IMG

PhD Conference

AND AFTERPARTY

12th PhD Conference @ Institute of molecular genetics of the ASCR

Friday 24th May

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CONTENT

PROGRAM

	9:00 – 9:15	**Opening**
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KEYNOTE SPEAKER	**11:00 – 12:00**	**Dr. Boris Cvek** Repurposing of Drug Repurposing
	12:00 – 13:00	**Lunch**
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KEYNOTE SPEAKER	**15:30 – 16:30**	**Kristin Tessmar-Raible** Timing Physiology and Behavior With Sun and Moon
	16:30 - 16:45	**Stanislav Kukla (Merck)** Rethink Western Blotting
	17:00 - 18:00	**Poster session**
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BORIS CVEK (keynote lecture)

Repurposing of Drug Repurposing

Univerzita Palackého , Olomouc , Czech Republic

In this presentation, I will start with short discussion of recent arguments why the current model of drug discovery is a failure, including a mention of reproducibility crisis and intrinsic limitations of cell & animal models of human diseases. In fact, major breakthroughs in history of medicine, even in last decades, have been rather serendipitous than "rational" as current model of drug discovery names itself. Often suggested as an alternative model, the drug repurposing and rescuing is based on the serendipitous, unexpected observations. The examples of paromomycin and disulfiram will demonstrate, at the end of the presentation, a further step ahead in shaping drug discovery for better future: the idea of "nonprofit drugs", i.e. the idea of repurposing of unpatentable drugs.

Your notes:

KRISTIN TESSMAR RAIBLE (keynote lecture)

Timing Physiology and Behavior With Sun and Moon

Max F. Perutz Laboratories , Wien , Austria

The moon is an important timing cue for numerous marine species, ranging from brown and green algae to corals, worms, fishes and turtles. Such lunar timing typically controls the gonadal maturation and behavioral changes associated with reproductive rhythms. Despite the fundamental nature and widespread occurrence of these lunar-controlled rhythms and oscillators, little is known about their principle molecular mechanisms, their interplay with rhythms and oscillators of different period lengths, or their modulation in changing environments.
The marine bristle worm Platynereis dumerilii and the midge Clunio marinus harbor light-entrained circadian, as well as a monthly (circalunar) clocks and also exhibit seasonal behaviors. Our work in Platynereis suggests that the circalunar clock persists even when circadian clock oscillations are disrupted.
In order to study the molecular and cellular nature of the circalunar clock, as well as its interactions with other timing, Platynereis and Clunio can be used for complementary experimental approaches:
For Platynereis we established transient and stable transgenesis, inducible specific cell ablations based on the integration of a nitroreductase-cassette, as well as TALEN/Crispr-Cas-mediated genome engineering. Using these techniques on candidate light receptors provides us with insight into the genes required for solar vs. lunar light detection.

Your notes:

ALICE ABBONDANZA

Understanding the role of nicotinic cholinergic receptors in striatal-based behaviour

Abbondanza A[1], Höfflin J[2], Janickova H[1]

[1] Department of Neurochemistry, Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic
[2] Rheinische Friedrich-Wilhelms-Universität Bonn

Striatum is the basal ganglia nucleus responsible for motor control, reward related processes, learning and motivation. The vast majority of its cell population is represented by medium spiny neurons (MSNs), which form the striatal output. The fine regulation of MSNs activity is given by cholinergic transmission, exerted by cholinergic (CINs) and GABAergic interneurons (GABAINs). While CINs modulation trough nicotinic and muscarinic receptors has been extensively studied, the role of nicotinic receptors on GABAergic interneurons (GABAINs) is still largely unknown.

To determine the function of nicotinic acetylcholine receptors (nAChRs) expressed by GABAINs, we selectively deleted β2 subunit in the dorsal striatum by injecting β2-floxed mice with Cre-expressing AAV viral vector. After confirming that the deletion only occurs in the injected area, we tested the mice in a battery of behavioral tasks focused on striatal-based behavior such as open field test, cued Morris water maze, cross maze task, nest building and social preference test, followed by histological and biochemical measurements.

First data obtained with one cohort of mice consisting of both males and females showed the effect of deletion might differ between sexes. While male mutants showed impairment of goal-directed behavior, motivation and sociability, females did not differ from the non-deleted controls.

We conclude that nAChRs expressed by GABAINs in the striatum have a functional role in the control of striatal-based behavior and we will mainly focus on male mice in our future experiments.

This work was supported by the Grant Agency of the Czech Republic grant 19-07983Y . J.H. was supported by DAAD RISE program during her internship.

Your notes:

ŠIMON BORNA

Outcome Predictor of Acute Leukemia 1 regulates CXCR4 signaling and murine hematopoiesis

The rediscovery of Mendel's laws in 1900 had started a new wave of discoveries such as associations between genes and enzymes/proteins, DNA structure determination and many others. Those have encouraged people enough to join efforts in the biggest collaborative project in the history of science. As a result, the whole sequence of Human genome was published in 2004. Since then, more than 15 years passed and yet the knowledge about the 90 percent of protein coding genes is none or minimal. Scientific community seems to be rather focused on only about a 10% of all these genes. In my short talk I would like to introduce you to a one of those unknown. It is an Outcome predictor of acute leukaemia 1 (OPAL1) a gene overexpressed in most common type of childhood acute lymphoblastic leukaemia. Using both in vivo and in vitro strategies we have shown that acute loss of OPAL1 has a positive effect on CXCR4 signalling a major chemokine receptor in bone marrow cell homing and retention. It is also a negative regulator of haematopoietic stem cell function. The precise molecular mechanism of its action is not completely defined yet. So far, we know that it includes a regulation of Nedd4 family ligases. Simply stated, the work presents a new function of an unknown gene.

Your notes:

MARKÉTA SCHMIDT - ČERNOHORSKÁ (invited speaker)

C-terminal tail of tubulin mediate assembly of flagellar microtubule doublet in vitro

Schmidt-Cernohorska M, Zhernov I, Le Guennec M, Achek R, Demurtas D, Mouawad L, Lansky Z, Hamel V, Guichard P.

In centriole, microtubules are assembled in conserved structure of nine-fold blades in triplets that elongates as doublets to the axoneme and provide scaffold for intraflagellar transport. While tough structure of these blades has long been known, the mechanism by which they form remained unknown. The most recent work on flagellar doublets using cryo-electron tomography (Ichikawa et al., 2017) suggested two possible hypotheses. First, B-microtubule is bound to A-microtubule via small microtubule inner proteins penetrating through the microtubule wall, and second, both microtubules might be bound directly via unique side-to-side contacts. In order to reveal the nature and mechanism of microtubule doublet assembly, I employed a cell-free assay combined with limited proteolytic digestion. This approach showed for the first time that microtubule doublet is assembled solely from tubulin. In this talk I will explain our discovery of the inhibitory role of tubulin C-terminus.

Your notes:

ZUZANA CIHLÁŘOVÁ

The role of BRAT1 protein in DNA damage response and its links to neurodegeneration

Zuzana Cihlářová[1], Hana Hanzlíková[1,2], Grace Yoon[3] and Keith W. Caldecott[1,2]

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[3] Division of Clinical and Metabolic Genetics, and Division of Neurology, The Hospital for Sick Children, University of Toronto, Canada.

The human genome is continuously under attack from different agents leading to breakage of one or both strands of DNA. Our cells have developed mechanisms to rapidly repair these lesions, highlighting the importance of genetic integrity. DNA repair defects can lead to a variety of human genetic diseases, with pathologies including growth and developmental defects, immunodeficiency, predisposition to cancer and neurodegeneration.

BRAT1 (BRCA1-associated ATM activator-1) has been recently identified as a sensor of DNA double-strand breaks induced by ionizing radiation. However, the exact mechanism/s by which mutations in BRAT1 gene trigger neurodegeneration and to what extent DNA breaks contribute to this are unknown. In my project, I examine two patients with novel, unpublished, mutations in BRAT1 to address this question. My preliminary data indicate that, contrary to published literature, BRAT1 mutated patients do not exhibit a defect in ATM kinase activation during the DNA damage response. Based on my preliminary findings, I propose that BRAT1 may function during a different DNA damage response, such as DNA single-strand breaks repair for example, which is implicated strongly in neurodegenerative disease.

Your notes:

KARL HOFFMAN

Quantitative Analysis of Oriented Structures: From Noisy Image Data to Robust Topological Defects

The life sciences often deal with ensembles of elongated objects like actin filaments or stretched cells within a tissue.
The orientation of these objects plays an important role for active force generation, tissue structure and behaviour, etc.

I present an automated analysis tool to quantify the locally dominant orientation in image data on user-defined length scales as well as the orientation's sensitivity to noise.
In the resulting orientation fields, distinguished points called topological defects are of particular interest, because they fully determine the topology of the field and their motion is informative of active force generation.

While topological defects remain unaltered under some even drastic changes in the underlying field, in some unfortunate conditions already tiny fluctuation in an orientation field cause aberrant topological charges.
To overcome this problem and capture the uncertainties, I propose a robustness measure for topological defects indicating which orientation changes are admissible without alteration of the topological defect.

The robustness measure quantifies uncertainty of defect identification, helps in defect tracking, and anticipates defect dynamics from a single static image.

Your notes:

VERONIKA KRCHLÍKOVÁ

Antiviral activity of chicken tetherin

Krchlíková, Veronika [1], Farkašová, Helena [1,2], Hron, Tomáš [1], Koslová, Anna [1,3], Hejnar, Jiří [1], Elleder, Daniel [1]

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Tetherin, known as BST-2 or CD317, is an antiviral restriction factor stimulated by interferon which inhibits the release of newly formed viral particles from infected host cells. Tetherin has a broad range of activity against many different enveloped viruses including retroviruses. Orthologues of tetherin were identified in various mammalian species, including human, monkey, mouse, cow, dog, sika deer or bat. Recent work identified tetherin genes in non-mammalian vertebrates, including some avian species. In this study we determine the sequence of chicken tetherin and describe its antiviral activity against the avian sarcoma and leucosis virus (ASLV), an alpharetrovirus, which poses a threat to poultry industry. Exogenous expression of chicken tetherin in DF1 chicken cell line mediated by transient or stable transfection revealed strong inhibition of viral particle release into the medium. Endogenous tetherin in DF1 cells can be stimulated by chicken interferon-α (IFNα), which results in more than 100-fold higher tetherin expression, leading to a reduction of viral particle release in comparison with untreated cells. Moreover, after CRISPR-mediated knock-out of endogenous tetherin in DF1 cells, the IFNα-induced inhibition of virion release from chronically infected cells is nearly undetectable. In line with this, electron microscopy demonstrates activity of chicken tetherin against HIV particles in HEK293T cells, which are tethered to the cellular membrane. Furthermore, we have assembled tetherin sequences from a wide variety of avian species. Molecular phylogenetic analysis of tetherin sequences from orders Passeriformes and Galliformes show patterns of positive selection, consistent with virus-host arms race typical for antiviral genes. This study is the first to report of cloning and characterization of chicken tetherin. Overall, our data indicate that chicken tetherin is a part of the interferon-induced response against viral infection.

Your notes:

JULIUS LUKEŠ

Chromosome 21 Gain Is Dispensable for Transient Myeloproliferative Disorder (TMD) Development

Julius Lukes Jr.[1,2,] Petr Danek[4], Eliska Potuckova[1,2,] Julia Starkova[1,2], Jan Stary[2,3,] Jan Zuna[1,2,3], Jan Trka[1,2,3] , Jan-Henning Klusmann[5] and Marketa Zaliova[1,2,3]

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Transient myeloproliferative disorder (TMD) is a hematopoietic disease, characterized by a clonal proliferation of immature megakaryoblasts in the neonatal period occurring in approximately 10% of newborns with Down syndrome (DS). Rarely, TMD occurs in non-DS newborns but then it is associated with somatic trisomy 21 (tri21). Tri21 together with in-utero gained mutations in the GATA1 gene encoding a myeloid transcription factor are thus considered essential in TMD. Recently, we have identified a TMD with a typical manifestation and course in a newborn without DS/somatic tri21, which admits that tri21 is dispensable for TMD development. To elucidate the alternative TMD pathogenesis, we performed comprehensive genomic/transcriptomic profiling of this TMD case. We utilized high-density SNP array and whole exome and transcriptome sequencing (WES/RNAseq) to detect copy number changes, mutations and fusion genes. We did not find any aberrations on chromosome 21 and any fusion genes. Two focal intronic losses, likely representing benign germline variants, were found on chromosome X. In addition to 6 missense mutations affecting genes without established roles in hematopoietic disorders, we found in-frame deletions in the GATA1 and JAK1 genes. Both mutations are novel. The GATA1 D65_C228del mutation is predicted to result in an internally truncated protein – GATA1aber. Unlike GATA1s (resulting from GATA1 mutations in DS-TMD) which lacks the transactivation domain (TAD) but retains both Zinc fingers (ZF), GATA1aber lacks part of TAD and the N-terminal ZF. Nevertheless, we hypothesize that GATA1aber substitutes the pathogenetic role of GATA1s. The JAK1 gene encodes a non-receptor tyrosine-kinase engaged in the JAK/STAT signaling pathway. The identified mutation results in the loss of phenylalanine 636 (F636del), which is located in the pseudokinase domain and belongs to a conserved amino acid triad (F636-F575-V658) that is believed to mediate a structural switch controlling the JAK1 catalytic activity (Toms et al., 2013). JAK1 mutations are implicated in various hematological malignancies including acute megakaryocytic leukemia, and we hypothesized that JAK1 F636del co-operates with GATA1aber on TMD pathogenesis via deregulation of cytokine/growth factor signaling. We cloned the coding sequences of GATA1aber and JAK1 F636del and transfected them into a model cell line in which we confirmed the expression of both in-silico predicted proteins. Their subcellular trafficking was analogous to that of their wild type counterparts; GATA1aber was found in the nucleus and JAK1 F636del in both the nucleus and cytoplasm. Next, we assessed the kinase activity of JAK1 F636del. To distinguish auto- from trans-phosphorylation, we utilized the JAK1 F636del construct harboring an inactivating mutation of an ATP-binding site (K908G). The JAK1 F636del (but not JAK1 F636del + K908G) was autophosphorylated on Y1034/Y1035 and induced STATs phosphorylation both under steady-state conditions and following non-specific stimulation with PMA. However, at all studied time points all phosphorylation levels were lower compared to wild-type JAK1. Moreover, unlike constitutively active JAK1 V658I, JAK1 F636del did not confer IL3-independent growth to the murine B-cell progenitor cell line BAF3. Interestingly, the transforming potential of double-mutated JAK1 (JAK1 V658I + F636del) was enforced compared to JAK1 V658I. These data show that F636del does not lead to constitutive activation, but in the same time it is not functionally neutral. Next we introduced the JAK1 constructs into CD34+ cells from mouse bone marrow and fetal liver using lentivirus. Sorted cells where used for colony forming unit assays, which didn't reveal any difference in colony-forming capacity of JAK1wt versus F636del. To study the impact of JAK1 F636del on Gata1S background we used CRISPR/Cas9 gene editing tools to induce Gata1S expression in knock-in-Cas9 mouse fetal liver cells. After 3 weeks of selection and differentiation a pure population of Gata1S-positive cells was acquired (Labuhn et al., accepted for publication). There was no overgrowth of the Gata1S+JAK1F636del compared to Gata1S+JAK1wt cells during the 4 weeks of measurement. To conclude, we describe an extraordinary tri21-independent TMD, where two novel mutations affecting GATA1 and JAK1 were identified. Hypothesized driver JAK1 F636del mutation did not impact phenotype of employed in-vitro models, which questions its role in this particular tri21-independent TMD pathogenesis. Support: GAUK 86218, EHA Research Mobility Grant

Your notes:

MIROSLAV PŘIBYL

Interferon-regulated suprabasin is essential for stress-induced stem-like cell conversion and therapy resistance of human malignancies

Miroslav Pribyl1, Sona Hubackova[1,2] , Lenka Kyjacova[1,$], Alena Moudra[1], Rastislav Dzijak[1], Barbora Salovska[1], Hynek Strnad[3], Vojtech Tambor[4], Terezie Imrichova[1], Jiri Svec[5,6], Pavel Vodicka[7], Radka Vaclavikova[8], Lukas Rob[9] , Jiri Bartek[1,10,11] and Zdenek Hodny[1]

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Radiation and chemotherapy represent standard-of-care cancer treatments. However, most patients eventually experience tumour recurrence, treatment failure and metastatic dissemination with fatal consequences. To elucidate the molecular mechanisms of resistance to radio- and chemo-therapy, we exposed human cancer cell lines (HeLa, MCF-7, and DU145) to clinically-relevant doses of 5-azacytidine or ionizing radiation and compared the transcript profiles of all surviving cell subpopulations, including low-adherent stem-like cells. Stress-mobilized low-adherent cell fractions differed from other survivors in terms of deregulation of hundreds of genes, including those involved in interferon response. Exposure of cancer cells to interferon-gamma but not interferon-beta resulted in the development of a heterogeneous, low-adherent fraction comprised of not only apoptotic/necrotic cells, but also live cells exhibiting active Notch signalling and expressing stem-cell markers. Chemical inhibition of MEK or siRNA-mediated knockdown of Erk1/2 and IRF1 prevented mobilization of the surviving low-adherent population, indicating that interferon-gamma-mediated loss of adhesion and anoikis resistance required an active Erk pathway interlinked with interferon signalling by transcription factor IRF1. Notably, a skin-specific protein suprabasin (SBSN), a recently identified oncoprotein, was among the top scoring genes upregulated in surviving low-adherent cancer cells induced by 5-azacytidine or irradiation. SBSN expression required the activity of the MEK/Erk pathway, and siRNA-mediated knockdown of SBSN suppressed the low-adherent fraction in irradiated, interferon-gamma- and 5-azacytidine-treated cells, respectively, implicating SBSN in genotoxic stress-induced phenotypic plasticity and stress resistance. Importantly, SBSN expression was observed in human clinical specimens of colon and ovarian carcinomas, as well as in circulating tumour cells and metastases of the 4T1 mouse model. The association of SBSN expression with progressive stages of cancer development indicates its role in cancer evolution and therapy resistance.

Your notes:

KRYŠTOF ŠTAFL

Resurrection of syncytins: fossils from the 3rd floor

Kryštof Štafl, Kateřina Trejbalová, Eliška Gáliková, Martin Trávníček, Ľubomíra Pecnová, Dana Kučerová & Jiří Hejnar

Laboratory of Viral and Cellular Genetics, Institute of Molecular Genetics of the CAS, Prague

During evolution, our genome has caught a lot of viral sequences that are proofs of previous defeats in host-pathogen fights. Most of these fossils are already mutated and do not encode functional proteins. But there are some exceptions and one of them are syncytins. Syncytins originally served as envelope glycoproteins of retroviruses. However they lost the function of mediating virus entry into the host cell, they perfected their fusion capacity and became a critical component in development of mammalian placenta. In humans, two syncytins are involved in this process: Syncytin-1 and Syncytin-2. Syncytin-1 belongs among gammaretroviral envelope glycoproteins of HERV-W family. The provirus was integrated approximately 25 million years ago.

We wondered whether it was possible to restore Syncytin-1 original function on the surface of infectious virus. We used a replication competent alpharetroviral genome with a fluorescent marker and modified it. We optimized retroviral splicing and shortened cytoplasmic tail of Syncytin-1 that regulates protein function. The modified vector consists of strong LTR sequences derived from Myeloblastosis-associated virus, gag and pol derived from Bryan high-titer strain of Rous sarcoma virus, syncytin-1 as the envelope gene, and dsRed fluorescent marker.

After transfection of chicken DF-1 cell line with this vector, an infectious virus was produced. This virus was able to infect DF-1 cells stably expressing human ASCT2 receptor and also various human cell lines, e.g. HEK293T. It reached the titer of ±1×104 IU/mL measured by flow cytometry.

These results demonstrate the compatibility of alpharetroviral core with gammaretroviral envelopes. We are using this approach for studies of other endogenous retroviruses and their interactions with host cell.

Your notes:

OKSANA TSYKLAURI

Bardet-Biedl Syndrome protein complex in the adaptive immune system

O. Tsyklauri, V. Niederlová, M. Huranová, O. Štěpánek
Laboratory of Adaptive Immunity, Institute of Molecular Genetics of the ASCR, v. v. i.

BBSome is a transport protein complex, which is important for normal formation and functioning of primary cilium. Mutations in BBSome subunits cause multiorgan disease called Bardet-Biedl Syndrome (BBS), major symptoms of which include rod–cone dystrophy, polydactyly, obesity, learning difficulties, abnormalities of the genitalia and renal dysfunction. Unexpectedly, patients' data analysis showed increased risk of autoimmune diseases in BBS cohort. In order to investigate the role of the BBSome in the immune system, BBS4 KO murine strain was generated. In a line with previously described mouse models of BBS, BBS4 KO mice are smaller than their littermates after birth, but due to high food consumption they gain weight faster and become obese. Further examination reveals that BBS4 KO mice have increased level of leptin, and more interestingly, increased level of proinflammatory cytokine IL-6. Moreover, BBS4 KO mice showed significantly lower percentage of the mature B cells in the bone marrow, but higher percentage of early B cell progenitors (Pre-pro-B, Pro-B, and Pre-B cells) which might indicate B cell developmental block. However, immune system of hematopoietic-specific conditional knock-out mouse seems to be unaffected. The results obtained might reveal the role of obesity in the immune phenotype of BBS4 KO mice, which also might be relevant for patients.

Your notes:

JULIE VACKOVÁ

Abrogation of IFN-γ signalling in tumour cells does not necessarily worsen sensitivity to PD-1/PD-L1 blockade

Julie Vacková, Ingrid Poláková, Adrianna Grzelak, Michal Šmahel
Faculty of Science, Charles University, BIOCEV, Prague, Czech Republic (julie.vackova@natur.cuni.cz)

Programmed cell death protein 1 (PD-1)/PD-1 ligand 1 (PD-L1) interaction blockade is used for therapy of several cancer types with efficacy about 30%. To maximise the number of responders, broadening of predictive biomarkers is necessary. Besides defective interferon (IFN)-γ signalling in tumour cells, low level of PD-L1 and/or major histocompatibility complex class I (MHC-I) expression have been found in non-responders. Though IFN-γ is considered to have a major role in enhancement of the PD-L1 or MHC-I expression in tumour cells, other cytokines might have a similar effect. Therefore, the aim of our study was to assess if a loss-of-function mutation in IFN-γ receptor 1 (Ifngr1) in tumour cells can interfere with anti-PD-L1 cancer therapy. For this purpose we used mouse oncogenic TC-1/A9 cell line (Šmahel et al., 2003) with reversibly downregulated PD-L1 and MHC-I expression. By the CRISPR/Cas9 we generated cells with deactivated Ifngr1 (TC-1/A9/dIfngr1). We found unreduced expression of PD-L1 and MHC-I in tumours induced by TC-1/A9/dIfngr1. Moreover, Ifngr1 deactivation did not reduced tumour sensitivity to anti-PD-L1 in vivo. Consequently, we detected cytokines in tumours that are potential inducers of PD-L1 and/or MHC-I (IFN-γ, IFN-α, IFN-β, interleukin (IL)-1α, tumour necrosis factor-α, IL-6, IL-27, chemokine CCL-2, granulocyte-macrophage colony stimulating factor and epidermal growth factor) by LEGENDplex assay and we demonstrated in vitro the significant effect of IFN-α and IFN-β and a mild contribution of TNF-α. Our results suggest that the only abrogation of IFN-γ signalling is not sufficient for PD-L1 and MHC-I reduction in tumour cells and it should not to be a contradiction for anti-PD-L1 cancer therapy.

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Šmahel, M. et al. (2003) Vaccine 21. 1125-1136

Your notes:

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