

OR Vývojové a buněčné biologie

Vypsáné doktorské práce pro akademický rok 2021/2022- II. kolo přijímacího řízení

Expression of Huntingtons disease markers in eye tissues

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ID 242255

Huntingtons disease (HD) is a genetic neurodegenerative disorder that affects muscle coordination (chorea) and leads to cognitive decline and psychiatric problems (dementia). It typically becomes noticeable in mid-adult life. This disease is caused by an expansion of a polyglutamine (polyQ) domain in the protein of huntingtin (htt). PolyQ expansion above 35–40 results in the disease associated with htt aggregation into inclusion bodies. The recent studies have shown that the illness arises not only in neurons, but also in non-neuronal cells. Purpose of this study will be to investigate the expression of selected HD markers (on htt and their aggregates into nuclear inclusions, micro- and microgliosis, proteolytic enzymes) in eye tissues (retina, corneas, conjunctivas) of minipig transgenic or knock-in for the mutated huntingtin and compare them with tissues of wild type (WT) minipig. Since mutant Htt (mtHtt) and especially their small proteolytic fragments are very toxic to all HD cells (particularly those of neuroectodermal origin such as neurons or retinal pigment epithelial cells), it has been suggested that upregulated proteolysis of mtHtt plays a crucial role in the HD pathogenesis including damage of the retina.

How epigenetic modulators influence phenotypic bi-stability

RNDr. Petr Bartůněk, CSc.

ID 234362

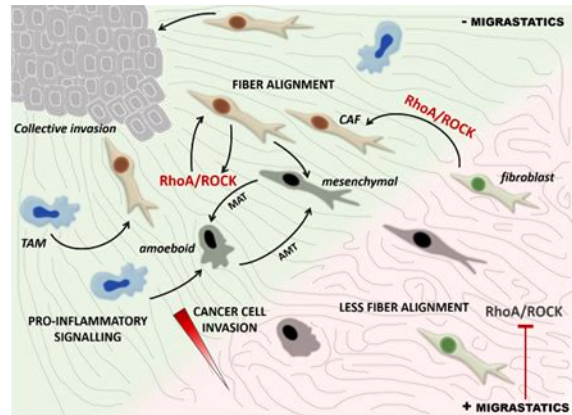
A dominant ENU mutagenesis screen in mice, conducted in the laboratory of Prof. Emma Whitelaw (Australia) has been quite successful in identifying genes which modify epigenetic state. Preliminary analysis of one of the earliest identified mutants, MommeD3, uncovered very unusual and unique phenotypic features in homozygous embryos, including biphenism, imprinting defects, neural degeneration and profound anemia. At that time, the underlying genetic point mutation could not be identified. More recently, taking advantage of next generation sequencing approaches, we have been able to revisit this project and successfully identify the causative mutation. Combining formal description of the embryonic MommeD3 phenotype with global analyses of RNA expression and DNA methylation, the overall aim of the project will be to link the observed stochastic bi-stable embryonic phenotype with potential alterations in genomic imprinting networks.

Plasticity of cancer cells invasiveness and its targeting by migrastatic drugs

doc. RNDr. Jan Brábek, Ph.D.

ID 241379

The malignancy of solid cancer is mainly caused by the ability of tumor cells to form metastases. The crucial step during metastasis is the invasion of the cancer cells through the ECM. To achieve this, cancer cells can utilize the protease-dependent mesenchymal invasion mode or more recently discover the amoeboid mode that relies on enhanced cell contractility. All modes of cancer cell invasiveness are interconvertible and could be employed by cancer cells in combination. A great deal of effort of the world wide scientific community has been devoted to studying various aspects of cell invasion and migration. However, despite all the effort, the so far incomplete understanding of the plasticity of cancer cells' invasiveness precluded successful development of clinically usable anti-metastatic treatment strategies.



The project aims to analyze various aspect of cancer cell invasiveness and to test migrastatic potential of approved drugs as well as other promising candidates. The successful candidate should have experience in mammalian cell cultivation techniques and basic fluorescence microscopy. Experience with live-cell microscopy and molecular cloning are of further advantage.

Characterization of the knock-in mini-pig model of Huntington's disease and identification of pre-manifest biomarkers.

Zdeňka Ellederová

ID 234925

The aim of this work will be to elucidate the molecular changes related to cell cycle regulation, metabolism and apoptosis in Huntington's disease.

Preliminary results have showed gradual accumulation of oxidative stress and aberrant regulation of the G2 / M phase in cells expressing mutated huntingtin (mHTT). mHTT has been shown to bind to ATM, a serine / threonine kinase that is activated by double-stranded DNA breaks. It phosphorylates several key proteins that initiate cell cycle arrest, DNA repair, or apoptosis.

The student will study and compare cell cycle regulation, metabolism and apoptosis in primary cell cultures isolated from the mouse (HD-Q175) and mini-pig (KI-85Q) model for Huntington's disease as well as in human induced pluripotent stem cells or primary cells (fibroblasts or blood cells) isolated from human patients. The mHTT binding to the key components of the cell cycle together with mHTT influence on its regulation will be studied.

Genome stability in mammalian oocytes and somatic cells

Mgr. Helena Fulková, Ph.D.

ID 241974

Genetic analyses show that the female germline has a low mutation rate. The reason for this is unclear, but the abundance of repair machinery, as indicated by results from the male germline, represents the easiest explanation. To analyse the impact of the abundance of the repair machinery in oocytes and in other cell types, we will directly correlate this with the capacity of the cells to sense and repair SSBs. To achieve this, we propose to use laser beam-induced DNA damage. Finally, using somatic cell nuclear transfer, we ask whether the repair component amount is the only factor. As KO animal models for the repair machinery components are not viable, one of the aims will be to prepare new models. The project will be carried out in collaboration with the Jagiellonian University, Krakow, Poland. Interest and experience with microscopy and molecular biology is desirable.

Evolution of eyes: insight from photoreceptors of invertebrate chordate amphioxus

RNDr. Zbyněk Kozmik, CSc.

ID 223147

Project aims to provide new insight into evolution of animal 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).

Molecular associations between recurrent mutations and immunoresistant phenotype of leukemia cells

RNDr. Kateřina Kuželová, Ph.D.

ID 234646

Imunitní systém patrně hraje zásadní roli v prevenci vzniku leukémií i při eliminaci tzv. zbytkové choroby - leukemických buněk přežívajících v těle pacienta po chemoterapii. Lidský organismus je často schopen spontánně vyvinout protinádorovou imunitní reakci, ta ale může být řadou různých mechanismů následně utlumena. Schopnost buněk unikat imunitnímu systému může být nedílnou součástí leukemogenní transformace. Aktivace signálních drah zajišťujících neregulovanou buněčnou proliferaci nebo odolnost vůči indukci apoptózy může zároveň vést například k redukci exprese HLA molekul, na kterých jsou T-buňkám prezentovány antigeny, ke snížení prezentace antigenů na HLA molekulách, k expresi inhibičních receptorů (např. PD-L1) nebo k sekreci látek tlumících funkci imunitních buněk (např. Gal-9, IDO, argináza). Cílem disertační práce bude hledání souvislostí mezi nejčastějšími mutacemi, které jsou v leukemických blastech nalézány, a přítomností markerů rezistence vůči imunitní odpovědi. Mezi kandidátní mutované proteiny patří nukleofosmin, kináza FLT3 nebo vybrané epigenetické regulátory u akutní myeloidní leukémie nebo fúzní kináza BCR-ABL u chronické myeloidní leukémie. Aktivita těchto proteinů nebo jejich efektorů bude ovlivňována pomocí inhibitorů, siRNA nebo genových modifikací, následky budou studovány na úrovni proteinové exprese (průtoková cytometrie, western blotting, imunofluorescenční mikroskopie, případně imunoprecipitace) a funkčních buněčných analýz (cytotoxické testy, měření metabolismu). Práce bude prováděna převážně na ustavených leukemických liniích, částečně také na primárních leukemických buňkách.

Functional characterization of variants of uncertain significance in cancer predisposing genes

MUDr. Libor Macůrek, Ph.D.

ID 235827

Cílem tohoto projektu je vyhodnocení funkčního významu variant vybraných genů nalezených u nádorových pacientů v české populaci. Zaměříme se především na varianty v genech zapojených do kontroly integrity genomu a kontroly buněčného cyklu, včetně CHEK2, FANCG, MRE11, NBN, PPM1D a TP53. Varianty těchto genů budou identifikovány na spolupracujícím pracovišti (Ústav biochemie a experimentální onkologie, 1. LF UK) metodou panelového sekvenování (CZECANCA) zahrnujícího přes 200 známých i kandidátních nádorových genů. Vybrané varianty budou testovány na školitelském pracovišti v Laboratoři biologie nádorové buňky na ÚMG AVČR. Základem funkčních analýz bude příprava buněčných modelů, kdy budou metodikou CRISPR/Cas9 inaktivovány vybrané geny. Metodami buněčné biologie bude určen defekt v signální dráze způsobený inaktivací daného genu a bude optimalizována vhodná technika pro detekci tohoto defektu. Například inaktivace genu zapojeného do opravy DNA povede k nahromadění jaderných fokusů značených fosforylovanou variantou histonu H2A.X. Tento parametr může být vizualizován a kvantifikován fluorescenční mikroskopií o vysoké propustnosti (Olympus ScanR). V dalším kroku budou metodou Gibson assembly připraveny expresní plasmidy nesoucí kódující sekvence pro fluorescenční marker (např. EGFP), cDNA vybrané varianty a selekční kazetu (např. Neomycin, Puromycin). Po transfekci do buněk bude porovnána funkce mutované a přirozené formy daného genu. Ve vybraných případech budou pomocí selekčního antibiotika zavedeny stabilní linie exprimující varianty genu a bude sledován jejich význam pro přežití buněk (například po vystavení DNA poškození). Pro každý studovaný gen bude vytvořena knihovna variant a jednotlivé varianty budou porovnány jak vzhledem k přirozené formě genu tak navzájem. Toto vyhodnocení umožní rozlišit funkčně významné oblasti proteinů, například nové vazebné nebo lokalizační domény. Jedním z dílčích cílů projektu je rovněž přispět k porozumění mechanismu funkce studovaných genů a jejich zapojení do známých signálních kaskád.

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

Mgr. Marie Macůrková, Ph.D.

ID 234618

Clathrin-mediated endocytosis is an important cellular process involved in uptake of molecules into cells, signalling or membrane homeostasis. It is rigorously controlled at several levels. At the plasma membrane the clathrin pit assembly and cargo selection is assisted by numerous adaptor proteins, among them the AP2 adaptor protein complex. In mammalian cells AP2 complex activity is regulated by AAK1 kinase phosphorylation. We utilized the nematode *Caenorhabditis elegans* to study the developmental role of AAK1 ortholog SEL-5 in this invertebrate model. SEL-5 activity seems to be dispensable for general regulation of endocytosis in *C. elegans*, however loss of SEL-5 activity leads to a defect in migration of neuronal precursor cells and also to a defect in cell growth of a particular cell type. Interestingly, defect in cell migration can also be observed in cultured mammalian cells after loss of AAK1 expression. AAK1 and SEL-5 thus seem to act in both similar and disparate mechanisms. The aim of this project is to dissect the mechanism of SEL-5 function in neuronal migration and in cell growth and in case of migration, compare the AAK1/SEL-5 mechanism of action between mammalian and *C. elegans* models. The project will combine advanced *C. elegans* genetic techniques including targeted CRISPR/Cas9 mutagenesis and *in vivo* protein tagging with biochemical and microscopic analyses in cultured mammalian cells.

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.

Gene expression regulation during early and late phases of embryonic wound healing

Mgr. Radek Šindelka, Ph.D.

Wound healing is a complex and still elusive process. One of the objectives within the field of animal and human medicine is to heal wounds without scars and to effectively replace defective parts of the body. Mammals have the capability of scarless healing, but it is lost after birth and is substituted by scarred healing. Contrastingly, animals such as fishes and amphibians are able to heal without scar formation and completely regenerate a variety of organs even as adults. We recently revealed an important factors for embryonic healing in early (AP1 transcription factors) and late phases (matrix metalloproteinases). We will study signaling pathways and cell types that are responsible for healing and processes that are dependent on these molecules. We will apply a variety of methods ranging from single cell analysis to functional assays using loss/gain of functions and phenotype characterization. We will utilize frog and fish embryo models. Our goal is to uncover mechanisms behind embryonic wound healing and provide clues for improvement of regeneration and healing capabilities.

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

oc. RNDr. Daniel Rösel, Ph.D.

doc. RNDr. Jan Brábek, 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.

Transient nuclear protein-protein interaction networks

Ing. Václav Veverka, Ph.D.

ID 212076

Many processes implicated in the regulation of DNA transcription are driven by highly modular protein-protein interaction networks that are not exclusively maintained by the well structured protein domains but also by highly dynamic regions of interacting proteins. The aim of the doctoral project will be an identification as well as investigation of the physiological and pathological roles of these transient interactions using highly interdisciplinary approach, including protein biochemistry, biophysics, cell and structural biology.

Role of normal and mutant huntingtin during neural development.

Mgr. Petr Vodička, Ph.D.

ID 234639

Huntington disease (HD) is a hereditary neurodegenerative disorder caused by an expansion of CAG tract in huntingtin (HTT) gene, leading to expression of over 36 glutamines in mutant huntingtin (mHTT) protein. HTT mutation leads to both the loss of function as well as toxic gain of function phenotypes. Gene therapies focused on total HTT lowering are currently in preclinical and clinical testing. As HTT plays important, but not yet fully elucidated role in the cell differentiation and embryo development, this may have important consequences for safety of HTT lowering therapies. The aim of this project is to study fetal brain development in presence of mHTT using unique knock-in minipig expressing HTT with 86Q *in vivo*. Human HD iPSC lines, normal HTT iPSC lines and possibly HTT null lines will be used as model of neural cell differentiation *in vitro*. Methods will include immunohistology, proteomics, tissue culture and live cell microscopy.