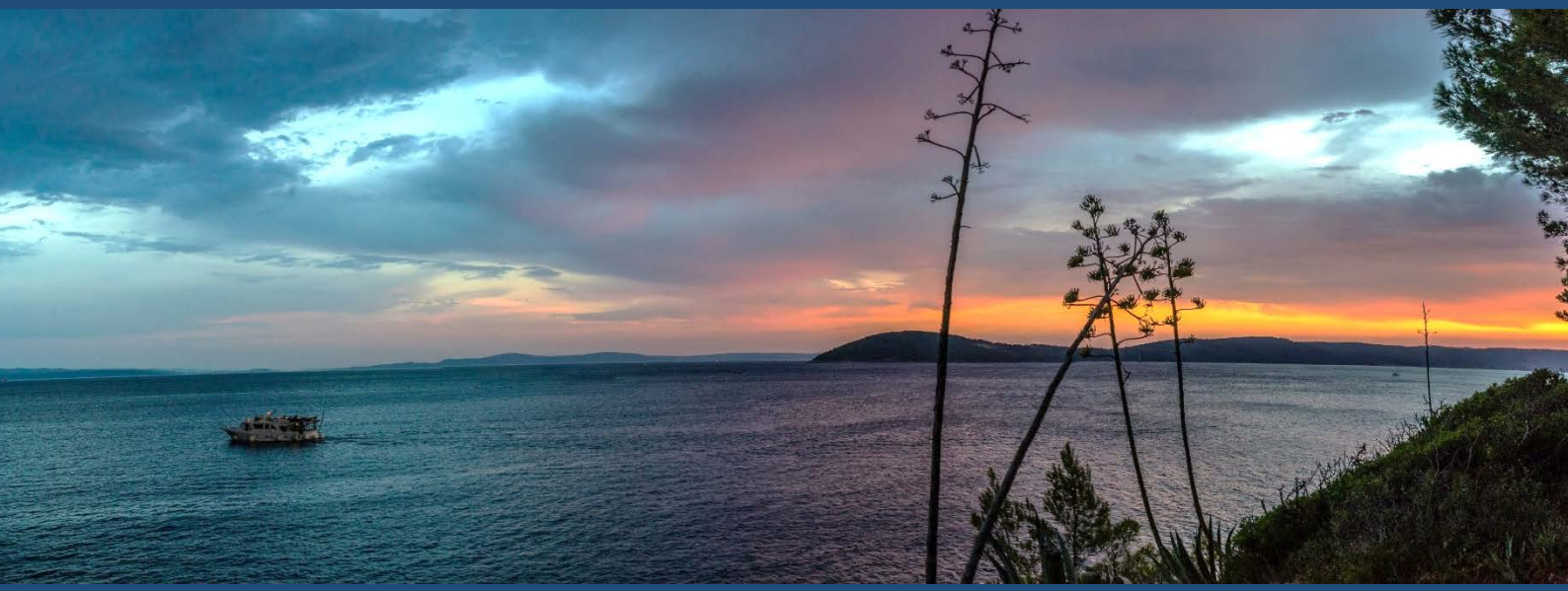
Advances in Biomedical Research III



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Contents

OPENING KEYNOTE

INTRODUCTION of the Speaker by IGOR ŠTAGLJAR

Regulation of microtubule dynamics: seeing proteins and drugs in action

Anna Akhmanova

Cell Biology, Department of Biology, Faculty of Science,
Utrecht University, the Netherlands

Tight control of microtubule dynamics is essential for many cellular processes, including cell division, migration and morphogenesis. Using in vitro reconstitution experiments, we explored the detailed mechanisms of such regulation by proteins that interact with the plus ends of cytoplasmic and centriolar microtubules. Furthermore, we used assays with fluorescent analogues of microtubule-stabilizing and destabilizing agents to directly visualize their effects on microtubule polymerization. We found that a single molecule of a microtubule-depolymerizing drug bound to the microtubule tip was sufficient to trigger a catastrophe, whereas microtubule rescue and stabilization required local accumulation of multiple drug molecules. Our results illustrate the diversity and complexity of mechanisms controlling microtubule growth and organization.

Session "Cancer I"

The Human Transportome

Giulio Superti-Furga[1,2,3], Ariel Bensimon[1], Alvaro Ingles-Prieto[1,3], Daniel Lackner[1,3], Andre Müller[1,3], Gernot Wolf[1,3], Ruth Eichner[1], Patrick Essletzbichler[1], Ulrich Goldmann[1,3], Leonhard X. Heinz[1], Mattia Pizzagalli[1], Vojtech Dvorak[1] , Tabea Wiedmer[1,3], Konstantinos Papakostas[1], Vitaly Sedlyarov[1,3], Manuele Rebsamen[1], Leonhard X. Heinz[1], Enrico Girardi[1]

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[3]RESOLUTE IMI consortium

Cells need to exchange nutrients, ions and metabolic products across membranes to survive and exert their function. Mitochondrial respiration, alcohol dependence, a sense of happiness, the colour of skin and hair, proper bone development, liver and kidney function and the drugs bioavailability are only some of the physiological processes and properties that heavily depend on the action of Solute Carrier Proteins (SLCs), the largest supergroup of transporters encoded in the human genome. Despite this fundamental physiological importance, it turns out that the majority of SLCs are still uncharacterized in terms of transport specificity and function. Moreover, we expect that the SLCs should be functionally integrated by mechanisms and principles that are not yet known but important to discover. We therefore have started to map systematically the protein and genetic interactions of human SLCs as key step to characterize key properties of the human transportome. This required the development of tailored approaches as: 1) traditional proteomics protocols are not very suitable for proteins with an unusual high proportion of membrane-embedded residues located in a variety of different cellular membranes and 2) there is a strong element of redundancy in SLC function. We will report on the initial findings. On top of these systematic approaches, we have specifically investigated the role of SLCs in drug transport, specific immune cell function and cancer cell survival.
Overall, this allowed for an integrated approach that not only is expected to lead to first functional annotation for a variety of human transporters but also insight into the principles governing metabolic empowerment of cellular processes. Dependencies of processes on specific SLCs is likely to offer unique pharmacological opportunities of disease modulation due to the exquisite drugability of this class of proteins.

Targeting protein-protein interactions to identify anticancer drugs

Marion Wiesmann, PhD

Novartis, Switzerland

Protein-protein interactions (PPI) are pivotal in a wide range of biological functions and frequently contribute to the oncogenic transformation of cells on their path to becoming cancer. PPIs are characterized by surface interactions of two or more proteins required for the determination of cell fate and have always been attractive targets for cancer drug discovery. The aim is to identify molecules that specifically interrupt a PPI to modify cell growth by inducing cell cycle arrest or apoptosis. There are successful examples of targeting the BCL2 family and the P53/MDM2 interaction with low molecular weight inhibitors resulting in anti-proliferative effects. The challenges to identify attractive PPIs and molecules to block specific interactions will be discussed.

Tumor Cell Plasticity as a Challenge for Targeted Therapies

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The Wnt and Hedgehog signaling pathways play critical roles during embryonic development and in adults through modulation of proliferation, cell migration and differentiation. Inappropriate activation of the these pathways through mutations in a resident stem cell can result in tumorigenesis, in particular in the gastrointestinal tract and in the skin. Targeting these oncogenic pathways or the stem cells compartment driving the tumorigenic process represent therapeutic opportunities for the treatment of these tumor types. However, we also uncovered that plasticity between cell compartments involved in tissue repair and regeneration processed poses new challenges for the efficacy of these therapeutic approaches in cancer.

Toward precision medicines with next-generation EGFR inhibitors

Igor Stagljar[1,2]

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The Receptor Tyrosine Kinase (RTK) family of transmembrane receptors has been of great clinical interest due to its role in many diseases, and, historically, therapeutic molecules directed against RTKs have been identified using *in vitro* kinase assays. In particular, there is an outstanding need for compounds that can specifically inhibit the activity of mutated but not the normal RTKs. I willl describe MaMTH-DS, a new live-cell, small-molecule screening platform, based on the Mammalian Membrane Two-Hybrid (MaMTH), for identification of compounds targeting the functional protein interactions of RTKs. We applied MaMTH-DS to screen a small molecule library against an oncogenic Epidermal Growth Factor Receptor (EGFR) mutant resistant tyrosine kinase inhibitor therapeutics. We identified four promising mutant-specific compounds, two of which would not have been identified by conventional *in vitro* kinase assays. One of these compounds targets mutant EGFR signalling via a novel mechanism of action, distinct from classical TKI inhibition. Our results demonstrate the utility of our MaMTH-DS platform as a powerful complement to traditional drug screening approaches.

Dynamic view of receptor tyrosine kinase signaling specificity

Lemmon, Mark A

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The 58 growth factor receptor tyrosine kinases (RTKs) provide a useful palette of signaling mechanisms used by receptors with a single transmembrane domain. Although initial studies of examples such as the epidermal growth factor receptor (EGFR) suggested a simple ligand-induced dimerization mechanism, it is now clear that RTKs are much more complex than this, with substantial diversity across the superfamily. Early work with *Drosophila* and *C. elegans* EGFRs suggested models for allosteric regulation of dimeric receptors. More recently, we explored how a given growth factor receptor like EGFR can promote different cellular outcomes depending on how it is activated. EGF provokes different signaling responses through EGFR than are seen with other ligands such as epiregulin and epigen. To investigate the origin of these differences, we solved crystal structures of the EGFR extracellular domain bound to epiregulin and epigen, which revealed that different ligands induce EGFR dimers with different structures. Further analysis suggested that epiregulin and epigen induce weaker EGFR dimers than does EGF. We confirmed that this is the case for the isolated extracellular region of the receptor, and – using FRET and single particle tracking studies – for the membrane-bound intact receptor, arguing that epiregulin and epigen induce weaker (and shorter-lived) activated receptor dimers than does EGF. Comparison with other EGFR ligand suggests that this is likely to reflect dimer structure rather than ligand-binding affinity, since amphiregulin (a low-affinity EGFR ligand) induces strong dimers. Paradoxically, the shorter-lived EGFR dimers induced by epiregulin or epigen give rise to a more sustained signal when monitored at the level of EGFR autophosphorylation or Erk activation. We hypothesize that this reflects a kinetic proofreading effect in which short-lived receptor dimers fail to activate key negative feedback events, which may include trafficking/internalization and/or dephosphorylation. The altered EGFR signaling dynamics is propagated to the level of Erk signaling, and allows epiregulin and epigen (but not amphiregulin) to induce sustained Erk signaling and differentiation of cancer cells – whereas EGF induces proliferation of these same cells. Recent studies with other receptors, and mutated EGFR variants, suggest that this is a general phenomenon, linking signaling specificity to receptor activation kinetics and dynamics of network control.

Explaining Disease Models by Integrated Networks

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To fathom disease processes, we need to systematically integrate diverse omics data and link them using relevant annotations and networks. What may appear as noise in large datasets, could be a hidden signal, explainable by some existing relationships, obtained from these networks. We highlight approaches in integrative computational biology that identify and characterize such signals.
Across diseases, many deregulated genes reside on chromosomal regions with DNA copy number alteration opposite to the expression changes, resulting in paradoxical signal. Traditionally treated as noise, integrative analysis identified signal in such data. Specifically, most of these paradoxes appear to be a consequence of microRNA-mediated control of mRNA levels.
Using TCGA-LUAD data we have validated 70/85 identified paradoxical genes. Notably, 41/70 genes validated as prognostic on three independent datasets. We identified 24 consistently-deregulated microRNAs in LUAD, and 19 of these microRNAs explain differential expression of the paradoxical genes using TCGA data.
Our results highlight complex deregulation of prognostic genes in LUAD, and the need to integrate diverse data using high quality curated networks. Application of graph theory, data mining, machine learning and advanced visualization enables data-driven, precision medicine. In turn, this helps building explainable models for complex diseases.

This research was funded in part by Ontario Research Fund (#34876), Canada Foundation for Innovation (#225404, #30865), Natural Sciences Research Council (#104105), and Canada Research Chair Program (#225404).

Interplay of therapy and tumor microenvironment in human colorectal cancer

Bergmann M

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Advances in tumor immunology now calls for a novel understanding of the immunological consequence of standard cancer therapy. At the same time the expression of proteins mediating immunogenic cell death should have a positive predictive and prognostic impact. This molecular understanding of the disease will allow a more rational design of immunomodulating drugs and standard therapy.
Murine models clearly indicate that irradiation induced DNA damage can stimulates the innate and adaptive immune system. However, there is little evidence that irradiation leads to apiscopal effects in the clinic. We here show that neoadjuvant irradiation applied in rectal cancer patients induces the polarization of tumor associated M2-like macrophages to an M1-like phenotype in surgical resection specimen. Ex vivo primary cultures and organotypic assays were used to better dissect this re-polarization. Using ex-vivo cultures we further show that the shift of irradiation-induced macrophage polarization could be mediated by exosomes. Those data clearly indicate that radiotherapy induced-DNA damage using 25 Gy actively stimulates the innate immune system. This pro-inflammatory effect of radiotherapy might now be complemented by immunomodulating drugs targeting the adaptive part of the immune system.
In contrast, when analyzing the prognostic and predictive impact of spontaneous DNA damage and associated pathways in colorectal liver metastases we demonstrate that DNA damage had a strong negative impact on response to neoadjuvant applied chemotherapy but also on disease free and overall survival. Spontaneous DNA damage was not associated with an induction of the innate immune response in this setting and inversely correlated with infiltrates of CD8+ or CD45RO+ cells . This calls for a more detailed understanding of spontaneous DNA damage induced pathways in colorectal liver metastases as their blockade might enhance prognoses.

Stary et al., submitted
Laengle et al., Theranostics 2018

Session "Cancer II"

Targeting RIP kinases provides therapeutic benefit in inflammatory diseases

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RIP1 kinase is a critical mediator of multiple signaling pathways that regulate inflammatory responses and cell death. RIP1 regulates caspase-dependent apoptotic and caspase-independent necroptotic cell death. In vivo, the kinase activity of RIP1 has been implicated in tissue damage and numerous inflammatory, neurodegenerative and oncogenic diseases. Using RIP1 kinase-dead knockin (KD KI) mice and the murine-potent RIP1 kinase inhibitor, GNE684, we show that RIP1 inhibition can efficiently block collagen antibody-induced arthritis, skin inflammation caused by Sharpin mutation, and colitis triggered by intestinal NEMO deficiency. Conversely, genetic or pharmacological RIP1 inhibition had no effect on tumor growth or survival in Kras mutant genetically-engineered pancreatic tumor models. Similarly, RIP1 kinase inactivation or RIP3 gene ablation did not impact lung metastases in a B16 melanoma model. Together these data emphasize the instrumental and protective role for RIP1 kinase inhibition in inflammatory disease, as compared to its tangential relevance for tumor progression and metastases.

Protein ADP-ribosylation in Health and Disease

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Besides its role as electron transporter within the mitochondria, nicotinamide adenine dinucleotide (NAD^+) is an important co-factor for enzymatic reactions, such as ADP-ribosylation. Protein ADP-ribosylation is a reversible posttranslational modification (PTM) that results from the transfer of the ADP-ribose (ADPr) moiety from NAD^+ to specific amino acid residues or to ADPr itself. The covalent attachment of ADPr is governed by ADP-ribosyltransferases (ARTs), it is recognized by ADPr-binding domains and removed by ADP-ribosylhydrolases (ARHs). Transferring one ADPr to the target protein is called mono-ADP-ribosylation (MARylation). These initial modifications can serve as the acceptor sites for poly-ADP-ribosylation (PARylation) where additional ADPr moieties are appended to the MARylated protein to form ADPr chains. This PTM can affect the structure and the function of the modified proteins.

ADP-ribosylation is predominantly induced during conditions of cellular stress or danger signaling. The development of ADP-ribosylation inhibitors (i.e. PARPi) was originally initiated by the pharmaceutical industry based on the early discovery that protein ADP-ribosylation plays a role in genotoxic stress signaling (via ARTD1, also known as PARP1). These compounds are currently used as stand-alone therapeutics, or in combination with other cancer therapy drugs, to help potentiate the cytotoxic effects. Studies from us and others have revealed that ADP-ribosylation inhibitors also have a strong anti-inflammatory effect in several inflammation-associated disease models (e.g. atherosclerosis and metabolic dysfunction).

One of the greatest needs and challenges within the ADP-ribosylation signaling field is to further improve the discovery tools, e.g. for visualization, target identification and ADP-ribose (ADPr) acceptor site localization for different cellular compartments to enable researcher to uncover the functional meaning of this modification. Due to recent technological breakthroughs, we are now in a unique position to address so far unanswered biological questions. These breakthroughs include the establishment of ADP-ribosylation-specific mass spectrometry (MS) workflows (i.e. the identification and quantification of modified proteins and accurate prediction of ADP-ribose acceptor site) or the chemical synthesis of ADP-ribosylated peptides based on identified endogenous modified peptides.

Together these findings allow us to start resolving the functional implications of protein ADP-ribosylation in different physiological and pathological processes as well as the potential to target them therapeutically.

Microbiota-immune cell crosstalk in the progression of multiple myeloma

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Clonal proliferation of plasma cells in the bone marrow (BM), monoclonal protein in biological fluids and organ dysfunction characterize multiple myeloma (MM), which is often preceded by asymptomatic smoldering MM (SMM). Whereas 10% of SMM patients each year progress to MM, reliable criteria to predict disease progression are lacking, also because of poor knowledge on the biological and molecular characteristics of the BM microenvironment in SMM patients. We have hypothesized that the nature of the immune response during the SMM phase could already have profound consequences in determining whether neoplastic plasma cells are eliminated or allowed to expand. Thus, we have implemented multiparameter flow cytometry, immunohistochemistry, ELISA, gene knock out and *in vitro* assays to investigate the BM microenvironment during disease progression in Vk*MYC mice, in which the activation of the transcription factor *MYC*, whose locus is found rearranged in half human MM tumors including SMM, occurs sporadically through the exploitation of the physiological somatic hypermutation process in germinal center B cells. We have found in Vk*MYC mice that disease appearance and progression were characterized by substantial modifications of the BM microenvironment, including accumulation of selected populations of the innate and adaptive immunity, release of specific cytokines and chemokines, which promoted phenotypic skew of the immune cells, angiogenesis and plasma cell survival and proliferation (1). We also obtained evidence that similar events occur in those SMM patients that more rapidly progress to MM (1). Finally, we have found an unexpected link between commensal bacteria and immunity in the gut and in the BM of Vk*MYC mice. More in details, we collected evidence that *Prevotella heparinolytica* promotes the differentiation of Th17 cells colonizing the gut and migrating to the bone BM of transgenic Vk*MYC mice, where they favor progression of MM. Lack of IL-17 in Vk*MYC mice, or disturbance of their microbiome delayed MM appearance. Similarly, in smoldering MM patients, higher levels of BM IL-17 predicted faster disease progression. IL-17 induced STAT3 phosphorylation in murine plasma cells, and activated eosinophils. Treatment of Vk*MYC mice with antibodies blocking IL-17, IL-17RA and IL-5 reduced BM accumulation of Th17 cells and eosinophils and delayed disease progression (2). Altogether, our data suggest that the environment and the BM microenvironment substantially impact on the history of the disease, and specific immunomodulating strategies can be designed to arrest progression to symptomatic MM.

This work has been supported by Associazione italiana per la Ricerca sul Cancro (AIRC IG21808).

(1) Calcinotto A. et al. Oncoimmunology. 2015 May 7;4(6):e1008850.
(2) Calcinotto A. et al. Nat Commun. 2018 Dec 3;9(1):4832.

Systems biology approaches to investigate cancer signaling

Julia Petschnigg[1], Noël Malod-Dognin[2], Nataša Pržulj[2], and Robin Ketteler[1]

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Signal transduction pathways are often derailed in cancer cells to adapt to the specific needs of a fast growing tumour and represent one of the most promising avenues for anti-cancer drug development. Despite significant advances in our understanding of basic signal transduction pathways, there is still a lack of understanding how cancer signaling is hi-jacked by tumour cells in order to facilitate their specific needs. We have taken two systematic approaches to identify re-wired signaling nodes in cancer: an unbiased cDNA expression screen to identify novel interaction partners of oncogenic forms of the Epidermal Growth Factor receptor (EGFR) (1), and siRNA-based validation of a computational method to predict cancer related genes in multiple cancers (2).

First, we were interested in identifying protein interaction partners that specifically interact with oncogenic forms of the Epidermal Growth Factor Receptor. We used a systematic genome-wide screening approach combining high-content imaging and a mammalian membrane two-hybrid protein-protein interaction (MaMTH) method to identify 8 novel interaction partners of EGFR, out of which 4 strongly interacted with oncogenic, hyperactive EGFR variants. One of these, Tacc3, stabilizes EGFR on the cell surface, which results in an increase in downstream signaling via the MAPK and AKT pathway. Depletion of Tacc3 from cells using shRNA knockdown or small molecule targeting reduces mitogenic signaling in non-small cell lung cancer cell lines, suggesting that targeting Tacc3 has potential as a new therapeutic approach for non-small cell lung cancer.

In addition, we used a novel computational model for the identification of cancer driver genes. The model integrates three tissue-specific molecular interaction networks of four cancers to identify the most rewired genes in cancer cells compared to controls. Using siRNA screening, we biologically validated that the identified genes have a role in these four cancers by cell viability assays and propose that they may be novel biomarkers of these cancer types.

In summary, our approaches allow the systematic identification of novel signaling nodes that are specifically required for signaling of mutant oncogenic EGFR, as well as a more general computational method for predicting cancer driver genes that can be applied to a wide range of cell models and cancer types.

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(1) Petschnigg et al. J Mol Biol 429:280-294, 2017.
(2) Malod-Dognin et al. Nature Communications 10:805, 2019.

Competitive Inhibitors of Ras Effector Binding

Svenja Wiechmann[1], Pierre Maisonneuve[2], Britta M. Grebbin[3], Henner F. Farin[3], Frank Sicheri[2] and Andreas Ernst[1]

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The small GTPases H, K and NRAS are molecular switches transmitting signals from growth factor receptor tyrosine kinases to transcription factors. In cancer, mutations often cause constitutive activation of Ras leading to an uncoupling of MAP kinase activation from receptor signaling. Consequently, their prominent role in regulating cellular proliferation in healthy and diseased cells has made them a major focus in the last decades for the development of anti-proliferative drugs albeit with limited success. In our study, we have engineered high affinity variants of the Rasbinding domain (RBD) of C-Raf kinase that bind with improved affinity the effector binding site of activated Ras. Structural analysis of the mutated RBDs in complex with GTP bound HRas shows that the improved binding is a consequence of a subtle rewiring of the hydrogen bond pattern between the modified RBD variant and the effector binding site of Ras. To study intracellular effects and specificity of the engineered RBD variants, we established several cancer and primary cell lines that allow a doxycycline dependent expression of HA-tagged fusion proteins. Coupled Co-IP/Mass-spectrometry experiments demonstrate that the engineered variants efficiently bind endogenous levels of Ras in HCT116 cells and lead to a specific, up to 5000-fold enrichment of KRasG13D-GTPase peptides in comparison to RBD wt binding. Importantly, cell-proliferation assays show that the variants inhibit cellular growth and block MAPK and PI3K signaling in several cancer cell lines and induce apoptosis in HCT116 cells. In order to translate the RBDvs to a clinical research question, we transduced several patient-derived cell lines from resected colon tumors that form 3D organoid intestinal crypts. Expression of the inhibitory RBD variants leads to a reduced growth of intestinal crypts revealing that our
engineered high affinity RBD variants are also functional in patient derived samples. In summary, the engineered RBD variants are a new class of Ras inhibitors that directly compete with effector binding leading to apoptosis in cancer cells. Importantly, the RBD variants are also effective in patient-derived 3D cancer tissue indicating that Ras-signaling remains one of the prime intracellular targets to therapeutically interfere with the proliferation of cancer cells.

L3MBTL3 is a novel suppressor of medulloblastoma tumorigenesis

Honglai Zhang[1], Ester Calvo Fernandez[1], Claire Peabody[1], Rork Kuick[2], Sung-Soo Park[1], Thomas Saunders[3], Sandra Camelo-Piragua[1], Jean-François Rual[1]

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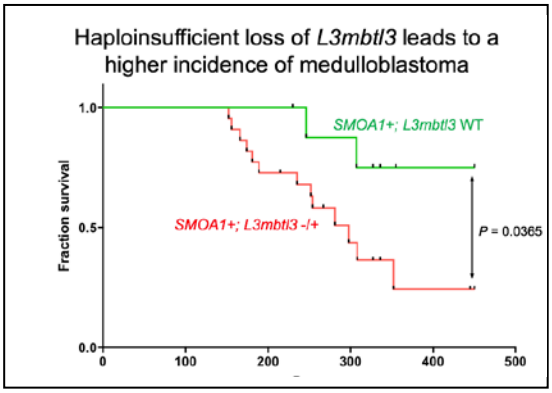
Background: Medulloblastoma is the most common malignant brain tumor of childhood. Therapeutic approaches to medulloblastoma have led to significant improvements but are achieved at a high cost to quality of life. Alternative therapeutic approaches are needed and molecular stratification of patients with medulloblastoma has yet to be routinely implemented in the clinic. The Notch pathway governs cell proliferation in many biological contexts, including SHH and Group#3 medulloblastoma tumorigenesis. Using our proteomic platform, we discovered an interaction between RBPJ, a key co-factor of Notch for the modulation of Notch signals, and L3MBTL3, a methyllysine reader. L3MBTL3 is recruited by RBPJ on chromatin at the enhancers of Notch/RBPJ target genes to repress their expression. Deletions of the *L3MBTL3* locus are observed in patients with WNT and Group#3 medulloblastoma and expression of L3MBTL3 in the SHH medulloblastoma-derived cell DAOY inhibits cell growth, suggesting a putative tumor suppressor role for L3MBTL3 in medulloblastoma.

Methods: To investigate the putative role of L3MBTL3 as a suppressor of medulloblastoma tumorigenesis, we used our *L3mbtl3* KO mouse in combination with a genetically engineered mouse model of SHH medulloblastoma, i.e. ND2:SmoA1, in a survival analysis.

Results: Our survival analysis validated *in vivo* our hypothesis that L3mbtl3 is a tumor suppressor in this disease context. Indeed, our data show that [*ND2:SmoA1; L3mbtl3+/-*] mice have a significantly lower survival rate than *ND2:SmoA1* mice (*P* = 0.0322; Log-rank test). Hence, the RBPJ-L3MBTL3 interaction is at the heart of a molecular mechanism governing the repression of Notch/RBPJ target genes and malfunction of this molecular mechanism likely contributes to L3MBTL3's tumor suppressor role in medulloblastoma through aberrant "de-repression" of Notch/RBPJ target genes.

Conclusion: L3MBTL3 is a novel suppressor of medulloblastoma tumorigenesis. Our discovery provides insights into the role of the L3MBTL3 in medulloblastoma that could be harnessed in the future for the therapeutic benefit of patients with medulloblastoma.



Session "Neuroscience and trafficking"

The link between gut infection, autoimmunity and Parkinson's disease

Matheoud D, Cannon T, Voisin A, Penttinen AM, Ramet L, Fahmy A, Ducrot C, Laplante A, Bourque MJ, Zhu L, Cayrol R, Le Campion A, McBride HM, Gruenheid S, Trudeau LE and Desjardins M*

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Parkinson's disease (PD) is a neurodegenerative disorder with motor symptoms linked to the loss of dopaminergic neurons (DNs) in the substantia nigra compacta (SNc). Although the mechanisms triggering the loss of DNs are unclear, mitochondrial dysfunction and inflammation are viewed as playing a key role. An early-onset form of PD is associated with mutations in the *PINK1* kinase and *PRKN* (*Parkin*) ubiquitin ligase genes. While PINK1 and Parkin have been shown to drive mitophagy and clearance of damaged mitochondria in cultured cells, recent evidence obtained using knock-out and knock-in mouse models have led to contradictory results regarding the contribution of PINK1/Parkin to mitophagy *in vivo*. We recently showed that PINK1 and Parkin play a role in adaptive immunity by repressing mitochondrial antigen presentation (MitAP), suggesting that autoimmune mechanisms participate in the aetiology of PD. Here, we present evidence that intestinal infection with Gram-negative bacteria in *Pink1*$^{-/-}$ mice engages MitAP and autoimmune mechanisms eliciting the establishment of cytotoxic mitochondria-specific CD8+ T cells in the periphery and in the brain. Remarkably, infection in these mice leads to the emergence of motor impairment, reversed by L-DOPA treatment, accompanied by a sharp decrease in the density of dopaminergic axonal varicosities in the striatum. These data support a role for PINK1 as a repressor of the immune system and provide a new pathophysiological model where intestinal infection acts as a triggering event in PD, highlighting the relevance of the gut-brain axis in the disease.

Mitochondrial Connectivity Landscape During Neuronal Reprogramming and Its implication in Neurodegeneration

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Mitochondrial protein assemblies undergo alterations during neurogenesis, a complex process vital in brain homeostasis and disease. Yet which mitochondrial macromolecular assemblies remodel during differentiation remains unclear. Here, using mass spectrometry-based cofractionation profiles, we generated mitochondrial interaction maps of human pluripotent embryonal carcinoma stem cells and terminally differentiated neurons. The resulting networks, encompassing 6,442 high-quality associations among 600 human mitochondrial proteins, revealed widespread changes in mitochondrial interactions. By leveraging the high-quality mitochondrial protein-protein interaction (PPI) network, we provide evidence that an orphan mitochondrial protein, which has a rare heterozygous 3'UTR variant in patients with mitochondrial respiratory chain deficiencies, functions as an assembly factor, causing a marked reduction in respirasome levels when disrupted. Our results provide new insights into the dynamic reorganization of mitochondrial networks during neuronal differentiation, and highlight novel mechanisms for mitochondrial proteins in respirasome and mitochondrial diseases.

Applying Barcoded Split TEV Assays to Dissect Molecular Specificities of ERBB4 Directed Kinase Inhibitors

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Most pharmaceutical companies have ceased drug discovery programs for psychiatric diseases given many costly failures in the last decades. Nonetheless, there is still a strong demand in novel pharmaceutical approaches since many symptoms e.g. of schizophrenia (SZ) cannot be targeted adequately. Human genetics and basic research have provided a plethora of genetic risk factors for SZ which seem to converge on a set of molecular and cellular pathways including the control of gene expression, synapse function and excitation-inhibition (E/I) balance. The Neuregulin1-ERBB4 signaling pathway has been shown to be implicated in the control of cortical E/I balance and thus represents a promising target for the development of novel drugs. Therefore, we have developed high-throughput screening compatible cellular assays based on the split TEV system. We used these assays to screen (a) collections of approved drugs in a repurposing approach and (b) huge compound libraries with high structural variety to identify novel molecular entities. In the repurposing approach (a), we identified Spironolactone as a modulator of the Neuregulin1-ERBB4 signaling pathway and showed in a pre-clinical study with dedicated transgenic mouse models that Spironolactone ameliorated cognitive deficits likely by rescuing E/I dysbalances. These results led to the initiation of a clinical trial to assess Spironolactone as a potential add-on therapy for schizophrenia and validated the pharmacological validity of the targeted pathway. (b) In the de novo drug discovery approach, we identified a series of kinase inhibitors as the most potent modulators of the Neuregulin1-ERBB4 signaling pathway. ERBB4 expression in the brain is highly specific for a subset of interneurons and interference with its function in adulthood seems not to be critical with respect to side effects. In contrast, kinase inhibitors targeting ERBB1/EGFR have been shown to be associated with severe side effects affecting the skin and gut. Towards the development of ERBB4 directed drugs that should be compatible be a chronic treatment of adult psychiatric patients, a high level of selectivity of ERBB4 over ERBB1/EGFR is critical. Therefore, we have developed barcoded split TEV assays to monitor the activity of many compounds in parallel and could contribute to the characterization of novel highly ERBB4 selective kinase inhibitors. These compounds outperform Spironolactone in in vitro assays monitoring E/I balance and thus represent promising candidates for further clinical development.

Control of Mitochondrial Respiration and Resistance to Ionizing Radiation via Cytokine Receptors

Arezu Jahani-Asl

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Glioblastoma Multiforme (GBM) contain a rare population of self-renewing, multipotent, brain tumor stem cells (BTSCs), which are endowed with properties to survive, proliferate, spur the growth of new brain tumor cells, and at the same time evade ionizing radiation and chemotherapy. BTSCs possess a unique metabolic phonotype, with a distinct upregulation of oxidative phosphorylation (OXPHOS) and a low glycolytic rate. Thus, BTSCs more closely resemble neurons as opposed to the majority of cells in the bulk of the tumour relying on aerobic glycolysis. This phenotype can also make BTSCs resistant to therapeutic interventions targeting the Warburg effect. Identification of mechanisms that contribute to BTSC resistance is urgently required to develop better treatment for GBM.
Cytokines and their receptors play important roles in the regulation of cell fate, inflammation and immunity. The cytokine receptor for Oncostatin M (OSMR) is upregulated in classical and mesynchymal subtypes of GBM and correlates significantly with poor patient prognosis. OSMR drives BTSC proliferation and glioblastoma tumorigenesis. OSMR orchestrates a feed forward mechanism with the oncogenic epidermal growth factor receptor variant III (EGFRvIII), and the transcription factor STAT3 to amplify receptor tyrosine kinase signaling. Genetic knockdown of OSMR impairs brain tumors and expands the life span of animals harbouring patient derived xenografts. Strikingly, we have established that OSMR controls cellular respiration and defines the metabolic profile of BTSCs. OSMR promotes mitochondrial biogenesis via PGC1$\propto$ signaling. Knockdown of OSMR sensitizes BTSCs to DNA damage-induced ionizing radiation. This mechanism is conserved in post-mitotic neurons. Thus, while OSMR is required for BTSC proliferation and brain tumor growth, OSMR confers resistance to therapy via strengthening mitochondria. These findings define a mechanism to differentiate between slow cycling resistant tumor stem cells compared to their highly proliferative progenies within the brain tumor niche.

Alterations in Rab-mediated membrane trafficking in neurological disease

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Membrane trafficking controls the levels and localization of proteins, and thus cellular function, and alterations in trafficking pathways contribute to human disease. Rab GTPases are key switches turning trafficking on and off. Our discovery that the differentially expressed in normal and neoplastic cells (DENN) domain functions enzymatically as a guanine-nucleotide exchange factor (GEF) to activate Rabs provided new understanding in the regulation of Rabs in membrane trafficking. There are 26 DENN domain (DENND) proteins in humans making them a critical new class of trafficking regulators. Here I will describe how alterations in DENN domain proteins and their Rab substrates contribute to neurological disease. Specifically I will discuss the role of DENND1 and its substrate Rab35 in the development of brain tumors and our recent discovery that mutations in DENND5A cause a severe neurodevelopmental disorder called epileptic encephalopathy.

Curing Protein Trafficking Diseases

:Graeme Carlile, Gregor Jansen, John W. Hanrahan, David Y. Thomas,

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Protein trafficking diseases arise from mutant proteins that are recognized as misfolded by cellular protein quality control systems and retained in the (ER). Cystic Fibrosis class II mutations (such as F508del-CFTR) cause such a disease. We have used cell-based assays to scren chemical libraries and identified chemically diverse "correctors" that allow the mutant CFTR protein to traffic to the plasma membrane. Two classes of "corrector" have been identified, pharmacological chaperones and proteostasis modulators.

Currently the clinically most advanced correctors are pharmacological chaperones that bind to the mutant protein, correct its folding, trafficking and function. The corrector VX-809 gives about 15% correction of F508del-CFTR trafficking in human bronchial epithelial cells and this translates to only 4% correction in the clinic. The goal is to correct at least 50% of mutant CFTR as F508del-CFTR heterozygotes are asymptomatic. We have identified 3 sites on the CFTR molecule that bind different pharmacological chaperone correctors that have additive level of correction in human bronchial epithelial cells of 35%.

Pharmacological chaperone correctors have their limitations as not all CFTR type II mutant proteins are corrected and we have shown that patient response to VX-809 is variable.

Proteostasis modulators are chemically diverse and have varied ways to modulate the stringency of the cellular protein quality control systems. They are useful starting points for the investigation of cellular proteostatic mechanisms and also for the development of therapies. We have identified as proteostatic correctors of mutant CFTR trafficking Nonsteroidal Anti-Inflammatory Drugs (NSAIDS) that target the ER stress protein cyclooxygenase 2(Cox2). They are known to act as analgesic and anti-inflammatory drugs and we have show that in addition to these properties some NSAIDs are correctors of F508del-CFTR trafficking. In addition we have data that shows that several NSAIDS can correct a wide spectrum of CFTR class II mutations that are not corrected by VX-809.

Supported by the Canada Research chairs Tier1 programme, Canadian Institutes of Health Research.

Testimonial, the diagnosis and activity that prolonged my life

Nenad Bach, Dr. Art Dubow

Ping Pong Parkinson

As a composer, singer-songwriter and a peace activist, I circled the planet Earth in 40 days (Sept-Oct 2010) for the first time. 2 months later I was diagnosed with Parkinson's. I always wanted to slow down, but I hoped that it would be on my own terms. I lived too fast. My body asked me to slow down but I didn't listen, and that is how PD saved my life.In April 2014, I was introduced to the Westchester Table Tennis Center here in Pleasantville, New York, where I played sporadically, but after 6 months noticed an improvement in my PD symptoms. A year later, I started to play the guitar publicly again**.**

Introduction and Objectives: On March 1st 2017, Ping Pong Parkinson, a non-profit organization was established with the goal of halting the progression of Parkinson's Disease by utilizing ping pong and an eclectic mix of other exercises as a form of physical therapy. Our model is based on the research-proven concept of neuroplasticity – the brain's capacity to make new neurons and connections through challenging physical exercise. Our program is underpinned by compelling evidence for the need for a regular exercise program for people with Parkinson's Disease (PwP). A cadre of volunteers serve as support and instructors for the various activities (principally Ping Pong).

Methods: Our approach in achieving the aforementioned goal revolves around ping pong, a sport which is ideally suited in targeting, not just the motor symptoms of PD, but some non-motor symptoms, as well (depression, anxiety). By the nature of the activity, ping pong is fun but also addresses such Parkinson's issues as balance, agility and some aerobic activity. Our weekly 75 minute sessions begin with specific exercises specifically adapted and suited for PwP, including some Tai qi and yoga derived callisthenics, as well as specific aerobic drills (e.g., marching to music). Our sessions conclude with juggling, another dopaminergic activity, and a group sing which enhances the volume of speech - and which also acts as a morale booster in fostering camaraderie. (Unanticipated, has been the evolution of our exercise therapy group into a concurrent support group - a significant and serendipitous discovery.)

Results: Videos of our "Ping Pong Pongers" (PwP) engaged in playing ping pong has been ongoing and provides an objective method of validating the positive motor effects of our program. Improvement has been cited by some of the treating neurologists of members of our group. Positive changes in coordination are clearly apparent. For example, initially, we would count the number of times a "Ponger" (PwP) could strike a ball safely, but soon this became pointless as the rally could continue almost without end, even with those afflicted with advanced symptoms. Similarly, in juggling there are some "Pongers" who can do a Three Ball Cascade, repeating the pattern numerous times. (This is a considerable achievement considering none of the group members knew anything about juggling to begin with and considering how difficult this is for anyone to juggle - with or without PD.)
Our module has evolved and been improved through trial and error. Our approach and model can be used as a basis for the nucleus of an exercise program designed for PwP.

Session "Cancer immunotherapy & signalling"

Harnessing innate immunity for immunotherapy of solid cancer

Adelheid Cerwenka

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The innate immune response serves as the first line of immune defense against invading pathogens and tumors. The focus of our research is to dissect activation modules of innate immune cells, in particular Natural Killer (NK) cells and Innate Lymphoid Cells (ILC) and the influence of the microenvironment on their function in tissues during homeostasis and disease. NK cells are major cytolytic and cytokine producing effector cells that can efficiently kill tumors. In contrast to T cells that rely on the recognition of tumor cells via MHC I/tumor associated antigen, NK cells become activated upon integration from signals originating from inhibitory receptors specific for self MHC class I molecules and activating receptors. Thus, NK cells can kill tumors that resist to T cell recognition. So far, NK cell-based therapies such as the adoptive transfer of NK cells or the application of NK engaging mAbs showed benefits in leukemia patients. Adoptive transfer of IL-2-activated NK cells in patients suffering from solid tumors, however, only resulted in poor clinical responses. NK cells in solid tumor tissues frequently display impaired functionality compared to peripheral blood NK cells. There is emerging evidence that tumor infiltrating NK cells express decreased levels of activating receptors, increased levels of inhibitory receptors, are poor producers of IFN-g and exert low cytotoxicity. In our study, we elucidated the molecular landscape of tumor-infiltrating NK cells using high throughput single cell sequencing. We demonstrate that the transcription factor Hypoxia Inducible Factor-1a (HIF-1a) was highly upregulated in tumor infiltrated NK cells and serves as a key modulator of NK cell function. The functional consequences of HIF-1a expression in NK cells will be discussed in the talk.

https://www.umm.uni-heidelberg.de/immunbiochemie/

Harnessing Innate Immunity for new generation cancer therapies

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Over the last decade, new therapies have been developed to promote antitumor immunity. Most of these immunomodulatory therapeutic approaches, including Immune Checkpoint Inhibitors, have focused on enhancing T-cell responses. However, despite unprecedented clinical success, only a minority of patients respond to these therapies highlighting the need to identify new strategies for immunotherapeutic approaches. Given its crucial role in immune responses, harnessing innate immunity opens up new possibilities for mounting efficient antitumor immunity. We report, here, 2 examples of molecules developed to harness either both innate and adaptive immune cells or exclusively innate immune cells to induce anti-tumor efficacy. First, Monalizumab, a humanized antibody blocking the inhibitory NKG2A receptor enhanced tumor immunity by promoting both natural killer (NK) and CD8+ T cell effector functions in mice and humans. Monalizumab performed anti-tumor efficacy when used as a single agent or in combination with other therapeutic antibodies, such as durvalumab, blocking PD-L1, or cetuximab, directed against the epidermal growth factor receptor (EGFR), which is expressed by tumor cells. Second, we report the generation and efficiency of trifunctional antibodies engaging NK cells that target two activating receptors, NKp46 and CD16, on NK cells and a tumor antigen on cancer cells. Trifunctional NK cell engagers (NKCEs) were efficient to eliminate tumor cells both in-vitro and in-vivo, and were more potent than clinical therapeutic antibodies targeting the same tumor antigen. Therefore, efficacy of Monalizumab and NKCEs emphasizes the potential of triggering innate responses in immunotherapeutic approaches and demonstrate that they constitute the next generation of molecules for fighting cancer.

AcTakines: a novel class of immunocytokines

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Although pro-inflammatory cytokines such type I and type II interferons (IFN), interleukin 1 (IL-1) and tumor necrosis factor (TNF) have major translational potential, they all met with limited, if any, clinical success due to their systemic toxicity. These cytokines are typically very pleiotropic immunoregulatory proteins that have evolved to act locally. Systemic cytokine exposure is therefore non-physiological and often associated with sometimes severe unwanted side-effects. To impose spatial control of cytokine activity, we are developing AcTakines (Activity-on-Target cytokines). AcTakines consist of genetic fusions of mutant cytokines, with strongly reduced binding affinity for their receptor complex, and a targeting moiety that binds a cell-specific surface marker. Importantly, these mutant cytokines are inactive “en route” through the body, and only unveil their activity by avidity effects as a result of the cell-specific targeting. AcTakines can thus be administered systemically, yet they recapitulate the local action that is typical for cytokines, which allows separating desired from undesired effects and hence safe exploitation of their clinical potential.
Data will be presented on the application in different tumor models of type I and type II interferon-derived “AcTaferons”, and a TNF-derived “AcTafactor”.

AMPK promotes chemotherapy resistance by direct phosphorylation and transcriptional activation of TFEB/TFE3

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Increased autophagy and lysosomal activity promotes tumor growth, survival, and resistance to chemotherapy. During acute starvation, autophagy is rapidly activated by AMPK and mTOR inhibition to maintain energy homeostasis and cell survival. The transcription factors TFEB and TFE3 are master regulators of autophagy and lysosomal activity. Their shuttling between cytoplasm and nucleus is controlled by mTOR-dependent multisite phosphorylation. However, it is not known whether and how their transcriptional activity is controlled. Here, we show that AMPK directly phosphorylates TFEB on three serine residues, mediating TFEB transcriptional activity upon nutrient starvation, FLCN depletion, and pharmacological manipulation of mTOR or AMPK. Importantly, we show that resistance to chemotherapy is mediated by AMPK-dependent activation of TFEB which is abolished by pharmacological inhibition of AMPK or mutation of serines to alanines. These results place AMPK as a key regulator of TFEB/TFE3 activity and validate AMPK as a promising target in cancer therapy to evade chemotherapeutic resistance.

Complex roles of a mitochondrial SUMO ligase in cancer

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Notwithstanding their pivotal role in regulation of metabolism and survival, the mechanisms underlying the role of mitochondrial signaling in metabolic syndrome and neoplasia are still unclear. We here identify a new function for the Mitochondrial Anchored Protein Ligase (MAPL, also known as MUL1), in the regulation of gluconeogenesis during the post-prandial state and establish its tumor-suppressive role in the liver. Following insulin mediated activation of AKT and phosphorylation of GSK3□ cyclin D1 is stabilized and translocates to the nucleus where it represses genes required for gluconeogenesis when nutrients are high. During the post-prandial state, cyclin D1 is degraded through the action of the ubiquitin E3 ligase Fbx4, thereby initiating gluconeogenesis. Strikingly, MAPL ablation abrogates oscillation in cyclin D1 levels within liver during the fed to fasted transition. Mechanistically, MAPL SUMOylates Fbx4 which is essential for the ubiquitination and degradation of cyclin D1. Loss of MAPL leads to a liver specific, chronic elevation of cyclin D1, which results in the development of hepatocellular carcinoma with near 100% penetrance after 12 months. These data reveal a role for mitochondrial SUMOylation in stabilizing a signaling platform essential to drive the metabolic feed/fast transition in liver and when disrupted results in hepatocellular carcinoma.

Novel Stress Response Signaling Complexes of *Saccharomyces cerevisiae*

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In the wild, the budding yeast *Saccharomyces cerevisiae* encounters different types of environmental stress that can compromise cell wall integrity and threaten cell survival. These stresses may include nutritional starvation, pH/oxidative stress, dehydration, hyper- and hypo-osmotic conditions, extreme temperatures, or antibiotics that can damage the cell wall, the plasma membrane, or other essential components within the cell.

In yeast, signaling components that contribute to the activation and modulation of the Cell Wall Integrity (CWI) Pathway are especially important because they contribute to their survival under conditions of cell wall stress, and other forms of environmental stress. During this talk, we will focus primarily on the role of the Pkc1-Mpk1 signaling module in the response to cell wall stress in *Saccharomyces cerevisiae*. Activation of this CWI mechanism is regulated at the plasma membrane level by a family of five transmembrane proteins Wsc1, Wsc2, and Wsc3 known as the "Wsc family", and Mid2 and Mtl1, two related sensor proteins, which share functions and structural features with the Wsc family, that we will collectively refer to as stress receptors.

The main hypothesis for this study is that: Novel protein partners of the Wsc family, Mid2 and Mtl1 proteins that function as regulators of Pkc1-Mpk1 signaling and confer resistance to cell wall stress will be identified. We show that a subset of novel protein partners (interactors) discovered in this study using iMYTH technology, interact with the cytosolic domains of the stress receptors and can directly or indirectly regulate the accumulation of phospho-Slt2/Mpk1, a readout of the Pkc1-Mpk1 pathway, as well as cellular resistance to cell wall and oxidative stress.

Because of the evolutionary conservation of these stress receptors among different types of fungi, *Saccharomyces cerevisiae* represents an excellent model system for working out the mechanistic details of their molecular interactions with potential translation of this knowledge to understanding drug-resistance in other fungi.

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THE ROLE OF RIBOSOMAL PROTEINS L5 AND L11 IN TUMOR SUPPRESSION

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Impairment of ribosome biogenesis can activate the p53 tumor suppressor through the RPL5/RPL11/5S rRNA complex-mediated inhibition of HDM2. This, together with a well-established causal link between inherited and acquired impairments of ribosome biogenesis and cancer, led to the suggestion that the RPL5/RPL11/5S rRNA/HDM2/p53 checkpoint might constitute an important anti-cancer barrier. To gain a deeper insight into this issue in humans, here we analyze somatic mutations in *RPL5* and *RPL11* coding regions, reported in The Cancer Genome Atlas and International Cancer Genome Consortium databases. Using a combined computational, statistical and biochemical approach, we demonstrate the existence of several mechanisms by which *RPL5* mutations may impair wild-type p53 upregulation and ribosome biogenesis. In contrast, the same approach provides only modest evidence for a similar role of RPL11. Furthermore, we find that several functional cancer-associated *RPL5* somatic mutations occur as rare germline variants in general population. There findings may have strong implications for understanding the tumor suppressive role of the RPL5/RPL11/5S rRNA complex in human cancers.

Session "Cancer III"

“Targeting Wnt signaling in cancer and regenerative medicine”

Stephane Angers, PhD.

Leslie Dan Faculty of Pharmacy and Department of Biochemistry, University of Toronto

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Secreted Wnt proteins regulate development and adult tissue homeostasis. Mutational activation of Wnt-beta-catenin signaling is pervasively observed in cancer. Identification of therapeutics blocking the activity of this pathway for cancer treatment is needed but has been difficult due to the absence of easily druggable target downstream of these activating mutations and toxicity associated with broad inhibition of this pathway. We have performed a series of CRISPR-Cas9 functional genomic screens to identify novel therapeutic targets and delineate synthetic lethal interactions specific to oncogenic Wnt pathway mutations and we are developing new drugs targeting these vulnerabilities. Insufficient Wnt signaling is also the cause of degenerative conditions, such as IBD, osteoporosis and several neurodegenerative conditions. The hydrophobicity of Wnt proteins has complicated their purification and limited their use in basic research and as therapeutics. We describe modular tetravalent antibodies that can recruit Frizzled and LRP6 in a manner that phenocopies the activities of Wnts both *in vitro* and *in vivo*. The modular nature of these synthetic Frizzled and LRP6 agonists, called FLAgs, enables tailored engineering of specificity for one, two or multiple members of the Frizzled family. We show that FLAgs underlie differentiation of pluripotent stem cells, sustain organoid growth and activate stem cells in vivo. Activation of Wnt signaling circuits with tailored FLAgs will enable precise delineation of functional outcomes directed by distinct receptor combinations and could provide a new class of therapeutics to unlock the promise of regenerative medicine.

β1 integrin signaling is required to inactivate both Rb and p53 tumor suppressor check-points in a luminal B model of breast cancer progression.

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Breast cancer progression is a multistep process involving distinct pathological stages and adaptation to support tumor growth and metastatic dissemination. Acquisition of genetic alterations underlies initiating events and loss of cellular homeostasis. However, cells with perturbed homeostasis due to such oncogenic events are often kept in check by growth arrest, cell death and immunity. Overcoming these tumor suppressive barriers is a crucial step in permitting transition from benign lesions into malignant carcinomas. By utilizing a mouse model that faithfully reflects the multistep progression of human disease, we investigate the role of a cell adhesion protein β1 integrin and identify paradoxical effects of its deletion on different stages of mammary tumor progression. Our data shows that while β1 integrin signalling promotes tumor initiation by preventing growth arrest and apoptosis, its long-term loss promotes a hyperactive tumor microenvironment correlating with chronic inflammation, that also facilitates malignant progression. Our findings implicate Rb and p53 as key targets of the β1 integrin signalling axis that are involved mammary tumor progression.

Chasing the not so elusive histidine phosphorylation and their signaling in Neuroblastoma

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Phosphorylation is an important post-translational modification. In living organisms, four types of phosphate-protein linkage (SONAtes) exist on nine different phosphoresidues and histidine phosphorylation is established for 50 years but is unexplored. The acid-lability of phosphohistidine (pHis) means that techniques applied to study phosphate esters (phospho-Ser/Thr/Tyr) must be modified for the phosphoramidate (phospho-His/Arg/Lys). Advances, including anti-pHis monoclonal antibodies, mass spectrometry methods and stable mimics of pHis, facilitate the study of phosphoramidate bonds. The use of chemically phosphorylated synthetic peptides, confirmed to contain pHis by peptide dot blot, thin-layer chromatography and MS/MS analysis, allowed us to test a method for purifying some pHis-containing peptides of a human cancer cell line (HeLa). This purification is a strictly non-acidic method compatible with hydroxyapatite (HAP) for phosphoresidues enrichment, preserving phosphoramidate bonds. Subsequently, 1- and 3-pHis immunoaffinity purification is done, then pHis-enriched peptides are analyzed by LC-MS/MS and identified using MaxQuant software. It reveals conventional and non-conventional phosphorylation sites allowing a wider coverage of potential SONAtes phosphorylation. Specifically, the known pPGAM H11 peptide was enriched by 3-pHis IAP from human cells and validated by mass spectrometry with a b/y ions series as well as the correct immonium ion. It strongly suggests that a non-acidic enrichment method allows the detection of N-phosphate residues by mass spectrometry. On another aspect, several His kinases are defined in prokaryotes, especially those involved in two-component system (TCS). However, in higher eukaryotes, NME/NM23 is the only known protein–histidine kinase. This ubiquitous and conserved His kinase autophosphorylates its active site His118 and transfers this phosphate either onto a nucleoside diphosphate or onto a protein His residue. Studies of NME targets using anti-pHis antibodies should enlighten on potential His phosphorylation-based signaling pathways. Recently, a first sequence-specific pNME1/2 (H118) polyclonal antibodies was developed. These new antibodies detect pHis118 NME1/2 by immunoblotting and establish a precedent for generating other sequence-specific pHis antibodies. This ubiquitous kinase, first considered as a non-metastatic protein, is related to cancer and was shown to be upregulated in some tumors. Collaborative works are ongoing to study the involvement of this kinase in different models like in neuroblastoma. Recently, orthotopic xenografted tumors from mice were already harvested to progress toward tumor tissue analyses.

Cancer Drug Sensitivity Screening and Precision Cancer Medicine for B-Cell Malignancies

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Chronic Lymphocytic Leukemia (CLL) is the most common leukemia in adults and is currently considered incurable. Although current treatment regimens prolong life for patients, CLL eventually relapses. Efficient therapies may require a personalized approach that combines targeting cancer cells and the tumor microenvironment by restoring the patient's own anti-tumor immunity. However, a major limitation is that no efficient approach exists to identify the most effective drugs for each patient and cancer stage.

With the aim to support future introduction of individualized treatment for patients, we assessed the sensitivity of CLL patient samples to drug candidates using our *in vitro* functional drug sensitivity screening platform. We also examined signaling patterns by phosphoflow cytometry.

We have established novel *in vitro* culture settings that mimic the CLL tumor microenvironment and allow proliferation of CLL cells for 5 days. Using our unique method, we performed drug screening on B cells from 24 patients and 10 healthy donors against a customized, annotated library of 516 drugs including kinase inhibitors, proteasome inhibitors, B-cell pathway inhibitors and several other approved drug classes. A similar approach was taken also for multiple myeloma (MM), another B-cell malignancy, using a smaller focused library of 32 clinically used drugs.

Our analyses show that several drugs may be effective for CLL and MM and can be tested in drug combinations in order to identify synergistic effects. The signaling analyses have identified potential biomarkers for prediction of drug responses. As a future perspective, we want to combine machine learning strategies with the experimental drug screening strategies to identify effective drug combinations, and validate drug candidates by xenografting and in precision medicine clinical trials.

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Functional characterization of a novel p97:ASPL protein complex

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In terms of understanding cellular functionality and disease pathology at the molecular level protein-protein interactions (PPIs) and the networks they form *in vivo* are a major target of analysis. One particularly good example of a protein that plays a central role in a large interaction network is p97, an essential AAA+ ATPase in all eukaryotic cells. p97 interacts with more than 30 proteins[1] which are important for its versatile functionality, including processes like protein homeostasis and ubiquitin-dependent degradation[2]. To deepen our understanding of the fundamental cellular pathways p97 is involved in, we focused on the characterization of the heterooligomeric p97:ASPL protein complex[3]. Affinity purification of endogenous ASPL from HEK293 cells followed by mass-spectrometry revealed a group of putative ASPL interaction partners, among which RNA-binding proteins involved in splicing and mRNA-maturation were enriched. In the follow-up validation study using the LuTHy[4] method (double-readout bioluminescence-based two-hybrid technology), it was demonstrated that the early splicing factor U2AF2 (U2 auxiliary factor 65 kDa subunit) is the most promising hit for further characterization.
Interestingly, no signal was measured when testing the binary interaction between p97 and U2AF2 by LuTHy but increasing amounts of wild-type ASPL in the cells led to the detection of the interaction. However, mutated versions of ASPL that do not bind to p97 were unable to promote the interaction between p97 and U2AF2 in LuTHy assays. This indicates that ASPL might function as a bridging factor that induces the association between p97 and U2AF2, enabling the formation of a trimeric complex. Furthermore, RNA-Seq analysis of HEK293 cells upon ASPL knock-out revealed significant changes in the pattern of splicing events. Taken together, we now can speculate about the existence of a new p97 protein complex, which might play a critical role in mRNA splicing.

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Mechanisms of activation of receptor tyrosine kinases with membrane proximal FNIII domains

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Ligand induced dimerization is a central component in activation of most RTKs, but it is increasingly clear that the 20 distinct RTK families exploit an array of complex mechanism to regulated receptor activity and signaling outcome. We are focusing on the 23 RTKs that have fibronectin type III domains located proximal to the membrane of their extracellular regions (ECRs). In particular, we are investigating features of two families from this group: the Tie and the TAM (for Tyro3, Axl and Mer) receptors. These two families have very different biological roles – regulation of vascular homeostasis and angiogenesis for the Tie receptors, and of immune homeostasis and inflammation for the TAMs. We are, however, discovering that they share common features in their mechanisms of regulation. Notably, we have evidence to suggest that members of both these RTK families exist as dimers in the absence of ligand, and that higher order oligomerization, or clustering, may be key in directing the specific signaling outcome for different ligands and/or different receptor combinations.

Our studies are more advanced for the Tie family of RTKs, where we have proposed a structure based model for an FNIII domain mediated Tie2 ECR dimer. We also find that Tie1 and Tie2 interact via their ECRs to form a complex with a very different conformation than that of Tie2 homodimer. We are now investigating alterations induced by ligand. The Tie ligands, the angiopoietins (Angs), bind only to Tie2, and are known to exist in a range of different oligomeric states. It is clear that Ang ligands must be oligomers to induce Tie2 activation, but the mechanism of this is unclear. We propose that binding of ligand leads to formation of higher order Tie2 oligomers or clusters, and that the precise nature of these clusters is important in defining signaling outcome. Using Ang ligands of defined oligomeric state, we are investigating how binding of different ligands alters Tie2 structure, and how this affects receptor activation and signaling. It is also clear from cellular studies that the outcome of Tie2 activation is highly context dependent, and is influenced by the presence of Tie1 and other co-receptors. We hypothesize that this is due to direct interaction of the Tie1 and Tie2 ECRs that modulates the ligand induced oligomers or clusters that can form.

Recent studies suggest that the three different members of the TAM family (Tyro3, Axl and Mer) dimerize to different extents. Using isolated TAM ECRs, we are investigating whether these dimers are also mediated by the membrane proximal FNIII domains, as we observed for Tie2. TAM receptor activation is complex and poorly understood. For full biological responses, the receptor bound ligand must also engage phosphatidylserine on the outer leaflet of an adjacent cell membrane, which we propose induces receptor clustering. We are investigating the molecular changes that occur in TAM receptors upon ligand engagement that lead to receptor activation, and developing approaches to investigate these structural changes in a cellular context.

Session "Systems Approaches in Disease"

Charting genetic interactions in mammalian cells

Moffat, J[1,2,3]

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The genotype-to-phenotype relationship in health and disease is complex and influenced by an individual's environment and their unique genome. Personal genetic variants can modulate gene function to generate a phenotype either through a single gene effect or through genetic interactions involving two or more genes. The relevance of genetic interactions has been particularly clear in cancer research, where an extreme genetic interaction, synthetic lethality, has been exploited clinically. Therefore, the efficient and systematic mapping of the functions of genomic segments, and the comprehensive mapping of genetic interactions in mammalian cells, represent major challenges in biomedical research. Genome-wide pooled CRISPR and transposon mutagenesis screens have defined a core set of essential genes that are required for human cell proliferation and that share functional, evolutionary, and physiological properties with essential genes in other model systems. Continued genome-scale, loss-of-function genetic screens in diverse cancer cell lines have begun to resolve the human essential gene set in great detail and comparison of essential gene profiles identified subsets of genes that are specifically required for the viability of particular types of cancer cells. Importantly, cancer cell-line-specific essentiality profiles can uncover functional relationships because genes belonging to the same pathway or protein complex are often essential in the same subset of cancer cell lines.

Although a comprehensive understanding of the molecular vulnerabilities of different cancer cell types will undoubtedly provide a powerful roadmap to guide genetic interaction studies and therapeutic approaches, identifying relevant second site mutations that underlie differential essentiality remains a major challenge. Complementary approaches involve systematic testing of genetic interactions between defined pairs of genes. One approach uses gene editing methods to systematically introduce second site mutations into an engineered cell line carrying a stable query mutation of interest and subsequently identifies genetic interactions based on relative fitness of the resultant double mutant cells. My talk will outline our efforts to identify and map di-genic interactions and how this information can be used to help further annotate gene function(s) for the human genome.

The tumor microenvironment and the role of TGFbeta

Zadora PK, Dvornikov D, Engesser R, Szczygiel M, Lucarelli P, Foyer J, Boos E, Schilling M, Timmer J, Klingmüller U.

Transforming Growth Factor beta (TGFβ) signal transduction is deregulated in multiple pathophysiological conditions including cancer. TGFβ inhibits tumor development at early stages of tumor development and drives tumorgenesis at the later stages. The core TGFβ signaling pathway is a conceptually simple and linear pathway, but a mechanistic understanding of the pathway and its deregulation in the context of cancer remains limited.
We showed that TGFbeta treatment of the lung squamous carcinoma (LUSC) cell line SK-MES resulted in major morphological changes, induced the phosphorylation of SMAD2 and SMAD3, the expression of known TGFbeta target genes and enhanced the migratory as well as the invasive properties of the cells. The time-resolved analysis of TGFbeta induced target genes in SK-MES cells by RNAseq revealed an up-regulation of genes associated with the actin-cytoskeleton, migration, ECM and secretion. The analysis of the expression ratio of MYO10 mRNA in tumor tissue versus tumor free tissue showed that in LUSC patients a high-fold change in the MYO10 expression ratio correlated with shortened survival of the patients and resistance to chemotherapy. To further dissect the regulation of TGFβ signal transduction we combined a mathematical modeling approach with quantitative mass spectrometry data to elucidate which of the ten possible trimeric Smad complexes are indeed formed. Our analysis identified three crucial Smad complexes that influence target gene expression to different degrees providing a possible explanation for cell context-specific transcriptional responses. Next, we studied a set of NSCLC and healthy control cell lines that showed distinct dynamic behavior of TGFβ-induced Smad2/3 phosphorylation and differentially responded to inhibitor perturbations. Based on extensive time- and dose-resolved data we established a comprehensive dynamic pathway model describing the different dynamic features of TGFβ signal transduction in the NSCLC cell lines. Our results suggested a differential prevalence of negative feedback regulators that induce the degradation of the TGFβ receptors or reduce its ability to phosphorylate Smad2 and Smad3.
Taken together, these findings highlight that the TGFβ signal transduction is tightly regulated at the receptor and Smad complex formation steps. The work showcases the potential of a mathematical modeling approach combined with quantitative experimental data to uncover general principles of the regulation of TGFβ signal transduction, an important basis to elucidate deregulation in the context of cancer.

The Human Glycome Project - exploring the new frontier in personalised medicine

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The majority of proteins that evolved after appearance of multicellular life are glycosylated and glycans significantly affect structure and function of these proteins. However, due to structural complexity of glycans and the absence of a direct genetic template, the analysis of protein glycosylation is much more complicated than the analysis of DNA or proteins. Consequently, the knowledge about the importance of individual variation in glycans for both normal physiological processes and diseases is still limited. In the last few years it is becoming increasingly clear that variations in a DNA sequence are only a beginning of the understanding of complex human diseases. Genetic polymorphisms have to be put in the context of complex biology of life and a more elaborate approach that combines different 'omics phenotypes is needed to understand disease mechanisms and perform patient stratification that transcends genomics. Glycomics, as by far the most complex posttranslational modification, has an immense potential in this respect, which is only beginning to be investigated. By generating glycomic data for over 80,000 individuals from some of the best characterised clinical and epidemiological cohorts we enabled glycomics to meet other 'omics. The analysis of this rich gold mind is painting a picture of a very complex genetic and epigenetic regulation of glycosylation that fine tunes protein activity in multiple biological systems and, if altered, contributes to development of different complex diseases.

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Human Social Genomics: Recent Advances and Relevance for Human Health

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Although we generally experience our bodies as being biologically stable across time and across the different situations that we encounter in daily life, an emerging field of research is demonstrating that external social-environmental conditions, and especially our subjective perceptions of those conditions, can influence our most basic internal biological processes—namely, the expression of our genes. In 2013, we coined the term *human social genomics* to describe this phenomenon, which provides a multi-level framework for understanding how social-environmental stressors "get under the skin" to affect human health, behavior, and development (Slavich & Cole, 2013). In the present talk, I will describe recent research on human social genomics, which has now begun to identify the specific types of genes that are subject to social-environmental regulation, the neural and molecular mechanisms that mediate the effects of social processes on gene expression, and the genetic polymorphisms that moderate individual differences in genomic sensitivity to social context. The molecular models resulting from this research provide new opportunities for understanding how social and genetic factors interact to shape complex behavioral phenotypes and susceptibility to disease. This research also sheds new light on the evolution of the human genome, and challenges the fundamental belief that our molecular makeup is relatively stable and impermeable to social-environmental influence.

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Phenomenology of ageing and age-related diseases

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The rates of age-related diseases (ARD) and death increase with roughly 5th power of time as if they had a common root cause. Plausible common cause of ageing and ARD is age-related accumulation of malfunctioning oxidatively damaged proteins that display ageing-like phenotypes. Variable patterns of proteome damage can produce limitless phenotypic diversity, including genome instability. Proteins of aerobic organisms acquired structure-based resistance to oxidation such that oxidation damages mainly imperfectly folded proteins. Hence, when “silent” amino acid substitutions provoke oxidation-prone malfolding, some polymorphisms in particular proteins can predispose to specific ARD.
Ageing and ARD display species-specific latency that can be accounted for by phenotypic suppression of emerging cellular defects via molecular traffic among neighbouring cells, coined cellular parabiosis. Such cellular «solidarity» creates tissue homeostasis. Proteolytic interruption of cellular parabiosis by chronic inflammation leads to the expression of dormant pathologies, as in tumour promotion and inflammaging. Variation in oxidative protein damage and cellular parabiosis can account for inter-species correlation between body mass, ARD latency and longevity. Hence, predictive diagnostics, prognostics, prevention and healing of diseases by phenotypic reversion become conceivable.

Use of CRISPR/dCas9-based tools in studies of aberrant protein glycosylation in complex diseases

Vlatka Zoldoš

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Aberrant protein glycosylation has been observed in many physiological states, from aging and age-related diseases to inflammatory and autoimmune diseases as well as in cancer. In chronic inflammatory diseases (CID), including inflammatory bowel disease, systemic lupus erythematosus and rheumatoid arthritis, glycosylation of immunoglobulin G (IgG) is ubiquitously changed compared to a healthy state. Indeed, the effector function of IgG is regulated by the composition of the carbohydrate at its Fc region, thus affecting activity of the immune system. For instance, glycosylation acts as a switch between pro- and anti-inflammatory IgG functionality. Also, aberrant glycosylation of glucose transporters 1 and 2, present on the membrane of pancreatic β cells, is involved in improper glucose uptake and perturbed glucose stimulated insulin secretion (GSIS) leading to diabetes type 2. Using molecular tools, based on CRISPR/dCas9 technology, we try to uncover molecular mechanisms in order to understand how protein glycosylation is involved in CID and diabetes mellitus. For this purpose, we have developed a modular toolbox which uses the orthologous dCas9 proteins from two different bacteria for fusion with different effector domains such as KRAB and VPR for direct manipulation of gene expression, or DNMT3A and TET1 for epigenetic modulations. Our candidate genes are those that have been previously identified by genome wide association studies (GWAS) to be associated with IgG glycosylation and show pleiotropy with CID or diabetes. We analyse glycan phenotype following genetic and epigenetic manipulations of the GWAS hits.

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Genome-scale CRISPR-based approaches for identification of cell surface receptor-ligand interactions.

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Specific extracellular interactions enable a variety of biological processes including tissue organisation, immune regulation and host-pathogen recognition. Such interactions are of interest to the wider biomedical community due to their ease of targeting with therapeutic antibodies. Advances in forward genetic screening have allowed rapid functional characterisation of important intracellular pathways driving disease, but techniques to identify extracellular receptor-ligand interactions through which these pathways may be modulated remain elusive. Current approaches rely on libraries of recombinant proteins or cDNA constructs which can be costly to procure and maintain [1], and rarely represent the full complement of extracellular receptors. Here, we present two genome-scale approaches for identifying human cell surface receptors to defined ligands using CRISPR knockout (CRISPR-KO) and transcriptional activation (CRISPRa) screening systems [2, 3]. We show that both methods can identify known receptors-ligand pairs with high statistical confidence. In addition, CRISPR-KO screening is able to identify not only the direct receptor, but also other required gene products, such as co-receptors, post-translational modifications, and transcription factors contributing to receptor expression and subsequent receptor-ligand recognition on the surface of cells, whilst CRISPRa screening can identify multiple receptors to a single ligand. Using these approaches we identified IGF2R (cation-independent mannose-6-phosphate receptor) as a novel binding partner for the R2 subunit of GABAB receptors, as well as novel associations between ADGRB1 (Brain angiogenesis inhibitor 1) and all three Nogo receptors. We envision that such cost-effective, genome-scale approaches will facilitate ongoing characterisation of the extracellular interactome.

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Engineering protein-protein interactions to probe and rewire cell signaling

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Effective therapeutic strategies rely on our ability to interfere with cellular processes that are deregulated in human diseases. Thanks to the advance of genomic technologies in recent years, components essential for major biological pathways have been identified at the genetic level. Together they constitute signal transduction cascades relying on protein-protein interactions (PPIs) to elicit various biological functions. However, it is still poorly understood about the exact roles of individual PPI in controlling enzyme activity and complex assembly, especially in the context of diverse signaling networks. Traditional mutation-to-function studies have limitations in this regard due to unpredictable changes in protein folding and conformation, and difficulties in the identification of *bona fide* "separation-of-function" alleles. Hence, there is an urgent need for novel approaches that can selectively probe and investigate individual PPIs to dissect their biological roles.

To tackle this problem, I have devised a structure-based combinatorial protein design and engineering strategy to develop novel protein-based PPI modulators. In the past three years, we generated inhibitors and/or activators for more than 50 E3 ligases and deubiquitinases, enzymes that determine specificity of ubiquitination and deubiquitination, respectively (*1-8*). With the help of these synthetic molecules, we discovered new biochemical mechanisms and new biological functions of diverse protein families in the ubiquitination system. Importantly, we have established effective delivery methods for these intracellular probes and successfully target therapeutic-relevant genes in cells and organoids. I will present at the conference about our recent work on utilizing the protein engineering and synthetic biology platform to develop potent and highly specific PPI modulators to probe and rewire DNA repair signaling pathways with unprecedented precision for underlying molecular mechanisms and potential therapeutics.

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CLOSING KEYNOTE

INTRODUCTION of the Speaker by MIROSLAV RADMAN

Engineered Systems, Biological Networks and Relevance to Medicine

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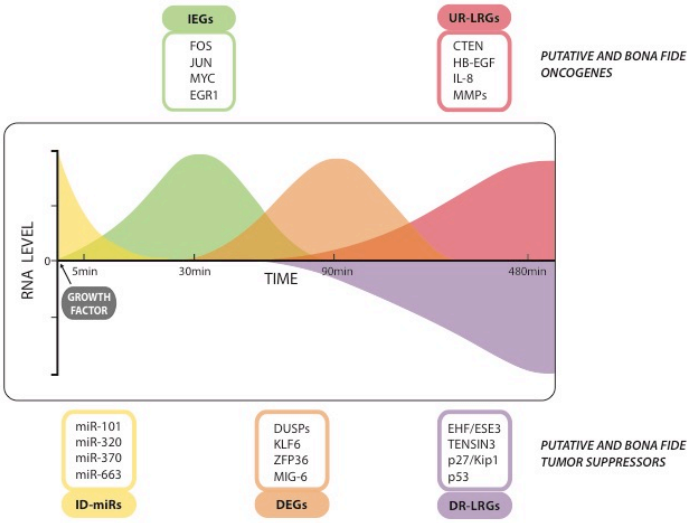
Engineered systems usually undergo an accelerated phase of increasing sophistication, prior to reaching an operational plataue that precedes their decline or replacement by competing technologies. Do biological system follow a similar path? Life is limited by external factors, such as physical parameters, and intrinsic factors, primarily the coding capacity of the genome. The need to frequently replicate the genome and protect it from mutagens or viruses have capped the size of the metazoan genomes. To code more cell lineages and instruct life in 4 dimensions, despite the limited genome size, evolution resorted to epigenetics and invented networks.
My lecture will exemplify these principles by adopting the group of EGF-like growth factors and their ERBB/HER family receptors. I will highlight the attributes of the network, such as modularity and feedback regulation, and focus on the complex transcriptional programs that underlay EGF-to-ERBB signaling (see scheme below). In the second part of my lecture I will describe the opportunities offered by pharmacological targeting of the network in cancer, including the use of clinically approved drugs and ways to overcome resistance to molecular targeted therapies.



Poster Presentations

Exploring the internal PDZ domain interactome and its involvement in disease and cancer

Jelena Čalyševa[1], Susanne Lüchow[2], Javier Rodríguez Balboa[3,4], Ylva Ivarsson[2], Toby Gibson[1]

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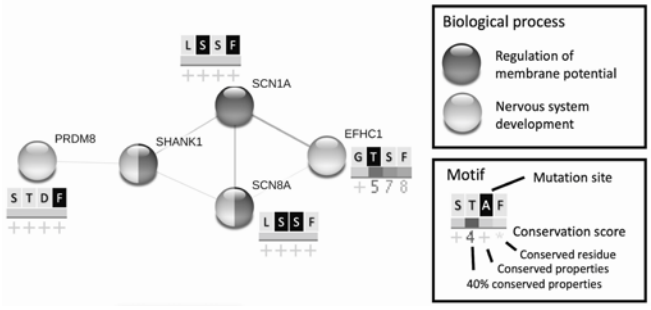
PDZ domains are highly abundant in Metazoa and are a part of cellular signaling and a variety of other crucial processes and diseases, including neurodegenerative diseases and cancer. PDZ domains are most commonly known to bind C-terminal motifs in proteins, but cases of internal protein binding are also known.
To investigate the internal PDZ binding, a bioinformatics assay was designed, utilizing proteomic peptide-phage display results obtained by collaborators in Uppsala University. One known internal PDZ-binding motif – TxF, where "x" is any amino acid – was validated; moreover, a few novel internal motifs were identified. Based on phage display peptide alignments, regular expressions and biological protein sequences, first internal binding predictions were made, covering the internal motif spectrum with bias towards the best phage display hits. Predictions were validated using SPOT arrays with collaborators in Novo Nordisk and refined using the obtained data. New refined predictions were validated using SPOT arrays and will serve as a basis for PDZ internal binding prediction.
Upon developing a reliable internal PDZ binding prediction method, the human proteome will be scanned for internal PDZ binders, and their involvement in biological processes and disease will be analyzed.



A network between the SHANK1 PDZ-containing protein and proteins associated with epilepsy containing disease-causing mutations within their SHANK1 internal PDZ binding motifs. The motif is also conserved among Metazoan species. The interactions were visualized using the STRING platform.

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COLORECTAL CANCER CELL MODEL FOR STUDYING THE ROLE OF THE CALCIUM-SENSING RECEPTOR IN THE COLON

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The Calcium-Sensing Receptor (CaSR) is a heterodimeric G protein-coupled receptor, first identified as key regulator of Ca^{2+} homeostasis in the blood [1]. It is a multi-functional protein able to sense different ligands spanning cations such as Ca^{2+}, amino acids such as phenylalanine and small peptides such as poly-l-lysine. In the colon the CaSR regulates fluid absorption and also seems to lead to cell differentiation and to inhibit epithelial inflammation. Interestingly, CaSR expression is down-regulated in colon cancer cells and patients with CaSR positive colorectal tumors have better prognosis. Furthermore, intestine-specific CaSR knock out mice are more susceptible to DSS-induced colitis [2]. Thus, we hypothesized that the CaSR is a targetable factor that can counteract intestinal inflammation and carcinogenesis.

We generated a colorectal cancer cell model where we induced the expression of the CaSR via a lentiviral system. We stably transfected the colorectal cancer cell line HT-29 with lentiviruses bearing either the CaSR-GFP construct ($HT\text{-}29^{CaSR\text{-}GFP}$) or only GFP ($HT\text{-}29^{GFP}$) as control. After antibiotic selection, we FACS-sorted the cells based on GFP intensity to discard GFP negative cells.

We assessed CaSR mRNA via RT-qPCR and protein via western blot and immunofluorescence, confirming CaSR expression in the $HT\text{-}29^{CaSR\text{-}GFP}$ cells. We further tested CaSR functionality in the presence or absence of CaSR-specific ligands (Ca^{2+}) or positive (NPS-R568) and negative (NPS-2143) allosteric modulators. We measured CaSR-dependent intracellular calcium mobilization with Fura2, confirming CaSR functionality upon extracellular calcium stimulation, an effect that was inhibited by the administration of the negative modulator NPS-2143. Activation of the CaSR led to reduction of cAMP synthesis. Furthermore, we demonstrated CaSR-dependent activation of the PLC-IP3 pathway by assessing the production of IP1, a metabolite of IP3. Increasing Ca^{2+} concentrations induced IP1 production and this effect was enhanced by NPS-R568 and inhibited by NPS-2143, revealing a CaSR-specific effect.

In conclusion, we generated a model to study CaSR functionality in colon cancer cells. With this model, we will be able to dissect further which cellular pathways are regulated by the CaSR in colon cancer cells and how the CaSR interferes with colorectal carcinogenesis and inflammation.

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This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 675228.

Effects of the antiepileptic valproate and of the flavonone naringenin on the antioxidative defence system in the brain of C57Bl6 mouse

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Valproate is a widely used antiepileptic drug. It is considered generally safe, but adverse effects, mainly in lipid metabolism are known to occur among patients. Similarly, adverse effects caused by valproate in the antioxidative defence system, in various tissues, including the brain, are known from *in vivo* and *in vitro* experimental systems. Our aim was to investigate, whether the use of polyphenylene naringenine, a known antioxidant and antilipidemic agent, together with valproate, affects the redox balance, and whether the synergistic application of naringenine and valproate, can reduce the potential negative effects in C57Bl6 mice. Lipid peroxidation, the concentration of total glutathione and the antioxidant activity of superoxide dismutase were measured in the prefrontal and central cortex, and in the cerebellum, after 15 days of exposure to PBS (control), Valproate 400 mg/kg bw, naringenin (25 mg/kg be) and joint valproate+naringenine treatment (in the same doses as individual groups). Our results showed, that the treatment with valproate and naringenine, and their combinations, caused increased lipid peroxidation in both males and females, in all three measured brain regions. The activity of the superoxide dismutase, and of the reduced glutathione in the brain, has been differentialy activated dependent on the tratment. The protective effect of the polyphenolic naringenine molecule, has not been demonstrated as expected.

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Extracellular vesicles as a potential diagnostic tool for neurodegenerative diseases and cancer

Teja Lavrin[1], David Badovinac[2], Hana Zavrtanik[2], Simona Sitar[3], Ema Žagar[3], Ana Plemenitaš[1], Rok Kostanjšek[4], Boris Rogelj[5], Nina Gunde Cimerman[4], Aleš Tomažič[2], Metka Lenassi[1]

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Introduction: Extracellular vesicles (EVs) are a heterogeneous population of membrane vesicles, which are shed from cells and accumulated in various body fluids *in vivo*. EVs protein, nucleic acid (miRNA) and lipid composition reflect the composition and the physiological/pathological state of the parental cell. EVs can be isolated from easy accessible body fluids and thus have a great potential for human diagnostic applications. In our study, we evaluated the biomarker potential of EV's molecular composition and morphological characteristics on two different disease models: neurotropic fungal infections and pancreatic ductal adenocarcinoma (PDAC).

Methods: To study the neurocytotoxic effect, EVs were isolated from the culture of neurotropic fungus *Exophiala dermatitidis* and tested *in vitro* on neuroblastoma cells SH-SY5Y. For the PDAC clinical study, EVs were isolated from the blood plasma of 50 subjects, monitored longitudinally at four time-points. Subjects were characterized with respect to age, gender and other factors. All subjects provided informed consent and the study was approved by the Slovenian Medical Ethics Committee. EVs isolated from fungal culture and human plasma were characterized for the presence of marker proteins and melanin (WB, A_{400}) and for their morphology, size and concentration (TEM, NTA, AF4-MALS).

Results: Fungal EVs showed typical EV characteristics: the average size of 90 nm (*R*g), the concentration of 2.8×10^{10} EVs/ml and the presence of marker proteins HSP70 and GAPDH, however, EVs showed a round-shaped morphology rather than cup-shaped. Interestingly, they showed higher buoyant density, probably due to melanin. EVs containing melanin were neurocytotoxic for neuroblastoma cells SH- SY5Y (20.9% viability compared to control), but the effect was lost in the presence of EVs without melanin due to presence of melanin-biosynthesis inhibitor in the culture (viability of 78.6% at twice the EVs concentration). EVs isolated from the plasma of PDAC patients showed the mode size range from 78.8–170.4 nm and the concentrations from $4.3–16.2\times10^{10}$/ml. Importantly, although there was some variability in EV size and concentration between the PDAC patients at zero time-point, the changes were also observed longitudinally over time. Currently, we are evaluating the association of EV size and concentration to the clinical characteristics of the PDAC cohort.

Conclusions: We showed that molecular composition and morphology of EVs can be specific to certain pathologies and could therefore be used as biomarkers of disease.

Profiling target selectivities and compound actions at early drug discovery in living cells using the safetyPROFILER

Lukša Popovic[1], Jan P Wintgens[1,2], Ben Brankatschk[1], Sven P Wichert[1,2], Moritz J Rosser[2], Michael C Wehr[1,2]

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The drug development process is long (currently 25 years or more), costly (5,5 billion $ per newly approved drug), and suffers from high attrition rates (1 marketed drug out of 50000 – 100000 compounds)[1-3]. Attrition rates during early drug discovery are largely due to low target selectivity, unwanted off-target effects, poor performance in ADME tests (absorption, distribution, metabolism, and excretion), and low efficiencies in preclinical studies. Furthermore, marketed drugs often still have off-targets effects, thus resulting in unwanted side effects that can lead to retracting the drug from the market[4]. To reduce the substantial amount of attrition rates, four leading pharma companies (Pfizer, Novartis, GlaxoSmithKline, AstraZeneca) identified common cellular targets that are associated with potential off-target effects and key are for making a compound safe[5]. They proposed developing an *in vitro* profiling tool to assess target selectivity based on an cross-over of impact (severity of side effect, i.e. off-target effect) and hit rate (frequency)[5-6]. These companies suggested that implementing this tool at the early stages of drug development would allow (1) the early discovery of unwanted off-targets and related effects; (2) a faster (*in vitro* vs *in vivo*) and cheaper (smaller amounts used *in vitro*) drug optimization process; (3) a more precise design of *in vivo* testings (free plasma concentration and affinities, possible drug dosage); (4) better understanding of polypharmacology and abnormal pathway mechanisms caused by a given compound[5]. Within the suggested list of common targets for such a profiling tool are G protein-coupled receptors (GPCRs), kinases, ion channels, nuclear receptors, proteases, epigenetic modifiers, enzymes, and transporters. However, current solutions are restricted to biochemical assays or single cell-based assay solutions. Therefore, we are developing a multiparametric cell-based assay platform to simultaneously assess the activities of multiple target classes within one experiment using barcoded reporters as readout. Previously, we have established multiplexed assays to examine drug effects on GPCRs[7], receptor tyrosine kinases (RTKs)[8], and proteases[9]. Currently, we are developing cell-based assays to dissect drug effects on nuclear receptors. The overarching goal will be to integrate the different assay types into one assay to test target selectivities and compound actions in living cells, a concept we call safetyPROFILER. In sum, we anticipate that the safetyProfiler will accelerate the drug discovery process by providing selective pharmacological profiles of compounds at the early stages of drug development by reducing attrition rates and associated costs at the clinical stages of drug discovery.

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Dynamic proteostatic and proteomic responses of breast cancer cells to high dose ionizing radiation

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Differential cellular responses of MCF7 breast cancer cell line luminal-like, estrogen, and progesterone positive, HER2/Neu receptor negative, responsive to chemotherapy and MDA-MB-231 mesenchymal-like, triple negative, highly invasive, metastatic, resistant to chemotherapy breast cancer cell to high dose ionizing radiation have been well studied at the genomics and transcriptomics level. Proteins are effector molecules of the genes that dynamically change in response to external stimuli. To understand the differences in biological responses of these cells to high dose ionizing radiation, we did a detailed analysis of proteome changes at different time points post-irradiation.
Differences in protein expression and levels of oxidative protein damage caused by radiation-induced oxidative stress were examined by a proteomic approach using 2D Oxi-DIGE method.
Further experiments analyzed changes in the radiation-impacted proteostasis networks by characterizing it in its prominent nodes, such as rate and fidelity of protein synthesis, the activity of protein quality control (ubiquitin-proteasome (UPS), autophagy and chaperone systems).
Proteomic and proteostasis analysis was followed by a selection of compounds targeting protein networks impacted by irradiation that could synergize with the effect of ionizing radiation in inducing the cancer cells death.
Various cellular processes impacted by irradiation in MCF7 and MDA-MB-231 breast cancer cell lines will be presented.

Bridging genotype-phenotype in cancer using data-driven causal networks

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Oncogenic selection manifests through dysregulated pathways, which are controlled by protein abundance and post-translational modifications. Therefore, proteomic measurements are essential for molecular dissection of cancer mechanisms. We have developed a causal network inference algorithm (Gabi) designed specifically for data from Reverse Phase Protein Array (RPPA) or Tissue Microarray (TMA) experiments and clinical variables. The Gabi algorithm includes a novel automated relevance thresholding procedure; facility to incorporate prior knowledge; as well as information-theoretic directionality inference based on conditional mutual information. In an evaluation on independent, blind test data, Gabi outperformed existing state-of-the-art-approaches in terms of both network connectivity and directionality. Gabi is available within an easy to install R package 'rTMA'. We analysed an invasive hormone-driven breast cancer cohort (n=284) from The Cancer Genome Atlas, comprising RPPA data for 106 proteins, including 20 phospho-forms and 11 clinical variables. Given the cohort was hormone-driven cancers, estrogen (ER) signaling is of particular interest. We found a switch-like expression state driven by ER-α and its phosphorylated form ER-α (S118), both highly connected, regulating multiple nodes and showing mutual antagonism by consistently opposing regulatory effects on common targets. The ER-α neighbourhood shows activation of growth pathways (e.g., mTOR) and increased adhesion (e.g., Claudin-7), whereas, the ER-α (S118) neighbourhood has proteins involved in Epithelial to Mesenchymal Transition (EMT) (e.g., N-Cadherin, fibronectin), suppression of apoptosis (e.g., NOTCH-1). Furthermore, we predicted causal associations between proteins and clinical parameters, which may inform response to therapy and disease progression.

The effect of membrane integrity disruption by freeze-thaw cycles on Na^+/K^+-ATPase activity and submembrane localization

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Na^+/K^+-ATPase (NKA), an enzyme expressed by all mammalian cells, is crucial for maintenance of ion homeostasis. Being a membrane protein, its positioning as well as different functions of active and inactive pools are highly dependent on the interactions with neighboring membrane lipids.
In this study we initially aimed to optimize conditions for spectrophotometric enzyme activity determination in brain tissue homogenates of wild type (wt) mice. More specifically, we analyzed the decrease in NKA activity after repeated freeze and thaw cycles. Dissected cortical tissue from wt mice was snap frozen in liquid nitrogen and stored at -80˚C until use and repeated spectrophotometric measurements showed changes of enzyme activity that corresponded to the number of freeze-thaw cycles. However, we detected an unexpected pattern of decreased followed by increased NKA enzyme activity after several freeze-thaw cycles. We hypothesized that this surprising rise in NKA activity is a consequence of NKA liberation from the inactive pool due to partial membrane integrity disruption caused by local interruption of intermolecular forces between NKA and lipids membrane's components. To test this hypothesis we performed Western blotting to detect total NKA quantity after every freeze-thaw cycle. Western blotting showed that the total immunoreactivity of NKA stays the same, even though the NKA activity is significantly different after a specific number of freeze-thaw cycles. To further investigate the potential submembrane redistribution of NKA, we performed lipid rafts isolation after specific number of freeze-thaw cycles. The isolation of lipid rafts was performed from wt mice cortical homogenate using the nonionic detergent Brij O20 and sucrose density gradients ultracentrifugation. Successful lipid raft isolation was confirmed by Western blotting for known lipid raft markers, flotilin and ganglioside GM1, in addition to NKA. The results of lipid raft analysis showed that the submembrane localization of NKA undeniably changes after a specific number of freeze-thaw cycles, which corresponds to changes in NKA activity.
In conclusion, this preliminary study revealed that modulation of membrane integrity causes the disruption of fine intermolecular interactions leading to changes in NKA localization to distinct membrane microregions and consequent change in NKA activity.
Keeping in mind that NKA not only serves as an electrogenic pump, but has multiple roles in signaling pathways, and the consequences of impaired NKA function are extensive and severe, the possibility to modulate NKA pumping and signaling properties is attractive and gives a new prospect in the research of NKA linked disorders.

The role of proteases in the repair of DNA-protein crosslinks

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DNA-protein crosslinks (DPCs) are DNA lesions formed by a protein irreversibly covalently bound to DNA which can arise after exposure to physical, chemical or chemotherapeutic agents and by faulty action of certain DNA metabolizing enzymes. DPCs are common lesions which present a physical blockage to all DNA transactions. If left unrepaired they cause genomic instability, premature aging and liver cancer in mice and humans. Recently, several groups have identified proteases Wss1 in yeast and SPRTN in mammals which initiate the removal of DPCs through the proteolytic digestion of crosslinked proteins. These discoveries led to recognition of DNA-protein crosslink repair (DPCR) as a separate DNA damage repair pathway. After SPRTN proteolysis of DPCs, peptide remnant of unknown size remains crosslinked to the DNA backbone, and is subsequently removed by unknown factors. Considering that SPRTN is a replication-specific protease, it is probable that another protease acts in lowly proliferative cells where DPCs pose a threat to transcription progression. Phylogenetic analysis of the SPRT family in metazoans indeed identified a SPRT-like protein family, ACRC (Acidic Repeat Containing) protein. In line with the phylogenetic proximity, the 3D structure of the protease core within the Sprt domain of ACRC is very similar to that of SPRTN. The putative protease core of ACRC includes two α-helices bearing three Zn-binding histidines and a catalytic glutamate residue which together form a HEXXH motif, a characteristic of all Zn-dependent metalloproteases. The goal of our study is to determine if ACRC is proteolytically active, if it bears a role in DPC repair and what is its relation to SPRTN using zebrafish model. We compared both proteases using phylogenetic and syntenic analysis and in regard to mRNA and protein expression across different tissues in human and zebrafish. To address functionality of ACRC, protein purification and protease functional assays are under way. Most importantly, we are addressing the role of ACRC *in vivo* using CRISPR/Cas9 gene manipulation to introduce mutation in the ACRC putative protease active site with the aim of creating an enzymatic dead version of ACRC. Our study will reveal actual contribution of ACRC and SPRTN to the DPC removal on the organismal level.

Ultrastructure of human sperm head vacuoles

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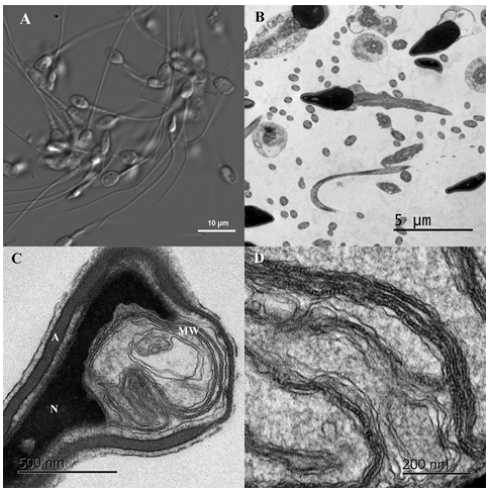
The ultrastructure of normal (normozoospermia) and abnormal (teratozoospermia) sperm cells was investigated with differential interference microscopy (DIC) and transmission electron microscopy (TEM). A detailed description of sperm head vacuoles, their size and number was studied in sperm samples of men involved in the in vitro fertilization program.The sperm head vacuoles observed under the high magnification of light microscopy were mostly described as "pocket-like" nuclear concavities related to failure of chromatin condensation [1]. The origin and dynamics of sperm head vacuoles is not well understood, some authors suggest that sperm vacuoles are normal features of sperm head and others describe them as degenerative structures related to male subfertility. In our study the ultrastructure of sperm head vacuoles is described and discussed in relation to sperm maturation and chromatin reorganization.

For ultrastructural investigation samples of normal and abnormal sperm cells were prepared for examination with Philips CM 100 transmission electron microscope [2].

The observation of sperm cells with DIC microscope showed several small and larger surface concavities termed sperm vacuoles, usually located in the head of sperm cells (Fig. A). In TEM micrographs sperm head vacuoles are seen as electron lucent areas of various sizes and positions with very variable structure (Fig.B).The results of this study indicate that sperm samples of men with teratozoospermia are characterized by increased number and size of sperm head vacuoles although they are present also in sperm samples of normospermic men [3]. Large sperm head vacuoles are mostly surface bulges which contain abundant membrane whorls and fine granular material.



Membrane whorls are composed of single and double membrane layers with septate electron dense molecular complexes which are continuous with the nuclear envelope (Figs. C, D). Acrosome (A) was fully developed in most spermatozoa with sperm head vacuoles. The degree of chromatin condensation differed between the sperm cells, with less condensed chromatin prevailing in nuclei (N) of abnormal sperm cells. Based on our ultrastructural findings we propose that sperm head vacuoles are the sites of removal of membranous material and proteins during chromatin reorganization. The exact origin and role of membrane whorls (MW) in sperm head vacuoles remains to be established in further ultrastructural research.

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Fluctuations in cell density alter protein markers of multiple cellular compartments, confounding experimental outcomes

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The life cycle of cultured proliferating cells is characterized by fluctuations in cell population density induced by periodic subculturing. This leads to corresponding changes in micro- and macroenvironment of the cells, accompanied by altered cellular metabolism, growth rate and locomotion. Studying cell density-dependent morphological, physiological and biochemical fluctuations is relevant for understanding basic cellular mechanisms and for uncovering the intrinsic variation of commonly used tissue culture experimental models. Using multiple cell lines, we found that expression levels of the autophagic markers p62 and LC3II, and lysosomal enzyme cathepsin D were altered in highly confluent cells as a consequence of nutrient depletion and cell crowding, which led to inactivation of the mTOR signaling pathway. Furthermore, both Lamp1 and active focal adhesion kinase (FAK) were reduced in high-density cells, while chemical inhibition or deletion of FAK led to alterations in lysosomal and autophagic proteins, as well as in the mTOR signaling. This was accompanied by alterations in the Hippo signaling pathway, while cell cycle checkpoint regulator p-cdc2 remained unaffected in at least one studied cell line. On the other hand, allometric scaling of cellular compartments in growing cell populations resulted in biochemically detectable changes in the plasma membrane proteins Na+K+-ATPase and cadherin, and nuclear proteins HDAC1 and Lamin B1. Finally, we demonstrate how treatment-induced changes in cell density and corresponding modulation of susceptible proteins may lead to ambiguous experimental outcomes, or erroneous interpretation of cell culture data. Together, our data emphasize the need to recognize cell density as an important experimental variable in order to improve scientific rigor of cell culture-based studies.

Electronic cigarette liquid exposure impairs bronchial epithelial cell homeostasis

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The whole effect of the use of electronic cigarettes on lung function is still largely unknown. Electronic cigarettes were introduced in use as a safer alternative to conventional cigarettes, with their safety only recently started being studied to a greater extent. Bronchial epithelium acts as a defensive barrier between the external environment and pulmonary parenchyma and has important role in maintaining the normal airway function. We aimed to test the effect of electronic cigarette liquids (ECL) on cell viability, cell-cell communication and epithelial mesenchymal transition (EMT) in human bronchial epithelial cell line BEAS2B.
BEAS2B cells were treated with electronic cigarette liquids (ECL) containing propylene glycol and glycerol as carriers, with and without nicotine, for up to 72h. Live cell imaging, EMT biomarker analysis and cell viability assays were used to characterize changes associated with ECL treatment.
Using MTT, EC liquid showed cellular toxicity with increasing concentration. Exposure of BEAS2B cells to EC liquid induced increase in formation of tunneling nanotubes for the duration of the treatment. After 48h of treatment there was a change in morphology characterized by acquisition of a fibroblast-like morphology which was accompanied by increase in the expression of fibronectin. We also detected presence of cell-free mitochondrial DNA (mtDNA) in culture medium of treated cells indicating cell injury and mitochondrial dysfunction.
Our findings indicate that ECL exposure can induce changes in normal human bronchial epithelial cells associated with cell injury, stress signaling and malignant transformation.

Identification of small molecule inhibitors of oncogenic FGFR4 tyrosine kinase mutant using MaMTH-DS platform

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Cells receive signals from beyond the cell membrane which are detected by specific receptors and that renders a cellular response. One of the systems that enable such signalling is through receptor tyrosine kinases (RTKs). RTKs are membrane proteins which have been proven as crucial regulators of pivotal cellular processes including proliferation, differentiation, cell survival, cell cycle control and metabolism. Consequently, constitutive and aberrant activation of RTKs and downstream pathways is shown to lead to tumorigenesis through disruption of these processes. Fibroblast growth factor receptor 4 (FGFR4) is an RTK whose aberrant regulation is associated with various cancers and the ability to metastasize. Its mutation V550E is considered activating in rhabdomyosarcoma, a childhood tumor with inadequate therapy for later stages. As knowledge of molecular basis of cancer expands it enables development of novel, targeted therapies. High-throughput screening approach to drug discovery involves biochemical and cell-based assays to screen collections of compounds in order to find specific hits on a target. Aim of this study was to identify small molecule inhibitors specific to the FGFR4 V550E using MaMTH assay, a recently developed technology for mapping interactions of membrane proteins, modified into a high-throughput drug screening method, MaMTH-DS. Collection of 5570 compounds was tested and five were identified as mutant specific inhibitory hits.

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Moderator: ***Igor Stagljar*** *(University of Toronto, Canada)*

14:00 – 15:00	**Mirella Bucci –** Nature Chemical Biology **Ursula Klingmüller –** DKFZ Germany **Heidi McBride –** McGill University **Marion Wiesmann**- Novartis **Anna Akhmanova**- Utrecht University

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June 20th 2019

2nd WORKSHOP - "Behind the Scenes of Scientific Publishing"

14:15 - 15:15	**Mirella Bucci –** Nature Chemical Biology

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Practical Info:

For the Bus No. 12 schedule, visit the Promet Split website http://www.promet-split.hr/

For bus departures and arrivals, visit the Bus station Split website http://www.ak-split.hr/

All information on flights to and from Croatia are available at the Croatia Airlines website http://www.croatiaairlines.com/hr/

See traffic information for Croatia can be found at the Jadrolinija website http://www.jadrolinija.hr/

Additional information on Split is available on our page The City of Split http://www.visitsplit.com/

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