroskopických analýz v savčích buněčných kulturách.

Role of underlying mesenchyme and neural network in the morphogenesis of epithelial placodes

RNDr. Ondřej Machoň, Ph.D.

ID 246289

Morphogenesis of some organs originating from the embryonic epithelium such as lens, hair, teeth or salivary glands is dependent on fine-tuned interactions between the surface ectoderm and the underlying mesenchyme, the neural tissue or the peripheral innervation. The peripheral innervation, which forms rather early in embryogenesis, may also serve to distribute signalling molecules influencing development of the neighboring epithelium. Molecular mechanisms of these interactions that determine spatiotemporal control of development of epithelial structures are still unclear. This project will analyze selected mouse embryo conditional mutants to unravel mechanisms of the formation of epithelial structures and the role of neighboring tissues. The project will employ single-cell RNA-seq analysis, spatial transcriptomics, in situ RNA hybridization and immunohistochemistry. Advance microscopy techniques such as light-sheet or spinning disc confocal microscopy will also be included.

Targeting cytoskeletal force-generation in *C. elegans* embryos using optogenetics

Teije Corneel Middelkoop, Ph.D.

ID 254363

Our group is interested in how forces arising in the cytoskeleton of embryonic cells drive embryonic shape generation in early *C. elegans* embryos. In this project the successful candidate will establish novel optogenetic methodology to experimentally control cytoskeletal force-generation with tight spatiotemporal precision. This project is part of an international collaboration with the Physical Chemistry of Mechanobiology group led by Prof. Arjun Narayanan at New York University, Abu Dhabi. In order for development to occur normally, cytoskeletal forces must be tightly regulated in space and time. Therefore, a mechanistic understanding of morphogenesis inevitably requires spatiotemporal control over these forces in experiment. In recent years, optogenetics has been used to facilitate spatiotemporal control over biological processes. This methodology makes use of light-sensitive protein domains that can either recruit proteins of interest or modulate enzymatic activity in a light-sensitive manner. In this project the candidate will adopt novel optogenetic methodology to gain spatiotemporal control over cytoskeletal force generation in *C. elegans*. To this end, the candidate will optogenetically target molecular-scale force generators, like myosins and actin polymerases, and their upstream activators and monitor the effect on morphogenesis. Importantly, our preliminary results already show proof-of-principle of this approach. The project will be conducted in close collaboration with the Physical Chemistry of Mechanobiology group (led by Prof Arjun Narayanan), who pioneered novel breakthrough methodology to visualize, measure and understand transient structures in living cells. As this project will involve molecular biology & genetics, fluorescence time-lapse imaging, quantitative image analysis and biophysical characterization, it will provide a highly versatile and cross-disciplinary training. At the same time, it will pave the way towards a detailed mechanistic understanding of the physical principles underlying morphogenesis.

More information

Visit the website(s):

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Molecular mechanisms of pathogenicity in ATP synthase disorders

RNDr. Tomáš Mráček, Ph.D.

ID 246649

Mutations in mitochondrial FoF1 ATP synthase responsible for severe inborn errors of metabolism. As is the case with other mitochondrial diseases, one of the striking features is the tissue specificity of symptoms associated with mutations in individual subunits. Thus, mutations in TMEM70 or ATP5E present primarily as myopathies, while *Usmg5* patients present with neurological disorders. While the primary biochemical features are generally characterised, mechanisms dictating tissue specificity are still poorly understood.

Recently, we have developed animal models for defects in TMEM70 as well as *Usmg5*. The aim of this project is to explore differences in tissue presentation as well as compensatory or regulatory mechanisms involved to mitigate pathogenic phenotype. The project should aim beyond the biochemical characterisation of mitochondrial function and dig further into the adaptations occurring at the whole-body level to understand the role of ATP synthase in modulation of metabolic plasticity. This project should take the advantage of wide array of phenotypisation techniques available at the Institute of Physiology and adapt them for the use on mitochondrial models.

References:

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2. Vrbacky M, Kovalcikova J, Chawengsaksophak K, Beck IM, Mracek T, Nuskova H, Sedmera D, Papousek F, Kolar F, Sobol M, Hozak P, Sedlacek R, Houstek J. Knockout of *Tmem70* alters biogenesis of ATP synthase and leads to embryonal lethality in mice. *Hum Mol Genet.* 2016;25(21):4674-85.

Role of metabolic switch in liver regeneration

prof. Ing. Jiří Neuzil, CSc.

ID 248135

Liver is a highly intriguing organ due to its capacity to regenerate after partial resection (partial hepatectomy, PHx). In this PhD project, the student will study altered metabolic pathways following PHx, which is linked very fast proliferation of liver during its regeneration. Assumptions as well as preliminary data point to a switch from salvage pathways of pyrimidine and purine synthesis to de novo pathways, critically involving the mitochondrial function. For increased biomass needs during liver regeneration, there is need for fast synthesis of amino acids. All these metabolites use glutamin as the substrate. This implies that its synthesis will be powered by diversion of the urea cycle to aminatino of glutamate. This project will utilise advanced methods of biochemistry and cell biology, as well as stable isotope labelling followed by metabolomics, exclusively using mouse models of PHx.

Regulation of mitochondrial oxidative phosphorylation by tissue-specific isoforms of cytochrome c oxidase

Mgr. Petr Pecina, Ph.D.

ID 246707

Mitochondrial cytochrome c oxidase (COX) is a key enzyme of oxidative phosphorylation (OXPHOS) system responsible for ATP production in mammalian cells. Expression of tissue-specific isoforms of COX subunits represents a crucial mechanism of OXPHOS regulation. Recently, our studies helped establish the lung isoform of regulatory subunit 4 (COX4I2) as a key component of functionally modified COX with reduced oxygen affinity dedicated to oxygen sensing. Our focus is now expanded to subunit COX6B that occurs either as ubiquitous isoform (COX6B1) or as a protein with exclusive testicular expression (COX6B2). Also, ectopic COX6B2 expression was associated with poor prognosis of lung carcinoma (LC). The project aims to explore the understudied phenomenon of COX6B isoform exchange and its effect on structure and function of OXPHOS. COX6B knock-out/knock-in models will be constructed in HEK293 and LC cell lines to characterize basic functional features of subunit isoforms and their impact on proliferation and tumorigenesis of LC. The role COX6B2 and its post-translational modification will also be studied in the physiological context of sperm maturation and capacitation. The proposed research will provide novel data on OXPHOS biogenesis and regulation and the role of these processes in carcinogenesis and male fertility.

The role of SURF1 protein in biogenesis of mammalian cytochrome c oxidase

Mgr. Petr Pecina, Ph.D.

ID 246710

Cytochrome c oxidase (COX) is a key enzyme of mitochondrial energetics providing ATP in mammalian cells. COX biogenesis integrates sequential assembly of 14 subunits, encoded by nuclear and mitochondrial genes. It depends on numerous ancillary factors, including SURF1 implicated in insertion of heme a into catalytic subunit COX1. However, its precise role remains unclear, although SURF1 dysfunction causes most frequent COX deficiency - fatal Leigh syndrome. Proposed project will follow up on our studies characterizing COX functional alterations in patients fibroblasts harboring SURF1 mutations, and on key preliminary discovery of incorrect cofactor (heme b) incorporation into COX in mouse SURF1 knock-out. We hypothesize that SURF1 represents an adaptor protein between heme a synthesis enzymes and COX1, that cooperates with putative homologues of assembly factors PET117 and Coa2 described in yeast, thus securing insertion of proper cofactor type. New data characterizing COX biogenesis may contribute to therapy of SURF1-associated neurodegeneration disease.

Structural basis of plexin signalling

Mgr. Daniel Rozbeský, Ph.D.

ID 234637

All that we know, and all that we are, comes from the way our neurons are connected. There are about 10¹¹ neurons in our brains, and each neuron makes on average thousand synapses with other neurons. Thus, the human brain contains about 10¹⁴ synaptic connections that give rise to our memory, intelligence, speech, movement, sensation or emotion. How are these highly organized neuronal networks correctly established? What molecules are involved in setting-up neuronal connections? What are the neuronal networks underlying specific behaviours?

Semaphorin ligands and their plexin receptors are one of the classical cell guidance factors that play essential roles in cell processes requiring discrete changes in the cytoskeleton. Although the field has made enormous advances in understanding semaphorin function at the level of genetic and cellular experiments, our knowledge of the molecular-level mechanisms of semaphorin signalling is still lacking.

As a PhD student, you will aim to understand structural basis through which the extracellular and cytoplasmic segments of plexin receptors communicate and dissect a long-standing question on how does a signal from outside the cell pass to the cytoplasmic plexin domain. You will use protein crystallography and cryoEM that will be further combined with biophysical and cellular experiments. Furthermore, you will build key oral and written communication skills, techniques for open science, and how to lead collaborative research projects. The project will give you opportunities to present your findings at scientific meetings. Our group has a strong commitment to both personal and professional development, and you will be encouraged to develop your own ideas within the scope of the group's interests and build your academic profile.

The work will be based at a new research centre, Biocev, which houses scientists of international repute in related fields and provides a broad range of frontline structural biology techniques.

You should hold or, be near completion of a master's degree in biochemistry, structural biology, or related discipline, and have hands-on experience in techniques relevant to the project. Particularly useful would be the experience of protein production and purification, plasmids construction, cell culture, protein crystallography or electron microscopy. Good communication and writing skills are essential.

Investigating the role of Src in osteoclasts

doc. RNDr. Daniel Rösel, Ph.D.

ID 246354

The product of the c-src proto-oncogene, tyrosine kinase Src, is an essential regulator of cellular physiological processes ranging from cell adhesion, migration to mitogenic and anti-apoptotic signaling. Although Src is ubiquitously expressed, targeted disruption of c-src in mice leads to only one major phenotype, osteopetrosis. This results in the excessive accumulation of bone matrix caused by defective osteoclast functions.

The project aims to analyze the role of Src kinase in the physiology of osteoclasts, especially in the formation of sealing zones. Src activity and dynamics in the osteoclast sealing zone will be analyzed in living osteoclasts using our Src-FRET biosensor. CRISPR/Cas9 knock-in strategies will be used to prepare monocytes expressing the Src-FRET biosensor under an endogenous promoter.

The PGS candidate should have experience in mammalian cell cultivation techniques and basic fluorescence microscopy. Experience with live-cell microscopy, FRET and/or CRISPR/Cas9 are of further advantage.

PKN3 signaling at the crossroad of Rho signaling and mitochondrial metabolism.

doc. RNDr. Daniel Rösel, Ph.D.

ID 246408

PKN kinases belong to family of PKC kinases and are involved predominantly in regulation of cytoskeletal organization as effector proteins of Rho family of small GTPases. Unlike other PKN kinases, PKN3 is physiologically expressed mostly in primary endothelial cells and osteoclasts but is also often overexpressed in cancer cells. Recently, PKN3 was found to be surprisingly enriched in mitochondria. The aim of the project will be to analyze a potential PKN3-mediated crosstalk of Rho signaling and mitochondrial physiology.

The PGS candidate should have experience in mammalian cell cultivation techniques and basic fluorescence microscopy. Experience with live-cell microscopy, and/or CRISPR/Cas9 are of further advantage.

The determination of p130Cas role in mechanics of Cell-ECM mechanosensing

doc. RNDr. Daniel Rösel, Ph.D.

ID 217751

The ability of cells to sense mechanical properties of surrounding environment is crucial for many physiological as well as pathological processes including morphogenesis, tissue homeostasis or cancer. Cells sense these mechanical cues through specialized mechanosensory proteins. One of such mechanosensory proteins is p130Cas. P130Cas is a major substrate of Src proto-oncogene, plays an important role in oncogenic transformation mediated by the v-crk and v-src oncogenes and increased levels of its human ortholog, BCAR1, are associated with exacerbated prognosis in breast cancer patients. The project aims to determine the mechanistic role of CAS substrate domain in mechanosensing and mechanotransduction and to prepare FRET-based p130Cas-derived biosensors of intracellular mechanical tension.

The PGS candidate should have experience in molecular cloning, mammalian cell cultivation techniques and fluorescence microscopy. Experience with live-cell microscopy, FRET and biophysical methods analyzing mechanical properties of cells are of further advantage.

Development of therapeutic exosomes and gene therapy for Diamond Blackfan Anemia (DBA)

doc. RNDr. Radislav Sedláček, Ph.D.

ID 253151

Development of Extracellular vesicle (EV)-based and AAV-based gene therapy of hematopoietic stem cells (HSC) for the treatment of the Diamond-Blackfan Anemia (DBA) model. Development of delivery/therapeutic protocols.

In vitro/ex vivo correction of HSC using genetic approach and site-specific nucleases. In vivo analysis of DBA disease mouse model after the application of the gene therapy.

Conditions of international mobility of researchers: The applicant must not have resided or carried out his or her main activity (work, studies, etc.) in the Czech Republic for more than 12 months in the 36 months immediately before the date of recruitment.

Link to the tender: <https://www.img.cas.cz/2022/09/66643-phd-position-in-exosome-mediated-gene-therapy-of-diamond-blackfan-anemia/>

Molecular mechanism of quality control during spliceosome assembly

doc. Mgr. David Staněk, Ph.D.

ID 246264

Small nuclear ribonucleoprotein particles (snRNPs) are conserved essential components of the RNA splicing machinery snRNPs undergo a complex maturation pathway, and defects in their biogenesis lead to various human diseases such as spinal muscular atrophy and retinitis pigmentosa. The aim of this PhD project is to elucidate the molecular mechanism of the quality control process that distinguishes between mature and defective particles. The project balances between biochemistry, molecular biology and cell biology and the candidate will learn to work with proteins, nucleic acids and to analyse cells using advanced fluorescence microscopy.

Investigating mechanisms of hematopoietic stem cells and their therapy resistance in hematological malignancies - cellular, murine, humanized models.

ID 246112

prof. MUDr. Tomáš Stopka, Ph.D.

The maintenance of human health is the result of a dynamic interaction of external and internal forces that include environmental cues, genetic background and its epigenetic realization into various cellular products that give rise to phenotypic expressions. Clonal disorders, such as solid tumors and hematologic malignancies, are the result of alteration of unique homeostatic interactions in stem cells. They involve early chromatin remodeling during differentiation and subsequent involvement of key transcription factors. Thus, chromatin represents a major barrier to the development of genetic variants and deregulated gene expression involved in hematopoietic cell diseases such as leukemia, lymphomas and myeloma. Modelling such phenomena involves intensive genetic research, cloning, development of transgenic models in cell lines or mice and, not least, the development of xenograft implantation of human tumor cells into immunocompromised mice. Testing of newly defined mechanisms includes, among other things, validation studies with inhibitors of newly identified target pathways.

The objectives of the proposed project:

- 1) to identify the mechanisms involved in the stem cell response to anticancer therapy;
- 2) to determine how epigenetic mechanisms influence the molecular causes of tumor transformation.

Our research program involves the design of innovative transgenic mouse and cell culture systems and advanced molecular methods, including next-generation genome sequencing, and the development and testing of novel modalities useful in therapy. We are looking for scientifically curious and talented predoctoral and postdoctoral fellows to join the modern laboratory environment in the Czech Republic (BIOCEV) and abroad (IARC) to develop conceptually new projects in the above research areas.

Reprogramming of translation during oogenesis and embryogenesis

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ID 254230

A subset of maternal transcripts is stored in a dormant state in the oocyte, and timely driven translation of specific mRNAs guides meiotic progression, oocyte-embryo transition, and early embryo development. In the absence of transcription, the regulation of gene expression in oocytes is controlled almost exclusively at the level of transcriptome/proteome stabilization and protein synthesis.

We recently showed that tightly controlled translational program is essential for proper meiotic maturation, fertilization and subsequent development of the offspring. Thus, understanding the key molecular regulators of translational control is very important. High resolution polysome fractionation and low-input ribosome profiling of mouse oocytes and preimplantation embryos has enabled us to define the translational landscape at an unprecedented level. We systematically and comparatively analyzed the transcriptome, polysome- and nonpolysome-bound RNA profiles of mouse oocytes and early embryos at 2-cell, 8-cell, morula and blastocyst stage. We defined four modes of translational selectivity: i. selective translation of non-abundant mRNAs, ii. active translation of highly expressed mRNAs, iii. translationally suppressed abundant mRNAs, and iv. mRNAs bound by monosomes. We found that translatoome positively correlates with transcriptome in oocytes, followed by a markedly different translational control in 2-cell embryos, which is gradually synchronized at the later developmental stages. We identified important novel cellular/embryonic functional regulators that are being utilized and prioritized for translation at every developmental stage accompanied by enigmatic embryonic developmental programming. Together, these data reveal a unique, stage-specific, temporal regulation of translation that accompanies oocyte and preimplantation development.

This project will unravel new mechanisms of regulation of development of mammalian egg and preimplantation embryo, including human.

Microtubule arrangement in axonemes of primary and motile cilia

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ID 246703

Cilia are cylindrical organelles on the surface of a majority of human cells. They have critical motility, signaling and sensory roles, and their malfunction causes diseases called ciliopathies. There are two principal types of cilia, motile and primary cilia. Their skeleton, the so called axoneme, is based on microtubules. However, recent studies indicated that the axoneme of the primary cilia significantly deviates from the classic pattern of 9 outer microtubule doublets with the central pair of microtubules. The project aims to investigate how are the microtubules patterns of axonemes in both motile and primary cilia established during ciliogenesis, and what are the implications of a particular axonemal arrangement for the ciliary function and transport of material along the cilium. To answer these questions advanced live cell imaging and high resolution approaches, such as electron microscopy and expansion microscopy, will be employed. Moreover, biochemical approaches will be used to identify proteins involved in regulating a particular arrangement of axonemal microtubules.