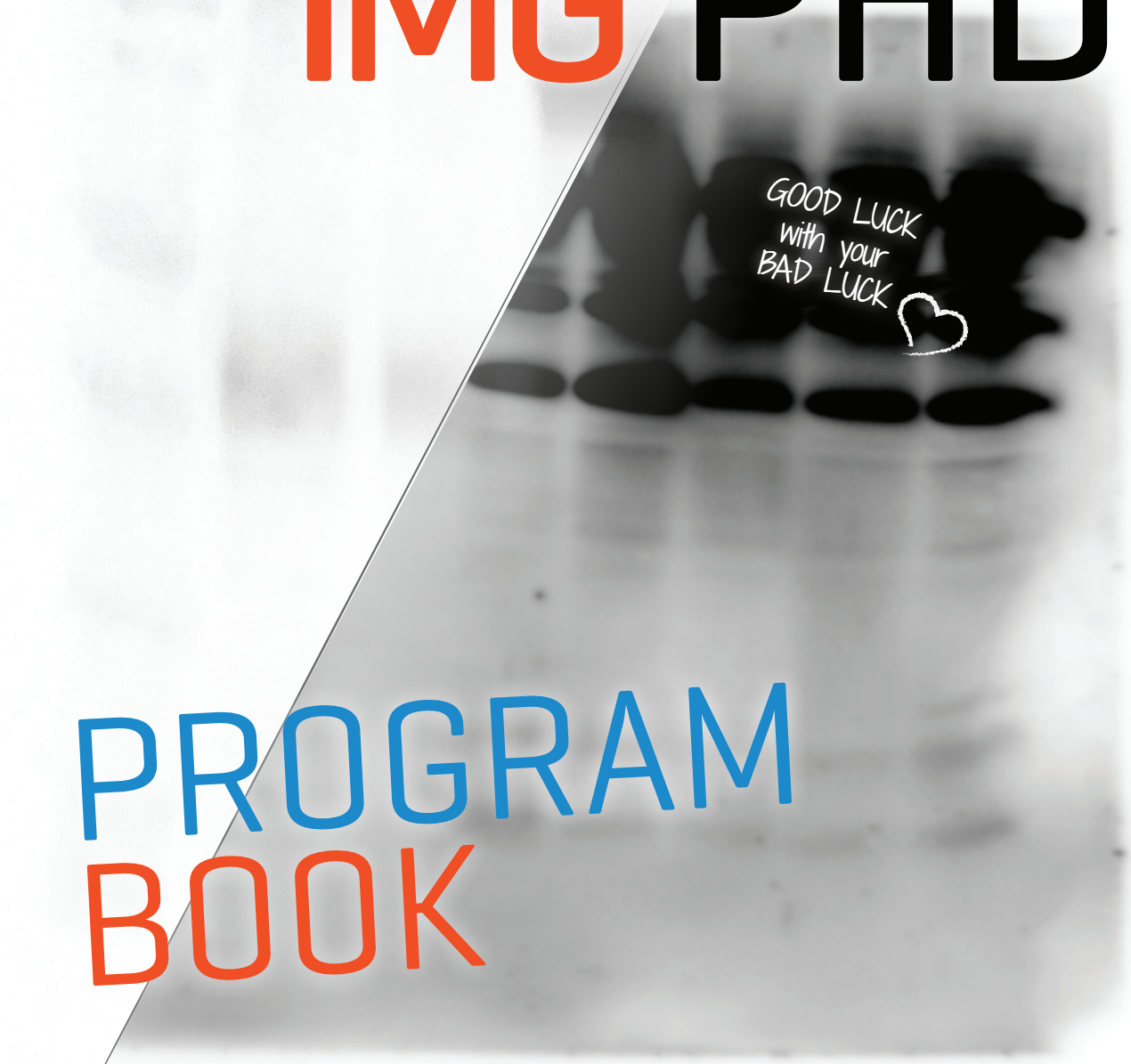
IMG

13th conference IMG PHD



PROGRAM BOOK

KEYNOTE: How to avoid drug resistance in cancer? [Michal Šimíček]

PRESENTATION SKILLS WORKSHOP: Sell your message! A few axioms of modern presenters. [Ondřej Staněk]

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PROGRAM

09:00 - 09:15	invitation
09:15 - 09:30	Srdjan Grusanovic
09:30 - 09:45	Martin Kovář
09:45 - 10:00	Jiří Březina
10:00 - 10:15	Maria Kuzmina
10:15 - 10:30	Šárka Janušová
10:30 - 11:00	coffee break
11:00 - 12:00	How to avoid drug resistance in cancer? [Michal Šimíček]
12:00 - 12:15	Felix Zimmann
12:15 - 12:30	Jana Uhlířová
12:30 - 12:45	Veronika Krchlíková
12:45 - 13:00	EastPort [Jaroslav Icha]
13:00 - 14:00	lunch time
14:00 - 14:15	Miroslav Stoyanov
14:15 - 14:30	Cecilia Perez Aquino
14:30 - 14:45	Miroslava Kolková
14:45 - 16:15	Sell your message! A few axioms of modern presenters [Ondřej Staněk]
16:15 - 16:45	coffee break
16:45 - 17:45	figure design workshop [Oldřich Beneda & Ivan Novotný]
17:45 - 18:00	Bio-Rad [Eddy van Collenburg]
18:00 - 18:30	closing remarks / best presenter award

Michal Šimíček, Ph.D.

Blood Cancer Research Group, University Hospital Ostrava

Michal šimíček, Ph.D. obtained his immunology MSc. Degree at Charles University, working on his research at IMG for 4 years. After that he moved to Belgium to University of Louvaine, where he graduated. He spend his postdoc at MRC LMB in Cambridge where he recieved several prestigious stipends and grants (e.g.: FWO and EMBO fellowship). His work was published in the best scientific journals (including Science and Nature) and was awarded by Neuron Endowment Fund Prize. Recently he is the head of international Blood Cancer Research Group in Ostrava, where he employs basic and applied reasearch approaches to investigate hematological malignancies.

How to avoid drug resistance in cancer?

Many highly effective anti-cancer drugs have been developed so far. However, emergence of therapy resistance remains one of the biggest obstacles in the fight with cancer. Multiple myeloma (MM) is a typical example of how cancer patients are benefiting from scientific discoveries. In the past decade, development of several specific anti-myeloma drugs let to dramatic improvement of patient survival. Selection of certain combinations for the right patient to achieve the best possible outcome is still very problematic and often based on overall clinical picture rather than precise molecular characteristics of the patient tumor.

MM is hematological cancer arising from an outgrowth of malignant plasma cells in the bone marrow. Typical feature of MM is a massive production of monoclonal immunoglobulins (Ig). An inevitable consequence of extensive Ig synthesis is overload of misfolded proteins that saturate proteasome capacity. Therefore, the myeloma cells are highly and uniquely sensitive to proteasome inhibitors (PI). Initial tremendous success of PI in the clinic is unfortunately followed by often relapses in majority of patients.

By applying wide spectra of methods including bioinformatic analysis, primary patient's samples, modern genetic tools in cell lines and animal models, advanced biochemistry and proteomics; we have discovered a novel regulatory mechanism for Ig production in plasma cells and its relation to PI-resistance. Finally, we performed a rational, integrative screening using the clinically approved drugs to design a new re-sensitization strategy for PI-resistant MM patients. Our findings will be very useful in the identification of patients benefiting the most from the selective, PI-based combinatory therapies.

Ondřej Staněk

stanekconsulting.cz

Ondřej Staněk is consultant and lecturer of presentation skills, presentation design, and communication. He specializes in creating design presentations, conducts trainings on public speaking, and provides consulting services to conference speakers. He works as an expert consultant for startup incubators and lecures on rhetoric and modern presentation skills at the University of Chemistry and Technology Prague.

Sell your message! A few axioms of modern presenters

Shortening attention spans, overconfidence and hypocrisy, general lack of interest in the audience's needs. These are just a few examples of potential problems speakers face, especially now, in the age of Snapchat, Instastories and TikTok. Times when audience members listened only because it was expected of them, are long gone, and are not coming back anytime soon. The audience has changed and so we, the speakers, need to change with them – or become utterly irrelevant. Sell your Message is talk about modern rhetoric, human mind, and hard truths about public speaking in the 21st century.

SCREEN OF G2/M EXPRESSED GENES IDENTIFIES FAM110A AS A REGULATOR OF MITOTIC PROGRESSION

Aquino Perez Cecilia [1], Monika Burocziova[2], Gabriela Jenikova & Libor Macůrek [3]

[1, 2, 3] Cancer Cell Biology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague CZ14220, Czech Republic

Eukaryotic cell cycle progression is driven and regulated by cyclin dependent kinases that control gene expression, orchestration and establishment of the mitotic spindle and a successful cell division in order to start the next cycle. In this work, we aimed to identify potential new regulators of the cell cycle and mitosis by comparing gene expression in human non-transformed cells. For this end, we implemented a Fluorescent Ubiquitination-based Cell Cycle Indicator and performed transcriptomic analysis of human non-transformed cells. We identified 701 differentially expressed genes in G1 and G2 cells. We further focused on the poorly studied Family with sequence similarity 110 member A (FAM110A) protein, which showed to be highly expressed in G2 cells with its expression further increased towards mitosis. In order to describe a preliminary profile of the protein, we performed immunofluorescence confocal microscopy and identified its endogenous localization at the mitotic spindle and spindle poles. RNAi-mediated depletion of FAM110A increased mitotic index and extended duration of mitosis. Live cell imaging revealed impaired positioning of the mitotic spindle and delayed metaphase to anaphase transition in cells with depleted FAM110A. Using mass spectrometry and immunoprecipitation, we identified casein kinase I in complex with FAM110A, finding a specific enrichment of the interaction during mitosis. We decided to further explore the interaction between CKI isoforms CK1δ and CKIε by generating a series of truncations in the FAM110A sequence, in order to map the interaction with said CKIs and other candidates identified in the mass spec assay. We identified that FAM110A C-terminal domain is necessary for the protein to be a proper phosphorylation substrate for CKIδ. We are further working on mapping the nature of this phosphorylation trough phenotype rescue after endogenous knock-down, assays assessing metaphase length duration and correct spindle positioning.

TIGHT JUNCTION PROTEINS AS DETERMINANTS FOR THE ESTABLISHMENT OF CENTRAL TOLERANCE

Březina Jiří[1,2] and Dominik Filipp[1]

[1] Laboratory of Immunobiology, Institute of Molecular Genetics of the AS CR, Prague, Czech Republic
[2] Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic

The presentation of self-antigens in the thymus by medullary thymic epithelial cells (mTECs) and thymic dendritic cells (DCs) is fundamental for the establishment of T cell central tolerance as its failure results in the development of autoimmunity. While mTECs produce and present self-antigens in an autonomous manner, DCs can acquire these self-antigens from mTECs by the process of Cooperative Antigen Transfer (CAT). It has been well documented that CAT is essential for the establishment of central tolerance, however, the molecular mechanism and the heterogeneity of thymic DCs that participate in CAT remain unclear.

Utilizing single-cell RNA sequencing, we discovered the previously unappreciated heterogeneity of DCs whereby at least six different subsets participate in CAT. Using fluorescent tdTomato protein as a model, mTEC-produced antigen, we compared the transcriptome of the cells from each thymic DC subset which recently acquired the tdTomato protein from mTECs to those cells of each subset which did not. This approach identified candidate genes which determine the efficacy of the CAT mechanism. We found that CAT-experienced DCs highly expressed tight junction proteins such as EpCAM and Claudin 1. Using publically available databases, we found that the expression of these two genes in DCs is anatomically restricted to those DCs that reside in the thymus as compared to peripherally localized DCs. To experimentally verify their contribution to CAT, we assessed the efficacy of CAT using thymic versus splenic DCs. Using an *in vitro* model of CAT, we have shown that the thymic DCs are much more efficient in antigen acquisition. When the function of EpCAM was blocked by antibody-mediated neutralization, the CAT in the thymic DCs dropped down to the levels observed in the splenic DCs. This implies that EpCAM endows thymic DCs with the capacity for enhanced antigen transfer. Taken together, our data suggests that EpCAM and also likely Claudin 1 are key molecular determinants of CAT.

CHRONIC INFLAMMATION INSTRUCTS HEMATOPOIETIC STEM CELLS TO LOSE THEIR FITNESS

Grusanovic Srdjan[1, 2], Petr Danek[1, 2], Miroslava Kardosova[1], and Meritxell Alberich-Jorda[1]

[1] – Laboratory of Hemato-oncology, Institute of Molecular Genetics of the Czech Academy of Science, Videnska 14220, Prague, Czech Republic
[2] – Department of Molecular and Cellular Biology, Genetics and Virology, Faculty of Science, Charles University

Chronic inflammation is a condition that affects one-third of the human population and it is characterized by excessive production of cytokines and chemokines. Recent evidence showed that hematopoietic stem cells (HSCs) directly respond to these signaling molecules. In order to investigate how chronic inflammation may affect HSCs, we employed a unique mouse model. The mice carry a point mutation in the *proline-serine-threonine phosphatase-interacting protein 2* (*Pstpip2*) gene, resulting in a progressive autoinflammatory disorder called chronic multifocal osteomyelitis (CMO). Symptoms of the disease include swollen paws and tail kinks.

It was previously published that symptomatic CMO mice have increased bone marrow (BM) cellularity and increased number of granulocytes. Recently, we observed that expansion of the HSC compartment in the BM occurs while the mice are still asymptomatic. To assess whether phenotypical expansion of HSCs affects their functionality, we performed limiting dilution assays from sorted WT and CMO long-term HSCs (LT-HSCs). Our analysis showed that CMO LT-HSCs have significantly impaired engraftment ability. Furthermore, we investigated whether the BM niche contributes to this phenotype. We exposed WT HSCs to WT or CMO BM niche, after which we re-transplanted them into secondary recipients. Our results indicate that after WT HSCs have been exposed to the CMO BM niche, they no longer have the same engraftment capabilities as the control group. RNAseq analysis showed that these cells have upregulated several signaling pathways related to inflammatory response, most prominently the IL6/Jak/Stat3 signaling pathway. Altogether, our data indicate that chronic inflammation has a detrimental effect on HSCs and that the BM niche, possibly through the production of IL-6, contributes to this effect.

ANALYSIS OF GITR SIGNALING PATHWAY AND ROLE OF ABIN1 IN T CELL DEVELOPMENT

Janusova Sarka[1], Paprckova Darina[1], Drobek Ales[1], Draber Peter[2], Stepanek Ondrej[1]

[1]Laboratory of Adaptive Immunity, Institute of Molecular Genetics, Czech Academy of Science, Videnska 1093, Prague, 14220, Czech Republic
[2]Laboratory of Immunity & Cell Communication, BIOCEV, First Faculty of Medicine, Charles University, Vestec, Czech Republic

T-cell immune response is initiated upon the recognition of an antigen presented on MHC glycoproteins by specific T cell receptors. However, for proper immune response, additional costimulatory signal is required. Several members of the TNF-receptor superfamily, such as GITR, CD137 or OX40, can provide the required costimulatory signal, which affects T-cell survival, proliferation, and effector functions. These receptors are expressed on activated T cells and after engagement with their ligands, they trigger downstream signaling events, such as activation of NF-κB or MAPK signaling pathways. The precise understanding how the costimulatory signaling is propagated would provide a potential target(s) to modulate T-cell response in autoimmune diseases or cancer. In this study we analysed molecular mechanisms of signaling pathway triggerd after GITR stimulation using mass-spectrometry. We aimed to find novel molecules participating in these pathway and elucidate how they affect GITR-induced signaling responses. One of the identified molecules is Abin1 that was determined as an inhibitor of GITR signaling and also was proposed to have a role in T-cell development *in vivo*. Mice lacking this protein were published to be lethal, but our model is viable and showed modified ratios between different T cell compartements. We aimed to determine the importance of Abin1 in T-cell development and their activation.

INSIGHT INTO THE ROLE OF PAX6 GENE IN EYE DEVELOPMENT OF PLATYNEREIS DUMERILII.

Kolková Miroslava[1], Jovana Doderović [1], Iryna Kozmiková[1] & Zbyněk Kozmik[1]

[1]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague

The role of Pax6 as a key regulatory gene of eye development was mainly studied in prominent model systems such as Drosophila or mouse. Marine annelid *Platynereis dumerilii* has recently become one of the popular model organisms in evolutionary and developmental biology. *Platynereis* larvae develop two sets of eyes adult eyes, which are precursors to the visual pigment-cup eyes and distinct larval eyes which eventually degenerate. Pax6 is known to be expressed in developing larval eyes but also at lower levels in the developing adult eyes. To assess whether Pax6 gene is essential for visual system development and if so, which eye and cell type is actually needed for and also which genes are dependent on its function, we used the Pax6 knock-out line. Simple behavior assay revealed phototactic deficiency in homozygote mutants, which do not survive past three weeks. We analyzed the expression of transcription factors, eye markers, cholinergic markers and neuropeptides present in the Pax6 expression domain and differentially expressed genes provided by RNA-seq analysis of 4 days old wild type and homozygote larvae. Our data confirmed the previous results, suggesting an essential role of Pax6 in visual function and helped to pinpoint the missing parts of visual circuit in Pax6 knock-outs.

HEMATOPOIESIS IN SEA LAMPREY

Kovář Martin[1], Petr Bartůněk[1]

[1] Institute of Molecular Genetics of the ASCR, Prague, Czech Republic

Hematopoiesis is specific process in vertebrates during which terminally differentiated blood cells with specific functions arise from a hematopoietic stem cells (HSC) through different progenitors. Now, we have a deep knowledge about the hematopoiesis in standard vertebrate models, but in the field of the evolution of hematopoiesis our knowledge is limited. The main reason is that process of the hematopoiesis is well conserved. In all standard vertebrate models, we can identify the same key regulatory genes/proteins involved in the regulation of hematopoiesis as well we can observe the same blood cell types. Therefore, in order to get to the roots of hematopoietic cell lineage evolution, we need to employ an unconventional animal model(s) such as sea lamprey, which is the representative of a jawless taxon, also being the most ancestral vertebrate living up to date. The aim of this project is to map the hematopoiesis in sea lamprey on cellular and genetic level with special attention to the embryonic development.

THE CHICKEN ASLV-A RECEPTOR, TVA, SERVES AS A RECEPTOR FOR CELLULAR UPTAKE OF COBALAMIN (VITAMIN B12)

Krchlíková Veronika[1], Cyril Bařinka[2], Josef Geryk[1], Jan Kosla[1], Jiří Hejnar[1], Daniel Elleder[1]

[1]Laboratory of Viral and Cellular Genetics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
[2]Laboratory of Structural Biology, Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic

Deficiency of vitamin B12 (cobalamin) caused by malnutrition or mutations in the cobalamin pathway genes is associated with haematological and neurological pathologies in mammals. Cobalamin is transported from blood into cells in complex with carrier protein transcobalamin (TCN) via a receptor encoded by the *CD320* gene. In mammals, CD320 is highly upregulated in tumour cells and is used experimentally as tumour-specific marker for drug delivery and tumour visualization experiments. In birds, the *tva* genetic locus encodes protein belonging to low density lipoprotein receptor superfamily and is predicted to be orthologous to the human *CD320* gene. TVA receptor in avian cells also determines susceptibility to Avian sarcoma and leucosis viruses subgroup A (ASLV-A), however its physiological function has not been determined.

In this study we investigated whether the physiological function of the two orthologs, human CD320 and chicken TVA, is conserved despite diversification of mammals and birds more than 300 million years ago. We produced and purified recombinant chicken TCN which was then incubated with radioactively or fluorescently labelled B12. The complex was then used for the import assays of B12 into chicken DF-1 cells. Ectopic over-expression of TVA receptor in DF-1 cells by transient or stable transfection caused higher cellular uptake of the labelled B12. CRISPR-mediated knock-out of endogenous TVA resulted in significantly lower cobalamin import. Further, import of vitamin B12 into cells chronically infected with ASLV-A was reduced in comparison with uninfected cells. This can be explained by lower number of TVA receptor molecules available on the infected cell membrane due to occupancy with viral envelope proteins (analogous to receptor interference). Moreover, preincubation of DF-1 cells with TCN-B12 complex caused a partial block of infection with subgroup A ASLV, but infection with other virus subgroups that do not use TVA for entry was unaffected. In line with this, fluorescence microscopy indicated import of fluorescently labelled B12 into chicken cells, and its dependence on TVA. This study is the first to report of physiological function of the chicken TVA receptor. Overall, our data indicate that TVA is a part of the cobalamin metabolic pathway in chicken cells.

CHRONIC INFLAMMATION INDUCES MOBILIZATION OF HSPCs

Kuzmina Maria[1], Srdjan Grusanovic[1], Meritxell Alberich-Jorda[1]

[1]Laboratory of Hemato-oncology, Institute of Molecular Genetics, CAS, Prague

Bone marrow transplantation (BMT) is a well-known method used in the treatment of malignant and hematopoietic diseases. Successful transplantation requires sufficient amount of hematopoietic stem and progenitor cells (HSPCs) that can be harvested from peripheral blood by using mobilization agents such as granulocyte colony stimulating factor (G-CSF) or AMD3100.

Chronic low-grade inflammation (CI) is a crucial contributor to various diseases but little is known about how CI affects HSPC mobilization. We hypothesize that donors which suffer from CI might have different response to HSPC mobilization agents which could in turn affect yields of HSPCs for further transplantation.

To investigate the possible effect of CI on mobilization of HSPCs we employed a unique mouse model, referred as CMO. CMO mice suffer from CI, leading to the development of chronic multifocal osteomyelitis (CMO), a progressive inflammatory disease developed due to the mutation in *Pstpip2* gene. To study the effect of CI on HSPC mobilization, we applied several *in vitro* and *in vivo* approaches including whole blood transplantation, flow cytometric analysis, and colony forming unit assays.

Together, these results demonstrated that CI promotes mobilization of HSPCs from the bone marrow to peripheral blood, unravelling novel factors affecting the harvesting of HSPCs in human BMT.

ROLE OF WIP1/PPM1D IN GENOME INSTABILITY

Stoyanov Miroslav [1], Andra Stefania Martiníková [1], Libor Macůrek [1]

[1]Affiliation 1 (Laboratory of Cancer Cell Biology, Institute of Molecular Genetics, Prague, Czech Republic)

Upon DNA damage the DNA repair machinery is activated and cells are halted from progressing further into the cell cycle in order to prevent mutations and their subsequent spreading that could lead to cell transformation. However, as every biological process DNA repair is also a balance between activation and deactivation. In order for cells to progress through the cell cycle once the DNA is repaired, the checkpoint that had activated the DNA repair machinery have to be switched off. This is done by Wip1/PPM1D, which is a member of the PP2C family of Ser/Thr protein phosphatases. PP2C family members are known to be negative regulators of cell stress response pathways. Here we demonstrate that mutations in exon 6 of Wip1 produce truncated form of the protein, which retains its biological function and is much more stable than the full length. This leads to excess accumulation of the truncated Wip1 and permanent shut off of the G1/S and G2/M phase checkpoints, allowing cells to go through the cell cycle with damaged DNA. This leads to micronuclei formation, accumulation of mutations and possibly – cell transformation.

NUCLEOPORIN TPR REGULATES GENE EXPRESSION DURING MYOGENIC DIFFERENTIATION

Uhlirova Jana[1], Lenka Sebestova [1], Karel Fiser[2], Tomas Sieger[3], Jindriska Fiserova[1] and Pavel Hozak[1]

[1] Department of Biology of the Cell Nucleus, Institute of Molecular Genetics of the CAS, Videnska 1083, Prague, Czech Republic
[2]CLIP-Childhood Leukaemia Investigation Prague, Department of Pediatric Hematology/Oncology, Second Medical Faculty of the Charles University and University Hospital Motol, V Uvalu 84, Prague, Czech Republic
[3]Department of Cybernetics, Faculty of Electrical Engineering, Czech Technical University in Prague, Technicka 1902/2, Prague, Czech Republic

In the past decade, nuclear pore complex (NPC) emerged as a hub for transcriptional regulation, as a few transcriptional factors (TFs) and super enhancer sequences were shown to localize to NPC proximity and activate subsets of target genes. This type of transcriptonal regulation was also described as crucial in the context of differentiation. Nucleoporin TPR is crucial for the enrichment of open chromatin around nuclear pore complexes (NPCs) and thus likely affects the transcriptional regulated at NPCs. However, the role of TPR in gene expression and cell development has not been described. Here we show that depletion of TPR results in aberrant morphology of murine proliferating C2C12 myoblasts (MBs) and differentiated C2C12 myotubes (MTs). Our ChIP-Seq data revealed that TPR binds to Myosin heavy chain (*Myh4*), Myocyte enhancer factor 2C (*Mef2c*) and majority of olfactory receptor (*Olfr*) genes in C2C12 MBs, and its binding decreases upon differentiation into MTs. *Myh4*, *Mef2c* and some *Olfrs* are expressed in muscle cells and regulate muscle formation and morphology. We show that TPR affects expression of both *Myh4* and *Olfr376*, but not *Mef2c*. We discuss lysine specific demethylase 1 (LSD1) as a possible co-player in regulation of *Myh4* and *Olfr376* expression.

HERE'S LOOKING AT YOU, KID – ESTABLISHMENT OF A NEW DISEASE MODEL FOR SPLICE FACTOR-ASSOCIATED RETINITIS PIGMENTOSA

Felix Zimmann[1], Zuzana Cvačková[1], Tomáš Bárta[2], David Staněk[1]

[1]Laboratory of RNA Biology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
[2]Department of Histology and Embryology, Faculty of Medicine, Masaryk University Brno, Czech Republic

Retinitis pigmentosa (RP) is one of the leading causes of inherited blindness. Currently, more than 90 genes are known to be involved in the pathogenesis of RP that is characterized by a progressive loss of retina cells. While most of these genes are expressed in the retina and associated with retinal function, surprisingly, the second largest group of mutations causing RP affects proteins that are crucial for RNA splicing. However, it remains elusive how these mutations affecting a ubiquitous and essential process for almost every cell give rise to such a tissue-specific phenotype. To unravel the pathogenic mechanism underlying RP we work on the establishment of a disease-relevant model. Using CRISPR/Cas9 we introduced an RP-linked mutation in the splicing factor Prpf8 in human induced pluripotent stem cells (hiPSC). We then differentiated these hiPSCs to cells of the retinal pigment epithelium (RPE) and 3D retinal organoids. Our future goal is to unravel the molecular mechanism by analysing alterations in splicing and interactions between RNA binding proteins and RNA using RNA-seq and iCLIP methods. Additionally, we plan morphological and functional analysis of RPE cells and retinal organoids to integrate potential effects on splicing with the tissue-specific retinal phenotype. The knowledge gained from our analysis will provide new insights into how splicing factor mutations are involved in the pathology of RP.