Faces of Industrial Research *Symposium* 2019

The 1st Swiss National Symposium on Biomedical Research in Industry.

Tuesday, 19th February 2019

EPFL Campus (room SV1717)

Symposium Booklet

Contents

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PROGRAMME

09:45	**Registration**
10:30	**Prof Kei Sakamoto**, Department Head - Nestlé Research *Introduction of Nestlé Reseach and the PhD programme*
10:45	**Dr Carles Cantó,** Team Leader - Nestlé Research *Targeting mitochondria in metabolic disease: the told and the untold*
11:10	**Dr Paulina Rowicka**, Researcher - Roche *Understanding oligodendrocyte precursor cell differentiation in vitro*
11:30	**Dr Silvia Candido**, RPF Representative - Roche *Roche Postdoctoral Fellowship (RPF) Programme*
11:45	**Dr Nikhil Pandya**, Postdoc - Roche *A Retroviral Twist to Angelman Syndrome?*
12:00	**Lunch break**
13:00	**Dr Jan Tchorz**, Team Leader - NIBR *Liver stem cells: fact or fancy?*
13:25	**Dr Stefan Kustermann**, Lab Head - Roche *Human cellular models for drug safety assessment: focus on CNS*
13:50	**Dr Antoine deWeck**, Senior Scientist - NIBR *Functional screening for target identification and drug discovery in oncology*
14:15	**Dr Gabriele Civiletto**, Postdoc - Nestlé Research Zebrafish as model to measure autophagy in vivo
14:30	**Dr Omid Maschinchan**, PhD Student - Nestlé Research *Myogenic Specification of Pluripotent Stem Cells using Three-Dimensional Multicellular Microenvironments*
14:45	**Dr Christian Pasquali**, Head of Preclinical Res. - Vifor Pharma *"Escaping oriented science" A example of translational medicine with Broncho Vaxom*
15:10	**Coffee break**
15:30	**Dr Nicolas Mercado**, Postdoc - NIBR *Transcriptional network analysis identifies key regulators of human epidermal stem-cell fate*

PROGRAMME

15:45	**Dr. Cong Tang**, Postdoc - NIBR *MoA compound library screening reveals novel LMW molecules with anti-diabetic potential*
16:00	**Dr Hanna Sundström**, Senior Scientist - Vifor Pharma *Pharmacological Inhibition of the Iron Exporter Ferroportin*
16:25	**Closing of the symposium**

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Targeting mitochondria in metabolic disease: the told and the untold

Dr Carles Cantó

Nestlé Research

Mitochondria are a group of mighty organelles in the cell playing multiple vital functions. Even if the most well recognized mitochondrial function is ATP synthesis through oxidative phosphorylation, mitochondria also play key roles in fatty acid metabolism, steroidogenesis, calcium homeostasis and ROS signaling. Not surprisingly, mitochondrial functions can critically determine cell fate and viability. Much evidence from model organisms and clinical samples suggest a strong link between mitochondrial function, mitochondrial shape and age-related diseases, including metabolic complications and neurodegeneration. Such a nodal role turns mitochondria into a hotspot for therapeutic targeting. Here, we will dissect the intricate and often contradictory observations linking mitochondrial function and metabolic diseases. In our evaluation of the success or failure of current and past mitochondrial therapeutic agents, we will ask ourselves what were the key limitations for these agents and how can we overcome them.

Understanding oligodendrocyte precursor cell differentiation *in vitro*

Dr Paulina Rowicka

Roche Innovation Centre Basel, Pharmaceutical Research and Early Development

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease with secondary neuro-degeneration. Remyelination is able to keep disease progression below the clinical threshold for around 10 years post-diagnosis. However it ultimately becomes defective, leading to axonal degeneration and increased disability. Thus, there is an urgent need for new drugs targeting the remyelination process as current treatments are unable to delay the clinical threshold progression. Remyelination is a crucial multistep repair process that requires the recruitment of oligodendrocyte precursor cells (OPCs) to the demyelinated site, their differentiation into oligodendrocytes, and the subsequent ensheathment and wrapping of axons by myelin. In later stages of MS, there seems to be a block in the differentiation of OPCs, despite their sufficient migration to the site. Thus, the aim of this project is to investigate possible regulators of OPC differentiation in vitro. We hypothesise that by knocking down specific microRNAs we will be able to identify potential targets by RNAseq that could overcome this block and induce the differentiation of OPCs.

Roche Postdoctoral Fellowhship (RPF) programme

Dr Silvia Candido

Roche Fellowship program representative

The Roche Postdoctoral Fellowship (RPF) Programme was initiated in November 2008; since then over 350 research projects, from all areas of Roche Pharma Research and Early Development (pRED) have been initiated, 270 of which have been successfully concluded and 80 are currently on-going. The Programme was introduced within Roche with the ambition to facilitate in the creation and education of the "Scientific Leadership" of the future, by fostering creative science and technology, strengthening and expanding Roche's academic network, and engaging highly talented Postdocs who are actively encouraged to publish in high-impact, peer-reviewed, scientific journals and to present their work at international symposia. RPF Projects guarantee an initial work period of 2-years with the possibility of a 1-year extension; dependent on progression of the project. RPFs focus on exploratory projects with a dual mentoring approach; whereby a Roche Scientist (Roche Mentor) collaborates with an internationally recognized academic expert (Academic Mentor) to formulate the project design. Based on the specific needs of the project, experiments are performed either at one of the pRED Innovation Centers (EU/US/AP), the collaborating University, or a mixture of both. Whilst RPF projects are initiated on the basis of scientific and technological excellence and fit to Roche's long-term strategic interests, they are also considered as a reward to eminent Roche scientists to enable such creative projects. Moreover, through the identification of highly qualified postdoctoral fellows, the RPF Programme has been instrumental in the successful recruitment of extremely talented new members of the Roche scientific family.

A Retroviral Twist to Angelman Syndrome?

Dr Nikhil Pandya

Roche Pharma Research and Early Development

Angelman syndrome (AS) is a neuro-developmental disorder caused by neuronal loss of E3 Ubiquitin ligase UBE3A leading to a plethora of severe intellectual disabilities. Although neuronal loss of UBE3A causes AS, there is a paucity of knowledge of downstream molecular and cellular dysfunction, ultimately hampering drug discovery. To this end, protein and RNA expression profiling was performed on AS patient and healthy control human induced pluripotent stem cell (iPSC)-derived neurons. UBE3A and proteins and pathways were deregulated across patient lines. Using RNAi molecules, reducing UBE3A protein in control lines or restoring it in patient lines, by knocking down the sense or anti-sense transcript respectively, reciprocally modulated a subset of these proteins. This included a subset of LTR retrotransposon-derived genes containing GAG capsid domains. UBE3A formed a complex with these Gag proteins and directly regulated its ubiquitination to modulate proteosomal degradation. Since these proteins have similarities to viral GAG proteins, we compared the exosomal proteome of AS and control hIPSCs derived neurons. This analysis revealed changes in exosomal content in a UBE3A dependent manner. Thus, we identify a novel function of UBE3A in exosomal neuronal physiology. Experiments are ongoing to elucidate the functional consequences of this axis. Ultimately, this work will lead to a better understanding of the role of UBE3A function in health and disease, critical to both drug and biomarker discovery.

Liver stem cells: fact or fancy?

Dr Jan Tchorz

Novartis Institutes for Biomedical Research, CBT-DAx

It has been established in many organs that tissue stem cells maintain homeostasis and promote regeneration in response to injury. In contrast to organs with high rates of cell turnover that harbor constitutive active tissue stem cell niches, the identity and roles of cellular niches that enable liver regeneration during homeostasis and injury are still controversial. Several publications claimed different subsets of hepatic cells to be `the` liver stem cell niche, while other groups proposed that liver cell across the lobule can serve as facultative stem cells on demand. Given the rising incidence of liver diseases worldwide, deciphering the mechanisms promoting liver regeneration is critical to address the high unmet medical need in restoring liver architecture in these patients. Using an integrated approach of in vivo lineage tracing, transgenic mice, CRISPR screening in liver organoids and single cell transcriptomics, we have now revisited proposed liver stem cell compartments and identified novel mechanisms regulating liver repair. Hepatocytes throughout the liver lobule can act as facultative stem cells by re-entering the cell cycle and replacing hepatocytes. In addition, we identified the mechanisms by which atypical ductal cells (also termed oval cells in rodents) promote a ductular reaction in response to drug-induced liver injury (DILI). While our data highlight the enormous plasticity of epithelial cells throughout the liver and mechanisms that enable their regenerative potential, it argues against the existence of previously proposed liver stem cell compartments highlighting the importance of validating published findings.

Human cellular models for drug safety assessment: focus on CNS

Dr Stefan Kustermann

Pharmaceutical Sciences, Roche Innovation Center

During the last years targets and therapeutic modalities for new drug candidates got increasingly complex and sometimes for example antibody based drugs do not any longer cross-react with any other species than human. This triggers a strong need for improved human cellular models enabling safety testing in a human model before entering clinical trials. Drug induced toxicities of the CNS, i.e. the brain and the eye are of concern as they could lead to devastating conditions for patients. But how could human CNS safety of novel, complex modalities be addressed in vitro? The nervous system is a highly complex, multicellular tissue which offers a challenge for in vitro modelling. For example in the case of the retina, a 2D model might not sufficiently recapitulate the complex physiology as in vivo the retina has very distinct multilayered, 3D cyto-architecture. Fortunately, recent developments in in vitro tissue engineering came up with improved and novel procedures on how to enable 3D tissue reconstruction in vitro in the field of neurosciences by e.g. 3D organoids or microfluidic systems. Encouraged by these developments we are currently investigating new human models with increasing complexity to mimic human physiology of the retina and brain. Examples will be presented on current developments of human complex cellular models of the retina and the brain as well as how such models are applied for safety testing of new drug candidates in a pharmaceutical industry setting.

Functional screening for target identification and drug discovery in oncology

Dr Antoine deWeck

Oncology Disease Area, Novartis Institutes for Biomedical Research

The systematic translation of cancer genomic data into knowledge of tumor biology and therapeutic opportunities remains challenging. This effort has been greatly aided by robust preclinical model systems, such as the Cancer Cell Line Encyclopedia (CCLE), for which detailed genetic annotation has been generated. However, a comprehensive mapping of cancer dependencies has lagged behind. Firstly I will present Project DRIVE, a large-scale RNAi screen in which viability effects of mRNA knockdown were assessed for 7,837 genes in 398 cancer cell lines. Secondly I will compare RNAi and CRISPR screens and show evidence that CRISPR-based screens have a significantly lower false-negative rate compared with RNAi-based screens, but have specific liabilities particularly in the interrogation of regions of genome amplification. I will describe computational means to correct for this artifact in order to leverage the full information available in CRISPR screens. Time permitting I will show results leveraging transposon-based functional screens to elucidate mechanism of resistance to HDM201, a MDM2-TP53 protein-protein interaction inhibitor.

Zebrafish as model to measure autophagy *in vivo*

Dr Gabriele Civiletto

Nestlé Research

Autophagy is an intracellular catabolic process that promotes the recycling of organelles and cytoplasmatic components, acting as regulator of homeostasis and cellular metabolism. Several pathways can modulate different steps of autophagosome formation and maturation, making autophagy a highly dynamic process. Due to its critical role in cellular quality control and metabolism, autophagy modulation has raised interest as possible therapeutic target for various human conditions ranging from age related diseases, such as neurodegeneration and muscle frailty, to immunity and cancer. Despite the great potential, no molecules specifically targeting autophagy have been used for human interventions so far; however, chemical agents exist that are known to modulate autophagy, and that are in clinical use for independent indications (i.e. rapamycin for immunosupression). The limited number of autophagy modulators known to date have limitations: some of them target key metabolic sensors that control energy status and cell growth such as AMPK and mTOR, showing low specificity and off-target effects. In addition most of the known autophagy modulators have been identified by in vitro screening, which is not sufficiently predictive and informative for in vivo implementation. Here, we describe a zebrafish transgenic autophagy reporter, which expresses ZsGreen-tagged map1lc3 protein specifically in skeletal muscle, hereafter termed actc1b:lc3-ZsGreen. Treatment of transgenic zebrafish larvae with well-established autophagy inducers clearly induced autophagic flux quantified by confocal microscopy. To further automate the analysis of autophagy dynamics in vivo, we developed a method for high-content image acquisition and analysis of actc1b:lc3-ZsGreen larvae. This new platform represents a high-throughput technology for the identification of new autophagy inducers.

Myogenic Specification of Pluripotent Stem Cells using Three-Dimensional Multicellular Microenvironments

Dr Omid Mashinchian

Nestlé Research

One of the most fundamental problems associated with stem cell therapy of skeletal muscle is the limited availability of cells that can robustly engraft into the stem cell compartment. It has extensively been attempted to isolate adult muscle stem cells (MuSCs) and expand them in culture to obtain sufficient cell numbers for such treatments. The challenge associated with this approach is that, once isolated from their niche and maintained in culture, MuSCs become terminally committed to myogenic differentiation and show a dramatically reduced engraftment potential. The recent discovery of induced pluripotent stem cells (IPSCs) has opened new avenues for the in-vitro derivation of cell types that are more suitable for transplantation. Thus, IPSCs hold great promise as a source of cell replacement therapy for muscular dystrophy. Here, we report a highly efficient approach for the scalable derivation of uncommitted MuSCs from human iPSCs in a biologically faithful 3D environment. We employed human iPSCs and a spectrum of immortalized cell lines to generate 3D-aggregation conditions promoting mesoderm formation and subsequent specification to the myogenic lineage in-vito/vivo without the parallel upregulation of myogenic commitment markers. Taken together, our work reveals a fourteen-day derivation protocol for the generation of uncommitted MuSCs that can easily be scaled up to the bioreactor level. Our novel protocol has fundamental implications for cell therapy of muscular dystrophy and will inspire future preclinical studies that will pave the way towards trails in human patients.

"Escaping oriented science" A example of translational medicine with Broncho Vaxom

Dr Christian Pasquali

Vifor Pharma

Broncho-Vaxom® (OM-85) was developed over 30 years ago to foster the immune defense against respiratory infections. It is an oral treatment of biological origin, composed of soluble extracts of lysates from 21 bacterial strains representing 5 pathogenic genera responsible for respiratory tract infections (RTIs). Since first launch in 1979, Broncho-Vaxom® has continuously been on the market in a number of EU member states and other countries worldwide and safely administrated to more than 80 million patients from 8 month to old adult populations. Early work from the 1980 to 2000 conducted in an "oriented science basis" aiming at demonstrating the "vaccine like" effect of OM-85 evidenced its capacity to stimulate immunoglobulins. This comprised immunogen properties in experimental animal as well as human cellular models. In particular, an cell-mediated humoral response evidenced by IgA production in saliva, serum and broncho alveolar liquid (BAL). The proposed mode of action (MoA) at that time was mostly based on "vaccine-like" properties where specific-bacterial antibodies against OM-85 content where identified. Recent non-clinical findings performed in "non-oriented science basis" brought more light in this regards and in particular, a mode of action which is based on two main arms: an humoral, non-specific polyclonal activation of the host immune response as well as a selective, cellular-based, receptor-mediated innate signaling. Protection in asthma airway inflammation models and anti-viral properties are MyD-88-Trif-dependent, thus promoting its use in potential new therapeutic areas. The presentation is aimed at demonstrating how to unravel new findings by using and conducting non-oriented science in proven safe products.

Transcriptional network analysis identifies key regulators of human epidermal stem-cell fate

Dr Nicolas Mercado

Chemical Biology & Therapeutics, Novartis Institutes for Biomedical Research

Resident adult epithelial stem cells precisely maintain tissue homeostasis by balancing self-renewal and provision of differentiated cells. Human epidermal keratinocytes retain stem cell potential in vitro but this is highly variable and lost over time suggesting extrinsic and intrinsic regulation. Transcription factor-controlled regulatory circuitries govern cell identity and are sufficient to induce pluripotency or transdifferentiate cell types. We asked whether transcriptional circuitry also governs phenotypic changes within a given cell type by comparing human primary keratinocytes with intrinsically high versus those with low stem cell potential. Exploiting the loss of epidermal stem cell function in vitro, two populations with widely differing stem cell potential and function were isolated and subjected to comprehensive whole genome transcriptional and epigenetic profiling (RNA-seq, H3K27ac-ChIP, BRD4-ChIP, ATAC-seq). Computational reconstruction of core regulatory circuitry predicted distinct transcriptional hierarchies operational in keratinocytes with high and low stem cell potential. Validation of the biological relevance of these predictions was performed by CRISPR-Cas9 mini-pool screen. We implicated the transcription factor "X" as antagonistic to stemness and show that CRISPR-KD of "X" in keratinocytes with low stem cell potential is sufficient to increase self-renewal, migration and epidermis formation. These data demonstrate that transcription factor regulatory circuitries, in addition to maintaining cell identity, control plasticity within cell type and offer potential for therapeutic modulation of cell function.

MoA compound library screening reveals novel LMW molecules with anti-diabetic potential

Dr Cong Tang

Novartis Institutes for Biomedical Research

Type 2 diabetes is a world-wide health threat, with diminished insulin/GLP-1 secretion from beta cell/L cell and insulin resistance as a hallmark. Typical therapies based on GLP-1 aim to boost insulin secretion from beta cells, however, due to the low effectiveness and high risk of developing pancreatic cancer of current clinic drugs, more effective therapies to restore insulin/GLP-1 secretion are desired. For this purpose, the MoA box compound library was screened using Glutag, a neuroendocrine GLP-1-secreting cell line. 42 compounds that increase GLP-1 secretion were spotted, and among of them 37 were validated in a repeated assay. Confirmed hits were then tested on primary colonic culture as well as on beta cell line and primary pancreatic islets. Here, we present one compound exhibiting sound stimulatory effect on GLP-1 and insulin secretion from primary cells without obvious toxicity. The effect of this compound will be then evaluated on both healthy and diabetic mice. At the meantime we generated Cas9-expressing Glutag cells and demonstrated efficient gene editing, this cell line is used for target identification using CRISPR approach.

Pharmacological Inhibition of the Iron Exporter Ferroportin

Dr Hanna Sundström

Vifor Pharma

Ferroportin (Fpn) is the only cellular efflux channel for iron and it exports iron from intestinal epithelial, hepatic and splenic cells as well as from erythrocytes. At the systemic level, Fpn is regulated by the peptide ligand hepcidin, which is expressed by hepatocytes and upregulated in conditions of iron overload. Hepcidin binds Fpn on the plasma membrane, induces ubiquitination, internalization and subsequent degradation or alternatively occlusion of Fpn, inhibiting iron export.

Blocking iron export by Fpn would be beneficial for patients with iron overload diseases. For the development of an orally available small molecule Fpn inhibitor (FI) we investigated and compared mechanisms of inhibition by FI and hepcidin. Both showed similar potency in blocking iron transport and to compete with tetramethylrhodamine labeled hepcidin (TMR-hepcidin) for Fpn internalization in human T47D cells and mouse J774 cells, respectively, both expressing endogenous Fpn. Hepcidin induced internalization of Fpn with higher potency than FI, as showed in MDCK cells constitutively expressing human Fpn with a fluorescent Halo tag. FI displaced TMR-Hepcidin from recombinant Fpn with higher potency than hepcidin. Additionally FI and hepcidin triggered ubiquitination and degradation of Fpn in J774 cells with comparable kinetics.

These data indicate that FI blocks Fpn-mediated iron export additionally to Fpn internalization. Both FI and hepcidin utilize similar pathways of Fpn internalization and degradation. The FI showed efficacy in several iron overload disease models and is currently tested in phase I clinical study.

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