Akos Heinemann, MD

Otto Loewi Research Center for Vascular Biology, Immunology and Inflammation (Pharmacology), Medical University of Graz

Mitochondrial dysfunction and altered metabolic adaptations in pulmonary fibrosis

Background:

Idiopathic pulmonary fibrosis (IPF) is a devastating lung disease with poor prognosis and limited therapeutic options. While a role of mitochondrial dysfunction in the pathogenesis of IPF has been proposed (Bueno et al., 2020), the exact molecular mechanisms by which altered mitochondrial function and subsequent metabolic maladaptations contribute to IPF require further investigation.

Objectives:

Within this project, we aim to thoroughly investigate the functionality and dynamics of mitochondria in structural (such as epithelial cells) and immune cells (such as alveolar macrophages) of the lung and how they might be altered in IPF. Indeed, evidence supports that dysfunction of alveolar epithelial cells (particularly type II) is an initial event in the development of fibrosis. Additionally, in fibrotic lungs, macrophages seem to be pivotal in promoting fibrosis (Ogawa et al., 2021). Alterations in cellular metabolism that might accompany mitochondrial dysfunction (such as in the Krebs cycle) will be explored. Understanding how these alterations reflect on cellular fate and behaviour will expand our knowledge on the disease and might provide novel strategies to approach it.

Methodology:

To investigate mitochondrial dynamics, main proteins regulating mitochondrial fusion and fission will be monitored. Mitochondrial function will be evaluated by investigating mitochondrial membrane potential, superoxide production, oxygen consumption and others. Altered metabolic reprogramming will be addressed by investigating the metabolic activity of Krebs cycle enzymes and associated metabolite levels will be measured (Atallah et al., 2021) (Rajesh et al., 2023). Cellular fate and behaviour will be investigated according to particular cell type. Hence, numerous methodologies and techniques including mass spectrometry, live-cell imaging, flow cytometry, and numerous molecular biology assays will be employed. Established animal models are intended to validate promising findings in-vivo (Bärnthaler et al., 2020).

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Ellen Heitzer

Research Center for Molecular BioMedicien (Institute of Human Genetics Medical), University of Graz

Project Title:

***PAN*creatic *CA*ncer *I*nitial *D*ectection via liquid biopsy (PANCAID**

Background:

PDAC (Pancreatic Ductal Adenocarcinoma) will soon become the second leading cause of cancer-related death in Western societies. Europe has the highest burden of PDAC in the world, with 150 000 new cases in 2018 and 95 000 deaths/year and worldwide half a million deaths (2020 [Globocan]). Moreover, PDAC has the lowest survival of all cancers in Europe (median survival time 4.6 months, with patients losing 98% of their healthy life expectancy). The biggest challenge in the management of PDAC is the inability to diagnose it in time. Studies for biomarkers in early pancreatic cancer are promising but have not yet produced any tangible results suitable for clinical diagnostics. Thus, there is an urgent need to establish a biomarker panel that is able to reliably detect PDAC at its earliest stages.

The development of next-generation sequencing (NGS) methods, together with technical advances in molecular biology, has sparked interest in liquid biopsy, which involves sampling blood and/or other body fluids for the presence of ctDNA or CTCs, as a tool for the early detection of cancer (summarized in1-5). In particular, the analysis of ctDNA (cell-free circulating tumour DNA) is a very promising tool as a minimal-invasive method of diagnosing pancreatic cancer.

Being a clonal oncogenic driver present in ~95% of pancreatic cancer, KRAS is a candidate gene for early detection; however, a major shortcoming of liquid biopsies as a cancer screening tool is the low abundance of individual tumor makers. In particular for early stage tumors with sizes smaller than 10-15 mm in diameter the expected plasma VAF of ctDNA is in the range of 0.1 %-0.01% (one tumor DNA molecule admixed with 10,000 normal DNA molecules)6. Therefore, a single gene/marker analysis can only achieve limited sensitivity and specificity which highlights the need for ultrasensitive detection techniques and combinatorial approaches.

There are some intrinsic features of ctDNA such as methylation status, size fragment patterns, or occupancy of nucleosomes that may help enhance its ability to be used as an independent biomarker that go beyond the exploitation and evaluation of targeted tumor-specific mutations. Genome-wide epigenetic changes are common in cancer and somatic methylations are considered as early cancer-driving events7, which make them a perfect candidate for early detection. As cfDNA/ctDNA is primarily a byproduct of cell death, fragment length distributions follow a biased, non-random pattern and differ between tumor-derived fragment and ctDNA8,9. Therefore, certain size patterns as well as fragment end motifs and preferred ends might be enriched in tumor-derived DNA and enhance the sensitivity9-11. Nucleosome occupancy patterns in cfDNA are rather newly studied features12-14 and can be obtained from (high coverage) WGS data. Recently, several groups have thoughtfully

characterized open chromatin landscapes in human cancer15-17, allowing further extrapolations to the cfDNA fragmentation footprints.

This PhD project is part of the EU-funded IHI project PANCAID that combines the expertise of world leaders in LBx including circulating tumour and host cells as well as cellular products released from tumour lesions into the blood such as DNA, EVs, proteins and metabolites.

Hypothesis and Objectives:

The major aim of the project is an orthogonal integration of cell-free DNA data types, including genetic, epigenetic, and fragmentomics markers. All these features enable the interrogation of multiple features, which has been demonstrated to improve the limit of detection. First, we aim to assess the utility of each level for early detection of pancreatic cancer, followed by a combined assessment of the tested ctDNA features in order to gain sensitivity by integration of multiple assays that are not sufficient on their own. This work will be done in close collaboration with the groups of Nitzan Rosenfeld (Cancer Research UK Cambridge Institute) and Yuval Dor (The Hebrew University of Jerusalem, Israel).

Hyopthesis: The evaluation of patient risk profiles by integrating multiple orthogonal ctDNA assays will have the potential to further enhance the utility of ctDNA as an early cancer detection tool.

Methodology:

We will comprehensively analyse cfDNA from patients with pancreatic cancer, individuals at risk for panctrativc cancer and healthy controls. To this end, the PhD candidate will employ a variety of NGS based methodologies. Moreover, the candidate will make use of sophisticated bioinformatics and statistics approaches.

Competences required for the position:

The student will employ and further develop a broad range of next generation sequencing (NGS) approaches, which is why experience with NGS is desirable. Moreover, the candidate will make use of bioinformatic approaches such as read depth analysis, pathway analysis, or machine learning technologies in order to characterize fragment size, tissue of origin and nucleosome occupancy patterns in plasma DNA samples from in healthy individuals and cancer patients. Therefore, knowledge of Linux and programming skills are beneficial. If desired, the PhD candidate might also employ next generation sequencing based methodologies, such as whole genome sequencing, bisulfite sequencing, etc. Therefore, experience with lab work is an advantage, but no requirement.

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Sereina HERZOG, PhD (PI)
Kirsten MAERTENS, PhD (Co-PI)

Institute for Medical Informatics, Statistics and Documentation,
Medical University of Graz, Austria

Vaccine & Infectious Disease Institute, University of Antwerp, Belgium

For informal inquiries, please contact:
sereina.herzog@medunigraz.at or +43-316-385-14263

Vaccine preventable diseases in pregnant and lactating women - Kinetics of vaccine-induced antibodies (Tdap and COVID-19)

This PhD position is part of an international research project between the Medical University of Graz (Austria) and the University of Antwerp (Belgium). For the Austrian part, we seek a PhD candidate with strong background in (bio)statistics, mathematics or related fields who would like to gain research experiences in mathematical and statistical models in the field of infectious diseases.

Background:

Due to the high susceptibility and vulnerability of pregnant women, foetuses and infants from infectious diseases, maternal immunization has gained interest in recent years. Nevertheless, the optimal time point for vaccination in pregnancy is still unknown. Also, dynamic changes in immune function occur during pregnancy if a vaccine is given at a different gestational age. This may affect vaccine response and kinetics of vaccine-induced maternal antibodies in blood and in breastmilk. Antibody kinetics describes the change in antibody titers or concentrations over time.

Additionally, since several recommendations for vaccination in pregnancy are currently in place or being in discussion, the question arises whether the administration of different vaccines in pregnancy has an impact on the immune responses to these vaccines. In addition, administrating of multiple vaccines potentially leads to an interaction in the kinetics of antibodies induced by these different vaccines

Hypothesis and Objective:

Within this project, we aim to determine the optimal time point for vaccination in pregnancy and we compare antibody kinetics and investigate potential interactions in immune responses when administering different vaccines (pertussis, COVID-19) during pregnancy. We develop conceptual frameworks in which we combine statistical approaches with mathematical models of infectious diseases in order to improve the design and the analysis of maternal immunization

studies.

The objectives are 1) to describe and compare kinetics of antibodies to two different vaccines (pertussis, COVID-19) and to investigate potential interactions; 2) to develop a framework to plan maternal immunization studies.

Approaches and Methods:

In two ongoing trials in Belgium, serum and breastmilk samples are collected at several timepoints during and after pregnancy (Tdap vaccine trial for pertussis, COVID-19 vaccine trial). In the Tdap trial, three cohorts of women are included differing in gestational age at which women are vaccinated with Tdap vaccine. In the COVID-19 trial, the effects of vaccinating pregnant and lactating women with a COVID-19 vaccine are investigated.
The Belgium collaborator has the main responsibility for the conduct of the clinical trials, the laboratory work and the interpretation of findings on maternal immunization. We will concentrate on tasks related to methodology development and data analysis.
This means that the PhD candidate in Graz (Austria) will focus on

1) Methodological frameworks, i.e. to develop frameworks to analyse antibody kinetics in pregnant and lactating women; and to develop frameworks to plan maternal immunization studies.
2) Analysing clinical datasets - antibody kinetics, i.e. to describe and analyse antibody kinetics in pregnant and lactating women for antibodies against Tdap and COVID-19.
3) Planning maternal immunization studies, i.e. to use the developed framework in order to make recommendation on best time points/best way to conduct a clinical trial.

The focus of the project will be on pertussis and COVID-19, but findings can be applied to other infectious diseases for which vaccines can be administered in pregnancy.

Specific qualifications required for the PhD positions:

- Background in field of (bio)statistics, mathematics or equivalent
- Interest to develop and analyse mathematical models in combination with statistical methods
- Programming skills, e.g., in statistical software such as R.

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Dagmar KRATKY, PhD (PI)
Nemanja VUJIC, PhD (Co-PI)

Gottfried Schatz Research Center, Molecular Biology and Biochemistry, Medical University of Graz

Transfer and uptake of lysosomal acid lipase and its consequences on atherosclerosis

This PhD position is advertised in the frame of the SFB Lipid Hydrolysis: Cellular Lipid Degradation Pathways in Health and Disease, a research network between the Medical University of Graz, the University of Graz, the Medical University of Vienna, and the Technische Universität Wien. The selected candidate will be enrolled in the PhD program *Molecular Medicine* at Med Uni Graz.

Background:

Lysosomal acid lipase (LAL) is required for efficient degradation of cholesteryl esters (CE) and triacylglycerols (TG) from endocytosed lipoproteins in lysosomes, resulting in the release of unesterified cholesterol, glycerol, and fatty acids (FA) for membrane assembly, steroidogenesis, energy production, and signaling. Thus, LAL is critically involved in catabolic, anabolic, and signaling pathways (1). In humans, LAL deficiency (LAL-D) leads to lysosomal accumulation of CE (and TG) with ectopic lipid deposition, most pronounced in liver, intestine, and macrophages (2). Despite complete loss of LAL activity, LAL-D mice phenotypically resemble late-onset LAL-D based on their life expectancy of one year (3). The striking accumulation of CE crystals in Kupffer cells, the drastic expansion of lipid-laden macrophages in the lamina propria of the small intestine (4), their ectopic deposition in various organs with aging, and the massive amount of CE in peritoneal macrophages suggest that this cell type carries the greatest burden of LAL-D pathology.

Hypothesis and Objective:

We hypothesize that some cell types, but not all, can take up functional LAL secreted from LAL-expressing cells. In addition, we postulate that increased LAL expression in macrophages enhances lipoprotein degradation via multiple pathways, thereby potentiating lipoprotein uptake by macrophages, stimulating foam cell formation and intravascular inflammation, which in turn aggravates atherogenesis.

Methodology:

To follow the fate of functional LAL, the student will fuse LAL with the mKeima fluorophore, an acid-stable fluorescent protein whose excitation wavelength depends on the environmental pH

(5). The student will use primary wild-type macrophages in a co-culture model with mKeima-LAL-transfected RAW264.7 macrophages to determine enzyme transfer, localization, and its compartmentalization in an active form to lysosomes. Fluorescence microscopy and live-cell imaging of primary macrophages incubated with conditioned medium, transwell co-cultures, or co-incubation with mKeima-LAL-transfected RAW264.7 macrophages will identify the source of LAL taken up by primary macrophages and the half-life of LAL. To examine whether phagocytes can take up LAL present in apoptotic cells, the student will use mKeima-LAL-transfected RAW264.7 cells irradiated with UV light as substrate for LAL-D macrophages and determine hydrolase activities or track LAL in the cell using fluorescence microscopy. To determine which of the cells affected by LAL-D are able to take up the functional enzyme, the student will isolate primary cells (hepatocytes, adipocytes, enterocytes) from LAL-D mice and perform uptake/secretion studies as described above. This will clarify the mechanisms involved in LAL transfer to inform potential gene therapy approaches.

LAL-D patients suffer from metabolic complications and are more prone to develop cardiovascular diseases. However, studies have not found associations between reduced LAL expression in metabolic tissues and atherogenesis. Paradoxically, individuals carrying the common risk allele for cardiovascular disease susceptibility have increased LAL expression in macrophages (6). To delineate the consequences of varying LAL expression in the most affected tissues, the student will use tissue-specific LAL overexpressing and LAL-D mice and assess lipid parameters, lipoprotein profiles, and characterize and quantify atherosclerosis in these mouse models.

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Med Uni Graz

PhD Program
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SINGLE CELL ANALYSIS

Thomas KRONEIS, PhD.
Division of Cell Biology, Histology and Embryology
Gottfried Schatz Research Center

Principal Investigator / Deputy Chair

For informal inquiries, please contact:
thomas.kroneis@medunigraz.at or +43 316 385 71904

Microchimeric Cells: Distribution of microchimeric cells in offspring of genetically identical and non-identical parents (mouse model)

Background:

During pregnancy, some cells manage to traffic from the fetus into the mother and vice versa giving rise to microchimerism (MC) – a phenomenon in which an individual hosts a small number of cells originating from another (genetically different) individual. In humans this microchimeric cells can persist beyond pregnancy thereby founding the basis for a lifelong MC. Microchimeric cells can differentiate into almost any cell type in the host, and implant in almost any tissue type. The consensus is that MC plays a paradoxical role in host health: some studies show benefits, others suggest it may play a role in the development of diseases. MC has been proposed to play a role in maternal wound healing, but may also be associated with pregnancy complications, such as pre-eclampsia and spontaneous abortion, as well as cancer and auto-immune diseases. MC may also play a role in providing immunological protection for the developing fetus, but also has been associated with offspring autoimmune disorders.

We launched a John Templeton Foundation-funded project on microchimerism connecting experts in evolutionary medicine, reproductive immunology, biochermistry, and single-cell analysis to address basic scientific questions in microchimerism research. One approach is to assess the distribution of MC within an entire individuum using a mouse model to identify (1) potential preferential sites of homing to host tissues and to (2) assess differences in the homing pattern related to the immune system. Within the project we cross transgenic mice of different background (corporation partner: Dr. Frank Schildberg, University Hospital Bonn/GER) and body-tome offspring.

In this project we will test for the presence of microchimeric cells in host tissues adapting recently developed methods allowing identification of haplo-identical cells. In addition, we use spatial histology based on *in situ* techniques to characterize (immunophenotype) tissues at the

single cell level. The envisaged analysis will help us understanding how differences in the immune system contribute to presence of MC and its biology.

Objectives:

Objective 1: Development and improvements of assays identifying microchimeric cells based on mismatch markers e.g., sex chromosomes (e.g., X, Y, Xist) or transgenes (e.g., RFP, tdTomato, ovalbumin).

Objective 2: Assess MC cell distribution in offspring of in bred (C57BL/6 x C57BL/6) and immunologically different mice (e.g. BALB/c x C57BL/6).

Objective 3: Immunophenotyping of tissues potentially positive for MC using *in situ* sequencing.

Hypotheses:

Hypothesis 1: MC cell numbers are higher in offspring from immunologically identical mice (e.g., C57BL/6 x C57BL/6 vs BALB/c x C57BL/6).

Hypothesis 2: In case we detect MC cells in offspring from immunologically non-identical mice (e.g., BALB/c x C57BL/6) we hypothesize that MC sites are linked to differences in the immune cell environment.

Methodology:

In the project the candidate will use histological (e.g., tissue pre-analytics, sectioning, staining), molecular biology techniques (ddPCR, qPCR, *in situ* techniques) to identify and characterize fetal and maternal cells and its environment in mouse models.

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Katharina LEITHNER, MD, PhD

Division of Pulmonology, Department of Internal Medicine

Metabolic adaptation of cancer cells to glucose deprivation

Background: Glucose is a major fuel of cancer cells and a high glycolytic activity promotes cancer growth. However, cancer microenvironments typically contain reduced glucose levels due to insufficient supply. Diets suppressing blood glucose are studied as an adjunct to anti-tumor therapies and further aggravate glucose deprivation of tumor cells. Despite the enormous progress in the understanding of cancer cell metabolism in the past decade (1), cancer-specific adaptive strategies to low physiological levels of glucose are still poorly understood. Specifically, the preferential glycolysis downstream pathways fuelled from glucose under low glucose conditions and the underlying mechanisms re-directing glucose to the different pathways according to the needs under low glucose are still unclear. Adaptive responses to overcome nutrient deprivation potentially may reduce the benefits of glucose lowering adjunct therapies and promote tumor growth.

Hypothesis and Objectives:

We hypothesize that glucose deprivation elicts responses in cancer cells that are tailored to sustain their proliferative capacities and are distinct from low glucose responses in non-cancerous, respiring cells.

Methodology:

The candidate will utilize panels of lung cancer cells, immortalized bronchial epithelial cells, as well as primary bronchial epithelial cells in standard and plasma-like culture media containing different contentrations of glucose or ^{13}C-glucose and assess glycolysis and anabolic downstream pathway activities as well as Krebs cycle activity using gas chromatography-mass spectrometry, a well-established method in our lab (2). The interaction with gluconeogenesis (as core interest of the group) will be investigated by additional tracers. Furthermore, the expression and activities of the relevant glycolytic and gluconeogenic enzymes and splice variants, a well as metabolite transporters and potentially involved signaling pathways,

including the Akt pathway, will be measured. Oligomerization and cellular localization will be monitored as appropriate. Candidate genes will be manipulated by genetic tools including CRISPR-mediated knockdown, inhibitors and by plasmid-mediated overexpression of wild-type and mutant alleles. Cell survival, apoptosis and proliferation as well as 3D growth capacity will be explored. Since cancer cells exhibit marked intratumoral metabolic heterogeneity *in vivo* (3), the candidate will utilize specific subpopulations of cells to assess their glucose deprivation responses. The relevance of the obtained findings for low glucose adaptation will be addressed in short-term explants from mouse xenografts or lungs and surgically resected human lung cancers and lungs.

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Roland MALLI, PhD

Molecular Biology and Biochemistry
Gottfried Schatz Research Center for Cell Signaling, Metabolism, and Aging
Medical University of Graz

Squeezing Mitochondria to Unravel Cancer Cell Metabolic Dynamics

Background: We are at the forefront of pioneering investigations, specializing in advanced live cell imaging and genetically encoded biosensors[1-3]. Our mission is to uncover the mysteries of metabolic activity and signaling in single cells, focusing on complex diseases like cancer. A recent breakthrough revealed that hexokinase 1 (HK1) forms intriguing protein clusters that squeeze mitochondria when glucose is limited[4]. These findings hint at a potential link between HK1 and changes in cellular energy metabolism during energy stress. We are gearing up for a game-changing project to engineer HK1-like proteins and control mitochondrial squeezing, unraveling the secrets of cancer cell metabolic switches!

Hypothesis: We hypothesize that we can better understand cancer cell metabolic flexibility by utilizing HK1 mutants and HK1-like engineered proteins to induce controlled squeezing of mitochondria. The localized changes in mitochondrial activity resulting from squeezing will enable us to unravel novel insights into cellular responses under altered metabolic conditions.

Objectives:

1. Develop HK1 mutants, and HK1-like engineered proteins and protocols that facilitate controlled squeezing of mitochondria in cancer cells without compromising overall cellular function.
2. Investigate the impact of mitochondrial squeezing on cellular energy metabolism and signaling pathways using genetically encoded fluorescent biosensors to monitor real-time changes in metabolic activity.
3. Analyze the consequences of manipulated mitochondrial squeezing on cancer cell proliferation, survival, and response to energy stress conditions.

Methodology: We will employ a multidisciplinary approach, beginning with the genetic engineering of HK1 mutants and HK1-like engineered proteins specifically tailored to induce mitochondrial squeezing without interfering with the overall cellular homeostasis. These mutants will be integrated into cancer cells using advanced transfection techniques. Through live-cell imaging, we will visualize and quantify the changes in mitochondrial morphology and dynamics resulting from the engineered HK1 constructs. To monitor cellular metabolic activity, we will employ state-of-the-art genetically encoded fluorescent biosensors, allowing us to observe real-time changes in energy metabolism within squeezed mitochondria. Concurrently, we will assess signaling pathways and cellular responses impacted by these changes. We will perform proliferation and survival assays under various energy stress conditions to evaluate the functional consequences of mitochondrial squeezing. This will provide valuable insights into the effects of controlled mitochondrial squeezing on cancer cell behavior and adaptation to altered metabolic states. The comprehensive data from this research will pave the way for a deeper understanding of cancer cell metabolic dynamics and the potential development of novel therapeutic strategies targeting mitochondrial activity. Together, as a fearless Ph.D. candidate, you will be an integral part of this exciting journey, pushing the boundaries of knowledge in cellular biology and making significant contributions to cancer research.

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Monika OBERER, PhD
Institute of Molecular Biosciences – Structural Biology, University of Graz

This PhD position is advertised in the frame of the SFB Lipid Hydrolysis: Cellular Lipid Degradation Pathways in Health and Disease, a research network between the Medical University of Graz, the University of Graz, the Medical University of Vienna, and the Technische Universität Wien.

Project Title: Structure-function relationship of lipolytic regulation

Background:

Over the last decades, the number of obese individuals has drastically increased and with it the risk for susceptibility to cardiovascular diseases, type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), and cancer. For potential therapeutic intervention, it is of immense importance to identify the players involved in lipid hydrolysis and lipid synthesis and to understand their dynamic interplay in detail. Our work focuses on expanding our knowledge of key mammalian lipid hydrolases at the molecular level. In adipose tissue, neutral lipolysis is primarily carried out by consecutive action of adipose triglyceride lipase (ATGL), hormone sensitive lipase, and monoacylglycerol lipase [1]. ATGL initiates intracellular lipid mobilization, yet has also newly discovered functions as a transacylase in the biosynthesis of lipids with anti-diabetic and anti-inflammatory effects [2]. Insights in ATGL regulation have been emerging over the last years of intensive research from different laboratories worldwide along with significant contributions from the Oberer-group [3–6].

Hypothesis and Objective:

Our work focuses on expanding our knowledge of ATGL, and its regulation by proteins at the molecular level. These include its co-activator comparative gene identification-58 (CGI-58), officially annotated as ABHD5, and its inhibitory protein G0/G1 switch protein (G0S2). Biochemical studies with variants of ATGL, CGI-58 and G0S2 will identify individual residues that are essential for co-activation and inhibition as evaluated by activity and interaction assays. MS-based approaches will be used to experimentally map the protein-protein interfaces of ATGL. *In-vitro* and *in-silico* approaches are carried out to characterize the proteins in lipid droplets and lipid-droplet-mimicking systems. We will continue to optimize protein production of highest quality suitable for structural studies using cryoEM and protein crystallography.

Methodology:

The PhD student of this project will predominantly focus on lipases that are involved neutral lipid hydrolysis and its interaction with regulatory proteins. Plasmids encoding proteins, expression protocols for bacteria and the mammalian Expi293F™ system, as well as purification protocls using ÄKTA chromoatography systems are already available in the group [3,4]. Biochemical activity/inhibition assays will be performed partly also with isotope-labelled substrates. The PhD student will also carry out biophysical methods (e.g., circular dichroism spectroscopy, small-angle X-ray scattering, dynamic light scattering, mass-spectrometry). Protein crystallization setups will be done with manual set-ups or robotic equipment. Cryo-EM studies are carried out in an international collaboration (United States). The PhD student will perform most of the work in the international Structural Biology group at the IMB at the University of Graz, where 6 PIs and their groups have been working together in a "laboratory without walls" concept with shared knowledge, seminars and equipment since many years. We will continue collaboration within the SFB Lipid Hydrolysis and especially with the groups of Dr. R. Zimmermann, Dr. K. Gruber, Dr. R. Schreiber at the University of Graz, Dr. R. Birner-Grünberger at TU-Vienna, and Dr. A. Winkler at TU Graz.

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Florian REICHMANN, MD, PhD

Division of Pharmacology, Otto Loewi Research Center

Effects of altered microbiota-gut-brain axis signaling on aggressive behaviour

Background:

Aggression is an innate behaviour expressed throughout the animal kingdom for various reasons including foraging, fighting for territories or defending offspring. However, in humans, excessive aggression has been found as part of many major neuropsychiatric disorders such as attention deficit hyperactivity disorder, schizophrenia or dementia.

Hypothesis and Objectives:

While genetic factors play an important role for aggression, twin and animal studies suggest a substantial contribution of various environmental factors as well (1), (2). Among the latter an emerging, but so far understudied, field is the influence of an environmental factor within the body on aggression: the microbiota and its microbiome. Constituting a huge ecological community of microorganisms within the vertebrate gastrointestinal tract, the gut microbiota is able to influence host cells including distant organs such as the brain via the so-called “microbiota-gut-brain axis” (3). We hypothesize that (i) enhanced aggressive behavior is linked to specific changes in the gastrointestinal microbiome, (ii) that microbial alterations via antibiotics, prebiotics, probiotics and/or germ-free conditions affects aggression and neurobiology and that (iii) targeted microbial interventions can rescue excessive aggressiveness.

Methodology:

The recruited PhD student will use zebrafish as a model system to investigate the connections between the intestinal microbiota and aggression. Interventions include antibiotics/prebiotics/probiotics to modify the intestinal microbiota, germ-free animals that lack microbes, fecal microbiota transplantations between aggressive and not aggressive fish and transgenic zebrafish lines. A battery of behavioural tests will be used to assess the effects of the experimental interventions on aggression, but also on other behaviours such as anxiety, cognition and social behaviour. Bacterial community profiling (16S and/or shotgun metagenomics) within the gut will be used to characterize microbial alterations and metabolomics techniques will be used to identify potential mediators of the behavioural effects.

To investigate whether microbial alterations affect neurobiology, we will measure neurotransmitter levels via HPLC, neuronal activation via rpS6-immunohistochemistry, microglial polarization via qPCR and/or flow cytometry and global gene expression changes via RNAseq.

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PhD Program

MOLMED

Beate Rinner, PhD
Division of Biomedial Research,
Core Facility Alternative Biomodels & Preclinical Imaging;

The role of extracellular vesicles as emerging modulators of anticancer drug resistance

Background: As one of the leading causes of death, cancer remains a serious threat to human health, with anticancer drug efficacy, toxicity, and resistance being key concerns. Previous research has shown that extracellular vesicles (EVs) contribute to tumor growth, drug resistance, metastasis, and remodeling of the tumor immunological microenvironment (1,2). EVs are a heterogeneous population of nano-sized, membrane-delineated vesicles involved in the interactions between tumor and tumor microenvironments and cell-to-cell communication. They can be found in various body fluids and are used to transfer DNA, mRNA, microRNAs, long non-coding RNAs, and proteins from the originating cells to neighboring and distant cells (3,4). Cell lines/cell models that critically represent the tumor microenvironment and better reflect the human in vivo situation (5,6) serve as a basis to study the evolution of resistance to chemotherapeutic agents at the extracellular vesicle level.

Hypothesis and Objectives: The number of EVs released, the composition of tetraspanins, and especially the EV cargo are significantly altered by chemotherapies. These changes can subsequently lead to resistance formation and treatment failure. The aim is to reduce the release of EVs by different EV inhibitors to significantly improve chemotherapies' effect.

Methodology: Establishment and detailed characterization of patient-derived tumor models. Culturing will be performed in advanced dynamic 3D co-culture systems. EVs are isolated using ultracentrifugation, size exclusion, and tangential flow filtration and characterized using NanoSight, flow cytometry, western blot, and PCR technologies. Differences in EV cargo are mainly filtered out by proteome and RNA analysis. Size, structure, tetraspanin expression, and specific EV markers will be detected using the ExoView chip-based technology platform.

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PhD Program

MOLMED

Matteo VILLA, PhD
Martin STRADNER, MD

Division of Rheumatology and Immunology, Department of Internal Medicine, Medical University of Graz

Understanding the adaptation of immune cells to the tissue environment to treat rheumatoid arthritis

Background:

Rheumatoid Arthritis (RA) affects up to 1% of the population. Novel therapies improved its treatment, but they are seldom resolutive and around 20% of patients are refractory to them. While RA is a systemic autoimmune disease, clinical symptoms are primarily apparent in the joint. A better understanding of the local environment of the joint, and how this affects immune cell function, will be key to improve treatment of RA. Recent work revealed the heterogeneity of the immune populations in the RA joint (1, 2). However, it is not yet understood how immune cells sense and adapt to the joint environment, how this fuels the dysregulated immune response, and if the cross-talk between environment and immune cells can be hijacked to control RA progression.

Hypothesis and Objectives:

We hypothesize that the sensing of the joint environment by infiltrating immune cells shapes the acquisition of their inflammatory profile in the early stages of RA. Indeed, upon migration from the blood, immune cells encounter and adapt to the joint environment to perform their effector functions. We will assess how T cells, B cells and monocyte/macrophages infiltrating the RA joint sense and adapt their transcriptional and metabolic profiles to the surrounding environment (3). By understanding the metabolic requirements of immune cells, and how they change upon infiltration in the RA joint, we will pinpoint novel targets to manipulate the adaptation of immune cells to the joint environment, and prevent RA inflammation.

Methodology:

Our group focuses on translational immunology, working at the interface between basic research and clinical practice. We investigate questions that are clinically relevant, combining the analysis of human samples with state-of-the-art techniques such as flow cytometry, single cell transcriptomics, metabolic profiling and CRISPR-Cas9 gene editing. We also use mouse models of RA to mechanistically validate our human data.

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PhD Program

MOLMED

Matteo VILLA, PhD
Martin STRADNER, MD

Division of Rheumatology and Immunology, Department of Internal Medicine, Medical University of Graz

Exploring the cross-talk between fibroblasts and tissue microenvironment in rheumatoid arthritis

Background:

Rheumatoid Arthritis (RA) is an autoimmune disease that affects 1% of the population. Fibroblasts have been recently suggested to be important drivers of the joint inflammation characteristic of RA. In the healthy joint, fibroblasts constitute a barrier to protect and nourish the synovium, and provide lubrication to the joint. However, during RA synovitis, fibroblasts acquire an aggressive phenotype: they sustain local inflammation by recruiting immune cells to the joint, and promote remodelling of the extracellular matrix, a process that may ultimately progress to joint damage (1-3). While cytokines such as tumor necrosis factor and interleukin-1 have been implicated in the reprogramming of fibroblasts in RA, this phenomenon is far from being fully understood.

Hypothesis and Objectives:

The composition of the RA joint microenvironment is not extensively characterized. It is likely that besides inflammatory stimuli such as cytokines, other metabolites are actively or passively released by immune cells during the inflammatory process. We hypothesize that fibroblasts sense changes in the joint microenvironment during onset and progression of RA, and in turn adapt their transcriptional, metabolic and functional properties. We want to comprehensively assess how the joint environment reprograms the metabolic choices and function of fibroblasts, to ultimately identify strategies to disengage them from the vicious cycle that sustains RA joint inflammation.

Methodology:

Our group focuses on translational immunology, working at the interface between basic research and clinical practice. We investigate questions that are clinically relevant, combining the analysis of human samples with state-of-the-art techniques such as flow cytometry, single cell transcriptomics, metabolic profiling and CRISPRCas9 gene editing. We also use mouse models of RA to mechanistically validate our human data.

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Martin WAGNER, MD

Division of Gastroenterology and Hepatology, Department of Internal Medicine

FXR liver-adipose axis

Background:

Ligand activation of the bile acid receptor FXR is a promising therapeutic option for patients with non-alcoholic fatty liver disease (NAFLD), of which many are obese. In clinical trials, various FXR agonists improved biomarkers of NAFLD, partly induced mild weight loss and partly improved insu-lin sensitivity. In our previous project, we performed unbiased genome-wide approaches in obese patients with NAFLD treated with the clinically most advanced FXR agonist, obeticholic acid (OCA), in order to unravel FXR signaling pathways in livers of obese patients with NAFLD. A distinct finding of these genomic tests was that the hepatic FXR binding profile in ChIP-seq assays was strikingly different in obese patients compared to non-obese patients, suggesting different and/or additional FXR signaling capacities for obese individuals and a dependency of the FXR cistrome from the metabolic background. Surprisingly, when we integrated the FXR ChIP-seq data with the corre-sponding liver transcriptome (RNA-seq data), the top deregulated pathways in obese patients were enriched for mitochondria-related pathways including heat production by uncoupling reactions. Among the top FXR regulated genes/pathways in the liver we also found activation of FNDC5/irisin and the Slit pathway, two signaling pathways known to induce browning of white adipose tissue (WAT) and subsequently thermogenesis. A preliminary experiment in visceral and subcutaneous WAT samples of exactly the same human study population showed induced surrogate markers of browning in subcutaneous WAT after OCA treatment.

Hypothesis and Objectives:

Overall, we hypothesize that hepatic FXR stimulation activates a novel liver-adipose signaling axis preferentially in obese patients, which induces adipocyte browning and thermo-genesis.

The main research objectives are:

1. Does treatment with the FXR agonist OCA induce browning and modify the cellular microenvironment of the adipose tissue in obese patients?
2. Is hepatic induction of FNDC5/irisin and/or Slit2 triggering adipocyte browning (“liver-adipose axis”) and what are the direct effects of the FXR agonist OCA on adipose tissue?

Methodology:

In aim 1 we will use unbiased single nuclei transcriptomics to delineate the cellular

microenvironment and interrogate major deregulated pathways in subcutaneous and visceral adipose tissue from obese and non-obese patients treated with OCA. We will apply targeted lipidomics and NMR based metabolomics to reveal the energetic consequences of OCA treatment in these patient samples. In a complementary experiment in mice we will investigate the requirements of hepatic FXR using liver specific FXR knockout mice.
In aim 2 we will use in vitro and ex vivo sandwich culture systems of FXR-stimulated human hepatocytes and primary murine hepatocytes together with pre-adipocytes with genetically silenced FNDC5, Slit2 and/or their receptors.

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Lukas Groschner, MD, PhD
Gottfried Schatz Research Center, Medical University of Graz
Max Planck Institute for Biological Intelligence

Project Title:

Temporal signal processing in a small brain

Background: Brains process temporal signals over at least nine orders of magnitude, ranging from action potentials that last only milliseconds to circadian rhythms and beyond. While the biophysical mechanisms that act at both ends of this spectrum are well characterized, little is known about the processes that operate at intermediate time scales.
We use the fruit fly as a model in which numerical simplicity, well-charted connectivity, and our ability to control nervous activity have aligned to make mechanistic ideas precise and testable. Our work is based on the premise that nervous systems across species employ a common set of circuit architectures to delay, accumulate, and store signals over this time range. Bridging biophysics and behaviour, we describe these circuits in molecular detail and search for general principles of how they compute.

Hypothesis and Objectives: This project focuses on temporal patterns of neural activity that unfold over hundreds of milliseconds up to minutes. Successful applicants will address one of the following three research questions:
1) What ion channel make-up and what circuit motifs allow neurons to delay signals by hundreds of milliseconds? To compute the direction of visual motion, signals from adjacent photoreceptors need to be differentially delayed and processed in a nonlinear fashion. In the visual system of Drosophila, we have a good understanding of the nonlinear processing step1,2, but not of the temporal filters. We want to find out how a small circuit of nine morphologically and transcriptionally similar neurons gives rise to a filter bank of widely varying time constants.
2) How does visual information accumulate over time to inform behavioural choice? The noisy and fleeting nature of sensory inputs requires the brain to construct time-averaged representations when making decisions3,4. In the visual circuit of Drosophila, motion-sensitive neurons provide noisy momentary information about visual motion. These signals are integrated over multiple seconds in the lead-up to the animal's response. This process of temporal integration takes place in a matter of just three synapses; we want to know how.

3) How does a brain construct a memoriy that are stable during times of immobility, but exquisitely malleable—sensitive to every step—during locomotion? When moving about, animals keep track of their current speed and heading in allocentric coordinates5. Temporal integration of these momentary travel vectors allows insects to generate and maintain a mental representation of the shortest route to the point of origin. We ask how the brain encodes the length of such a vector.

Methodology: The project relies on a set of experimental and computational approaches, which include behavioural assays, recordings and manipulations of neural activity *in vivo*, transcriptomic profiling of neuronal populations, and biophysically realistic modelling of neurons and circuits.

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