PhD positions in the field of molecular medicine, cancer research, immunology, molecular and lipid metabolism, cardiovascular research and biostatistics

Projects starting October 1, 2024

Application deadline: March 24, 2024

Join our cutting-edge PhD programs at one of Europe's leading Universities in the field of medical research. We offer the unique opportunity to work with state-of-the-art techniques and link basic research with clinically-oriented sciences. The close cooperation with the facilities of the Medical University and the infrastructure of the University Hospital Graz will allow you to push the boundaries of classical medical research. It will give you all the tools to publish in high-ranking journals.

POSITIONS

The PhD Program "Molecular Medicine" (Medical University of Graz), offers 18 PhD positions with a special focus on cancer research, immunology, lipid metabolism, cardiovascular research and pregnancy. As a PhD student, you will be embedded in an interdisciplinary community of supervisors, fellow students and administrative support. PhD students receive a 3-year contract (1 initial year + 2 years extension) including a competitive salary and social benefits. The possibility of a contract extension for 1 year (4th year) may be possible upon successful completion of a research stay abroad.

THE UNIVERSITIY

The Medical University of is a vibrant future-oriented University in the heart of Austria. Our researchers are highly motivated, future-oriented and innovative. The Medical University has been repeatedly named one of the top universities in the world (among the top 200 universities worldwide according to THE). It is located in the Medical Science City Graz, merging space, content, and strategy of basic research institutions, applied clinical research, and patient care at the University Hospital. As a result, Graz is becoming one of the leading medical centers in Europe where science and research is entangled with practical implementation.

THE CITY

Graz is located at the heart of the beautiful region of Styria. It is Austria's second-biggest city and enjoys a rich history yet is at the same time filled with modern life. As a university town (there are 6 universities located in the city), Graz offers a young and international atmosphere with a lively cultural scene.

APPLICATION

Applications are exclusively accepted through the online platform https://phd-recruiting.medunigraz.at. You do not need to contact the supervisors and do not send any documents by regular mail or e-mail unless you are explicitly asked to do so by our staff. There is no application fee. Our Welcome Center is happy to support you with your visa application and entry into Austria.

For further information about the programs, open projects, and the online application procedure, please see: https://www.medunigraz.at/phd/application/

Application deadline: March 24, 2024

Mahmoud Abdellatif

Division of Cardiology, Department of Internal Medicine, Medical University of Graz

Project Title: Energizing the failing heart

Background:

A significant proportion of heart failure patients, accounting for at least 50%, do not manifest a major deficit in left ventricular systolic function. Instead, they present with diastolic impairment, leading to what is known as preserved ejection fraction heart failure (HFpEF). This condition becomes more prevalent with age and currently stands as a major cause of hospitalization among the elderly. Despite its substantial socioeconomic burden, HFpEF has limited evidence-based therapies capable of enhancing patients' survival and quality of life.

Hypothesis and Objectives:

We hypothesize that HFpEF is an age-related disease driven by compromised cellular quality control due to impaired autophagy. Therefore, our objectives include investigating: (i) recently identified targets for the regulation of autophagy, known as autophagy checkpoints; (ii) the extent of impairment of these checkpoints during HFpEF development; and (iii) the potential for targeting these checkpoints as a therapeutic approach for HFpEF.

Approaches and methods:

The research will employ a comprehensive array of cutting-edge techniques, encompassing autophagic flux and mitochondrial assays, echocardiography, invasive hemodynamics, as well as multiomics and data integration. Both genetic and pharmacological interventions will be applied in vivo using relevant mouse models and human HFpEF tissue. The selected PhD candidate will collaborate closely with other fellows in the Cardiovascular Aging group, led by M. Abdellatif. Additionally, the PhD student will have the opportunity to conduct parts of the study abroad, collaborating with distinguished international research groups involved in this European project. These collaborations include the labs of Prof. Guido Kroemer (Paris), Prof. Maria Mitterbrunn (Madrid), and Prof. Rudolf de Boer (Rotterdam).

References:

1. Abdellatif *et al*. Actionable Autophagy Checkpoints in Cardiovascular Ageing. **European Heart Journal** (2023)
2. Abdellatif *et al*. Hallmarks of Cardiovascular Aging. **Nature Reviews Cardiology** (2023)
3. Abdellatif *et al*. Fine-Tuning Cardiac Insulin-Like Growth Factor 1 Receptor Signaling to Promote Health and Longevity. **Circulation** (2022)
4. Abdellatif *et al*. Nicotinamide for the treatment of heart failure with preserved ejection fraction. **Science Translational Medicine** (2021)

Nerea Alonso Lopez

Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz

Project Title: CXCR4 role in the anabolic response of teriparatide in bone

Background:

Osteoporosis is a common disease causing major public health and patient burdens. It is characterised by reduced bone formation and increased bone resorption; a process yet not fully understood. Patients with established osteoporosis are treated with teriparatide (TPTD), a highly effective anabolic agent. However, it is a costly treatment and the individual response is variable. Our preliminary data suggest that the genetic background plays a key role in the success of the treatment, appointing to a locus in chromosome 2, where the CXCR4 is located. In vitro experiments suggest that CXCR4 is involved in osteoblast migration and mineralisation in response to TPTD. Moreover, we found for the first time that TPTD could also exert a direct effect on osteoclasts via CXCR4.

Hypothesis and Objectives:

This grant proposal aims to investigate the role of CXCR4 in bone metabolism in response to TPTD and to identify novel pathways that could have therapeutic potential for patients with established osteoporosis.

Approaches and methods:

We will use a *CXCR4* haploinsufficient mouse model (Cre+*CXCR4*$^{flox/+}$) model and analyse changes in bone tissue by microCT, histomorphometry and 3-point bending test and serum biomarkers in response to TPTD. We will also investigate in vitro the role of CXCR4 in the osteoblast and osteoclast response to TPTD to identify cellular changes. At this stage, both cell types will be analysed separately and also in combination to assess not only individual response but also cross-talk changes. mRNAseq and proteomics will be performed to identify novel pathways involved in bone turnover that could be used in future as markers for osteoporosis appearance and/or as therapeutic targets to improve bone health.

References:

Alonso, N., Albagha, O. M. E., Azfer, A., Larraz-Prieto, B., Berg, K., Riches, P. L., Ostanek, B., Kocjan, T., Marc, J., Langdahl, B. L., & Ralston, S. H. (2023). Genome-wide association study identifies genetic variants which predict the response of bone mineral density to teriparatide therapy. *Ann Rheum Dis*. https://doi.org/10.1136/ard-2022-223618

Canalis, E., Giustina, A., & Bilezikian, J. P. (2007). Mechanisms of anabolic therapies for osteoporosis. *N Engl J Med*, *357*(9), 905-916. https://doi.org/10.1056/NEJMra067395

Shahnazari, M., Chu, V., Wronski, T. J., Nissenson, R. A., & Halloran, B. P. (2013). CXCL12/CXCR4 signaling in the osteoblast regulates the mesenchymal stem cell and osteoclast lineage populations. *FASEB J*, *27*(9), 3505-3513. https://doi.org/10.1096/fj.12-225763

Tian, Y., Xu, Y., Fu, Q., & He, M. (2011). Parathyroid hormone regulates osteoblast differentiation in a Wnt/beta-catenin-dependent manner. *Mol Cell Biochem*, *355*(1-2), 211-216. https://doi.org/10.1007/s11010-011-0856-8

Zhu, W., Liang, G., Huang, Z., Doty, S. B., & Boskey, A. L. (2011). Conditional inactivation of the CXCR4 receptor in osteoprecursors reduces postnatal bone formation due to impaired osteoblast development. *J Biol Chem*, *286*(30), 26794-26805. https://doi.org/10.1074/jbc.M111.250985

Katharina Artinger

Division of Nephrology, Department of Internal Medicine, Medical University of Graz

Project Title: Atypical chemokine receptors in kidney disease

Background:

This project aims to evaluate the roles of two atypical chemokine receptors (ACKRs), ACKR1 and ACKR4, in murine kidney disease. Atypical chemokine receptors are involved in the regulation of immune cell movement through their interaction with chemokines, although they do not transmit conventional intracellular signals [1]. So far, ACKR1 has been described on venular endothelial cells, erythroid cells and cerebellar neurons [2]. Interestingly, the majority of individuals of West African ancestry carry a single nucleotide polymorphism of ACKR1 which disrupts transcription of ACKR1 in the erythroid lineage only, thus resulting in "erythroid silent" ACKR1 phenotype. It has been well-documented that people of West African ancestry have a higher incidence of kidney diseases resulting in more severe disease parameters and more unfavorable outcomes. In line with this, it was shown previously that ACKR1$^{-/-}$ mice develop more severe kidney disease as compared to control mice [3]. So far, there was no knowledge on the renal expression pattern of ACKR1 in diseased kidneys. Therefore, one goal of this project is to evaluate whether expression of ACKR1 in the kidney changes in response to renal disease. Furthermore, this project is set to discern whether endothelial expression of ACKR1 or its erythroid cell expression would be responsible for the observed aggravated renal phenotype in ACKR1$^{-/-}$ mice. For this work, a well-established and reliable murine model of experimental glomerulonephritis (nephrotoxic serum nephritis, NTSN) will be used. The second part of the project will be centered around another atypical chemokine receptor, ACKR4. Our preliminary data using ACKR4^{eGFP} reporter mice revealed a polar ACKR4 expression of only few glomerular cells, which until now has not been reported. ACKR4 scavenges chemokines like CCL21, a classical CCR7 ligand. The fact that CCR7 is implicated in the pathogenesis of NTSN [4] and our preliminary data on the expression of ACKR4 in the kidney suggest a potential role for this atypical chemokine receptor in kidney disease. One main goal of the project will therefore be to define the cells expressing ACKR4 in the kidney in health and disease. The contribution of ACKR4 to the development of kidney disease will be evaluated using the murine model of NTSN. A central part of this project will also be to establish the role of ACKR4 as expressed in the kidney as compared to ACKR4 expressed lymphoid tissues.

Hypothesis and Objectives:

This project aims to map molecular and cellular pathways involving atypical chemokine receptors ACKR1 and ACKR4 and evaluate their pathogenic contributions to experimental murine glomerulonephritis. Regarding ACKR1, our preliminary experiments show that the loss of this receptor on either endothelial cells or bone marrow erythroblasts results in an aggravated NTSN phenotype. Building on this data, we plan to investigate the pathomechanisms and involvement of ACKR1

expressed by erythroblasts versus endothelium, contributing to leukocyte recruitment into the kidney. Furthermore, this project aims to establish how ACKR4 contributes to the development of NTSN including the potential involvement of the newly discovered ACKR4-expressing subset of glomerular cells.

Approaches and methods:

Mice will be preimmunized with rabbit IgG and three days thereafter, injected with rabbit anti-mouse glomerular basal membrane (GBM) serum leading to the induction of NTSN. Flow cytometry, confocal microscopy, scRNAseq, and spatial transcriptomics as well as conventional ELISA, histopathology and quantitative real-time PCR will be applied. Global ACKR1, ACKR4 deficient mice and ACKR4-eGFP reporter mice and their appropriate controls will be used in these experiments as required. The PhD student will learn all needed methods including animal experiments under the supervision of the PI and our technician.

References:

1. Ulvmar MH, Hub E, Rot A. Atypical chemokine receptors. *Exp Cell Res*. 2011;317(5):556-568. doi:10.1016/j.yexcr.2011.01.012

2. Rot A, Andrian UH von. Chemokines in Innate and Adaptive Host Defense: Basic Chemokinese Grammar for Immune Cells. *Annu Rev Immunol*. 2004;22(1):891-928. doi:10.1146/annurev.immunol.22.012703.104543

3. Vielhauer V, Allam R, Lindenmeyer MT, et al. Efficient Renal Recruitment of Macrophages and T Cells in Mice Lacking the Duffy Antigen/Receptor for Chemokines. *Am J Pathol*. 2009;175(1):119-131. doi:10.2353/ajpath.2009.080590

4. Eller K, Weber T, Pruenster M, et al. CCR7 Deficiency Exacerbates Injury in Acute Nephritis Due to Aberrant Localization of Regulatory T Cells. *J Am Soc Nephrol*. 2010;21(1):42-52. doi:10.1681/asn.2009020133

Eva Böhm

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

Project Title: The role of the circadian clock in pulmonary fibrosis

Background:

Pulmonary fibrosis s a chronic, progressive and often fatal lung disease characterized by excessive deposition of fibrotic connective tissue in the lungs. The onset of pulmonary fibrosis is usually initiated by the dysregulation of tissue repair mechanisms which can be induced by various causes, such as air pollution, cigarette smoke, antineoplastic drugs, and respiratory viral infections such as influenza A virus and SARS-CoV-2 infections. However, there is currently no curative treatment; the only approved drugs, nintedanib and pirfenidone, can only slow down the progression of pulmonary fibrosis (1). Thus, investigating new promising molecular pathways involved in fibrogenic responses is urgently needed. Circadian regulation causes the activity of biological processes to vary over a 24-h cycle. Various studies to date demonstrate the fundamental interactions of circadian clock molecules, such as REV-ERBα or BMAL1, with lung inflammatory responses and the development of chronic lung diseases (2). However, the mechanism and role of the circadian clock in lung fibrogenesis is still poorly understood (3, 4).

Hypothesis and Objectives:

Circadian clock molecules are involved in the development of pulmonary fibrosis and represent a promising therapeutic target to halt the progression of the disease. Therefore, we aim to investigate the relationship between the circadian clock and fibrogenesis and to pharmacologically target the circadian clock in a comprehensive translational approach that includes established in vitro assays, in vivo models and analysis of patient samples.

Approaches and methods:

The recruited PhD candidate will be trained in and perform isolation of different immune cell populations from blood of healthy donors and fibrosis patients. Further, *in vitro* (co-)culture and functional assays for immune cells, fibrocytes, fibroblasts, and epithelial cells will be employed for this study (1st and 2nd year). The student will use established mouse models (e.g. intranasal LPS, bleomycin) and precision cut lung slices, generated from mouse or human lungs, to validate her/his findings *in vivo*/*ex vivo* (2nd and 3rd year). Expression and activation of the circadian clock or disease-specific markers will be monitored in cells and tissue by multi-color flow cytometry, Western blot, qPCR, immunohistochemistry, *in situ* hybridization, and immunofluorescence microscopy. Changes in glycolytic *versus* oxidative metabolism of the cells will be determined by Seahorse analysis. To target the circadian clock, siRNA silencing, selective synthetic agonists/antagonists, and

knockout/transgenic mouse strains will be used. Inflammatory mediators will be measured by mass spectrometry or multiplex ELISA.

References:

1. Durheim MT, Bendstrup E, Carlson L, Sutinen EM, Hyldgaard C, Kalafatis D, Myllärniemi M, Sköld CM, Sjåheim T. Outcomes of patients with advanced idiopathic pulmonary fibrosis treated with nintedanib or pirfenidone in a real-world multicentre cohort. Respirology. 2021 Oct;26(10):982-988. doi: 10.1111/resp.14116.
2. Cunningham PS, Jackson C, Chakraborty A, Cain J, Durrington HJ, Blaikley JF. Circadian regulation of pulmonary disease: the importance of timing. Clin Sci (Lond). 2023 Jun 14;137(11):895-912. doi: 10.1042/CS20220061.
3. Cunningham PS, Meijer P, Nazgiewicz A, Anderson SG, Borthwick LA, Bagnall J, Kitchen GB, Lodyga M, Begley N, Venkateswaran RV, Shah R, Mercer PF, Durrington HJ, Henderson NC, Piper-Hanley K, Fisher AJ, Chambers RC, Bechtold DA, Gibbs JE, Loudon AS, Rutter MK, Hinz B, Ray DW, Blaikley JF. The circadian clock protein REVERBα inhibits pulmonary fibrosis development. Proc Natl Acad Sci U S A. 2020 Jan 14;117(2):1139-1147. doi: 10.1073/pnas.1912109117.
4. Wang Q, Sundar IK, Lucas JH, Park JG, Nogales A, Martinez-Sobrido L, Rahman I. Circadian clock molecule REV-ERBα regulates lung fibrotic progression through collagen stabilization. Nat Commun. 2023 Mar 9;14(1):1295. doi: 10.1038/s41467-023-36896-0.

Isabell Dorn

Department for Blood Group Serology and Transfusion Medicine, Medical University of Graz

Project Titel: Genetic variants of the mechanosensitive ion channel Piezo1 in red blood cells - their role in human physiology and evolution

Background:

Tissue oxygenation by **red blood cells (RBC)** is one key factor defining fitness and survival under stress conditions. Therefore, genetic variants of proteins controlling RBC number and properties are crucial for natural selection.[1] The mechano-sensitive ion channel **Piezo1** is such a protein. Gain of function (GOF) mutations affecting the channel pore cause the hemolytic disorder hereditary xerocytosis (HX).[2] Another GOF mutation, located in the less investigated blade part, protects against severe malaria and is prevalent in 30% of the African populations in malaria-rich areas.[3] The Piezo1 gene is one of 87 genes that differ between Neanderthals and modern humans.The **Neanderthal** variant of Piezo1 has a mutation, which according to simulations, also affects the blade part and is likely to modulate channel function.

Hypothesis and Objectives:

We aim to establish a link between RBC Piezo1 polymorphisms and tissue oxygenation. We hypothesize that present-day Piezo1 variants differ from the Neanderthal variant in their channel properties and alter tissue oxygenation. Therefore, Piezo1 may be subject to selection in ongoing human adaptive evolution. Research at the genome, protein, cellular and systemic levels complemented with mathematical modelling, will elucidate the link between Piezo1 polymorphisms, channel activity and tissue oxygenation in modern humans. It will be the first study of its kind to examine the physiological effects of an archaic versus a modern human protein variant on O_2 supply and tissue oxygenation. The results are expected to shed light on the fundamental mechanisms of human evolution and the role of Piezo1 in RBC physiology and pathologies.

Methodology:

1) PhD student Graz: Genome engineering of human induced pluripotent stem cells to produce iPSCs and finally RBCs expressing archaic Piezo1.[4, 5] Archaic and modern human RBCs will be compared for their cellular properties. 2) Saarland University, Germany: Systemic effects of Piezo1 variants will be investigated in a mouse model with genetically/pharmacologically modified channel activity. The influence of Piezo1 on human tissue oxygenation will be analyzed in a cohort of HX patients in comparison to healthy controls. 3) University Zürich, Switzerland: Obtained results and databases will be used for mathematical modelling to reconstruct the demographic history of Piezo1 polymorphisms and to model potential environmental effects on present-day Piezo1 polymorphisms.

Primary researchers involved:

It is an interdisciplinary project with partners from Switzerland and Germany. A strong scientific exchange with secondments to the project partners is planned. I. Dorn and A. Reinisch (Medical University Graz, Austria) are experts in RBC differentiation from hiPSCs and CRISPR/Cas9 genome editing. L. Kaestner (Saarland University, Germany) is specialized in RBC ion-channel characterization, RBC biophysics and experienced with transgenic animal models. N. Bender and P. Eppenberger (University of Zürich, CH) will provide their knowledge of human evolution and mathematical modelling.

References:

1. Rotival M, Quintana-Murci L. Functional consequences of archaic introgression and their impact on fitness. *Genome Biol*. Jan 2 2020;21(1):3. DOI: 10.1186/s13059-019-1920-z
2. Ridone P, Vassalli M, Martinac B. Piezo1 mechanosensitive channels: what are they and why are they important. *Biophys Rev*. Oct 2019;11(5):795-805. DOI: 10.1007/s12551-019-00584-5
3. Ma S, Cahalan S, LaMonte G, et al. Common PIEZO1 Allele in African Populations Causes RBC Dehydration and Attenuates Plasmodium Infection. *Cell*. Apr 5 2018;173(2):443-455.e412. DOI: 10.1016/j.cell.2018.02.047
4. Bernecker C, Matzhold EM, Kolb D, et al. Membrane Properties of Human Induced Pluripotent Stem Cell-Derived Cultured Red Blood Cells. *Cells*. Aug 9 2022;11(16). . DOI: 10.3390/cells1116247
5. Dever DP, Bak RO, Reinisch A, et al. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. *Nature*. 2016/11/01 2016;539(7629):384-389. DOI: 10.1038/nature20134

Julia Feichtinger

Gottfried Schatz Research Center for Cell Signaling, Metabolism & Aging (Cell Biology, Histology and Embryology), Medical University of Graz

Project Titel: The immunomodulatory effects of the NR4A1/p53 axis in diffuse large B cell lymphoma

Background:

Diffuse large B cell lymphoma (DLBCL) is the predominant lymphoid malignancy in the western world. While immune checkpoint blockade therapy is in clinical practice for a number of cancer types, the response rates for B cell lymphomas remain disappointingly low. Importantly, immune evasive mechanisms are still poorly investigated in B cell lymphomas, which has impeded the progress in developing new therapeutic approaches targeting the lymphoma immune landscape. This in-silico project aims to address this gap by investigating the immunomodulatory effects of the NR4A1/p53 axis, two pivotal transcription factors and tumour suppressors.

Hypothesis and Objectives:

Our preliminary data strongly suggests that NR4A1 and p53 build a functional axis to orchestrate immune evasive mechanisms in DLBCL. We therefore hypothesize that the NR4A1/p53 status results in distinct expression patterns of immunomodulatory factors (such as coinhibitory immune checkpoint receptors/ligands, MHC and cytokines), which in turn influences the anti-tumour response. Furthermore, we anticipate that the NR4A1/p53 status holds significant clinical relevance in human DLBCL. This project is part of a consortium initiative with objectives that include the investigation of mouse models with Nr4a1 and/or p53 knockout and the exploration of treatment options. Additionally, the aim is to translate findings of the Nr4a1/p53 axis in mice into a clinical setting using the GRAZ lymphoma cohort.

Methodology:

The PhD will focus on the *in-silico* part of the consortium initiative by leveraging bioinformatics methods, data analysis and mining techniques. The analysis of transcriptomics data (qPROseq, (single-cell) RNA-seq) will serve as the foundation for investigating the expression patterns of immunomodulatory factors, anti-tumour response and tumour microenviroment. To explore the clinical relevance of the NR4A1/p53 axis and inter-patient heterogeneity, a comprehensively annotated DLBCL dataset will be processed and analyzed. This dataset includes multiple read-outs such as the NR4A1/p53 status, mutations, expression data of immunomodulatory factors, composition of tumour-infiltrating immune cells, *ex vivo* response rates and clinical parameters.

References:

Fechter K, Feichtinger J, Prochazka K, Unterluggauer JJ, Pansy K, Steinbauer E, Pichler M, Haybaeck J, Prokesch A, Greinix HT, Beham-Schmid C, Neumeister P, Thallinger GG, Deutsch AJA: **Cytoplasmic location of NR4A1 in aggressive lymphomas is associated with a favourablecancer specific survival**. *Sci Rep* 2018, **8**:14528.

Deutsch AJA, Rinner B, Wenzl K, Pichler M, Troppan K, Steinbauer E, Schwarzenbacher D, Reitter S, Feichtinger J, Tierling S, Prokesch A, Scheideler M, Krogsdam A, Thallinger GG, Schaider H, Beham-Schmid C, Neumeister P: **NR4A1-mediated apoptosis suppresses lymphomagenesis and is associated with a favorable cancer-specific survival in patients with aggressive B-celllymphomas.** *Blood* 2014, **123**:2367–2377.

Pansy K, Fechter K, Wenzl K, Arra A, Novak JP, Szmyra M, Haingartner S, Ramsay AG, Novak AJ, Greinix HT, Beham-Schmid C, Neumeister P, Deutsch AJA: **Loss of Nr4a1 Causes an Elevated Expression of Co-Inhibitory Receptor: Ligand Axes and Results in a Diminished T Cell-Mediated Lymphoma Cell Killing in Aggressive Lymphoma**. *Blood* 2022, **140**(Supplement 1):2038–2039.

Pansy K, Uhl B, Krstic J, Szmyra M, Fechter K, Santiso A, Thüminger L, Greinix H, Kargl J, Prochazka K, Feichtinger J, Deutsch A: **Immune regulatory processes of the tumor microenvironment under malignant conditions**. *Int J Mol Sci* 2021, **22**:13311.

Wenzl K, Stokes M, Novak JP, Bock AM, Khan S, Hopper MA, Krull JE, Dropik AR, Walker JS, Sarangi V, Mwangi R, Ortiz M, Stong N, Huang CC, Maurer MJ, Rimsza L, Link BK, Slager SL, Asmann Y, et al.: **Multiomic Analysis Identifies a High-Risk Metabolic and TME Depleted Signature that Predicts Early Clinical Failure in DLBCL**. *medRxiv* 2023:2023.06.07.23290748.

Gernot Grabner (PI)
Dagmar Kratky (Co-PI)

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

Project Title: Deciphering the role of lipolysis in brain lipid and enery metabolism

Background:

Triacylglycerols (TAGs) are the major storage form of fatty acids in mammals and are deposited in specialized subcellular organelles called lipid droplets (LDs) [1]. Emerging evidence demonstrates the importance of TAG metabolism in normal brain physiology and LD accumulation in brain cells is a common hallmark in various brain pathologies [2]. TAG biosynthesis is an important mechanism for coping with cellular stress and LD formation is crucial for brain cell survival [3]. The process of TAG degradation, called lipolysis, is catalyzed by the consecutive activities of lipases [1]. Although accumulating evidence suggests an important role of TAG metabolism in brains [4], it remains unclear which lipases are responsible for degrading TAGs within different brain cells. How lipolysis affects downstream metabolic pathways as well as cellular and whole-brain (patho-)physiology remains unknown.

Hypothesis and Objectives:

Our preliminary results indicate an important role of a distinct lipase in TAG catabolism in brains of mice and humans. We hypothesize that TAG hydrolysis mediated by this lipase in neurons critically affects brain lipid metabolism, inflammation, energy substrate utilization and mouse behavior. The overall objective of this project is to understand how defective lipolysis in distinct brain cells affects cellular and whole brain physiology in mice.

In our specific aims we will address consequences of impaired lipolysis in neurons on

Aim 1: Lipid composition of isolated cells and mouse brains

Aim 2: Energy metabolism in isolated cells *in vitro*

Aim 3: Gene expression and inflammatory response in brains

Approaches and methods:

The Gottfried Schatz Research Center as host institution, provides an excellent environment for research in the fields of biochemistry, molecular and cell biology as well as lipid and energy metabolism. All proposed experiments are based on established methods at the host or collaborating institutes. The PhD candiate will isolate and cultivate primary cells from brains (neurons, astrocytes, microglia) and will perform lipid loading and lipid analysis experiments. This will invole sample preparation and analysis via LC-MS and GC-MS in collaboration with the Core Facility Mass

Spectrometry. Primary cells will be further analyzed via SeaHorse respiratometry using selective lipase inhibitors. The PhD candiate will characterize lipase-deficient mice which involves analysis of gene expression, lipid composition, and histology of brain sections. Thereby, effects of dietary interventions and proinflammatory stimuli will be investigated. The PhD candidate will have the opportunity to acquire a diverse set of methods to expand and enhance his/her scientific skills at the interface of lipid and energy metabolism and neuroscience.

References:

[1] G. F. Grabner, H. Xie, M. Schweiger, and R. Zechner, "Lipolysis: cellular mechanisms for lipid mobilization from fat stores," *Nat. Metab.*, vol. 3, no. 11, pp. 1445–1465, Nov. 2021.
[2] I. Ralhan, C. L. Chang, J. Lippincott-Schwartz, and M. S. Ioannou, "Lipid droplets in the nervous system," *J. Cell Biol.*, vol. 220, no. 7, pp. 1–18, 2021.
[3] A. P. Bailey *et al.*, "Antioxidant Role for Lipid Droplets in a Stem Cell Niche of Drosophila," *Cell*, vol. 163, no. 2, pp. 340–353, Oct. 2015.
[4] E. Islimye, V. Girard, and A. P. Gould, "Functions of Stress-Induced Lipid Droplets in the Nervous System," *Front. Cell Dev. Biol.*, vol. 10, no. April, pp. 1–12, 2022.

Ursula Hiden
Department of Obstetrics and Gynecology, Medical University of Graz

Project Title: Prone Adiposity: Programming of neonatal adipose tissue by maternal gestational diabetes

Background:

Influences in pregnancy form the susceptibility for non-communicable diseases later in life of the offspring (1). This 'fetal programming' occurs largely through epigenetic changes, such as altered DNA methylation patterns (2). Offspring exposed to gestational diabetes (GDM) have an increased risk for obesity and metabolic diseases associated with adipose tissue dysfunction, including type 2 diabetes or metabolic syndrome (3). Dysfunctional adipose tissue is characterized by altered paracrine activity, vascularisation, lipid/ganglioside composition and insulin resistance (4). Disturbances in adipocyte differentiation (adipogenesis) are suggested to play a key role in the development of adipose tissue dysfunction (5). Mesenchymal stem cells (MSCs) are the precursors of adipocytes, and the umbilical cord is a source of neonatal MSCs which can be isolated and differentiated to adipocytes *in vitro*.

Hypothesis and Objectives:

The intrauterine environment in GDM reprograms fetal MSCs. This affects adipogenesis and function of the differentiated adipocytes regarding paracrine activity, lipid composition and insulin resistance.

Approaches and methods:

Umbilical cord MSCs will be isolated after pregnancies with and without GDM. MSCs will be cultured and differentiated to adipocytes. Both cell types will be studied for their epigenetic differences, paracrine and pro-angiogenic activity, lipid and ganglioside composition and response to insulin. Data will be related to clinical characteristics of mothers and neonates.

References:

1. Gluckman, P.D., M.A. Hanson, C. Cooper, and K.L. Thornburg, *Effect of in utero and early-life conditions on adult health and disease*. N Engl J Med, 2008. **359**(1): p. 61-73. DOI: 10.1056/NEJMra0708473
2. Goyal, D., S.W. Limesand, and R. Goyal, *Epigenetic responses and the developmental origins of health and disease*. J Endocrinol, 2019. **242**(1): p. T105-T119. DOI: 10.1530/JOE-19-0009
3. Friedman, J.E., *Developmental Programming of Obesity and Diabetes in Mouse, Monkey, and Man in 2018: Where Are We Headed?* Diabetes, 2018. **67**(11): p. 2137-2151. DOI: 10.2337/dbi17-0011
4. Longo, M., F. Zatterale, J. Naderi, L. Parrillo, P. Formisano, G.A. Raciti, F. Beguinot, and C. Miele, *Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications*. Int J Mol Sci, 2019. **20**(9). DOI: 10.3390/ijms20092358
5. Marcelin, G. and K. Clement, *The multifaceted progenitor fates in healthy or unhealthy adipose tissue during obesity*. Rev Endocr Metab Disord, 2021. **22**(4): p. 1111-1119. DOI: 10.1007/s11154-021-09662-0

Philipp Jost

Division of Oncology, Department of Internal Medicine, Medical University of Graz

Project Title: Deciphering the role of necroptosis-induced inflammation in lung adenocarcinomas

Background:

Despite the success of immunotherapy in lung cancer (Herbst et al, Nature 2018), most patients experience relapse within months to years. This acquired resistance to immunotherapy illustrates our failure to comprehend the complex biology of the tumor microenvironment, the immune system, and its interaction with tumor cells. Importantly, the lack of biomarkers that can predict which patient will respond to immunotherapy and who will relapse, remains a critical roadblock for physicians for effective lung cancer treatment. Hence, understanding inflammatory processes in cancer cells and how they impact on the immune environment will critically contribute to our understanding of predictive and prognostic biomarkers in lung adenocarcinoma and beyond. Necroptosis represents a molecularly defined signaling pathway that ultimately results in cancer cell death and may amplify anti-tumor immunity of cancer therapy due to its highly inflammatory nature (reviewed in Linkermann and Green, NEJM, 2014). Hence, we need to understand how tumor cells repress necroptosis, what the effects of failed necroptosis are on tumor cell survival and how necroptosis, or the lack thereof, shapes the tumor-immune environment.

Hypothesis and Objectives:

We hypothesize that lung adenocarcinoma represses cancer cell-intrinsic necroptosis and associated inflammation to prevent immune cell infiltration and thereby secure its sustained tumor cell survival and tumor progression. Following aims will be addressed by this project.

Aim 1: Elucidate the role of necroptosis-associated transcriptional and immune cell profiles in human lung adenocarcinomas.

Aim 2: Dissect the mechanism of alterations of necroptotic genes.

Aim 3: Investigate functional consequences of necrosome signaling using clinically relevant endogenous murine model systems.

Approaches and methods:

To dissect transcriptional and immune cell profiles of both human and murine lung adenocarcinoma, the student will employ computational methods, patient samples and clinically relevant CRISPR-Cas9-based mouse model systems of lung adenocarcinoma harbouring *Kras*^{G12D} and loxP-flanked *Tp53*. Our mouse models allow us to functionally dissect the contribution of individual necroptotic genes to lung cancer development, maintenance and tumor-immune interaction. All proposed mouse models have

been approved by the Austrian Federal Ministry of Education, Science, and Research (Vienna, Austria) approved (2021-0.588.538).

References:

Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature. 2018;553(7689):446-54. Linkermann A, Green DR. Necroptosis. N Engl J Med. 2014;370(5):455-65.

Medical University of Graz

PhD Program MOLMED

Karl Kashofer

Diagnostic & Research Institute of Pathology, Medical University of Graz

Project Title: The initiation and evolution of rare cancers

Background:

Rare cancers are rare, but patients suffering from rare cancers are common.

A large part of global molecular pathology research is focused on big tumor entities like colon, lung and skin tumors. However, a substantial percentage of patients suffers from a variety of rare tumor types of different etiology and biology. Our research group focuses on rare tumor types which present unique challenges in diagnosis and treatment. Currently our work is mainly focused on urogenital cancers, mostly of the cervix, vulva and penis. Human papilloma virus (HPV)-induced invasive cervical squamous cell cancer (SCC) develops via the precursor lesion high-grade squamous intraepithelial lesion (HSIL). We are investigating the events leading to progression of the precursor lesions thin and thick HSIL to microinvasive and fully invasive SCC. Thin HSIL has a prevalence of 22.5% of non-high-risk HPV genotypes compared to less than 3% in thick HSIL and invasive SCC. Genetic mutations of driver genes are not apparent in precursor lesions but seem to play an important role in later stages and we have described a very high prevalence of TP53 and CDKN2a mutations in SCC of the cervix and related penile cancers. More recently we also published reports on the classification and molecular subtyping of intraepithelial neoplasia and the mutational landscapes in penile cancer and squamous cell cancers of the cervix.

Our lab is structured into a core diagnostic laboratory providing molecular pathology-based diagnostics in southern Austria and a research and development lab which strives to transition innovative and novel analysis methodologies from the basic research setting into clinical diagnostics. The prospective PhD student will be embedded in the research and development laboratory and work in close collaboration with our other PhD and diploma students.

Hypothesis and Objectives:

- Genetic analysis can lead to correct diagnosis and elucidation of the etiology in rare cancers.
- Genetic changes are the hallmark of progression from precursor lesion to invasive cancer.
- TP53 and CDKN2a mutations play an important role in carcinogenesis of rare cancers
- Structural DNA damage is contributing to the evolution of rare cancers

Methodology:

We have access to cohorts of penile, vulvar and cervical cancers which have been characterized by histology, immunohistochemistry and focused NGS panel analysis. We want to expand on this genetic analysis using exome sequencing based on a new NovaSeq 6000 instrument recently made available to our lab. Genetic analysis combined with multi-color and multi-cycle immunohistochemistry will

inspire hypotheses on the interplay of genetic mutations of driver and tumor suppressor genes with structural DNA damage and protein expression in these progressive stages of cancer development. We want to further establish a tissue culture-based system to test the oncogenicity of different genetic changes in-vitro and possibly also in-vivo in murine xenotransplantation models. Introduction of genetic mutations will be performed with the CRISPR/Cas system by recapitulating oncogenic mutations known from the genetic screenings, or by modifying the DNA repair system leading to structural DNA changes.

The successful PhD candidate will use cutting edge sequencing technology and bioinformatics to characterize and correlate mutation and gene expression profiles as well as genomic copy number changes in retrospective cohorts of rare cancers. Subsequently we will focus on the generation and characterization of meaningful tissue culture models to study the progression of these cancer types from precursor lesions to invasive cancer.

References:

The Spectrum of HPV-independent Penile Intraepithelial Neoplasia: A Proposal for Subclassification. Regauer S, Ermakov M, Kashofer K. Am J Surg Pathol. 2023 Dec 1;47(12):1449-1460. doi: 10.1097/PAS.0000000000002130. Epub 2023 Sep 28. PMID: 37768009

Different Mutational Landscapes in Human Papillomavirus-Induced and Human Papillomavirus-Independent Invasive Penile Squamous Cell Cancers. Ermakov MS, Kashofer K, Regauer S. Mod Pathol. 2023 Oct;36(10):100250. doi: 10.1016/j.modpat.2023.100250. Epub 2023 Jun 21. PMID: 37353203

Acquisition of Genetic Aberrations During the Progression of High-Grade Intraepithelial Lesions/Micro-Invasive Squamous Cancers to Widely Invasive Cervical Squamous Cell Cancer. Kashofer K, Reich O, Regauer S. Arch Pathol Lab Med. 2023 Dec 1;147(12):1438-1445. doi: 10.5858/arpa.2022-0310-OA.

Driver gene mutations in micro-invasive cervical squamous cancers have no prognostic significance. Kashofer K, Regauer S, Reich O, Petru E, Winter E. Gynecol Oncol. 2022 Apr;165(1):121-128. doi: 10.1016/j.ygyno.2022.01.020. Epub 2022 Jan 31. PMID: 35101299

Cervical Precancers Originate From Infected Proliferating Reserve Cells: A Comparative Histologic and Genetic Study of Thin and Thick High-grade Squamous Intraepithelial Lesions. Regauer S, Reich O, Kashofer K. Am J Surg Pathol. 2022 Apr 1;46(4):519-527. doi: 10.1097/PAS.0000000000001818. PMID: 34619706

HPV-negative Squamous Cell Carcinomas of the Cervix With Special Focus on Intraepithelial Precursor Lesions. Regauer S, Reich O, Kashofer K. Am J Surg Pathol. 2022 Feb 1;46(2):147-158. doi: 10.1097/PAS.0000000000001778. PMID: 34387215

Thin variant of high-grade squamous intraepithelial lesion - relationship with high-risk and possibly carcinogenic human papilloma virus subtypes and somatic cancer gene mutations. Regauer S, Reich O, Kashofer K. Histopathology. 2019 Sep;75(3):405-412. doi: 10.1111/his.13869. Epub 2019 Jun 28. PMID: 30927371

Time series analysis of TP53 gene mutations in recurrent HPV-negative vulvar squamous cell carcinoma. Regauer S, Kashofer K, Reich O. Mod Pathol. 2019 Mar;32(3):415-422. doi: 10.1038/s41379-018-0141-4. Epub 2018 Oct 5. PMID: 30291345

HPV-negative penile squamous cell carcinoma: disruptive mutations in the TP53 gene are common. Kashofer K, Winter E, Halbwedl I, Thueringer A, Kreiner M, Sauer S, Regauer S. Mod Pathol. 2017 Jul;30(7):1013-1020. doi: 10.1038/modpathol.2017.26. Epub 2017 Apr 7. PMID: 28387325

Michael Khalil

Department of Neurology, Medical University of Graz

Project Title: Temporal dynamics of blood biomarkers in relation to clinical and radiological disease worsening in multiple sclerosis

Background:

Multiple sclerosis (MS) is a chronic autoimmune mediated disease that is characterized by episodes of focal inflammation in the brain and spinal cord that affects both the white and grey matter.[1] From a traditional point of few, the disease typically starts with a relapsing-remitting MS (RRMS) phase and after approximately 10 to 20 years, a progressive clinical course develops in many of the persons affected, eventually leading to impaired mobility and cognition.[1] A growing body of evidence however suggests that the clinical course is rather considered as a continuum with contributions from concurrent pathophysiological processes that vary across individuals and over time.[2] The evolution of a progressive course seems to be related to a shift from predominantly localised acute injury to widespread inflammation and neurodegeneration.[2] Early diagnosis and treatment of disease progression in patients with MS (pwMS) is a critical unmet need to provide optimal care.[3] The value of magnetic resonance imaging (MRI) in pwMS for diagnostic, prognostic, and monitoring purposes is well established.[4] International consortia aim to translate novel research findings of MRI into clinical practice to improve the use of MRI in the clinical management of individuals with MS.[4] Apart from MRI, body fluid biomarkers and particularly blood based biomarkers hold the potential to assist in disentangling the complex interplay between neuronal, glial, and immune cells that ultimately cause irreversible disease progression.[3] In the last years, tremendous progress has been made in studying blood biomarkers in particular for neuro-axonal injury (serum neurofilament light chain – sNfL)[5-7] and astroglial markers (glial fibrillary acidic protein – GFAP)[8] that have been related to disease activity and progression in MS.[6-8] However, so far only scarce information is available on how temporal changes of these biomarkers are related to clinical and radiological disease worsening in pwMS.

Hypothesis and Objectives:

Recently it was shown that sNfL elevates in advance of clinical worsening and thus may indicate to a potential time frame of ongoing dynamic central nervous system pathology that precedes the diagnosis of clinical disease worsening in MS.[4] Apart from NfL, some evidence also exists for blood GFAP to reflect, and potentially also predict worsening of disability in pwMS.[8,9] However, it is still unclear how longitudinal changes of both blood biomarkers (sNfL and sGFAP) are related to clinical and radiological disease worsening in MS.

Methodology:

The PhD student will focus on investigating sNfL and sGFAP proteins in serial samples of pwMS and relate them to longitudinal clinical and MRI data obtained from a 3T scanner. The student will learn

to use the ultrasensitive single molecule array (Simoa) platform using the HD-X analyzer for quantification of blood biomarkers.

The student will further have the possibility to learn the routine diagnostic cerebrospinal fluid (CSF)/serum work up, including determination of CSF white cell count, total protein, lactate, albumin CSF/serum quotient, calculation of immunoglobulin G, A and M indices and kappa free light chains and the determination of oligoclonal bands by isoelectric focusing followed by immunoblotting, as well as isolation of DNA form whole blood and peripheral blood mononuclear cells.

The student will learn how to handle larger clinical data sets and merge them with biochemical and comprehensive MRI data prior to statistical analyses. All MRI analyses and data post-processing procedures will be performed in collaboration with our Neuroimaging Unit. Statistical analysis will be performed in close collaboration with a biostatistician.

References:

1. Reich, D. et al. Multiple Sclerosis. N. Engl. J. Med. 378, 169–180 (2018).

2. Kuhlmann, T. et al. Multiple sclerosis progression: time for a new mechanism-driven framework. The Lancet Neurology 22, 78–88 (2023).

3. Abdelhak, A. et al. Neurofilament Light Chain Elevation and Disability Progression in Multiple Sclerosis. JAMA Neurol (2023).

4. Wattjes, M. P. et al. 2021 MAGNIMS–CMSC–NAIMS consensus recommendations on the use of MRI in patients with multiple sclerosis. The Lancet Neurology 20, 653–670 (2021).

5. Khalil, M. et al. Neurofilaments as biomarkers in neurological disorders. Nat. Rev. Neurol. 14, 577–589 (2018).

6. Khalil, M. et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. Nat. Commun. 11, 812 (2020).

7. Benkert, P. et al. Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. Lancet Neurol 21: 246–257 (2022).

8. Abdelhak, A. et al. Blood GFAP as an emerging biomarker in brain and spinal cord disorders. Nat. Rev. Neurol. 18, 158-172 (2022).

9. Meier, S. et al. Serum Glial Fibrillary Acidic Protein Compared With Neurofilament Light Chain as a Biomarker for Disease Progression in Multiple Sclerosis. JAMA Neurol (2023).

Karin Kornmüller

Gottfried Schatz Research Center for Cell Signaling, Metabolism & Aging (Medical Physics and Biophysics), Medical University of Graz

Project Title: Structure and dynamics of apolipoprotein B-100 approached by cryo-EM

Background:

Cardiovascular diseases (CVDs) are the leading cause of death worldwide [1]. Low-density lipoprotein (LDL) is a key-player in the human cholesterol transport system and is intimately linked to the development of CVDs [2]. LDL is a 22 nm complex nanoassembly, consisting of phospholipids, a large monomeric protein, triglycerides, and its cargo: free- and esterified cholesterol [3, 4]. The endothelial uptake of LDL particles by specific receptors critically depends on LDLs sole protein moiety apolipoprotein B-100 (apo B-100). Structural deviations within LDL and apo B-100 are correlated with an enhanced risk for the development and progression of atherosclerosis [5]. Therefore, it is of high medical relevance to understand the structural, dynamical and molecular features of LDL and apo B-100.

Hypothesis and Objectives:

Given the immense advances in cryo-EM technology, as well as in algorithm development, we hypothesize that it is possible to achieve a well resolved cryo-EM 3D map of apo B-100. Insights into apo B-100's atomic structure will help to decipher disease-relevant functional protein domains. The identification of new molecular targets might offer new, effective and specific treatment options to regulate LDL levels and improve cardiovascular health.

Specific objectives of this PhD thesis are:

- To isolate, purify and stabilize apo B-100 in a lipid-free detergent-solubilized form from normoplipidemic, triglyceride-rich and oxidized LDL.
- To screen for and select environmental conditions where purified apo B-100 is evenly distributed on an EM grid
- To collect a cryo-EM dataset eligible for single-particle analysis and to obtain a high-resolution 3D model of apo B-100
- To complement cryo-EM studies with small-angle X-ray scattering (SAXS) and molecular dynamics (MD) simulation studies

Methodology:

The PhD candidate will isolate LDL from human plasma by sequential ultracentrifugation, followed by detergent-solubilization and chromatography to isolate lipid-free detergent-stabilized apo B-100 [6]. To assess the chemical composition and the quality of the sample colorimetric assays, as well as SDS-PAGE, negative-staining electron microscopy, circular-dichroism and infrared spectroscopy are applied on a routinely basis. Cryo-EM data collection and MD simulation studies will be done in close

collaboration with our partner institutes (ILL, IBS) in Grenoble, France. Cryo-EM single-particle analysis will be done using CryoSparc and Relion. The PhD candidate will gain broad expertise in structural biology and biophysics, will be provided access to state-of-the-art infrastructure, and will be part of a highly multidisciplinary group integrated in an outstanding international network.

References:

[1] The World Health Organization (WHO). https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (accessed 20.07.**2023**).

[2] Borén, J., et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur. Heart J.* **2020**, 41(24): 2313-2330.

[3] Prassl, R.; Laggner, P. Molecular Structure of Low Density Lipoprotein: Current Status and Future Challenges. *Eur. Biophys. J.* **2009**, 38, 145-58.

[4] Cisse, A., Desfosses, A., Stainer, S., Kandiah, E., Traore, D., Bezault, A., Schachner-Nedherer, AL., Leitinger, G., Hoerl, G., Hinterdorfer, P., Gutsche, I., Prassl, R., Peters, J., Kornmueller, K. Targeting structural flexibility in low density lipoprotein by integrating cryo-electron microscopy and high-speed atomic force microscopy. 2023. *Int. J. Biol. Macromol.* **2023**, 252:126345

[5] Sniderman, A. D., et al. Apolipoprotein B Particles and Cardiovascular Disease: A Narrative Review. *JAMA Cardiology*. **2019**, 4(12): 1287-1295.

[6] A. Cisse, AL. Schachner-Nedherer, M. Appel, C. Beck, J. Ollivier, G. Leitinger, R. Prassl, K. Kornmueller*, J. Peters*. Dynamics of Apolipoprotein B-100 in Interaction with Detergent Probed by Incoherent Neutron Scattering. *J. Phys. Chem. Lett.* **2021**. 12, 51, 12402–12410.

Gunther Marsche

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

Project Title: Pioneering Inflammation Resolution with Engineered High-Density Lipoproteins

Background:

Lipoproteins are intricate nanoscale structures that are pivotal in cellular function, lipid metabolism, and disease progression. These complex assemblies, composed primarily of phospholipids and apolipoproteins, form a protective shell encasing a hydrophobic core containing triglycerides and cholesterol esters. Among these, high-density lipoproteins (HDL), primarily composed of apolipoprotein A1 (apoA-I) and phospholipids, are crucial mediators of reverse cholesterol transport. Beyond their lipid transport role, HDL actively interacts with and regulates the functions of immune cells and endothelial cells [1]. This dual functionality of HDL offers a promising avenue for the development of novel therapeutic strategies. In the quest for alternatives to the use of full-length apoA-I as the basis for HDL, short synthetic peptides - termed apoA-I mimetics - have emerged as potential candidates [2]. These apoA-I mimetics share similar amphipathic α-helical structures with apoA-I, suggesting their potential to replicate the beneficial effects of HDL.

Hypothesis and Objectives:

ApoA-I mimetics are molecules designed to mimic the beneficial effects of apolipoprotein A-I, the main protein found in HDL. ApoA-I mimetics are being studied as potential treatments for a variety of diseases, including atherosclerosis, lung disease, inflammatory bowel disease, sepsis, and cancer. One of the main goals of this research project is to understand how the structure and size of synthetic HDL (containing apoA-I mimetics) affect their biological activity. The anti-inflammatory capacity of synthetic HDL on activated blood cells, such as monocytes, neutrophils, and platelets, will be investigated, and their ability to neutralize endotoxin and modulate endothelial function. The effectiveness of apoA-I mimetics in animal models of chemotaxis and lung inflammation will be studied. This will enable us to design and engineer synthetic HDLs with optimized biological properties for therapeutic purposes.

Methodology:

The student will learn techniques such as preparing synthetic HDL using apoA-I mimetic peptides and phospholipids, isolating HDL from serum, performing native gel electrophoresis, and performing Western blotting. In addition, practical skills will be provided to isolate leukocytes and platelets from peripheral blood, perform flow cytometry and multiplex ELISA, and study the functional responses of

neutrophils and endothelial cells. This includes studying shape change, integrin upregulation, chemotaxis, Ca2+ signaling, and in vivo chemotaxis and lung inflammation.

References:

[1] Trakaki, A. Marsche, G. Current Understanding of the Immunomodulatory Activities of High-Density Lipoproteins. *Biomedicines* **2021**, *9*, 587. doi.org/10.3390/biomedicines9060587

[2] Rani A, Marsche G. A Current Update on the Role of HDL-Based Nanomedicine in Targeting Macrophages in Cardiovascular Disease. *Pharmaceutics*. **2023**, 15(5):1504. doi.org/10.3390/pharmaceutics15051504

Gerit Moser

Gottfried Schatz Research Center for Cell Signaling, Metabolism & Aging (Cell Biology, Histology and Embryology), Medical University of Graz

Project Title: LAMB3-DAG1 axis in early placental developement

Background:

Uterine glands fulfil major functions during implantation and placental development in early pregnancy by supporting histiotrophic prior to hemotrophic nutrition of the embryo. They provide glucose, lipids, glycoproteins and growth factors that stimulate rapid proliferation and differentiation of the villous trophoblast and thereby the developing placenta. The connection between maternal and fetal compartment is accomplished by invasion of fetal trophoblast cells (EVTs) invading the maternal endometrium, they also invade and thereby connect the uterine glands with the placenta and thereby guarantee the constant supply of the developing embryo with glandular secretions (so-called endoglandular trophoblasts). Only recently, tissue-clearing-based 3D imaging has unravelled the fundamental morphology of the human endometrial glands. Other than hitherto assumed, endometrial glands are not only tubular; about two thirds of the endometrial glands are branched and form a plexus network in the basal layer and expand horizontally along the myometrium Thus, many more glands and their secretions are in constant contact with the developing placenta than previously thought, which further strengthens their putative fundamental role in implantation and early pregnancy.

Hypothesis and Objectives:

It is likely, that the role of the uterine glands in general and their invasion by EVTs in particular for successful implantation and subsequent maintenance of a healthy pregnancy has been underestimated so far. Factors that may dictate this pattern of invasion and thus may influence reproductive success are currently unknown.

Preliminary bioinformatics analysis enabled identification of a first promising receptor ligand pair between uterine epithelial cells and EVTs, namely Laminin-Subunit Beta 3 (LAMB3) and Dystroglycan (DAG1). The localisation of LAMB3-DAG1 within first trimester decidua tissue reflects a potential role for endoglandular trophoblast invasion. This project aims to analyse the role of LAMB3-DAG1 in endoglandular trophoblast invasion and evaluate its contribution to fertility complications such as recurrent missed abortion (RM) and implantation failure (RIF).

Methodology:

- Isolate glandular epithelial cells from placental tissues and endometrial biopsies

- Optimize and apply novel *in vitro* model systems for endoglandular trophoblast invasion and for long-term culture of glandular epithelial cells, including a model for *in vitro* harvest of glandular secretions (organoid- and monolayer culture) for functional assessment of LAMB3-DAG1 axis

- Evaluate progress of cell growth and effects of treaments by standard techniques in our laboratory (tissue processing for histology, immunostaining, Western Blot, qPCR, etc)

- Evaluate LAMB3-DAG1 expression levels in larger cohorts (first trimester placentas, endometrial biopsies from RM/RIFpatients) and in specific cell culture experiments with various methods such as software-assisted analysis of immunostainings, padlock probe technique, qPCR and Western Blot, invasion assays

References:

Moser, G.; Weiss, G.; Gauster, M.; Sundl, M.; Huppertz, B. Evidence from the Very Beginning: Endoglandular Trophoblasts Penetrate and Replace Uterine Glands in Situ and in Vitro. *Human Reproduction* **2015**, *30*, 2747–2757, doi:10.1093/humrep/dev266.

Turco, M.Y.; Gardner, L.; Hughes, J.; Cindrova-Davies, T.; Gomez, M.J.; Farrell, L.; Hollinshead, M.; Marsh, S.G.E.; Brosens, J.J.; Critchley, H.O.; et al. Long-Term, Hormone-Responsive Organoid Cultures of Human Endometrium in a Chemically Defined Medium. *Nat Cell Biol* **2017**, *19*, 568–577, doi:10.1038/ncb3516.

G.J. Burton, T. Cindrova-Davies, M.Y. Turco, Review: Histotrophic nutrition and the placental-endometrial dialogue during human early pregnancy, *Placenta* **2020**, doi: 10.1016/j.placenta.2020.02.008.

Burton GJ, Jauniaux E. The human placenta: new perspectives on its formation and function during early pregnancy. *Proc Biol Sci*. **2023** Apr 26;290(1997):20230191. doi: 10.1098/rspb.2023.0191.

M. Yamaguchi, K. Yoshihara, K. Suda, H. Nakaoka, N. Yachida, H. Ueda, K. Sugino, Y. Mori, K. Yamawaki, R. Tamura, T. Ishiguro, T. Motoyama, Y. Watanabe, S. Okuda, K. Tainaka, T. Enomoto, Three-dimensional understanding of the morphological complexity of the human uterine endometrium, *IScience*. 24 (**2021**) 102258. doi: 10.1016/j.isci.2021.102258.

Gerit Moser

Gottfried Schatz Research Center for Cell Signaling, Metabolism & Aging (Cell Biology, Histology and Embryology), Medical University of Graz

Project Title: Modeling reproductive failure in humans

Background:

In a successful pregnancy, the blastocyst attaches to and implants into the endometrium – the lining of the uterus. Altered receptivity and function of the endometrium may lead to fertility issues. Such issues are more common than one might think, more than 10% of all couples worldwide are affected at least once in their lifetime. In particular, the endometrial epithelial glands and their secretions are important for fertility. These glands fulfil major functions in reproduction by supporting nutrition of the early conceptus.

Investigating human reproduction is a particular challenge. Apart from ethical concerns, processes such as implantation and subsequent placentation differ tremendously between animals and humans. Endometrial organoids offer an elegant *in vitro* method for investigation, besides avoiding the use of animal models. These organoids expand long-term, are genetically stable and differentiate depending on the respective treatment with reproductive hormones. By modeling the endometrium in physiological and pathological condition, organoids can be used to identify biomarkers, evaluate treatment options and predict response.

Hypothesis and Objectives:

We hypothesize that (1) common treatment options for reproductive failure alter genotype and secretome of uterine glandular epithelium and (2) these alterations of the glandular epithelium in turn influence reproductive success.

Aim of this is to establish patient individual endometrial organoids from women with fertility issues, evaluate common clinical treatments options, predict response on an individual level, and thereby move a step towards personalized treatment options in future.

Methodology:

- Isolate glandular epithelial cells from placental tissues and endometrial biopsies

- Optimize respective long-term cultures (organoids and monolayer) and treatment

- Evaluate progress of cell growth and effects of treaments by standard techniques in our laboratory (tissue processing for histology, immunostaining, Western Blot, qPCR, etc)

- Isolate and prepare samples for tissue dissociation for single cell RNAseq for genotyping of glandular epithelium (bioinformatics analysis will be performed by Dr. Julia Feichtinger).

- Evaluate identified targets in larger cohorts (endometrial biopsies from patients with fertility issues) and in specific cell culture experiments with various methods such as software-assisted analysis of immunostainings, padlock probe technique, qPCR and Western Blot, invasion assays.

References:

Gnoth, C., Godehardt, E., Frank-Herrmann, P., Friol, K., Tigges, J., and Freundl, G. Definition and prevalence of subfertility and infertility. *Hum. Reprod.* Oxf. Engl. 20, **2005**. 1144–1147. 10.1093/humrep/deh870.
Nikolakopoulou, K.; Turco, M.Y. Investigation of Infertility Using Endometrial Organoids. *Reproduction* **2021**, 161, R113–R127, doi:10.1530/REP-20-0428.

Garcia-Alonso, L.; Handfield, L.F.; Roberts, K.; Nikolakopoulou, K.; Fernando, R.C.; Gardner, L.; Woodhams, B.; Arutyunyan, A.; Polanski, K.; Hoo, R.; et al. Mapping the Temporal and Spatial Dynamics of the Human Endometrium in Vivo and in Vitro. *Nat Genet* **2021**, *53*, 1698–1711, doi:10.1038/s41588-021-00972-2.

Turco, M.Y.; Gardner, L.; Hughes, J.; Cindrova-Davies, T.; Gomez, M.J.; Farrell, L.; Hollinshead, M.; Marsh, S.G.E.; Brosens, J.J.; Critchley, H.O.; et al. Long-Term, Hormone-Responsive Organoid Cultures of Human Endometrium in a Chemically Defined Medium. *Nat Cell Biol* **2017**, *19*, 568–577, doi:10.1038/ncb3516.

Moser, G.; Weiss, G.; Gauster, M.; Sundl, M.; Huppertz, B. Evidence from the Very Beginning: Endoglandular Trophoblasts Penetrate and Replace Uterine Glands in Situ and in Vitro. *Human Reproduction* **2015**, *30*, 2747–2757, doi:10.1093/humrep/dev266.

Thomas Pieber (PI)[1]
Gabriele Schoiswohl (Co-PI)[2]

[1]Division of Endocrionolgy and Diabetology, Department of Internal Medicine, Medical University of Graz

[2]Gottfried Schatz Research Center for Cell Signaling, Metabolism & Aging (Molecular Biology and Biochemistry), Medical University of Graz

Project Title: Novel approaches for ß-cell protection – On the path to cure type 1 diabetes

Background:

Globally, type 1 diabetes mellitus (T1DM) incidence is increasing by about 2-3% per year with about 8.4 million cases wordwide in 2021 (1). T1DM is characterized by a complete loss of endogenous insulin production, leading to the need of life-long insulin substitution. However, treatment with exogenous insulin does not stop disease progression, causing significant health complications that affect quality of life and reduce life expectancy (2). So far, disease-modifying therapeutic approaches have focused on single immune-modulating compounds to protect ß-cell function with varying long-term effects (3). Recently, non-immunomodulators that directly protect and restore ß-cell health and stimulate insulin secretion, have come into focus as a potential novel therapeutic concept for curing T1DM (4).

Hypothesis and Objectives:

We hypothesize that direct protection and/or restoration of ß-cell health will preserve insulin secretory ability and suppress immune cell-mediated destruction of ß-cells, ultimately leading to novel treatments of T1DM in humans. For this PhD project, we will utilize established ß-cell lines to investigate potential ß-cell protective compounds that directly modulate cellular processes within ß-cells to improve insulin secretion and metabolic health including ER stress and inflammation.

Methodology:

To investigate the therapeutic approach of protective compounds on ß-cells (and thus potential novel anti-diabetic drugs), the PhD candidate will use state of the art molecular-biological techniques including generation of ß-cells expressing intracellular biosensors, 2D- and 3D-cell culture as well as primary cell culture, flow cytometry, immunohistochemistry, and a next generation drug screening plattform. The student will cooperate with different research groups and institutions.

References:

(1) Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. Lancet Diabetes Endocrinol 2022 Oct;10(10):741-760
(2) DiMeglio, L.A.; Evans-Molina, C.; Orma, R.O. Type 1 diabetes. Lancet 2018; 391:2447-2462
(3) von Scholten, B.J.; et al. Current and future therapies for type 1 diabetes. Diabetologia 2021; 64:1037-1048
(4) Eizirik, D.L; Pasquali L.; Cnop, M. Pancreatic ß-cells in type 1 and type 2 diabetes mellitus: different pathways to failure. Nature Reviews Endocrinology 2020; 16:349-362.

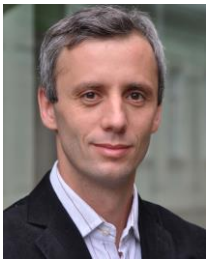
Thomas Pieber (PI)[1]
Julia Bandres-Meriz (Co-PI)[2]
Michael Khalil (Co-PI)[3]

[1]Division of Endocrionolgy and Diabetology, Department of Internal Medicine, Medical University of Graz

[2]CBmed – Center for Biomarker Research Graz

[3]Department of Neurology, Medical University of Graz





Project Title: Metabolic reprograming in glioblastoma in response to drug therapy

Background:

Glioblastoma is a highly aggressive and rapidly growing brain tumor with limited effective treatment options. Treating glioblastoma is complex because the tumor has the capacity to reprogram its metabolism to increase energy production and support rapid growth and proliferation1,2. Hence, current efforts to treat glioblastoma focus on understanding the intricate metabolic reprograming that takes place in the tumor. In this project, the PhD candidate will investigate mechanisms of efficacy and resistance to treatment with a special focus on metabolic reprograming in tumor tissue and biomarkers in tissue and plasma. The results will add mechanistical insights to the findings of a currently on-going clinical trial.

Hypothesis and Objectives:

We hypothesize that drug treatment modifies the tumor metabolome rendering it sensitive or resistant to therapy. We aim to identify biomarkers and metabolic vulnerabilities that can be further exploited in therapy and industry.

Methodology:

Patient derived cells (PDCs) will be isolated from brain tumor biopsies of patients with glioblastoma. The efficacy of a panel of drugs will be tested in a drug screening platform3,4. Intracellular tumor metabolism will be examined in biopsies, PDCs and cell lines using mass spectrometry (Q-exactive and LCMS-8060). Cell organelles will be isolated by differential centrifugation to investigate the subcellular localization of top identified metabolites5. Tumor tissue and plasma biomarkers will be identified using mass spectrometry and standard techniques such as RT-qPCR, western blot and immunohistochemistry. The candidate should be interested in cell biology and metabolomics. Previous experience in mass spectrometry and bioinformatics is not required but is a plus.

References:

(1) Le Rhun, E. *et al*. Molecular targeted therapy of glioblastoma. *Cancer Treat Rev* **80**, 101896 (2019).
(2) Minami, J. K. *et al*. CDKN2A deletion remodels lipid metabolism to prime glioblastoma for ferroptosis. **41**, 1048-1060.e9 (2023).
(3) Eun Kim, Y. *et al*. Glioblastoma patient-derived cell-based phenotypic drug screening and identification of possible action mechanisms through proteomic analysis. *STAR Protoc* **2**, 100849 (2021).
(4) Lee, J.-K. *et al*. Pharmacogenomic landscape of patient-derived tumor cells informs precision oncology therapy. *Nat Genet* **50**, 1399–1411 (2018).
(5) Qin, S., Zhang, Y., Tian, Y., Xu, F. & Zhang, P. Subcellular metabolomics: Isolation, measurement, and applications. *J Pharm Biomed Anal* **210**, 114557 (2022).

Thomas Pieber (PI)[1]
Christoph Magnes (Co-PI)[2]
Hansjörg Habisch (Co-PI)[3]

[1]Division of Endocrionolgy and Diabetology, Department of Internal Medicine, Medical University of Graz

[2]HEALTH Institute, JOANNEUM RESEARCH

[3]Otto Loewi Research Center for Vascular Biology, Immunology and Inflammation, (Medicinal Chemistry), Medical University of Graz


Project Title: An epigenetic-metabolic crosstalk reveals vulnerabilities of acquired drug resistance in glioblastoma

Background:

The main cause for disease relapse in patients with metastatic cancer is acquired drug resistance after an initially response to therapeutic intervention. While nearly half of disease recurrence ocurs by acquiring additional genetic alterations[1.],Fehler! Verweisquelle konnte nicht gefunden werden., evidence suggests an epigenetically- and metabolic- regulated and reversible drug-tolerance state prior to permanent resistance,[2.]. The impact of reversible cellular reprogramming has been shown in many solid cancers including melanoma[4.] or ovarian cancer[5.], the molecular mechanisms, however, facilitating the shift of a dormant to a proliferative state remain underinvestigated. We recently identified O-linked N-acetylglucosamine transferase (OGT) driving the transition of cancer cells from dormancy to proliferation[6.]. Current treatment options for glioblastoma have limited effectiveness, with a median survival of 18 months. Standard therapies include surgical resection, radiation therapy, and chemotherapy with temozolomide[6.]. Epigenetic and metabolic changes play a crucial role in regulating metabolic behavior of glioblastoma cells and both promote or inhibit acitivity of metabolic pathways[8.]. This project aims to determine epigenetic and metabolic features in dormant and proliferative drug tolerant patient-derived glioblastoma cells.

Hypothesis and Objectives:

Drivers of a drug tolerant and proliferative persister state remain to be underinvestigated in glioblastoma. Determing epigenetic and molecular markers as vulnerabilities of drug resistance in glioblastoma will identify novel markers and potential druggable targets. Investigations will allow to test intermittent treatment schedules for a re-challenge response of patients. In-vitro testing of immortalized patient-derived glioblastoma cell lines for identification of top transcription factor

binding motifs based on histone modifications (e.g. H3K4me3) and subsequent OGT involvement, combined with metabolome analysis of cell lines and their secretory products in the media supernatant to determine potential targets of key regulator sites. In vivo xenograft model[9.] to determine treatment efficacy of known treatment drugs with and without intermittent drug treatment with a constant monitoring using cerebral open flow microperfusion (cOFM) ISF sampling directly from inside the tumor to monitoring tumor and in-vivo drug resistance development. Immunohistochemistry of idenfied targets and markers in glioblastoma tissue.

Methodology:

- Induction of drug tolerant cells by continuous treatment of respective cell lines with indicated drug dosage followed by colony isolation.
- Metabolic analyses of cells and supernatant[10.] at set time-points,
- ATAC sequencing, ChIP sequencing or CUT&RUN and ChIP-qPCR analysis,
- Immunofluorescence and live cell imaging of organoids
- Xenograft mouse model with implanted cOFM probe to ascertain metabolic changes in the tumor during growth and potential in-vivo changes during resistance development.
- Immunohistochemistry in glioblastoma tissue.

References:

[1.] Manzano, J. L., et al. (2016). "Resistant mechanisms to BRAF inhibitors in melanoma." Ann Transl Med 4(12): 237.
[2.] Hammerlindl, H. and H. Schaider (2018). "Tumor cell-intrinsic phenotypic plasticity facilitates adaptive cellular reprogramming driving acquired drug resistance." J Cell Commun Signal 12(1): 133-141.
[3.] Hinohara, K., H. J. et al. (2018). "KDM5 Histone Demethylase Activity Links Cellular Transcriptomic Heterogeneity to Therapeutic Resistance." Cancer Cell 34(6): 939-953 e939.
[4.] Das, T. M., F. et al. (2013). "Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance." Nature 494(7436): 251-255.
[5.] Wang Y, et al. (2022) The Emerging Roles and Therapeutic Implications of Epigenetic Modifications in Ovarian Cancer. Front Endocrinol (Lausanne);13:863541. doi: 10.3389/fendo.2022.863541. PMID: 35620395; PMCID: PMC9127157.
[6.] Ravindran Menon, D., H. et al. "H3K4me3 remodeling induced acquired resistance through O-GlcNAc transferase." Drug Resist Updat. 2023 Nov;71.
[7.] Yalamarty, S. S. K., N. et al. Mechanisms of Resistance and Current Treatment Options for Glioblastoma Multiforme (GBM). Cancers (Basel). 2023 Apr 1;15(7):2116.
[8.] Park J, et al. Epigenetic and Metabolic Changes in Diffuse Intrinsic Pontine Glioma. Brain Tumor Res Treat. 2023 Apr;11(2):86-93. doi: 10.14791/btrt.2023.0011. PMID: 37151150; PMCID: PMC10172016.
[9.] Altendorfer-Kroath, T., et al. „Atraumatic access to human glioblastoma in a xenograft animal model by cerebral open flow microperfusion." J Neurosci Methods. 2023 Jun 1.
[10.] Vogel, F. C. E., et al. "Targeting the H3K4 Demethylase KDM5B Reprograms the Metabolome and Phenotype of Melanoma Cells." J Invest Dermatol. 2019 Dec;139(12).

Beate Rinner

Division of Biomedical Research, Core Facility Alternative Biomodels and Preclinical Imaging, Medical University of Graz

Project Title: Replace Mice Xenografts by Microfluidic CancerogEnesis Chips - ReplaceMICE

Background:

With personalized cancer treatment the detection of malignantly transformed cells by tumorigenicity testing is essential. For testing the tumorigenicity of patient derived tumor cells the use of in vivo animal models – especially xenograft mouse models - is currently standard. However, animal models suffer of several drawbacks: (i) the interspecies physiological and genetic differences, (ii) the incomparability or lack of an active immune system, (iii) the ethical concerns and also (iv) the high cost. This project promotes an alternative to animal testing that overcomes these restrictions. To replace animal testing we promote a human *in vitro* model on a bioelectronic sensor chip. Using only animal-free materials this human model will help to overcome all the previous cited problems. With a immune-competent, vascularized human tissue model on a microfluidic chip we will realize animal-free tumorigenicity testing.

Approach:

Using interconnected microchambers we will reproduce the sequence of tissues of the skin and will generate a human epidermis/dermis-equivalent on a chip. The four adjoining microfluidic chambers are a medium compartment for nutrient supply and drug supplementation, an epidermis compartment with a coculture of human keratinocytes and melanocytes, a dermis compartment with human fibroblasts and immune cells (CD4, CD8-T-cells, macrophages, monocytes), an adjoining vascular compartment with the walls lined with microvascular endothelial cells. The chambers are connected via fenestrated, micropermeable barrier sidewalls that enable the exchange of substances or signaling molecules. With this in vitro model on a chip, we will investigate the tumorigenic capacity of different tumor celllines. Starting with a healthy human model we will introduce human tumor cells via the medium chamber or the vascular chamber. As example, human melanoma cells will be seeded on the dermis compartment. Using microelectrode arrays on the chip we will continuously monitor the tumor promotion in real time by non-destructive bioimpedance sensing. For the first time the animal-free replacement of the mouse model for human tumorigenicity testing is realized. This tumorigenicity-chip features 100% human cells and features three innovations: (i) a well-defined dermal-vascular barrier (ii) the inclusion of human immune cells and (iii) the real-time bioimpedance measurement of tumor progression. With 3 defined tissue layers this animal-free model closely resembles the in vivo situation. The continuous bioelectronic monitoring of tumor progression is advantageous to achieve a personalized medicine without animal testing.

Primary researchers involved:

This proposal joins the expertise of a multidisciplinary team made of a Biomodel-specialist (B. Rinner, Meduni Graz), an immunologist (D. Wilflingseder, Meduni Innsbruck), a cell-chip specialists (P. Ertl, M an immunologist (D. Wilflingseder, Meduni Innsbruck), a cell-chip specialists (P. Ertl, M. Frauenlob, TU Wien), a biomedical & microelectronics engineer (H. Wanzenboeck, TU Wien) and a cancer-on chip biologist (S. Prado-Lopez, TU Wien).

References:

Byrne, A.T., et al., *Interrogating open issues in cancer precision medicine with patient-derived xenografts* Nature Reviews Cancer, 2017. **17**(4):p. 254-268.
Hidalgo, M., et al., *Patient-derived Xenograft models: An emerging platform for translational cancer research.* Cancer Discovery, 2014. **4**(9): p. 998-1013.
Michna, R., et al., *Vascularized microfluidic platforms to mimic the tumor microenvironment.* Biotechnology and Bioengineering, 2018. **115**(11): p. 2793-2806.

Christian Wadsack (PI)
Hanna Allerkamp (Co-PI)
Department of Obstetrics and Gynecology, Medical University of Graz

Project Title: Bioactive Lipids: Key Players in Shaping Placental Function in Pregnancies with Adverse Metabolic Profiles

Background:

Pregnancy requires remarkable adaptations of the maternal metabolic system to ensure the availability of nutrients for proper fetal development. These include changes in the maternal lipid metabolism to meet the changing energy demands, but a wide range of lipids also act as signaling molecules, so-called bioactive lipids (e.g., sphingolipids and ceramides). Several pregnancy complications, such as preeclampsia, gestational diabetes mellitus, or obesity, are associated with an adverse metabolic profile and abnormal changes in the maternal lipid profile, or dyslipidemia (1). Importantly, such pregnancy complications are linked to adverse fetal outcomes, such as increased risk of cardiovascular disease (2).

The placenta acts as a mediator between the maternal and fetal metabolism, and bioactive lipids also play an important role in supporting the function of the placenta itself – a process tightly regulated by specific enzymes. In particular, the balance between sphingosine-1-phosphate (S1P) and ceramides is an important regulator of essential pathways in most placental cells, including the placental vasculature (3, 4). However, placental sphingolipid signaling and its relation to the maternal metabolic profile in normal and complicated pregnancies still remains poorly understood.

Hypothesis and Objectives:

Previous and preliminary data from our group show that in preeclampsia, the placental sphingolipid profile, the expression of relevant sphingolipid receptors, and the activity of key regulating enzymes are altered (5). We hypothesise that such alterations are linked to an adverse maternal metabolic state beyond preeclampsia and adversely affect placental function and vascular reactivity and function. We aim to identify relevant maternal metabolic markers, relate them to impaired placental sphingolipid signatures, and identify the key enzymes and regulatory pathways involved. We will also investigate the functional impact of these findings on placental vascular function, with a particular focus on S1P, its *de novo* synthesis, and the potential involvement of disturbed “inside-out” signaling by S1P within the placental endothelium (6). Overall, this study will help to identify women at risk of placental sphingolipid dysfunction, who may particularly benefit from interventions to improve placental (vascular) function and thus long-term outcomes for their offspring.

Methodology:

A study cohort consisting of samples of term placental tissue and maternal plasma from normal and metabolically compromised pregnancies will be used and extended over the course of the project. Targeted lipidomics and profiling by NMR-based metabolomics will complement existing lipidomics datasets to identify relevant aberrations in maternal plasma and placental tissue. Placental enzyme activity of the sphingosine and ceramide *de novo* and catabolic pathways will be investigated using activity-based protein profiling. This will be complemented by using specific enzyme inhibitors *in vitro*, e.g., in placental explants, and measuring their secretome to dissect the relevant pathways in different metabolic settings. The effect on the placental vasculature will be studied by myography of the placental chorionic arteries. These experiments will be informed by the metabolomics part of the study. In addition to comparing overall vascular reactivity, this will also enable us to investigate the impact of the (patho)physiological placental environment on vascular function and signaling. These experiments will be complemented by cell-based assays using primary placental endothelial cells under flow and in endothelial barrier integrity assays (electric cell-substrate impedance sensing). This will allow us to investigate the functional consequences of changes within the sphingolipid profile, with a special focus on the role of autocrine/paracrine S1P signaling.

References:

1. **Kivelä J**, **Sormunen-Harju H**, **Girchenko P V.**, **Huvinen E**, **Stach-Lempinen B**, **Kajantie E**, **Villa PM**, **Reynolds RM**, **Hämäläinen EK**, **Lahti-Pulkkinen M**, **Murtoniemi KK**, **Laivuori H**, **Eriksson JG**, **Räikkönen K**, **Koivusalo SB**. Longitudinal Metabolic Profiling of Maternal Obesity, Gestational Diabetes, and Hypertensive Pregnancy Disorders. *J Clin Endocrinol Metab* 106: e4372–e4388, 2021. doi: 10.1210/clinem/dgab475.
2. **Ren Z**, **Luo S**, **Cui J**, **Tang Y**, **Huang H**, **Ding G**. Research Progress of Maternal Metabolism on Cardiac Development and Function in Offspring. *Nutrients* 15, 2023. doi: 10.3390/nu15153388.
3. **Berger N**, **Allerkamp H**, **Wadsack C**. Serine Hydrolases in Lipid Homeostasis of the Placenta-Targets for Placental Function? *Int J Mol Sci* 23, 2022. doi: 10.3390/ijms23126851.
4. **Fakhr Y**, **Brindley DN**, **Hemmings DG**. Physiological and pathological functions of sphingolipids in pregnancy. *Cell Signal* 85: 110041, 2021. doi: 10.1016/j.cellsig.2021.110041.
5. **Del Gaudio I**, **Sasset L**, **Di Lorenzo A**, **Wadsack C**. Sphingolipid signature of human feto-placental vasculature in preeclampsia. *Int J Mol Sci* 21, 2020. doi: 10.3390/ijms21031019.
6. **Del Gaudio I**, **Rubinelli L**, **Sasset L**, **Wadsack C**, **Hla T**, **Di Lorenzo A**. Endothelial Spns2 and ApoM regulation of vascular tone and hypertension via sphingosine-1-phosphate. *J Am Heart Assoc* 10, 2021. doi: 10.1161/JAHA.121.021261.