Large animal in vivo characterization of designated full size magnesium implants by clinical CT in a juvenile sheep model

Summary

Annelie-Martina Weinberg, Department of Orthopedic and Traumatology, Medical University of Graz

Supervisor:	Prof. Dr. Annelie-Martina Weinberg
Availability:	This position is available.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2018 00:00 and September 23, 2018 23:59 (Europe/Zurich)

Description

Background: In children, surgery has become the state-of-the-art treatment for displaced instable fractures in childhood, thereby reducing hospitalization costs and improving psychological outcomes for children and parents. Typical examples of surgical implants are k-wires or screws in the elbow and elastic stable intramedullary nailing (ESIN) for diaphyseal fractures. Surgical techniques have been adapted to the needs of pediatric fractures to optimally support bone healing (1). Conventional alloying systems including titanium or stainless steel are currently used for osteosynthesis in pediatric orthopedic surgery. In contrast to adults, implants must be removed in children otherwise impeding longitudinal bone growth. Therefore, the need for novel strategies is increasing and resorbable magnesium (Mg)-based implants display a good biocompatibility and mechanical properties. Several studies have demonstrated the osteoinductive properties of Mg implants, which promote callus formation and reduce complications associated with bone fractures. Mg ions released by Mg-based alloys have been demonstrated to influence bone formation, osteoblast proliferation and adhesion (2, 3). In the last decades, biomedical imaging has gained a significant technological push – a development that is of high relevance in practically all fields of medicine. For instance, computed Tomography (CT) or hybrid technologies (e.g. PET-CT, PET-MRI) are the mainstay of diagnosis and therapy monitoring.

Overall aim of the PhD project: Quantitative and qualitative evaluation of Mg-based implants in juvenile growing sheep.

Objectives: (i) Quantitative and qualitative analysis of implantation site with attention to clinically relevant aspects, (ii) determination of the suitability of clinically relevant implant shape and Mg amount with respect to local side effects (osteolysis, necrosis, pseudoarthrosis), (iii) analysis of degradation behavior (volume and surface changes) and gas evolution in sheep.

Methods: (i) surgical implantation into tibiae of sheep; (ii) *in vivo* clinical CT and *ex vivo* microCT; (iii) serum/plasma characteristics; (iv) RNA/protein isolation; (v) qRT-PCR; (vi) western blot; (vii) NMR-based metabolomics phenotyping

Planned and obligatory academic/industrial training: (i) HZG, R. Willumeit-Römer, 1 month, implant alloying and manufacturing, surface observation and characterisation; (ii) HiOA, P. Mirtaheri, 2 months, NIRS measurement for application in sheep; (iii) Bri.Tech, N. Grün, 1 month, Quality management and certification in industry; (iv) CNR-IFC, L. Menichetti, 1 month, large animal PET, µCT, etc.; (v) VSI, J. Jose, 2 months, training in USPA; (vi) UiO, H. Haugen, M34, 3 months, sample preparation and establishment in immunohistochemical stainings using ovine bone.

References:

1. A.-M. Weinberg and H. Tscherne, *Unfallchirurgie im Kindesalter*. Springer Science & Business Media, 2006
2. Cai YL, et al., Osteoblastic cell response on fluoridated hydroxyapatite coatings: the effect of magnesium incorporation. *Biomed Mater.* 2010;5(5):054114

3. Park J-W, et al., Osteoblast response to magnesium ion-incorporated nanoporous titanium oxide surfaces. *Clin Oral Implants Res*. 2010;21(11):1278–87

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In vivo characterisation of designated magnesium materials by µCT and fluorescence imaging in rats

Summary

Annelie-Martina Weinberg, Department of Orthopedic and Traumatology, Medical University of Graz

Supervisor:	Prof. Dr. Annelie-Martina Weinberg
Availability:	This position is available.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2018 00:00 and September 23, 2018 23:59 (Europe/Zurich)

Description

Background: In the last decades, biomedical imaging has gained a significant technological push – a development that is of high relevance in practically all fields of medicine. For instance, computed Tomography (CT) or hybrid technologies (e.g. PET-CT, PET-MRI) are the mainstay of diagnosis and therapy monitoring. Amazing progress in computational tools, sophisticated image fusion approaches and explorations into big data management are the catalysts for the advancement of biomedical imaging. Ageing populations, an ever-increasing incidence of obesity and a rapid rise in osteoporosis-related fractures, along with increasing high-risk sport activities make improvements in implants used in orthopedic interventions imperative.

Conventional alloying systems including titanium or stainless steel are currently used for orthopedic and trauma surgery. However, drawbacks associated with conventional alloys including “stress-shielding” or refractures, increase the need for novel strategies. In the last decade, resorbable magnesium (Mg)-based implants have been demonstrated as an interesting alternative with good biocompatibility and mechanical properties. Several studies have demonstrated the osteoinductive properties of Mg implants, which promote callus formation and reduce complications associated with bone fractures. Mg ions released by Mg-based alloys have been demonstrated to influence bone formation, osteoblast proliferation and adhesion (1, 2).

Overall aim of the PhD project: Establishing and combining of imaging techniques to evaluation of Mg-based implants by in young and old rats.

Objectives: (i) Quantification of implant degradation and bone response *in vivo* together with molecular activities of osteoblast, osteoclast and inflammatory cells, (ii) histological correlation with fluorescence signal of osteoblast, osteoclast and inflammatory response, (iii) *ex vivo* NMR-based metabolic phenotyping of bone and surrounding tissue, (iv) delivery of data, explants and tissue to network partners.

Methods: (i) surgical implantation into femur of rats; (ii) *in vivo* and *ex vivo* microCT; (iii) biocompatibility assays; (iv) serum/plasma characteristics; (v) RNA/protein isolation; (vi) qRT-PCR; (vi) western blot and/or ELISA; (vii) NMR-based metabolomics phenotyping; (viii) histological evaluation

Planned and obligatory academic/industrial training: (i) HZG, R. Willumeit-Römer, 1 month, training in implant alloying and manufacturing process and in surface observation and characterization; (ii) UGOT, P. Thomsen, 3 months, training in molecular analysis; (iii) CNR-IFC, L. Menichetti, 1 month, training PET, µCT, etc.; (iv) Bri.Tech, N. Grün, 2 months, Quality management and certification in industry; (v) HZG, R. Willumeit-Römer, 2 months, synchrotron tomography and SAXS measurements.

References:

1. Cai YL, et al., Osteoblastic cell response on fluoridated hydroxyapatite coatings: the effect of magnesium incorporation. *Biomed Mater.* 2010;5(5):054114

2. Park J-W, et al., Osteoblast response to magnesium ion-incorporated nanoporous titanium oxide surfaces. *Clin Oral Implants Res*. 2010;21(11):1278–87

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Light-triggered ion channel regulation

Summary

Rainer Schindl, Gottfried Schatz Research Center for Cell Signaling, Metabolism and Aging, Biophysics, Medical University of Graz

Supervisor:	PD Dr. Rainer Schindl
Availability:	This position is available.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2018 00:00 and September 23, 2018 23:59 (Europe/Zurich)

Description

Background: Optical techniques represent a powerful tool to precisely control signaling of excitable cells for neuroscience application. Electrical responses of neurons can be triggered and shaped by light, and enables interventions to treat neurological and psychiatric diseases. Recently, we have generated and used organic semiconductors that can be stimulated by laser light pulses[1]. These semiconductors have the size of single cells with thin needles in nanometer dimensions. These needles function as perfect contact sites with the plasma membrane of living single cells. A laser light beam is then focused on the semiconductor structure in order to stimulate the attached cell. The electrical signals Induced by laser stimulation are recorded in the target cells with the patch clamp technique. Hence, the developed organic semiconductors present a powerful and novel tool to manipulate electrical activity of cells by light.

Alternatively, we design photopharmacological tools for activation of specific ion channels. Herein, lipid messengers are modified to enable conformational changes in cation conductances by light and thereby to induce cellular Ca^{2+} signals. Specifically, the lipid messenger diacylglycerol was chemically modified with a light-sensitive molecular switch that can be efficiently controlled by the wavelength of the applied light pulse[2].

Hypothesis and Objectives: Optical control of ion channels: The goal of this PhD thesis is to efficiently induce ion channel regulation, and specifically voltage-gated ion channels and neuronal signaling by light. The opto-tools will include photoactive organic semiconductors and light controlled lipid messengers. The patch-clamp technique allows visualizing electrophysiological cell responses in a millisecond time range. Specific, voltage-gated ion channel will be heterologously expressed in single living cells to record their activation efficiency due to attached organic semiconductors or incubated photo-lipid messengers by light pulse. Successful stimulation of ion channel recordings will then be extended to primary cultured neurons. These cells generate action potentials, a transient depolarization to send the activation signal to the axon. Hence, the long term goal is to induce neuronal stimulation for physiological applications. These opto-tools will be designed to trigger visual responses in retinal ganglion cells or the recovery of neuronal networks after traumatic brain injury.

Methodology: The PhD student requires motivation and patience to perform single cell patch-clamp recordings. Additonally, the work of the PhD student will include fluorescence imaging, cell culture and neuronal tissue preparation. Prospective students should be able to plan experiments independently and like to work in an interdisciplinary research team. The PhD candidates with a technical master degree are prefered. Strength of the research team is intense collaboration with leading laboratories ranging of organic chemistry, computer simulations to neurosurgery. Experiments will be performed at the institute for biophysics and with collaboration partners within the Medical University of Graz. Semiconductors will be provided by two international collaboration partners and the Isolation of neuronal cells and cell culture will be important to achieve live cell recordings in contact with semiconductor structures.

References:

1. Cellular interfaces with hydrogen-bonded organic semiconductor hierarchical nanocrystals. Sytnyk M, Jakešová M, Litviňuková M, Mashkov O, Kriegner D, Stangl J, Nebesářová J, Fecher FW, Schöfberger W, Sariciftci NS, Schindl R, Heiss W, Głowacki ED. Nat Commun. 2017 Jul 21;8(1):91. doi: 10.1038/s41467-017-00135-0

2. An optically controlled probe identifies lipid-gating fenestrations within the TRPC3 channel. M. Lichtenegger, O. Tiapko, B. Svobodova, T. Stockner, T. N. Glasnov, W. Schreibmayer, D. Platzer, G. G. de la Cruz, S. Krenn, R. Schober, N. Shrestha, R. Schindl, C. Romanin, K. Groschner, *Nat Chem Biol* 14, 396-404 (2018)

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Cannabinoid receptors in gastrointestinal inflammation and cancer

Summary

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

Supervisor:	Prof. Dr. Rudolf Schicho
Availability:	This position is available.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2018 00:00 and September 23, 2018 23:59 (Europe/Zurich)

Description

Background: Cannabis has been traditionally used as a remedy against gastrointestinal (GI) diseases. Since the discovery of cannabinoid receptors and the endocannabinoid system, a modern pharmacological therapy of GI disorders with cannabinoids has become a potentially new option. Cannabinoids have been shown to activate or modulate a variety of receptors such as cannabinoid receptor 1 and 2 (CB1, CB2), GPR55, TRPV1 and PPARs. These receptors are present in epithelial cells and/or leukocytes of the GI tract and play an important part in the pathophysiology of inflammatory bowel diseases and colon cancer. We recently identified GPR55 as a proinflammatory and procarcinogenic receptor that opposes the behavior of CB1 receptors[1,2]. The mechanisms how cannabinoid receptors and the endocannabinoid system regulate tumor growth are still elusive.

Hypothesis and Objectives: Depending on their localization, cannabinoid receptors are thought to regulate cell differentiation and regeneration. Using knockout mice of several cannabinoid receptors, we will investigate the role of CB1 and CB2 in models of colon carcinogenesis and intestinal inflammation. In vivo models will be complemented by primary cell culture techniques.

Methodology: In tissue obtained from the in vivo experiments, the content of infiltrated leukocytes and inflammation markers as well as the pathological status will be determined using flow cytometry, immunoassays and immunohistological techniques. Primary cells and organoids isolated from colon mucosa will be used to determine the role of the receptors in cell proliferation and differentiation. The PhD candidate will quantify receptor expression by real-time PCR, Western blot, fluorescence microscopy and flow cytometry. Functional responses of immunocytes will be investigated in assays of cell migration and adhesion, integrin up-regulation, and Ca^{2+} signaling. Epithelial barrier integrity will be evaluated by cell impedance assays.

References:

1. Hasenoehrl C, Feuersinger D, Sturm EM, Barnthaler T, Heitzer E, Graf R, Grill M, Pichler M, Beck S, Butcher L, Thomas D, Ferreiros N, Schuligoi R, Schweiger C, Haybaeck J, Schicho R (2018) G protein-coupled receptor GPR55 promotes colorectal cancer and has opposing effects to cannabinoid receptor 1. Int J Cancer; 2018;142:121-132.

2. Stancic A, Jandl K, Hasenohrl C, Reichmann F, Marsche G, Schuligoi R, Heinemann A, Storr M, Schicho R: The GPR55 antagonist CID16020046 protects against intestinal inflammation. Neurogastroenterol Motil 2015;27:1432-1445.

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Regulation of nuclear import and phase separation

Summary

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

Supervisor:	Prof. Dr. Tobias Madl
Availability:	This position is available.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2018 00:00 and September 23, 2018 23:59 (Europe/Zurich)

Description

Background: Motifs rich in arginine and glycine residues were recognized several decades ago to play functional roles in RNA-binding and were termed RG/RGG motifs[1,2]. More than 1000 proteins harbor the intrinsically disordered RG/RGG motif, and these proteins play essential roles in a plethora of physiological processes such as transcription, pre-mRNA splicing, DNA damage signaling and mRNA translation[2], and very recently in neuroprotection[3]. We have shown that the RG/RGG-motif of FUS is involved in transportin-1 – mediated nuclear import, and that transportin-1 acts as a molecular chaperone regulating FUS phase separation[4-6]. Arginine methylation of the RG/RGG motif in combination with RNA-binding and mutations regulate phase separation of FUS and determine the formation of pathogenic inclusions in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) patients. Very recently we have discovered a novel regulator of phase separation of not only FUS but many other proteins which will be the focus of this project (Madl lab, unpublished).

Hypothesis and Objectives: Based on our recent studies and supported by our preliminary data, we propose an alternative mechanism for chaperoning phase separation involving a novel regulator. We propose that this regulator acts in coordination with transportin-1 and that a code of post-translational modifications regulates import of the large class of RG/RGG proteins and a new class of proteins and that disease mutations found in cancer and neurodegeneration modulate these interactions. We propose to use newly discovered target proteins as model systems to reveal the structural and functional mechanisms of nuclear import and phase separation by:

1. 1) studying interaction, structure and function of the novel protein complexes
2. 2) studying regulation of the novel protein complexes by post-translational modifications, disease mutations, and co-factors

This might set the base for the discovery of new potential druggable targets in the future for the treatment of a plethora of diseases with different phenotypes, though caused by the same molecular disease mechanisms.

Methodology: The PhD candidate will make use of our recent methodological achievements for studying structure of large protein complexes by combining solution Nuclear Magnetic Resonance (NMR) spectroscopy, and molecular modeling[7-12], and extend it with complementary approaches such as Mass Spectrometry (MS) and cell biology.

References:

1. Kiledjian, M. & Dreyfuss, G. Primary structure and binding activity of the hnRNP U protein: binding RNA through RGG box. The EMBO journal 11, 2655-2664 (1992).
2. Thandapani, P., O'Connor, T. R., Bailey, T. L. & Richard, S. Defining the RGG/RG motif. Molecular cell 50, 613-623, doi:10.1016/j.molcel.2013.05.021 (2013).
3. Peretti, D., Bastide, A., Radford, H., Verity, N., Molloy, C., Martin, M. G., Moreno, J. A., Steinert, J. R., Smith, T., Dinsdale, D., Willis, A. E. & Mallucci, G. R. RBM3 mediates structural plasticity and protective effects of cooling in neurodegeneration. Nature, doi:10.1038/nature14142 (2015).
4. Dormann, D., Madl, T., Valori, C. F., Bentmann, E., Tahirovic, S., Abou-Ajram, C., Kremmer, E., Ansorge, O., Mackenzie, I. R., Neumann, M. & Haass, C. Arginine methylation next to the PY-NLS modulates Transportin binding and nuclear import of FUS. The EMBO journal 31, 4258-4275, doi:10.1038/emboj.2012.261 (2012).

5. Suarez-Calvet, M., Neumann, M., Arzberger, T., Abou-Ajram, C., Funk, E., Hartmann, H., Edbauer, D., Kremmer, E., Gobl, C., Resch, M., Bourgeois, B., Madl, T., Reber, S., Jutzi, D., Ruepp, M. D., Mackenzie, I. R., Ansorge, O., Dormann, D. & Haass, C. Monomethylated and unmethylated FUS exhibit increased binding to Transportin and distinguish FTLD-FUS from ALS-FUS. Acta neuropathologica 131, 587-604, doi:10.1007/s00401-016-1544-2 (2016).
6. Hofweber, M., Hutten, S., Bourgeois, B., Spreitzer, E., Niedner-Boblenz, A., Schifferer, M., Ruepp, M. D., Simons, M., Niessing, D., Madl, T. & Dormann, D. Phase Separation of FUS Is Suppressed by Its Nuclear Import Receptor and Arginine Methylation. Cell 173, 706-719 e713, doi:10.1016/j.cell.2018.03.004 (2018).
7. Gobl, C., Madl, T., Simon, B. & Sattler, M. NMR approaches for structural analysis of multidomain proteins and complexes in solution. Progress in nuclear magnetic resonance spectroscopy 80C, 26-63, doi:10.1016/j.pnmrs.2014.05.003 (2014).
8. Huang, J. R., Warner, L. R., Sanchez, C., Gabel, F., Madl, T., Mackereth, C. D., Sattler, M. & Blackledge, M. Transient Electrostatic Interactions Dominate the Conformational Equilibrium Sampled by Multidomain Splicing Factor U2AF65: A Combined NMR and SAXS Study. Journal of the American Chemical Society 136, 7068-7076, doi:10.1021/ja502030n (2014).
9. Karagoz, G. E., Duarte, A. M., Akoury, E., Ippel, H., Biernat, J., Moran Luengo, T., Radli, M., Didenko, T., Nordhues, B. A., Veprintsev, D. B., Dickey, C. A., Mandelkow, E., Zweckstetter, M., Boelens, R., Madl, T. & Rudiger, S. G. Hsp90-Tau complex reveals molecular basis for specificity in chaperone action. Cell 156, 963-974, doi:10.1016/j.cell.2014.01.037 (2014).
10. Lorenz, O. R., Freiburger, L., Rutz, D. A., Krause, M., Zierer, B. K., Alvira, S., Cuellar, J., Valpuesta, J. M., Madl, T., Sattler, M. & Buchner, J. Modulation of the Hsp90 chaperone cycle by a stringent client protein. Molecular cell 53, 941-953, doi:10.1016/j.molcel.2014.02.003 (2014).
11. Madl, T., Gabel, F. & Sattler, M. NMR and small-angle scattering-based structural analysis of protein complexes in solution. Journal of structural biology 173, 472-482, doi:10.1016/j.jsb.2010.11.004 (2011).
12. Muller, R., Grawert, M. A., Kern, T., Madl, T., Peschek, J., Sattler, M., Groll, M. & Buchner, J. High-resolution structures of the IgM Fc domains reveal principles of its hexamer formation. Proceedings of the National Academy of Sciences of the United States of America 110, 10183-10188, doi:10.1073/pnas.1300547110 (2013).

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Triggers and protectors of vascular calcification in patients with coronary artery disease

Summary

Philipp Eller, Institute of Internal Medicine, Intensive Care Unit, Medical University of Graz

Supervisor:	Prof. Dr. Philipp Eller
Availability:	This position is available.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2018 00:00 and September 23, 2018 23:59 (Europe/Zurich)

Description

Background: The massive burden of cardiovascular disease in chronic kidney disease and diabetes mellitus is strongly associated with extensive media calcification, reduced vascular compliance, left ventricular hypertrophy, and sudden cardiac death. Media sclerosis and media calcification are regulated by a complex interaction of systemic and local triggers of vascular calcification such as hyperphosphatemia and hyperglycemia, but also critically dependent on diverse physiological protectors from vascular calcification such as fetuin A or vitamin K[1-3]. These triggers and protectors modulate the phenotype of vascular smooth muscle cells, which are not terminally differentiated cells. In this manner they can eventually react to stress, inflammation or injury by transdifferentiating from contractile to proliferative and/or osteoblastic phenotypes.

Hypothesis and Objectives: We postulate that the local microenvironment plays a central role in the phenotypic modulation of vascular smooth muscle cells. Preliminary data from our lab indicate that macroautophagy is not only essential for cellular hemostasis, but also an important protector from vascular calcification. The main objective of this project is to analyse phenotypic modulation of vascular smooth muscle cells in different arteries of patients undergoing coronary bypass surgery.

Methodology: The PhD candidate will learn how to evaluate ectopic vascular calcification using histology, molecular biology, and mass spectrometry, respectively[1-3]. The PhD student will investigate the molecular genetic determinants of phenotypic modulation in vascular smooth muscle cells. The focus of these molecular genetic analyses will be on the specific role of genes involved in vascular calcification. Ultimately we aim to modulate the vascular smooth muscle cell behaviour in primary cell culture experiments and in an *in vivo* mouse model of vascular calcification and thus prevent/treat media sclerosis and media calcification that are associated with heavy burden of morbidity and mortality in patients suffering from diabetes mellitus or end-stage renal disease.

References:

1. Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. Eller K, Kirsch A, Wolf AM, Sopper S, Tagwerker A, Stanzl U, Wolf D, Patsch W, Rosenkranz AR, Eller P. Diabetes. 2011 60(11):2954-62.
2. Regulatory T cells improve nephrocalcinosis but not dystrophic cardiac calcinosis in DBA/2 mice. Kirsch AH, Smaczny N, Riegelbauer V, Sedej S, Hofmeister A, Stojakovic T, Goessler W, Brodmann M, Pilger E, Rosenkranz AR, Eller K, Eller P. Am J Pathol. 2013 183(2):382-90.

3. Heterogeneous susceptibility for uremic media calcification and concomitant inflammation within the arterial tree. Kirsch AH, Kirsch A, Artinger K, Schabhüttl C, Goessler W, Klymiuk I, Gülly C, Fritz G, Frank S, Wimmer R, Brodmann M, Anders HJ, Pramstaller P, Rosenkranz AR, Eller K, Eller P. Nephrol Dial Transpl 2015 30(12):1995-2005.

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Shining a light on the subcellular potassium homeostasis of cancer cells

Summary

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

Supervisor:	Prof. Dr. Roland Malli
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: We recently developed a series of novel genetically-encoded potassium ion (K^+) indicators, which for the very first time enable real-time monitoring of K^+ in single cells and subcellular compartments [1]. Our data point to cell type specific K^+ fluxes within distinct cellular organelles including the nucleus and mitochondria [1]. Within this thesis project the mechanisms responsible for controlling the subcellular K^+ homeostasis of cancer cells will be explored using state-of-the-art techniques. Moreover, subcellular K^+ fluctuations to defined stresses and stimuli will be investigated using high resolution fluorescence microscopy. Finally, the imapct of local and global K^+ alterations for cell proliferation and induction of cell death pathways in cancer cells will be scrutinized.

Hypothesis and Objectives: We hypothesize that subcellular K^+ fluctuations of cancer cells essentially control cell metabolism, proliferation and fate. Accordingly, pharmacological and/or genetic interventions which specifically affect K^+ levels within cancer cells and their organelles have the potency to impact cancer cell growth, proliferation, and viability.

Methodology: In addition to classical cell culture, biochemistry- (WB) and molecular biology techniques (PCR, siRNA library screens) the Ph.D. candidate will work with genetically encoded tools and probes [1,2,3] and use state-of-the-art fluorescence imaging techniques [1,2,3] to visualize subcellular K^+ signals in real-time on the level of individual cells.

References:

1. Helmut Bischof, H., Rehberg, M., Stryeck, S., Artinger, K., Eroglu, E., Waldeck-Weiermair, M., Gottschalk. B., Rost, R., Deak, A.T., Niedrist, T., Vujic, N., Lindermuth, H., Prassl, P., Pelzmann, B., Groschner, K., Kratky, D., Eller, K., Rosenkranz, A.R., Madl, T., Plesnila, N., Graier, W.F. & Malli, R. Novel genetically encoded fluorescent probes enable real-time detection of potassium in vitro and in vivo. Nat. Commun. 8:1422, 2017
2. Eroglu, E., Gottschalk, B., Charoensin, S., Blass, S., Bischof, H., Rost, R., Madreiter-Sokolowski, C.T., Pelzmann, B., Bernhart, E., Sattler, W., Hallström, S., Malinski, T., Waldeck-Weiermair, M., Graier, W.F. & Malli, R. Development of novel FP-based probes for live-cell imaging of nitric oxide dynamics. Nat. Commun. 7: 10623, 2016

3. Madreiter-Sokolowski, C.T., Klec, C., Parichatikanond, W., , Stryeck, S., Gottschalk, B., Pulido, S., Rost, R., Eroglu, E., Hofmann, N.A., Bondarenko, A.I., Madl, T., Waldeck-Weiermair, M., Malli, R. & Graier, W.F. PRMT1-mediated methylation of MICU1 determines the UCP2/3-dependency of mitochondrial Ca^{2+} uptake in immortalized cells. Nat. Commun. 7:12897, 2016

Development of a personalized model of rheumatoid arthritis

Summary

Martin H. Stradner, Division of Rheumatology and Immunology, Department of Internal Medicine, Medical University of Graz

Supervisor:	Prof. Dr. Martin Stradner
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: Rheumatoid arthritis (RA) is a systemic chronic autoimmune disease. RA patients suffer from painful and destructive inflammation of their joints. The exact pathogenesis of RA is still elusive. Underlying genetic factors and the strong association of RA with certain HLA class II molecules, such as HLA-DRB1*004 (HLA-DR4), suggest defects in CD4+ T cell function and their interaction with B cells(1, 2). This is further supported by the fact that autoantibodies have been detected in blood samples of RA patients years before the onset of clinical disease(3). Although RA is still a chronic incurable disease, significant therapeutic improvements could be achieved in the last decades by introducing novel biological disease modifying anti-rheumatic drugs (bDMARD)(4). Rodent models of RA have contributed considerably to the development of these therapies. The basic immunological concepts underlying bDMARDs are derived mainly from experiments in mice. Rodent models of RA were used to test the efficacy and savety beforen clinical phase I studies. Although invaluable for the development of new therapies, rodent models of RA have clear limitations. Thus, an animal model with a functional human immune system could be an important step in further understanding and treating RA. The transgenic NOD, SCID, interleukin- 2 receptor γ knockout (NSG) mouse is highly immunodeficient(5). Human hematopoietic stem cells or peripheral blood mononuclear cells (PBMC) can be injected into these mice to populate and multiply in the blood and lymphoid organs. This leads to a humanization of the immune system in these mice with functional properties such as antibody production, anti-viral immunity and rejection of allografts(5, 6). Recently, NSG mice with additional transgenic expression of the human HLA-DR4, instead of the mouse-MHC class II (NSGAbDR4) have become available(7). This genetic modification reduces the occurrence of graft-versus-host disease (GvHD) and improves the function of HLA-DR4 positive human CD4+ T cells(7). Given that 45% of RA patients are positive for HLA-DR4 and RA is considered to be a disease initiated and driven by CD4+ T cells, these mice represent an ideal platform for the establishment of a patient-personalized, humanized mouse model of RA.

Hypothesis and Objectives: The central hypothesis of this project is that the humanization of NSGAbDR4 with PBMCs of HLA-DR4 positive RA patients leads to the development of RA-like disease in these mice. We are aware that development of GVHD impairs the utility of the model. Thus, we propose that further genetic modifications of NSGAbDR4 mice, such as deficiency of MHC class I will reduce the incidence of GvHD.

Furthermore we hypothesize that a defined population among the donor cells induces arthritis in these mice. Thus, we will deplete and/or enrich the donor cells for defined cell populations We propose that this will lead to consistent development of arthritis in our model. Importantly, NSGAbDR4 mice humanized with PBMCs from healthy HLA-DR4 positive donors will not develop arthritis. Upon completing these aims we will have generated a novel patient-specific humanized mouse model of RA. It will for the first time be possible to test the effect of therapeutic interventions on a human immune system in vivo without putting humans at risk. Furthermore, the individual immunological characteristics of a certain RA patient and its response to possible therapies could be evaluated in our model. Finally, our model will allow new and exciting insights into the pathogenesis of RA which may ultimately result in novel treatment or even cure of this painful and disabling disease.

Methodology:

- Breeding and genotyping of mice.
- Isolation of PBMCs from HLA-DR4 positive RA patients
- Cell sorting by MACS and FACS technology.
- Analysis of the T cell receptor repertoire by PCR.

- Assessment of humanization and development of arthritis by histology, immunohistochemistry, flow cytometry and qPCR.

References:

1. G. N. Goulielmos et al., Genetic data: The new challenge of personalized medicine, insights for rheumatoid arthritis patients. Gene 583, 90-101 (2016).
2. J. Holoshitz, The rheumatoid arthritis HLA-DRB1 shared epitope. Current opinion in rheumatology 22, 293-298 (2010).
3. M. M. Nielen et al., Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 50, 380-386 (2004).
4. J. S. Smolen, D. Aletaha, Rheumatoid arthritis therapy reappraisal: strategies, opportunities and challenges. Nat Rev Rheumatol 11, 276-289 (2015).
5. L. D. Shultz, M. A. Brehm, J. V. Garcia-Martinez, D. L. Greiner, Humanized mice for immune system investigation: progress, promise and challenges. Nat Rev Immunol 12, 786-798 (2012).
6. Z. Rong et al., An effective approach to prevent immune rejection of human ESC-derived allografts. Cell stem cell 14, 121-130 (2014).
7. L. Covassin et al., Human peripheral blood CD4 T cell-engrafted non-obese diabetic-scid IL2rgamma(null) H2-Ab1 (tm1Gru) Tg (human leucocyte antigen D-related 4) mice: a mouse model of human allogeneic graft-versus-host disease. Clin Exp Immunol 166, 269-280 (2011).

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Role of monoglyceride hydrolases in pregnancy related inflammatory diseases

Summary

Christian Wadsack, Department of Obstetrics and Gynecology, Medical University of Graz

Supervisor:	Prof. Dr. Christian Wadsack
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: Pregnancy represents a state of heightened oxidative stress and inflammation, and these processes are further increased in pregnancy complications. The focus of our research is on understanding the cellular and molecular changes of bioactive lipid metabolites affecting placental tissue inflammation and functionality and to understand the mechanisms which are needed to counteract these changes.

Background: Many bioactive lipids derive from polyunsaturated fatty acids (PUFA) that are mobilized by intracellular lipases. These enzymes liberate free arachidonic acid (AA) and other PUFA for metabolization by cyclooxygenase (COX) and lipoxygenase (LOX), leading to synthesis of pro- or anti-inflammatory lipids. The present project focuses on two lipases, monoglyceride lipase (MGL) and alpha/beta hydrolase domain-containing 6 (ABHD6), which both possess monoglyceride (MG) hydrolase activity and have been associated with a number of diseases. Studies with mutant mice and with pharmacological inhibitors of the enzyme demonstrate that MGL-deletion causes strongly increased MG and reduced AA levels in liver, brain, and other tissues (*1*). This distinct metabolic function has a major impact on cellular signaling events since MGL determines the availability of monoacylglycerol (MG) and free fatty acids species acting as signaling molecules. The human placenta produces large quantities of prostanoids and leukotrienes, and the concentrations of prostaglandins in the fetal circulation exceed those in the maternal circulation with unknown consequences. Placental PUFA metabolism affects pregnancy, fetal development and outcome (*2*). However, very little is known about the role of MGL/ABHD6 in the degradation or generation of lipid signaling molecules in the human placenta. Given that both ABHD6 and MGL are considered as therapeutic targets for a number of diseases, it is highly important to understand their role in placental lipid metabolism.

Hypothesis: We hypothesize that MG hydrolases play critical roles in the metabolism of PUFA and its metabolites modulating inflammation in pregnancy. This can be achieved by degrading bioactive lipid species, such as 2-arachidonoylglycerol (2-AG), and prostaglandin glycerol esters, and by providing PUFAs for conversion via COX and LOX pathways.

Experimental approaches: In order to understand the role of MGL in placental lipid metabolism, primary cultures of human trophoblasts and endothelial cells will be challenged with specific inhibitors for MGL and ABHD6 and to test their effects on PUFAs and other lipid metabolites in normal pregnancies. In comparison, as an inflammatory model placentas of pre-eclamptic pregnancies will be used to determine the effects of inhibitors on specific lipid metabolites. Furthermore, we have the possibility to perform *ex vivo* placenta perfusion experiments allowing the investigation of MGL/ABHD6 function in the intact tissue. The used methodologies comprise isolation and culturing of cells from placental tissues, characterization of cells by flow cytometry and laser-scanning microscopy. The student will isolate RNA and protein, perform transcriptomics, real time PCR analyses, and Western blotting experiments to determine expression changes in particular pathways, analyze hydrolase activities, and perform cell culture experiments with primary trophoblasts and endothelial cells. The student will also be trained to characterize and quantitate specific lipid subclasses from maternal/cord blood and placental tissue by mass spectroscopy.

Collaborations within DP-iDP:

- D. Kratky will educate students in the field of lipases with respect to their function and the underlying signaling pathways.
- A. Heinemann will train students how metabolic stimuli like cytokines, eicosanoids or lipids trigger cellular function.
- G. Marsche will introduce students to functional assays of bioactive lipid mediators.

- M. van Poppel will train students in fundamentals of statistics and applications of bioinformatics.

Know-how and infrastructure of the research group: The laboratory has longstanding expertise in the field of lipids in normal and pathophysiological pregnancies with a focus on the human placenta. In order to study transport/transfer of lipids across intact placental barrier the *ex vivo* placental perfusion system was assembled. The laboratory uses a wide range of techniques to follow either biologicals or lipids across the human placenta including inflammatory conditions like pre-eclampsia, diabetes or antiphospholipid syndrome. All required equipment is available at the department, including a team of research nurses which provide the laboratory with placentas from the delivery room including all subject characteristics. The group comprises two post-doctoral fellows, three technicians, three master-students and two PhD students.

References:

1. G.F. Grabner, T.O. Eichmann, B. Wagner, Y. Gao, A. Farzi, U. Taschler, F.P.W. Radner, M. Schweiger, A. Lass, P. Holzer, E. Zinser, M.H. Tschop, C.-X. Yi, R. Zimmermann, Deletion of Monoglyceride Lipase in Astrocytes Attenuates Lipopolysaccharide-Induced Neuroinflammation, J. Biol. Chem. 291 (2015) 913–23.
2. R.M. Lewis, C. Wadsack, G. Desoye, Placental fatty acid transfer, Curr Opin Clin Nutr Metab Care. 2017 Dec 4.

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Mutual crosstalk between feto-placental macrophages and endothelium in the human placenta

Summary

Christian Wadsack, Department of Obstetrics and Gynecology, Medical University of Graz

Supervisor:	Prof. Dr. Christian Wadsack
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: The laboratory is interested on the molecular mechanisms of the human placenta which are potentially responsible for pregnancy diseases associated with inflammation such as gestational diabetes or pre-eclampsia. A central focus of these studies has been on the role of lipid mediators during placental development and their regulation in the feto-placental vasculature. Recent studies have suggested that, during fetal development macrophages regulate the vasculature in the central nervous system by establishing cell-to-cell contacts.

Background: Pregnancy complications such as preterm birth, miscarriage, maternal and/or neonatal morbidities, and mortality can be manifestations of underlying placental pathology. Hofbauer cells (HBC) refer to a heterogeneous population of fetal macrophages that reside within the functional unit of the placenta known as the chorionic villus. HBCs cells can be detected within the connective tissue matrix of the placenta as early as 4 weeks post-conception and are present throughout pregnancy. These cells are implicated in a wide array of functions important for a successful pregnancy including placental morphogenesis, immune regulation, control of stromal water content, and the transfer of ions and serum proteins across the maternal-fetal barrier. Derangements in HBC-homeostasis are associated with placental pathologies involving infection, inflammation, and inadequate placental development. Macrophages represent the first line of defense in numerous human tissues and are crucial to both acute and resolving immune responses (1). These remarkably plastic cells are able to adapt to their micro-environment in response to various exo- and endogenous stimuli. We and others have shown that HBCs have an M2 anti-inflammatory, regulatory phenotype (2). Finally, a handful of studies indicated that HBCs contribute to feto-placental angiogenesis.

Hypothesis: Collectively, we hypothesize that HBCs play a major role in maternal immunological tolerance against the fetus by representing a regulatory, tissue-remodeling rather than an inflammatory macrophage phenotype.

Experimental approaches: In a first set of experiments, placental tissue will be examined immunohistochemically for the presence and subcellular localization of relevant molecules enabling HBC-EC-crosstalk. Placental tissue from first trimester and term placentae will be used, enabling us to study the important time windows of expression of these proteins as it is known that gestational age affects expression of many angiogenic factors. Putative direct cell-cell-contacts will be visualized by immune fluorescence techniques. For the proposed study, the student will establish a co-culture model of HBCs and feto-placental endothelial cells (fpECs). To study the effects of fpECs on HBC functionality, the co-culture system can be exploited to study chemo-attraction and transmigration of HBCs towards ECs. The effect of HBCs on angiogenesis by fpECs will be determined by different angiogenesis assays. Since tissue hypoxia is known to stimulate angiogenesis student will examine the effect of hypoxia on expression of angiogenic factors in HBCs and fpECs cultured individually and in co-culture. Once that pro-/anti-angiogenic factors, ligand/receptor axes and downstream signaling pathways have been identified in HBCs and ECs, siRNA mediated silencing of their expression will be used to study how individual proteins affect the angiogenic potential of HBCs and fpECs. Pre-eclampsia is associated with changes in the placental vasculature leading to insufficient perfusion of the placenta, fetal growth restriction, and maternal hypertension.

Collaborations within DP-iDP:

- G. Desoye will provide tissue and primary trophoblasts isolated from human first trimester placenta
- Martin Gauster will support the project with placental explant approaches
- M. van Poppel will train students in fundamentals of statistics and applications of bioinformatics.

Know-how and infrastructure of the research group: The laboratory has longstanding expertise in the field of lipids in normal and pathophysiological pregnancies with a focus on the human placenta. In order to study transport/transfer of lipids across intact placental barrier the *ex vivo* placental perfusion system was assembled. The laboratory uses a wide range of techniques to follow either biologicals or lipids across the human placenta including inflammatory conditions like pre-eclampsia, diabetes or antiphospholipid syndrome. All required equipment is available at the department, including a team of research nurses which provide the laboratory with placentas from the delivery room including all subject characteristics. The group comprises two post-doctoral fellows, three technicians, three master-students and two PhD students.

References:

1. Schliefsteiner, C. et al. Human Placental Hofbauer Cells Maintain an Anti-inflammatory M2 Phenotype despite the Presence of Gestational Diabetes Mellitus. Front. Immunol. **8,** 1–17 (2017).
2. Young, O. M. et al. Toll-like Receptor-Mediated Responses by Placental Hofbauer Cells. A Potential Pro-Inflammatory Role for Fetal M2 Macrophages. Am J Clin Obs. 73, 22–35 (2015).

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Maternal lifestyle in pregnancy and neonatal body composition

Summary

Mireille van Poppel, Institute of Sport Science, University of Graz

Supervisor:	Prof. Dr. Mireille van#Poppel
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: The research group of Prof. van Poppel is interested in improving physical activity behavior in general, and in pregnant women specifically. Valid and reliable measurement of physical activity is important when studying health consequences of this behavior. Thus, efforts are made to improve tools for the measurement of physical activity in pregnancy. Furthermore, trying to understand consequences of physical activity and sedentary behavior in pregnancy for health outcomes of both mother and offspring is a main research interest of the group. This translational research is not limited to establishing associations of maternal behavior with health outcomes of mother and offspring, but also involves conceptualizing and investigating underlying pathways.

Background: In the DALI study (Jelsma et al. BMC Pregnancy and Childbirth 2011), effects of three different lifestyle interventions on maternal metabolism and neonatal outcomes were evaluated. The interventions consisted of counseling on healthy eating (HE), physical activity (PA), or a combination of HE&PA. It was found that the HE&PA intervention was effective in reducing gestational weight gain of the women (Simmons et al. JCEM 2017), and reduced neonatal adiposity, but did not influence maternal glucose metabolism.

Objectives and Hypothesis: Trying to elucidate the underlying mechanisms behind the intervention effects on neonatal adiposity is the primary goal of this PhD project. We hypothesize that maternal lifestyle (physical activity & sedentary behavior) in pregnancy affects neonatal fat mass through changes in the maternal-placental-fetal dialogue. Specifically, the role of various cytokines, adipokines, oxidized lipids and other inflammatory and oxidative stress parameters will be studied in this context.

Experimental approaches: Data and samples from the EU-wide DALI randomized trial, consisting of 436 obese pregnant women, will be used. Data on maternal PA and diet, blood samples and ultrasound scans were collected before 20 weeks, and at 24-28 and 35-37 weeks of gestation. Cord blood and placenta samples were collected and neonatal anthropometry was measured after birth.

In addition to getting acquainted with various laboratory methods, the PhD student will learn several statistical methods to analyze the available data. Intervention effects on various parameters will be analyzed by multilevel regression analyses, taking clustering by study site into account. Mediation of intervention effects by maternal, placental, and/or neonatal parameters will be assessed using the PROCESS module of Hayes. Further possible mechanistic pathways will be studied using path-analyses in Mplus or AMOS.

Successful candidate's profile: The PhD candidate has an education that includes courses on lifestyle, prevention, and/or public health, in combination with more basic courses in biochemistry or cell biology. PhD candidates should have at least basic knowledge of statistics and interest in learning more advanced methods.

Collaborations within DP-iDP:

- Akos Heinemann will provide the PhD student with his expertise on cytokines in maternal and cord blood in relation to neonatal body composition;
- Gunther Marsche will help with the measurement of oxidized lipid proteins in maternal and cord blood;
- Berthold Huppertz will help with morphological analyses of placenta samples;
- Gernot Desoye will provide expertise with analyzing oxidative stress parameters in placenta samples and blood samples.

Know-how and infrastructure of the research group: The research group has extensive statistical expertise (e.g. multi-level and longitudinal analyses, mediation analyses) and expertise in meta-analysis and systematic reviews, which is applicable for different research topics and designs. This expertise will be made available to all PhD students of DP-iDP. Furthermore, the group has expertise in the measurement and analysis of physical activity and sedentary behavior in general and in pregnancy.

The research group consists of two senior scientists, a postdoc researcher, and several PhD students. Access to an exercise physiology laboratory is available, including technical support staff.

References:

1. Jelsma JG, van Poppel MN, Galjaard S, Desoye G, Corcoy R, Devlieger R, van Assche A, Timmerman D, Jans G, Harreiter J, Kautzky-Willer A, Damm P, Mathiesen ER, Jensen DM, Andersen L, Dunne F, Lapolla A, Di Cianni G, Bertolotto A, Wender-Oegowska E, Zawiejska A, Blumska K, Hill D, Rebollo P, Snoek FJ, Simmons D. DALI: Vitamin D and lifestyle intervention for gestational diabetes mellitus (GDM) prevention: an European multicentre, randomised trial -- study protocol. BMC Pregnancy Childbirth. 2013 Jul 5;13(1):142.
2. Simmons D, Devlieger R, van Assche A, Jans G, Galjaard S, Corcoy R, Adelantado JM, Dunne F, Desoye G, Harreiter J, Kautzky-Willer A, Damm P, Mathiesen ER, Jensen DM, Andersen L, Lapolla A, Dalfrà MG, Bertolotto A, Wender-Ozegowska E, Zawiejska A, Hill D, Snoek FJ, Jelsma JG, van Poppel MN. Effect of Physical Activity and/or Healthy Eating on GDM Risk: The DALI Lifestyle Study. J Clin Endocrinol Metab. 2017 Mar 1;102(3):903-913. doi: 10.1210/jc.2016-3455.

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Characterisation of morbidly adherent placenta

Summary

Daniela Ulrich, Department of Obstetrics and Gynecology, Medical University of Graz

Supervisor:	Prof. Dr. Daniela Ulrich
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: Pathologic placental invasion is an increasing problem in the industrial world due to rising caesarean section rates. The research group of Prof. Ulrich is particularly interested to give new insights in this interesting phenomenon. The aim of this project is to combine the knowledge of the faculty to allow a holistic analysis of this pathology combining histology, tissue culture work, and stem cells characterization to hopefully understand pathologic placental invasion better at the end of this project.

Background: Placenta creta is defined as a pathologic attachment of the placenta to the uterus. There is little doubt that the worldwide caesarean delivery epidemic has led to an increased incidence of abnormally adherent and invasive placentation, so called morbidly adherent placenta (MAP). The physiological separation of placenta from the uterus at time of delivery is impossible. This condition is associated with severe peripartum and postpartum haemorrhage and consecutively with maternal morbidities due to intraoperative complications. Manual removal of the placenta is often required and in severe cases like placenta increta or percreta hysterectomy is necessary. The risk for MAP is around 3% after one caesarean section and increases up to 60% in women with ≥ 4 caesarean sections.

To date it is not clear which pathomechanisms are the underlying cause for the pathological placental invasion. Only few reports have been published regarding basic histological and immunological parameters in MAP [1-9]. It seems that the lack of a functional decidua, dysregulated inflammatory reactions are likely to lead to pathological placentation caused by excessive trophoblast proliferation and invasion of the placenta into the uterine wall. Vascular abnormalities, uteroplacental underperfusion, decidual hemosiderosis and infarction, as well as acute or chronic inflammation have also been postulated as possible risk factors [2]. To date, no paper reports a comprehensive analysis of MAP tissue combining histological, immunological and stem cell data.

Hypothesis: The aim is to find a correlation of histological, immunological and endometrial stem cell properties in MAP.

Experimental approaches: Uterine cells and tissue will be examined at time of caesarean section and compared between women with a normal pregnancy and planned caesarean section, to women with previous uterine scars without MAP, and to women with MAP. The aim is to analyse and compare histological parameters of uterine and placental tissue between the above mentioned groups including further analysis of immunological parameters, MSC parameters, trophoblast behaviour and immunological serum markers. The PhD student will learn histological analysis, immunohistochemistry, tissue culture work with stem cells, and protein analysis.

Collaborations within DP-iDP:

- Berthold Huppertz- histology
- Ute Panzenböck- immunology
- Ursula Hiden- trophoblast characterization
- Karoline Mayer-Pickel- patient recruitment

Know-how and infrastructure of the research group: Our laboratory has a long-standing expertise in the field of placenta. Studies are routinely carried out with human primary cells isolated from both placental trophoblast. The present thesis will capitalize on this knowledge combined with the know-how from Dr. Ulrich's tissue culture expertise. The collaborators as mentioned above will contribute long standing knowledge in the field of histology and immunology.

References:

1. Endler, M., S. Saltvedt, and N. Papadogiannakis, Macroscopic and histological characteristics of retained placenta: A prospectively collected case-control study. Placenta, 2016. 41: p. 39-44.
2. Ernst, L.M., et al., Placental Pathologic Associations With Morbidly Adherent Placenta: Potential Insights Into Pathogenesis. Pediatr Dev Pathol, 2017. 20(5): p. 387-393.
3. Gozukara, I., et al., YKL-40 expression in abnormal invasive placenta cases. J Perinat Med, 2017. 45(5): p. 571-575.
4. Linn, R.L., et al., Adherent basal plate myometrial fibers in the delivered placenta as a risk factor for development of subsequent placenta accreta. Placenta, 2015. 36(12): p. 1419-24.
5. Timor-Tritsch, I.E., et al., Cesarean scar pregnancy and early placenta accreta share common histology. Ultrasound Obstet Gynecol, 2014. 43(4): p. 383-95.
6. Adler, E., et al., Increased placental trophoblast inclusions in placenta accreta. Placenta, 2014. 35(12): p. 1075-8.
7. Chantraine, F., et al., Abnormal vascular architecture at the placental-maternal interface in placenta increta. Am J Obstet Gynecol, 2012. 207(3): p. 188.e1-9.
8. Schwede, S., J. Alfer, and U. von Rango, Differences in regulatory T-cell and dendritic cell pattern in decidual tissue of placenta accreta/increta cases. Placenta, 2014. 35(6): p. 378-85.
9. Duzyj, C.M., et al., The invasive phenotype of placenta accreta extravillous trophoblasts associates with loss of E-cadherin. Placenta, 2015. 36(6): p. 645-51.

Modeling genetic syndromes with induced pluripotent stem cells

Summary

Michael Speicher, Diagnostic and Research Center for Molecular BioMedicine, Institute of Human Genetics, Medical University of Graz

Supervisor:	Prof. Dr. Michael Speicher
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: The conversion of adult somatic cells into induced pluripotent stem cells (iPSC) provides a technology to generate disease- and patient-specific stem cell lines, therefore facilitating translational applications including disease modeling, drug discovery assays, drug development applications, toxicology screening or functional testing of putative disease causing mutations. Since first reported by Yamanaka and Takahashi in 2006, the ability to derive iPSCs from somatic cells has become one of the most attractive methods for modeling and investigating human diseases in cell culture (Takahashi and Yamanaka, 2006). Somatic cells are reprogrammed to iPSCs by the enforced overexpression of a defined set of transcription factors (TF), e.g. by the original TF cocktail from Yamanaka, which was composed of OCT4, SOX2, KLF4 and c-MYC (OSKM) (Liu et al., 2008). However, in recent years several other transcription factor mixes have also been used for successful reprogramming of somatic cells.

In this project the iPSC technology will be used to functionally characterize putative disease causing mutations. A scientific main interest of our institute is the detailed characterization of patients with distinct germline mutations, which increase the susceptibility for malignant diseases (i.e. BAP1, POLE, POLD1, BRCA1, BRCA2). These genes are established high-penetrance tumor susceptibility genes. The iPSCs may provide insight into the pathogenicity of variants within these genes and furthermore, they may contribute to an improved understanding of the pathomechanism of certain mutations. Using iPSC cells similar efforts for the high-penetrance tumor susceptibility gene TP53 were recently published (Lee et al., 2015). The mode of inheritance for the aforementioned genes is autosomal dominant; however, at the cellular level these gene are recessive, meaning that the wild-type allele needs to get lost for the initiation of tumorigenesis. For this reason, we will also conduct gene knock-out of the wild-type allele using the CRISPR/Cas9 method. In our lab, we have already experiences with iPSC cells generated from individuals with BAP1 germline mutations. BAP1 germline mutations predispose to a variety of tumors such as atypical nevi, cutaneous melanomas, uveal melanoms, mesotheliomas, and other tumors (Wiesner et al., 2011).

Hypothesis and Objectives: The central hypothesis of this project is that patient derived iPSC cell systems combined with induction of defined alterations by CRISPR/Cas9 within the genome of these cells allow modelling human diseases and will improve our understanding of disease mechanisms.

The objectives include the development of human disease models in vitro for various aims, such as proving the disease causing potential of certain mutations on a specific cell type, studying the cellular consequences of defined mutations, e.g. by analyses of molecular disease pathways in the affected cell type, or use of these models for testing pharmaceutical drugs.

Methodology:

- Donor cells will be isolated (either skin punch biopsies performed by a dermatologist or blood draw) and a primary cell culture will be established;
- Reprogramming of cells employing various vectors and TFs;
- Characterization of cells, e.g. with tests for pluripotency;
- Differentiation, i.e. the obtained pluripotent cells will be differentiated into cells from the three germ layers using in vitro differentiation assays;
- CRISPR/Cas9 editing of the cell's genome;
- Genetic analyses, (genome, epigenome and transcriptome) at different stages of the cell differentiation and prior and after CRISPR/Cas9;

- Functional analyses, which depend of the gene of question and characteristics of the cells.

References:

1. Lee, D.F., Su, J., Kim, H.S., Chang, B., Papatsenko, D., Zhao, R., Yuan, Y., Gingold, J., Xia, W., Darr, H., et al. (2015). Modeling familial cancer with induced pluripotent stem cells. Cell 161, 240-254.
2. Liu, X., Huang, J., Chen, T., Wang, Y., Xin, S., Li, J., Pei, G., and Kang, J. (2008). Yamanaka factors critically regulate the developmental signaling network in mouse embryonic stem cells. Cell Res 18, 1177-1189.
3. Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663-676.
4. Wiesner, T., Obenauf, A.C., Murali, R., Fried, I., Griewank, K.G., Ulz, P., Windpassinger, C., Wackernagel, W., Loy, S., Wolf, I., et al. (2011). Germline mutations in BAP1 predispose to melanocytic tumors. Nature genetics 43, 1018-1021.

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Metabolic and mechanistic effects of various calorie restriction regimens in healthy humans and subjects with type 2 diabetes mellitus

Summary

Harald Sourij, Division of Endocrinology and Metabolism, Medical Universität of Graz

Supervisor:	Prof. Dr. Harald Sourij
Availability:	This position has been occupied.
Offered by:	CBmed
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: Obesity and diabetes mellitus probably represent the most challenging threat to public health in the 21st century. While the pathophysiology of type 2 diabetes is complex, the two major drivers of the disease are insulin resistance and beta-cell dysfunction. Intermittent fasting (IF, a diet regime of extended time periods (16-48h) with little or no energy intake, followed by periods of normal food intake, on a recurring basis) was shown to improve the functional outcome in experimental models of various age-related disorders such as CVD and diabetes by reducing blood pressure and insulin resistance (2,3). However, studies are usually small, in most cases uncontrolled and provide little mechanistic insights.

Hypothesis and Objectives: First, we will use samples obtained in an already performed intermittend fasting study with healthy subjects to investigate the metabolic profile of subjects following IF and those on control diet. First data of this trial also demonstrate that a prolonged fasting significantly reduces beta-cell secretory response to oral glucose load in healthy subjects (Tripolt N. et al., submitted). Beta-cell secretory capacity and the role of counterregulatory hormones in prolonged fasting will be further investigated in healthy subjects, obese subjects and people with type 2 diabetes.

Within a newly set up clinical trial we will compare the effects of simple calorie restriction against intermittend fasting in insulin resistant subjects with type 2 diabetes on insulin sensitivity. We will further focus on immune cell profiling and metabolic fingerprinting, including amino acid composition in those subjects.

Methodology: Two human studies will be used to undertake the research outlaid above.

1. Healthy individuals who have implemented the alternate day fasting dietary regime in their lifestyle for at least 6 months will be age and sex matched to patients without previous ADF history, and a control group, which will continue a control eating pattern (study already performed).
2. Insulin resistant subjects with insulin treated type 2 diabetes will be recruited for a clinical trial. The patients will be randomized to either follow an intermittend fasting regimen that will consist of 70% calorie restriction on fasting days (3 days per week), or a moderate, daily calorie restriction. This diet plan will be implemented for 12 weeks.

During the basic human interventions, blood and faeces samples will be collected for further analyses.

The following parameters will be assessed:

- Plasma metabolome analysis for identification of metabolic biomarkers – Tragetet and Untargeted metabolomics analysis (High performance Liquid Chromatography (HPLC)-coupled mass spectrometry (MS))
- Stool microbiome analysis – (16s sequencing)

- Immune phenotyping using FACS analysis
- Micro-RNA involved in type 2 diabetes pathophysiology with a focus on pancreas and liver (including miRNA-9, -21, -24, -26, -29, -133a, -187, -375)

References:

1. Ogurtsova K, da Rocha Fernandes JD, Huang Y, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract*. 2017;**128**:40-50.
2. Mattson, M.P.et al. Impact of intermittent fasting on health and disease processes. *Ageing Res. Rev.*2017; **39,** 46-58.
3. Wan, R. Intermittent food deprivation improves cardiovascular and neuroendocrine responses to stress in rats. *J.Nutr.* 2003; **133,** 1921-1929.

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Information extraction from narratives in electronic health records for biomarker research

Summary

Stefan Schulz, Institute for Medical Informatics, Statistics and Dokumentation, Medical Universität of Graz

Supervisor:	Prof. Dr. Stefan Schulz
Availability:	This position has been occupied.
Offered by:	CBmed
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: One grand challenge in biomarker research is to complete the picture of information about potential biomarkers using a broad range of clinical data sources. This project addresses the reuse of documented information on clinical phenotypes, past diseases, findings, procedures, lifestyle data, drugs, family history, etc. As most of this routine data resides in clinical narratives such as findings reports and low-structured patient summaries in electronic health record systems, a semantic data extraction platform for the optimized retrieval of biobank samples and a customizable toolkit for content retrieval is currently being built. An important use case is the optimized retrieval of biobank samples, such as needed for biomarker research, which requires information about the clinical context of the patient from which the samples had been taken. This data requires a thoughtful selection of important features like diseases, signs, symptoms, therapies, clinical evolution and laboratory parameters, altogether put into a temporal context, for the best ranking on a cohort search engine.

Hypothesis and Objectives: The hypothesis is that there is a common core of information needs for biomarker research that can only be addressed by either labour-intensive manual reworking of routine data or by machine processing of the EHR. The objective is to lay the foundations for the development of a customizable toolkit for content extraction. Existing approaches like i2b2 will be capitalized on, as well as existing terminologies and data models (SNOMED CT, clinical models).

Principal objectives are to develop, customize and assess components of a processing pipeline that takes raw clinical texts as they are and enriches them by semantic metadata. The specific tasks are manifold: identification of short forms (abbreviations, acronyms), correction of misspellings, identification of attribute – number – unit triples, identification of temporal contexts, identification of epistemic contexts (diagnostic (un)certainty, intentions), negations. Main purpose of these processing steps are to accurately map textual content to clinical terminologies (SNOMED CT, LOINC, ICD) and pre-defined information models, which could affect, for example, how a patient is ranked on a cohort search engine.

Methodology: Diverse computational linguistics methods will be used (rule-based, machine learning-based, deep learning), together with different information retrieval approaches. The student should therefore have a background in computer science, with a focus on computational linguistics and text mining. Familiarity with the medical domain, as well as with medical ontologies and terminologies is desirable.

References:

1. Patterson O., Igo S., and Hurdle J. F. Automatic acquisition of sublanguage semantic schema: Towards the word sense disambiguation of clinical narratives. In AMIA Annual Symposium Proceedings, volume 2010, pages 612–616. American Medical Informatics Association, 2010.
2. Joachims T. Text categorization with support vector machines: Learning with many relevant features. In European Conference on Machine Learning (ECML), pages 137–142, Berlin, 1998. Springer.
3. Baharudin B., Lee L. H., and Khan K. A review of machine learning algorithms for text-documents classification. Journal of Advances in Information Technology, 1(1): 4–20, 2010.
4. Chiticariu L., Li Y., and Reiss F. R. Rule-based information extraction is dead! Long live rule-based information extraction systems! In EMNLP, pages 827–832, 2013.

5. Chapman W. W., Chu D., and Dowling J. N. ConText: an algorithm for identifying contextual features from clinical text. In Proceedings of the Workshop on BioNLP 2007: Biological, Translational, and Clinical Language Processing, pages 81–88. Association for Computational Linguistics, 2007.
6. Meystre S. M., Savova G., Kipper-Schuler K., and Hurdle J. Extracting information from textual documents in the electronic health record: A review of recent research. Yearbook of Medical Informatics, 35:128–144, 2008.
7. Patterson O., Forbush T., Saini S., Moser S., and DuVall S. Classifying the indication for colonoscopy procedures: A comparison of NLP approaches in a diverse national healthcare system. Studies in Health Technology and Informatics, 216:614–618, 2015.
8. Kreuzthaler M., Daumke P., and Schulz S. Semantic retrieval and navigation in clinical document collections. EHealth2015–Health Informatics Meets EHealth: Innovative Health Perspectives: Personalized Health, 212:9–14, 2015.
9. Savova G. K., Masanz J. J., Ogren P. V., Zheng J., Sohn S., Kipper-Schuler K. C., and Chute C. G. Mayo clinical text analysis and knowledge extraction system (cTAKES): architecture, component evaluation and applications. Journal of the American Medical Informatics Association, 17(5):507–513, 2010.
10. Friedman C., Hripcsak G., DuMouchel W., Johnson S. B., and Clayton P. D. Natural language processing in an operational clinical information system. Natural Language Engineering, 1(01):83–108, 1995.

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CRISPR/Cas9-mediated genome engineering of human hematopoietic stem and progenitor cells to decipher the pathomechanisms of common mutations in myeloproliferative neoplasms (MPNs)

Summary

Andreas Reinisch & Heinz Sill, Clinic for Internal Medicine, Medical University of Graz

Supervisors:	Prof. Dr. Heinz Sill Dr. Andreas Reinisch
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: MPNs encompass a spectrum of chronic hematological disorders that are characterized by the overproduction of one or more mature, terminally differentiated myeloid blood cell lineages. MPN include three main disease: polycythemia rubra vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (MF). Most patients with MPN have a genetic basis responsible for their excessive myeloproliferation.

The most common genetic alteration of MPN disorders was elucidated in 2005 with the seminal identification of a cardinal single point mutation in the gene JAK2 (Janus kinase 2) resulting in JAK2V617F, in the majority of patients with PV and half of those with ET and MF. Physiologically, JAK2 is associated with the cytoplasmic portion of various cytokine receptors, including key hematopoietic growth factors such as erythropoietin (EPO), thrombopoietin (TPO) or granulocyte colony-stimulating-factor (G-CSF). Upon ligand binding JAK2 mainly functions to activate intracellular signaling via signal transducer and activator of transcription (STAT). Mutant JAK2V617F is rendered constitutively active and results in cytokine hypersensitivity and cytokine-independent growth of mutant cells and excessive cell proliferation and differentiation (Kralovics et al., 2005).

Additional genetic abberations that perturb JAK-STAT signaling are found in JAK2-unmutated MPNs. Mutations in CALR were identified in the majority of JAK2-unmutated patients with ET or MF and mutations in the thrombopoietin receptor MPL are found in about 5 – 8% of ET and MF patients (Klampfl et al., 2013). MPL mutations result in conformational changes in the receptor that mimic TPO binding and therefore ligand-independent aberrant intracellular signaling. CALR mutations lead to a common alteration of the reading frame and acquisition of a novel C-terminus of the protein. Recent data suggested that mutant CALR can activate MPL leading to aberrant signaling and disease development similar to MPL mutations. Although investigation in mouse models provided some information about the pathomechanism involved in MPN development, there is still a fundamental gap in understanding how these mutations transform human hematopoietic stem and progenitor cells (HSPCs) and cause MPN. This knowledge gap represents an important problem because although the recently FDA-approved JAK2 inhibitors can provide palliative benefit to MPN patients, including those harboring MPL and CALR mutations, JAK2 inhibition does not preferentially target the MPN clone and therefore does not have curative potential. Furthermore there are currently no treatment strategies to specifically target MPL- or CALR-mutant cells in MPN. The long-term goal is to understand the mechanisms by which mutant JAK2V617F, MPL and CALR transforms human hematopoietic cells in order to exploit these insights for therapeutic gain.

Hypothesis and Objectives: A major goal in cancer research is the development of better systems that accurately mimic human cancer cell function and are highly predictive of cancer cell behavior observed in patients. The objective of this project is to apply CRISPR/Cas9 genome engineering technology (Bak et al., 2017, Dever et al., 2016) to precisely introduce JAK2V617F, MPL or CALR mutations into human HSPCs and subsequently characterize the

molecular and functional consequences caused by the mutations. Gene expression analysis will be used to reveal moleculare pathways that are commonly disregulated and therefore would provide specific targets for novel therapy. Transformation-potential and disease initiation capacity of the mutations will be employed in our recently developed humanized-niche xenotransplantation model in vivo (Reinisch et al., 2017, Reinisch et al., 2016).

Once we understand how these mutations transform human HSPCs and induce MPN, it will be possible to identify mutant-specific molecular dependencies that can be exploited therapeutically to develop novel treatment approaches.

Methodology: The PhD candidate shoul have a strong drive to learn various innovative techniques and enjoys collaborative work with leading laboratories in the field of genome editing and leukemia research. The candidate will apply CRISPR/Cas9 genome engineering technologies to manipulate primary human HSPCs via i) non-homologous end-joining (NHEJ)-mediated mechanisms and ii) homologous driven repair (HDR) in order to perform allel-specific knock-ins of JAK2, MPL and CALR mutations. The work includes designing and cloning of appropriate CRISPR/Cas9 reagents as well as the production of Adeno-Associated viral (AAV) vectors for DNA template delivery. Basic technologies in in our lab include isolation, culture, characterisation and manipulation of primary human hematopoietic cells (including HSPCs). The candidate will also employ standard molecular biology techniques including PCR, qRT-PCR, ddPCR and Western Blotting. In order to characterize the modified cells the candiated will employ in vitro methylcellulose-based colony formation assays as well as investigate differentiation and proliferation behavior of the cells. Furthermore, the candidate we will use xenotransplantation mouse models to study engraftment potential and disease-initiation potential of engineered mutant human cells. Polychromatic flow cytometry will be used for most in vitro and in vivo assays performed for this project.

References:

1. Bak, R. O., Dever, D. P., Reinisch, A., et al. 2017. Multiplexed genetic engineering of human hematopoietic stem and progenitor cells using CRISPR/Cas9 and AAV6. Elife, 6.
2. Dever, D. P., Bak, R. O., Reinisch, A., et al. 2016. CRISPR/Cas9 beta-globin gene targeting in human haematopoietic stem cells. Nature, 539, 384-389.
3. Klampfl, T., Gisslinger, H., Harutyunyan, A. S., et al. 2013. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med, 369, 2379-90.
4. Kralovics, R., Passamonti, F., Buser, A. S., et al. 2005. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med, 352, 1779-90.
5. Reinisch, A., Hernandez, D. C., Schallmoser, K., et al. 2017. Generation and use of a humanized bone-marrow-ossicle niche for hematopoietic xenotransplantation into mice. Nat Protoc, 12, 2169-2188.
6. Reinisch, A., Thomas, D., Corces, M. R., et al. 2016. A humanized bone marrow ossicle xenotransplantation model enables improved engraftment of healthy and leukemic human hematopoietic cells. Nat Med, 22, 812-21.

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p53 in the control of adipose tissue homeostasis

Summary

Andreas Prokesch, Gottfried Schatz Research Center, Cell Biology, Histology and Embryology, Medical University of Graz

Supervisor:	PD Dr. Andreas Prokesch
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: According to the WHO about 2 billion people are currently overweight or obese. Increased adiposity is a strong risk factor for the metabolic syndrome, a cluster of disorders including type 2 diabetes mellitus and cardiovascular diseases. Adipose tissue (AT) is a major determinant in the development of metabolic syndrome and the body's largest endocrine organ (Gesta, Tseng, & Kahn, 2007). In times of starvation, by provision of free fatty acids and glycerol as energy substrates, AT is crucial for systemic energy homeostasis (Zechner, Madeo, & Kratky, 2009). The tumor suppressor p53 is a transcription factor activated in cancerous cells by a variety of stress signals such as DNA damage, oncogene activation, nutrient deprivation, and hypoxia (Kastenhuber & Lowe, 2017). Once activated, the p53 pathway has a wide range of downstream effects among which cell death, cell cycle arrest, autophagy, and regulation of cellular metabolism are most prominent (Berkers, Maddocks, Cheung, Mor, & Vousden, 2013). While many functional aspects for the p53-mediated cellular stress response during tumorigenesis (and therefore in rapidly dividing cells) are well established, much less is known about the role of p53 in non-transformed, post-mitotic cells and tissues. We have shown an upregulation of p53 signaling by starvation in several tissues, including adipose tissue and liver (Schupp et al., 2013). In a recent publication, we have shown that p53 protein is stabilized in hepatocytes under starvation (Prokesch et al., 2017). Acute, liver-specific knock-out showed that p53 is necessary for amino acid catabolism and glucose maintenance under starvation, while in the fed state glycogen storage is reduced and lipids accumulate in hepatocytes. Hence, our work suggests that, beyond its role as tumor suppressor, p53 plays a role as metabolic regulator in normal cells and tissues. We recently acquired an FWF-DACH grant in collaboration with the Charite in Berlin (Prof. Michael Schupp) and the German Institute for Nutritional Research (Prof. Tim Schulz). In this consortium we will utilize novel tissue-specific, inducible knock-out mouse models to investigate the role of p53 in the metabolism of liver, white adipose tissue, brown adipose tissue, and skeletal muscle.

Hypothesis and Objectives: The aim is to determine the effects of p53 in AT on the acute physiological starvation response that is characterized by dynamic changes in metabolic gene expression patterns and rapid shifts of nutrient fluxes via processes such as lipolysis and lypophagy.

Methodology: The PhD candidate working in Graz will investigate AT metabolism after acute AT-specific ablation of p53, directly comparing the effects in white, brite, and brown ATs. For that, a novel mouse model (Adiponectin-CreERT2xp53flox) will be used, that will be phenotypically, histologically, and metabolically characterized in our lab. Furthermore, the candidate will work with cell lines and primary cells to decipher detailed molecular mechanisms, using classical cell culture work (protein overexpression, Crispr/cas9, RNAi, proliferation assays) followed by downstream analyses such as western blot, qPCR. Mechanisms under scrutiny will be crosstalk with other starvation-relevant pathways such as AMPK, mTOR, Sirt1, and PPAR singaling. Further, assays to investigate lipid metabolism (triglyceride hydrolase assay, assessment of lipophagy and de novo lipogenesis, etc.) as well as various omics approaches (e.g. transcriptomics, lipidomics) will be applied.

References:

1. Berkers, C. R., Maddocks, O. D. K., Cheung, E. C., Mor, I., & Vousden, K. H. (2013). Metabolic regulation by p53 family members. Cell Metabolism, 18(5), 617–33. https://doi.org/10.1016/j.cmet.2013.06.019
2. Gesta, S., Tseng, Y.-H., & Kahn, C. R. (2007). Developmental Origin of Fat: Tracking Obesity to Its Source. Cell, 131(2), 242–256. https://doi.org/10.1016/j.cell.2007.10.004

3. Kastenhuber, E. R., & Lowe, S. W. (2017). Putting p53 in Context. Cell, 170(6), 1062–1078. https://doi.org/10.1016/j.cell.2017.08.028
4. Prokesch, A., Graef, F. A., Madl, T., Kahlhofer, J., Heidenreich, S., Schumann, A., … Schupp, M. (2017). Liver p53 is stabilized upon starvation and required for amino acid catabolism and gluconeogenesis. The FASEB Journal, 31(2), 732–742. https://doi.org/10.1096/fj.201600845R
5. Schupp, M., Chen, F., Briggs, E. R., Rao, S., Pelzmann, H. J., Pessentheiner, A. R., … Prokesch, A. (2013). Metabolite and transcriptome analysis during fasting suggest a role for the p53-Ddit4 axis in major metabolic tissues. BMC Genomics, 14(1), 758. https://doi.org/10.1186/1471-2164-14-758
6. Zechner, R., Madeo, F., & Kratky, D. (2009). Cytosolic lipolysis and lipophagy: two sides of the same coin. Nature Publishing Group, 2009. https://doi.org/10.1038/nrm.2017.76

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Nuclear receptors regulating metabolism and inflammatory mediators in endothelial cells

Summary

Ute Panzenboeck, Otto Loewi Research Center, Immunology and Pathophysiology, Medical University of Graz

Supervisor:	Prof. Dr. Ute Panzenboeck
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: The present major focus of our laboratory is to define the role of nuclear receptors, in particular liver-X receptors (LXRs) as lipid sensors and regulators in endothelial cells representing an underestimated, potent part of the innate immune system. Fundamental molecular mechanisms of nuclear hormone receptor signalling in endothelial immune-metabolic functions are investigated. A current goal of our laboratory is to define the impact of LXRs on cholesterol-metabolic and inflammatory functions in the feto-placental vasculature in inflammatory disorders of pregnancy.

Background: Oxysterols, generated from cholesterol oxidation enzymatically by CYP450 reductases or by reactive oxygen species (ROS), are endogenous activators of LXRs, nuclear transcription factors centrally involved in important biological processes, including lipid/cholesterol and glucose metabolism (1). LXRs promote high-density lipoprotein (HDL) formation by activating several target genes of reverse cholesterol transport. LXR target genes (ABCA1, ABCG1 and PLTP) are also centrally involved in HDL mediated cholesterol efflux from fetoplacental endothelial cells (HPEC) (2-4). While HDL itself exerts anti-inflammatory actions on endothelial cells, LXRs also regulate immune regulatory functions and can reduce inflammation by sumoylation-dependent and -independent mechanisms (1).

Objectives and Hypothesis: Gestational diabetes mellitus (GDM) may affect placental cholesterol metabolism via LXR activation due to increased oxysterol levels in cells and in the fetal circulation (UP, CW, GD, unpublished; 1,2). Upon activation, LXRs can be sumoylated and as a monomer can stabilize repressor complexes present on the promoter sequence of proinflammatory pathways such as activator protein 1 (AP-1) and nuclear factor kB (NF-kB), thereby preventing the expression of proinflammatory factors (1). We hypothesize that endogenous and/or pharmacologic activation of LXRs may improve fetoplacental endothelial function in conditions of GDM and other inflammatory pregnancy complications related to metabolic diseases. To test this hypothesis, the student will investigate effects of the GDM/metabolically disordered environment as well as of LXR activation by oxysterols or synthetic agonist TO901317 on (reduced) expression of inflammatory genes along with altered cellular cholesterol metabolism (5) in term fetoplacental endothelial cells.

Experimental approaches: Techniques applied will include primary cell culture, RTQ-PCR, immunoblotting, immunoprecipitation, sumoylation assay, ELISA, IHC, RNAi, functional assays for ABC transporters, cholesterol efflux and synthesis assays, TLC, GC-MS.

Successful candidate's profile: Successful PhD candidates are familiar with molecular biological/biochemical methods and are eager to learn an array of new methods.

Collaborations within DP-iDP:

- G. Marsche will provide equipment required for isolation of lipoproteins.
- C. Wadsack will provide fetal and maternal plasma as well as clinically well definded placental tissue obtained from healthy and GDM pregnancies.
- G. Desoye will provide know-how and technology to perform in depth epigenetic analyses of fetoplacental endothelial cells.

Know-how and infrastructure of the research group: Our group has profound research experience in the field of lipid and lipoprotein metabolism of specialized endothelial barriers, with a focus on anti-atherogenic and anti-neurodegenerative activities of HDL. Expertise in my laboratory includes isolation and culture of primary endothelial cells,

in vitro models of the blood-brain barrier, co-culture models, qualitative and quantitative lipid and protein analysis, standard molecular biological methods including real-time PCR, transfection, standard and specific immunological methods, radiobiochemical methods, in situ hybridization histochemistry, and experience with animal studies. The local infrastructure comprises cell culture facilities, a fluorescence microscopy facility, a state of the art Fluorescence Activated Cell Sorting facility, HPLC laboratory, molecular biology and immunology laboratory, histology and laboratory, radionuclide laboratory, and an animal facility.

References:

1. Guillemot-Legris O, Mutemberezi V, Muccioli GG. Oxysterols in Metabolic Syndrome: From Bystander Molecules to Bioactive Lipids. Trends Mol Med. 2016; 22(7):594-614.
2. Stefulj J, Panzenboeck U1, Becker T, Hirschmugl B, Schweinzer C, Lang I, Marsche G, Sadjak A, Lang U, Desoye G, and Wadsack C. Human Endothelial Cells of the Placental Barrier Efficiently Deliver Cholesterol to the Fetal Circulation via ABCA1 and ABCG1. Circ Res. 2009; 104:600-608.
3. Scholler M, Wadsack C, Metso J, Chirackal Manavalan AP, Sreckovic I, Schweinzer C, Hiden U, Jauhiainen M, Desoye G, Panzenboeck U. Phospholipid transfer protein is differentially expressed in human arterial and venous placental endothelial cells and enhances cholesterol efflux to fetal HDL. J Clin Endocrinol Metab. 2012; 97:2466-74.
4. Scholler M, Wadsack C, Lang I, Etschmaier K, Schweinzer C, Marsche G, Dieber-Rotheneder M, Desoye G, Panzenboeck U. Phospholipid Transfer Protein in the Placental Endothelium Is Affected by Gestational Diabetes Mellitus. J Clin Endocrinol Metab. 2012; 97:437-45.
5. Schweinzer C, Kober A, Lang I, Etschmaier K, Scholler M, Kresse A, Sattler W, Panzenboeck U. Processing of Endogenous AβPP in Blood-Brain Barrier Endothelial Cells is Modulated by Liver-X Receptor Agonists and Altered Cellular Cholesterol Homeostasis. J Alzheimers Dis. 2011; 27(2):341-360.

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Probiotic dietary intervention in Polycystic Ovary Syndrome (PCOS) – crosstalk of microbiome, metabolism, hormones and more

Summary

Barbara Obermayer-Pietsch, CBmed Center for Biomarker Research in Medicine & Division of Endocrinology and Diabetology, Medical University of Graz

Supervisor:	Prof. Dr. Barbara Obermayer-Pietsch
Availability:	This position has been occupied.
Offered by:	CBmed
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: According to epidemiological estimates, the prevalence of polycystic ovarian syndrome (PCOS) is about 6-22% of all women worldwide dependent on ethnic and environmental factors, but a higher number is assumed [1]. A commonly used definition of PCOS [2] involves the identification of at least two of the three criteria: clinical and/or biochemical hyperandrogenism (incidence 40-92%), oligo- and/or anovulation (75-90%), and polycystic ovaries (22-92%), but there is also a significant proportion of insulin resistance and obesity among these patients (at least 30-40%). The etiology of the syndrome is not clear. Interactions between environmental and genetic factors, but also immunological and microbiomal influences [3] have been supposed.

Recent results from our group revealed differences in stool microbiome between PCOS patients and healthy controls including clinical symptoms and parameters such as androgens, anti-Müllerian hormone, hirsutism and parameters of insulin resistance, but also depression scores, gut permeability and inflammation which were related to the microbiome.

We are now investigating possible mechanisms by which the intestinal microbiome might govern systemic metabolic processes, e.g. via a disturbed intestinal barrier, which is known to develop in the context of inflammatory bowel disease, high-fat diet or obesity, accompanied by microbiomal changes (1).

As both the microbiome and intestinal permeability might interact with the body's immune system, the effects of a disturbed steroid hormone metabolism, which is present in women with PCOS on the immune system and e.g. on regulatory T cells as important immune markers, are still unclear.

Hypothesis and Objectives: Based on our recent publications and ongoing studies, we plan to perform a randomized, placebo-controlled study using probiotic dietary intervention in a defined group of PCOS women. Our aim is to investigate the dimensions of potential microbial changes in PCOS women and whether these changes might correlate with specific clinical, hormonal, immunological and metabolic parameters in the study population.

Probiotic intervention might decrease gut permeability as measured by systemic and functional tests as well as inflammation and hormonal/metabolic parameters, and increase gut microbiome diversity as analysed by NGS techniques. In addition, this approach might also be important for the quality of life in the study participants as documented by questionnaires.

Methodology: The PhD candidate will be directly involved in the preparations, recruiting and implementation of the clinical intervention study, the biobanking approaches, some laboratory measurements and the statistical analysis of the results. Samples will be analysed using enzyme-linked and radioimmunological assays and HPLC-MS for hormones and phytoestrogen metabolites together with scientists at the Endocrinology Lab Platform. Microbiome analyses after DNA extraction from samples via MiSeq sequencing will be processed using open-source software according to published protocols and bioinformatic tools. Relative abundances of bacteria will be confirmed by qPCR; flow cytometry, whole blood gene expression as well as immunological and functional tests will be performed. Studies will be complemented by cell culture models of hormonal and immunological interaction.

References:

1. Lindheim L, Bashir M, Münzker J, Trummer C, Zachhuber V, Leber B, Horvath A, Pieber TR, Gorkiewicz G, Stadlbauer V, Obermayer-Pietsch B. Alterations in Gut Microbiome Composition and Barrier Function Are Associated with Reproductive and Metabolic Defects in Women with Polycystic Ovary Syndrome (PCOS): A Pilot Study. *PLoS One. 2017 Jan 3;12(1):e0168390.*
2. Lindheim L, Bashir M, Münzker J, Trummer C, Zachhuber V, Pieber TR, Gorkiewicz G, Obermayer-Pietsch B. The Salivary Microbiome in Polycystic Ovary Syndrome (PCOS) and Its Association with Disease-Related Parameters: A Pilot Study. *Front Microbiol. 2016;7:1270.*
3. Pedro J. Torres, Martyna Siakowska, Beata Banaszewska, Leszek Pawelczyk, Antoni J.Duleba, Scott T. Kelley , Varykina G. Thackray. Gut Microbial Diversity in Women with Polycystic Ovary Syndrome correlates with Hyperandrogenism. J Clin Endo Metab 2018, Epub ahead of print, DOI: 10.1210/jc.2017-02153.

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The mother – microbiome – child interplay during pregnancy

Summary

Christine Moissl-Eichinger, Department of Internal Medicine, Medical University of Graz

Supervisor:	Prof. Dr. Christine Moissl-Eichinger
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: The human body carries billions of microorganisms that influence health and well-being. Dysbioses (i.e. an imbalance of the human microbial community) has been linked with many disorders, including inflammatory bowel diseases, diabetes, but also mental problems. In my group, we are interested in the interaction of the human microbiome and the host itself and how the microbes mirror the physiological constitution of the body. We specifically use OMICS methods and next generation sequencing, in combination with visualization methods and cultivation in order to identify, characterize and finally understand the microorganisms involved in interactive processes.

Background: Over the course of a normal, healthy pregnancy, the body undergoes substantial hormonal, immunological, and metabolic changes (1,2). Since the human microbiome and the human body are interacting strongly with each other, such changes usually go along with changes in the microbial community (composition and abundances of certain members). For instance, it has been reported, that the bacterial load is increased during the course of gestation (3), or that the microbial composition reflects a “metabolic-syndrome like” status of the pregnant woman (4). Also the placenta harbors a unique and spatially distinct microbiome, representing the first platform that presents bacterial components to the unborn (5). However, the presence of living microorganisms in the placenta has been contrarily discussed and requires further studies.

Overall, studies focusing on the development of the microbiome during pregnancy are comparatively rare; this is particularly true for pregnancy disorders. In addition, many studies are based on standard techniques only, e.g. analyzing the entire microbial community without being able to distinguish between the active (living) and dead microbial community. Thus, the results obtained are often misleading and “blurred” by the background. Furthermore, the microbial community of the human body is composed of Bacteria, Archaea and Fungi. However, Archaea and Fungi are often neglected to be important members of the human microbiome, since they are difficult to detect, and thus require different methods and set-ups of the laboratory procedures.

Hypothesis:

a) The human placenta and amniotic fluid contains a distinct microbial community, which is alive and active. Biomarkers of this microbial community are detectable in circulating blood of highly-pregnant women (6).

b) Mother’s oral, urinary, vaginal and gut microbiome mirrors her health status, and allows predicting future medical issues, such as pre-term labor or pre-eclampsia.

Experimental approaches: We will use different methods in state-of-the-art microbiome research (metagnomics, metatranscriptomics, metabolomics), including 16S rRNA gene-based next generation sequencing, propidium monoazide treatment of the samples, fluorescence *in situ* hybridization to visualize the microorganisms. Data retrieved will be correlated with clinical information derived from the patients. Biomarkers for clinical conditions will be identified.

Collaborations within DP-iDP:

- C. Wadsack will provide clinically well-defined placenta samples and amniotic fluid.
- M. v. Poppel will support the students to link epidemiological aspects to microbiome data.
- B. Huppertz will support the students in creating a pregnancy- related microbiome culture and tissue-collection for subsequent microbiome analysis.
- Karoline Mayer-Pickel

Know-how and infrastructure of the research group: The laboratory of Christine Moissl-Eichinger has a long-standing expertise in the field of microbiology and microbiome research.

The group comprises 2 post-doctoral fellows, one technician and four PhD students. Various techniques are being used for a detailed analysis of the full microbiome, including Bacteria, Archaea and Fungi. The group is located at the Center for Medical Research, ZMF, at the Medical University, where numerous, state-of-the-art equipment is made available to the researchers. This includes: Mi-Seq Illumina sequencing facility, qPCR facility, flow cytometry, fluorescence microscopy, as well as an anaerobic work station for analyzing and cultivation of strictly anaerobic microorganisms. In addition, ZMF provides excellent support in bioinformatics and biostatistics and has established a Galaxy-based pipeline for the processing of complex NGS data.

References:

1. Mor, Gil, and Ingrid Cardenas. "Review article: the immune system in pregnancy: a unique complexity." *American journal of reproductive immunology* 63.6 (2010): 425-433.
2. Newbern, Dorothee, and Michael Freemark. "Placental hormones and the control of maternal metabolism and fetal growth." *Current Opinion in Endocrinology, Diabetes and Obesity* 18.6 (2011): 409-416.
3. Collado, Maria Carmen, et al. "Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women." *The American journal of clinical nutrition* 88.4 (2008): 894-899.
4. Koren, Omry, et al. "Host remodeling of the gut microbiome and metabolic changes during pregnancy." *Cell* 150.3 (2012): 470-480.
5. Aagaard, Kjersti, et al. "The placenta harbors a unique microbiome." *Science translational medicine* 6.237 (2014): 237ra65-237ra65.
6. Kowarsky, Mark, et al. "Numerous uncharacterized and highly divergent microbes which colonize humans are revealed by circulating cell-free DNA." *Proceedings of the National Academy of Sciences* (2017): 201707009.

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Effect of hydroxychloroquin on human placental function in severe preeclampsia

Summary

Karoline Mayer-Pickel, Department of Obstetrics and Gynecology, Medical University of Graz

Supervisor:	Prof. Dr. Karoline Mayer-Pickel
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: Preeclampsia is a pregnancy-specific multiorgan disorder, complicating 3-5 % of all pregnancies. Despite advances in feto-maternal management, preeclampsia is still a major cause of maternal and neonatal morbidity and mortality worldwide, especially in developing countries (1). Preeclampsia is associated with serious and especially long-term maternal and neonatal complications: for the child due to (iatrogenic) preterm delivery as well as intrauterine growth restriction (IUGR) and for the mother an increased risk of cardiovascular morbidity and mortality (2).

The aim of this project will be to investigate novel strategies for prevention and treatment of preeclampsia, especially anti-inflammatory therapy.

Background: Preeclampsia has been proposed being a two-step disease. The first step, starting at early gestation is characterized by an abnormal trophoblastic invasion with an impaired uterine spiral arteries remodeling leading to placental oxidative stress and hypoxic injury (3,4); additionally, systemic and excessive inflammatory processes lead to an abnormal Th1/Th2 cytokine balance with an increased secretion of pro-inflammatory cytokines leading to an angiogenic imbalance with an increase of anti-angiogenic factors such as soluble fms-like tyrosine kinase 1(sFlt-1) and Endoglin and a decrease of pro-angiogenic factors such as placental growth (5-7).

Autophagy, an important cellular process, which regulates the degradation of mostly dysfunctional cellular components in a lysosome-dependent manner (8) has also been described in the pathophysiology of preeclampsia (9-11). Excessive autophagy might promote cell dysfunction through excessive degradation of essential cellular components, characterized by an increase of autophagy markers such as LC3 and Beclin-1, thus leading to preeclampsia 12, 13)

One of the main problems in pregnancies complicated by preeclampsia, especially at early gestation is the lack of effective, especially causative therapeutic options.

Several attempts such as supplements and medications have unfortunately not reached its expectations for prevention of preeclampsia. Only low-dose aspirin (LDA) was found to have a modest benefit in reducing the rate of preeclampsia (14,15).

Hypothesis: Hydroxychloroquine (HCQ) has been used as treatment for several autoimmune diseases such as systemic lupus erythematosus (SLE) and Antiphospholipid Syndrome (APS). The mechanism of action is thought to be via interference with lysosomal activity, inhibition of antigen presentation in dendritic cells, cytokine production in macrophages, and calcium and Toll-like receptor (TLR) signaling in B, T and other immune cells (16,17). Additionally, HCQ is known as an autophagy inhibitor that impairs autophagic flux by blocking autophagic degradation (18).

HCQ has many antithrombotic effects, such as a reduction of blood viscosity and platelet aggregation, as well as anti-inflammatory effects including reduction of pro-inflammatory cytokines, effects on T-cells and neutrophils and a reduction in immune complexes (19). It has also been postulated that HCQ has immune-modulatory functions with complement pathway inactivation, TNF-alpha blocking, improvement of Th1/Th2 balance, inhibition of TLR and reduction of tissue factor (TF) (20-23).

The purpose of this project will be to investigate the effect of HCQ on first trimester primary trophoblast cells as well as on primary trophoblast cells obtained from term placentas, thus reveal a possible role of HCQ in the prevention and treatment of preeclampsia.

Experimental approaches: To test this hypothesis, we will examine if HCQ reduces sFlt-1-secretion and increases PlGF-secretion in first trimester primary trophoblast cells (7-12 weeks of gestation) as well as in primary trophoblast cells obtained from term placentas via ELISA analysis. We will further assess levels of IL-17 A and F as pro-inflammatory cytokines as well as the immunosuppressive cytokine TGF-ß via ELISA analysis. The next step will be to assess autophagic activity by evaluation of autophagy markers LC3B and Beclin-1 by immunohistochemistry.

Collaborations within DP-iDP:

- Christian Wadsack will provide expertise in the area of cell isolation laboratory techniques
- Gernot Desoye will assist the students in detailed analysis of endothelial cell function such as proliferation/cell cycle assays and train students in the isolation of placental endothelial cells and will provide primary trophoblasts isolated from human first trimester
- Akos Heinemann will train the students in isolating leukocytes from cord blood and assays of leukocyte-endothelial interaction, such as adhesion under flow and transendothelial migration
- Martin Gauster will train the students in immunohistochemical analysis
- Berthold Huppertz will train the students how to perform explant cultures of placental villi under different oxygen conditions
- Dagmar Kratky will train the students in autophagy assays

Know-how and infrastructure of the research group: The laboratory has a long-standing expertise in the field of the placenta. Studies are routinely carried out with human primary cells isolated from placental trophoblast and placental explants. The present thesis will capitalize on the large amount of placental tissue from the first trimester of pregnancy as well as term placentas from the delivery room/obstetric department in combination with the clinical expertise.

As one of Austria's largest obstetric tertiary care centers, the Department of Obstetrics at the Medical University of Graz has a broad expertise for many years in the management of high risk pregnancies, such as pre-eclampsia. The management contains of an extensive information about the disease and its propable complications during pregancy at early gestation as well a follow-up every 2 to 4 weeks during pregnancy in order to prevent, diagnose and treat certain complications such as pre-eclampsia.

References:

1. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013; 122:1122-31.
2. Epstein FH. Late vascular effects of toxemia of pregnancy. N Engl J Med 1964; 271:391-95
3. Young BC, Levine RJ, Karumanchi SA. Pathogenesis of preeclampsia. Annu Rev Pathol.2010; 5:173-92
4. Costantine M, Annath C. The early developments of preeclampsia drugs. Expert Opinion on Investigational drugs 2016-10-29
5. Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG. Synthesis of T helper 2-type catokines at the maternal-fetal interface.
6. De Groot CJ, van der Mast BJ, Visser W, de Kuiper P, Weimar W, van Besouw NM. Preeclampsia is asscociatedwith increased cytotoxic T-cell capacity to paternal antigens. Am J Obstet Gynecol 2010;203: e491-6
7. Rolfo A, Giuffrida D, Nuzzo AM, Pierobon D, Cardaropoli S, Piccoli E, et al. Pro-inflammatory profile of preeclamptic placental mesenchymal stromal cells: new insights into the etiopathogenesis of preeclampsia. PLoS One 2013;8: e59403
8. Lin NY, Beyer C, Giessl A, Kireva T, Scholtysek C, Uderhardt S, et al. Autophagy regulates TNFalpha-mediated joint destruction in experimental arthritis. Ann Rheum Dis 2013; 72:761-768
9. Chen B, Longtine MS, Nelson DM. Hypoxia induces autophagy in primary human trophoblasts. Endocrinology 2012; 153:4946-4954
10. Saito S, Nakashima A. Review: the role of autophagy in extravillous trophoblast function under hypoxia. Placenta 2013;34(suppl): S79-S84
11. Signorelli A, Avagliano L, Virgili E, Gagliostro V, Doi P, Braidotti P, et al. Autophagy in normal term placentas. Placenta 2011; 32:482-485
12. Choi J, Jo M, Lee E, Oh YK, Choi D. The role of autophagy in human endometrium. Biol Reprod 2012; 86:70; p. 1-10
13. Hutabarat M, Wibowo N, Huppertz B. The trophoblast survival capacity in preeclampsia. PLoS One 2017; 6:12, e0186909

14. Barton JR, Sibai B. Prediction and prevention of recurrent preeclampsia. Obstet Gynecol.112: 359
15. Askie LM, Duley L, Henderson-Smart DJ, Stewart LA; PARIS Collaborative Group. Antiplatelet agents for prevention of preeclampsia: a meta-analysis of individual patient date. Lancet 2007; 369:1791-1798
16. Lafyatis R, York M, Marshak-Rothstein A. Antimalarial agents: closing the gate on Toll-like receptors? Arthritis Rheum. 2006 ;54(10):3068-70
17. Al Bari MA. Chloroquine analogues in drug discovery: new directions of uses, mechanisms of actions and toxic manifestations from malaria to multifarious diseases. J Antimicrob Chemother. 2015;70(6):1608-21
18. Mizushima N, Yoshimori T, Levine B. Methods in mammalian autophagy research. Cell. 2010 Feb 5;140(3):313-26
19. Petri M. Use of hydroxychloroquine to prevent thrombosis in systemic lupus erythematodes and in antiphospholipid antibody-postive patients. Curr Rheumatol Rep 2011; 13:77-80
20. Kaplan YC, Ozsarfati J, Nickel C, Koren G. Reproductive outcomes following hydroxychloroquine use for autoimmune diseases: a systematic review and meta-analysis. Br J Clin Pharmacol 2016; 81:835-48
21. Meroni PL. Prevention & treatment of obstetrical complications in APS: Is hydroxychloroquine the Holy Grail we are looking for? J Autoimmunity 2016
22. Mekinian A, Lazzaroni MG, Kuzenko A, Alijotas-Reig J, Ruffatti A, Levy P, Canti V, Bremme K, Bezanahary H, Bertero T, Dhote R, Maurier F, Andreoli L, Benbara A, Tigazin A, Carbillon L, Nicaise-Roland P, Tincani A, Fain O. The efficacy of hydroxychloroquine for obstetrical outcome in anti-phospholipid syndrome: Data from a European multicenter retrospective study. Autoimmun Rev. 2015; 14:498-502
23. Sciascia S, Hunt BJ, Talavera-Garcia E, Lliso G, Khamashta MA, Cuadrado MJ. The impact of hydroxychloroquine treatment on pregnancy outcome in women with antiphospholipid antibodies. Am J Obstet Gynecol. 2016 ;214: 273.e1-8

Inflammation and fetal high-density lipoprotein composition, metabolism and function

Summary

Gunther Marsche, Otto Loewi Research Center, Pharmacology, Medical University of Graz

Supervisor:	PD Dr. Gunther Marsche
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: A major research interest of our laboratory is to understand the role of inflammation and oxidant stress in lipoprotein metabolism, with a focus on high-density lipoproteins (HDL). HDL is conserved and present in most species, suggesting an important biological role from an evolutionary standpoint. We assess the proteome (composition) and measure several metrics of function of HDL (and other plasma proteins of interest). We try to understand the molecular basis underlying the apparent loss of protective effects of HDL during inflammation. The overarching goal is to use this understanding to design new therapies aimed at enhancing the protective effects of HDL against human disease.

Background: HDL represents the major cholesterol carrying lipoprotein class in human cord blood, while in maternal serum cholesterol is mainly carried by low-density lipoproteins. HDL promotes numerous beneficial effects on the vascular system, including attenuation of the inflammatory response in the vascular endothelium and immune cells and stimulation of endothelial nitric oxide production In addition, HDL takes part in the regulation of the proliferation of haematopoietic stem cells from the bone marrow (1). Previous findings suggested that HDL isolated from cord blood differs from maternally derived HDL with respect to its proteomic composition, size and function (2).

Objectives and Hypothesis: Our preliminary data suggest that GD induces intense oxidation of fetal HDL. Interestingly, effects on maternal HDL were only minimal. Therefore, our results suggest that oxidatively modified HDL accumulates during fetal development and might affect fetal immune cells and the fetal vascular endothelium. The following aims should be addressed by the Ph.D student: (i) isolation of HDL from maternal and fetal serum (control and gestational diabetes (GD)), (ii) assessing compositional alterations (proteome and lipidome) and oxidative modifications of isolated HDL and (iii) to determine the impact of GD induced modifications on metrics of HDL function, including HDL cholesterol efflux properties, HDL endothelial regenerative activities (using primary placental endothelial cells), HDL anti-oxidative and paraoxonase activity, HDL mediated anti-inflammatory activities and several enzyme activities involved in HDL maturation and metabolism.

Experimental approaches: The PhD student will learn how to isolate lipoproteins, culture human placental endothelial cells, perform cholesterol efflux studies and perform leukocyte adhesion assays under flow conditions. Functional responses of endothelial cells will be investigated in apoptosis and proliferation assays, eNOS activity assays, multiplex cytokine ELISA, and endothelial barrier function tests. The PhD candidate will assess the impact of isolated fetal and maternal HDL (from controls and patients) on the synthesis, bioavailability of endothelium-derived vasorelaxing factors like nitric oxide and prostacyclin and on immune cell function.

Collaborations within DP-iDP:

- Karoline Mayer-Pickel: will provide maternal and fetal serum
- Gernot Desoye: will assist the students in detailed analysis of endothelial cell function such as proliferation/cell cycle assays and train students in the isolation of placental endothelial cells
- Christian Wadsack: will train the students in placental perfusion and isolation of fetal HDL
- Martin Gauster will train the students in immunhistochemical analysis
- Akos Heinemann will train the students in isolating leukocytes from cord blood and assays of leukocyte-endothelial interaction, such as adhesion under flow and transendothelial migration.

Know-how and infrastructure of the research group: The laboratory has a long-standing expertise in the field of lipoprotein research. Our laboratory uses a wide range of techniques spanning biochemistry, molecular biology, cell culture and biology, flow cytometry and mass spectrometry. The research group consists of two postdoc researcher, two PhD students and technical support staff. The Institute of Experimental and Clinical Pharmacology is equipped with the required infrastructure needed to execute the proposed investigations. This includes a cell culture laboratory, isotope facilities and flow cytometry.

References:

1. Yvan-Charvet L, Pagler T, Gautier EL, Avagyan S, Siry RL, Han S, et al. ATP-binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. Science. 2010 Jun 25;328(5986):1689-93.
2. Sreckovic I, Birner-Gruenberger R, Obrist B, Stojakovic T, Scharnagl H, Holzer M, Scholler M, Philipose S, Marsche G, Lang U, Desoye G, Wadsack C. Distinct composition of human fetal HDL attenuates its anti-oxidative capacity. Biochim Biophys Acta. 2013 Apr;1831(4):737-46. doi: 10.1016/j.bbalip.2012.12.015.

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15-deoxy-Δ12,14-PGJ2 (15d-PGJ2): a plausible therapeutic agent against osteosarcoma?

Summary

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

Supervisor:	Prof. Dr. Ernst Malle
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: Osteosarcoma (OS), a high-grade malignant bone tumour, is the most frequent malignant bone tumour found in children and adolescents. Despite of advancement in treatment modalities, the clinical outcome of OS remains poor. Thus, discovery of alternative strategies and development of novel therapeutic approaches are required. Prostaglandins (PGs) are pivotal modulators in bone patho(physiology) and tumourigenesis that activate various cellular signalling depending on the respective PG type. PGD_2 is highly unstable *in vivo*/*in vitro* and gets converted to the stable metabolite, 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) that exerts a panel of different biological activities due to its electrophilic character. In various cellular systems, including OS, 15d-PGJ_2 elevates generation of reactive oxygen species (ROS). Depending on the duration and magnitude of oxidative stress, cytoprotective or cytotoxic cellular responses may occur. However, upregulation of antioxidant levels in adaption to oxidative stress may confer OS cell resistance against therapeutic regime. Furthermore, human OS display a variety of genetic alterations where mutations of tumour-suppressor genes (e.g. p53 and Retinoblastoma [Rb]) are frequent. Therefore, cytotoxic and cytoprotective signalling events in OS cells against 15d-PGJ_2 will be investigated in three human OS cell lines with different genetic background. Primary OS cells will be used in parallel. Selective molecular targets will enter Chicken chorioallantoic membrane (CAM) assay. The major aim is to evaluate the anti-tumour activity of 15d-PGJ_2 in OS and plausible strategies to enhance its efficacy via impairment of cell defence system and/or acceleration of cell death signalling in vitro and in vivo.

Hypothesis and Objectives:

1. To investigate the cytotoxic signalling induced by 15d-PGJ_2 in MG-63 (deficient of tumour suppressor protein p53), U2OS (expressing intact p53 and Rb), and SaOS-2 OS cells (deficient of p53 and Rb). We will explore the involvement of ROS, Rb, E2F1, ATM, p53, PTEN and p21 to regulate cell growth arrest, metastasis and apoptosis. Selective experiments will be performed in primary OS cells.
2. To study the cytoprotective mechanisms (activation of MAPKs, PTEN-PI3K/Akt, Nrf2, Egr1, HO-1 and GCLc/m) against 15d-PGJ_2-induced cell death in MG-63, U2OS, and SaOS-2 cells. Selective experiments will be performed in primary OS cells.
3. To clarify the plausible involvement of candidate receptors (PPARg, DP1 and DP2) in 15d-PGJ_2-mediated cytotoxic and/or cytoprotective signalling in MG-63, U2OS, and SaOS-2 cells. To estimate 15d-PGJ_2 and PGD_2 levels in the microenvironment of human OS tissues with different aggressive behaviour as well as angiogenesis. Selective experiments will be performed in primary OS cells.
4. Selected molecular targets will be analysed for angiogenesis using CAM assay.

References:

1. A. Kovacevic, A. Hammer, E. Stadelmeyer, W. Windischhofer, M. Sundl, A. Ray, N. Schweighofer, G. Friedl, R. Windhager, W. Sattler, **E. Malle**, Expression of serum amyloid A transcripts in human bone tissues, differentiated osteoblast-like stem cells and human osteosarcoma cell lines, J Cell Biochem 2008 (103) 994-1004.

2. K. Kitz, W. Windischhofer, H.J. Leis, E. Huber, M. Kollroser, **E. Malle**, 15-Deoxy-Delta12,14-prostaglandin J2 induces Cox-2 expression in human osteosarcoma cells through MAPK and EGFR activation involving reactive oxygen species, Free Radic Biol Med 2011 (50) 854-865.
3. W. Windischhofer, E. Huber, C. Rossmann, M. Semlitsch, K. Kitz, A. Rauh, T. Devaney, H.J. Leis, **E. Malle**, LPA-induced suppression of periostin in human osteosarcoma cells is mediated by the LPA(1)/Egr-1 axis, Biochimie 2012 (94) 1997-2005.
4. C.N. Koyani, W. Windischhofer, C. Rossmann, G. Jin, S. Kickmaier, F.R. Heinzel, K. Groschner, A. Alavian-Ghavanini, W. Sattler, **E. Malle**, 15-deoxy-Delta(1)(2),(1)(4)-PGJ(2) promotes inflammation and apoptosis in cardiomyocytes via the DP2/MAPK/TNFalpha axis, Int J Cardiol 2014 (173) 472-480.
5. C.N. Koyani, K. Kitz, C. Rossmann, E. Bernhart, E. Huber, C. Trummer, W. Windischhofer, W. Sattler, **E. Malle**, Activation of the MAPK/Akt/Nrf2-Egr1/HO-1-GCLc axis protects MG-63 osteosarcoma cells against 15d-PGJ2-mediated cell death, Biochem Pharmacol 104 (2016) 29-41.

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Role of intracellular lipid hydrolases in lipid and energy metabolism in murine placenta and fetus

Summary

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

Supervisor:	Prof. Dr. Dagmar Kratky
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: The main subject studied in D. Kratky's group is the role of lipases with respect to their function and the underlying signaling pathways. Over the last years, the research group concentrated its effort on elucidating the role of metabolic lipases in various cells and tissues and the consequences of their deficiency on atherosclerosis development. Her laboratory utilizes transgenic and knockout mouse models with loss or overexpression of lipases to investigate the impact of the respective enzymes on systemic and cell-autonomous lipid and energy metabolism in cells/organs.

Background: Fatty acids are the most efficient substrates for energy production and are essential components of lipids that form biological membranes. Especially ω-3 long-chain polyunsaturated fatty acids are vital for the fetus as membrane lipids of the developing brain and central nervous system. The release of fatty acids from intracellular lipid stores requires their enzymatic hydrolysis by a process called lipolysis (1). Cytoplasmic lipases hydrolyze lipid droplet-associated triacylglycerols (TG) at pH~7 by the consecutive action of adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL) (neutral lipolysis). By contrast, lipoprotein-associated TG are transported to the lysosome, where they undergo acid lipolysis by the action of lysosomal acid lipase (LAL) at pH~4.5.

Hypothesis: The role of lipases in lipid metabolism and inflammation in the placenta and the fetus are incompletely understood. We have previously reported on lipase activities in human and murine placenta with extracellular lipases (lipoprotein lipase, endothelial lipase) being involved (2). The role of intracellular lipases in lipid and energy metabolism in placenta and fetus, however, is currently unknown. We hypothesize that loss of intracellular lipid hydrolases (including the monoglyceride hydrolase ABHD6) affects lipid and/or energy metabolism in the placenta and the fetus, thereby leading to alterations in cells (e.g. macrophages and endothelial cells) and tissues (e.g. placenta).

Experimental approaches: The DP-iDP student will utilize knockout mouse models with loss of the above mentioned intracellular lipid hydrolases to investigate the impact of the respective enzymes on lipid and energy metabolism in the placenta and the fetus. The student will perform tracer experiments to elucidate fatty acid and glucose uptake in placenta and fetus of the respective mouse model fed chow and high-fat diet (to trigger type 2 diabetes and inflammation). In addition, he/she will isolate RNA and protein, perform transcriptomics, real time PCR analyses, and Western blotting experiments to determine expression changes in particular pathways, analyze hydrolase activities, and perform lipid uptake and efflux experiments in trophoblasts and endothelial cells. We expect that the results from this study will advance our understanding of how lipolysis and the consequences of metabolic disorders affects the pathogenesis of inflammatory pregnancy-related diseases.

Collaborations within DP-iDP:

- G. Desoye: detailed analysis of endothelial cell function (e.g. proliferation/cell cycle assays)
- C.#Wadsack: strong interaction and discussions on the role of MGL and ABHD6 in murine and human placentae
- M.#Gauster: immunohistochemical analysis of placental tissue
- Á.#Heinemann: leukocyte–endothelial cell interaction; effects of cytokines and eicosanoids on cellular function
- G.#Marsche: (patho)physiology of lipoproteins
- M.#van#Poppel: biostatistics

Know-how and infrastructure of the research group: Dagmar Kratky is a biochemist and molecular biologist at the Gottfried Schatz Research Center for Cell Signaling, Metabolism, and Aging. Since 2006, she has been leading her own group, which currently comprises 1 senior scientist, 3 postdoctoral fellows, and 3 PhD students. Two technicians in the group with permanent positions are financed by the MedUni Graz. Dagmar Kratky coordinates the BioTechMed-Graz funded flagship project “Lipases and Lipid Signaling” (http://www.lipidsignalinggroup.at/index.html) and is Deputy Speaker of the international Ph.D. program “DK Metabolic and Cardiovascular Disease (DK-MCD” (http://www.medunigraz.at/DK_MCD/index.htm). Equipment and methodologies are available for lipid and lipoprotein analysis, real-time PCR, Western blotting experiments, and on-line metabolic cages for 12 mice (TSE PhenoMaster) including a temperature-controlled housing. The local infrastructure includes cell culture facilities, an isotope laboratory, fluorescent and deconvolution microscopes, GC-MS, FPLC, and HPLC systems, a proteomics platform, and an XF24 Extracellular Flux Analyzer (Seahorse) for simultaneous measurements of oxygen consumption and extracellular acidification rates in cells. The animal facility houses more than 20 different mouse models investigated in the group, including those relevant for the proposed experiments.

References:

1. Zechner R, Madeo F, Kratky D. Cytosolic lipolysis and lipophagy: two sides of the same coin. *Nat Rev Mol Cell Biol*. 2017; 18:671-84.
2. Lindegaard ML, Olivecrona G, Christoffersen C, Kratky D, Hannibal J, Petersen BL, Zechner R, Damm P, Nielsen LB. Endothelial and lipoprotein lipases in human and mouse placenta. *J Lipid Res*. 2005; 46:2339-46.

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Diagnostic and prognostic value of CSF and serum neurofilaments in neurological disorders

Summary

Michael Khalil, Department of Neurology, Medical University of Graz

Supervisor:	Prof. Dr. Michael Khalil
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: Neuronal degeneration is the key pathological substrate of sustained disability in various acute and chronic neurological disorders. To ensure optimal patient management it would be of great importance to reliably detect and follow neurodegenerative processes for diagnostic and prognostic purposes. In this respect several approaches have been followed so far; including analysis form cerebrospinal fluid (CSF) based proteins and magnetic resonance imaging (MRI) with varying insights and limitations. More recently, neurofilament (Nf) proteins have gained increasing attention, because abnormal high levels can be detected following neuroaxonal damage. However, up to now such measurements were solely possible in CSF, because available assays were not sensitive enough to detect the generally lower concentrations of this protein in blood samples. This has now changed with the introduction of more sensitive electrochemiluminescence (ECL) assay and ultrasensitive single molecule array (Simoa) technology. These blood based assays now facilitate investigating this marker in a broad range of neurological disorders, including stroke, dementia, multiple sclerosis, traumatic brain injury, amyotrophic lateral sclerosis and others.

Hypothesis and Objectives: Neurofilaments belong to the intermediate filament family of proteins and are the major components of the cytoskeleton of neurons. They consist of 3 isotypes: a neurofilament light (NfL) chain of 68 kDa, a neurofilament intermediate (NfM) chain of 150 kDa, and a neurofilament heavy (NfH) chain of 190 to 210 kDa. During the process of axonal injury, intracellular components, including neurofilaments, are released into the extracellular fluid and subsequently into the CSF and to a lower extent also to the peripheral blood. Ultrasensitive Simoa technology is currently the most sensitive assay available to measure serum NfL levels. In this PhD project we aim to exploit this new technology to determine the extent of axonal damage in different neurological disorders focusing on multiple sclerosis, stroke and dementia. Diagnostic and prognostic value of Nf will be analysed together with detailed MRI and clinical data.

Methodology: The PhD student will focus on investigating Nf proteins in serum and if available also in CSF of patients with multiple sclerosis, stroke and dementia. The student will learn to use the ultrasensitive Simoa platform for serum NfL measurements. Serum NfH is not yet available on the Simoa platform, the student will therefore try to transfer an in-house Luminex NfH assay onto the Simoa platform for assay development. CSF Nf levels will be measured by ELISA.

The student will further learn to perform diagnostic 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, determination of oligoclonal bands by isoelectric focusing followed by immunoblotting, as well as isolation of DNA and peripheral blood mononuclear cells.

The student will also learn to handle larger clinical data sets and merge them with biochemical and MRI data prior to statistical analyses. Basic scientific MRI analysis, including determination of normalized brain volumes and lesion volume will also be part of this project.

References:

1. Barro C, et al. Fluid biomarker and electrophysiological outcome measures for progressive MS trials. Mult Scler. 2017 Oct;23(12):1600-1613. Doi: 10.1177/1352458517732844.

2. De Marchis GM, et al. Serum neurofilament light chain in patients with acute cerebrovascular events. Eur J Neurol. 2017 Dec 27. doi: 10.1111/ene.13554.
3. Disanto G, et al. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol. 2017 Jun;81(6):857-870. doi: 10.1002/ana.24954.
4. Gattringer T, et al. Serum neurofilament light is sensitive to active cerebral small vessel disease. Neurology. 2017 Nov 14;89(20):2108-2114. doi: 10.1212/WNL.0000000000004645.
5. Khalil M, et al. CSF neurofilament and N-acetylaspartate related brain changes in clinically isolated syndrome. Mult Scler. 2013 Apr;19(4):436-42. doi: 10.1177/1352458512458010.
6. Khalil M, Salzer J. CSF neurofilament light: A universal risk biomarker in multiple sclerosis? Neurology. 2016 Sep 13;87(11):1068-9. doi: 10.1212/WNL.0000000000003107.
7. Meeter LH, et al. Neurofilament light chain: a biomarker for genetic frontotemporal dementia. Ann Clin Transl Neurol. 2016 Jul 1;3(8):623-36. doi: 10.1002/acn3.325.
8. Novakova L, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology. 2017 Nov 28;89(22):2230-2237. doi: 10.1212/WNL.0000000000004683.
9. Teunissen CE, Khalil M. Neurofilaments as biomarkers in multiple sclerosis. Mult Scler. 2012 May;18(5):552-6. doi: 10.1177/1352458512443092.
10. Weston PSJ, et al. Serum neurofilament light in familial Alzheimer disease: A marker of early neurodegeneration. Neurology. 2017 Nov 21;89(21):2167-2175. doi: 10.1212/WNL.0000000000004667.

Preeclampsia: Aponecrosis of placental trophoblast and release of subcellular fragments into the maternal circulation

Summary

Berthold Huppertz, Gottfried Schatz Research Center, Cell Biology, Histology and Embryology, Medical University of Graz

Supervisor:	Prof. Dr. Berthold Huppertz
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: Placental biology, especially trophoblast biology in healthy and diseased pregnancies. Focus on two major pregnancy pathologies, fetal growth restriction and preeclampsia. Major focus on interactive cell biological aspects.

Background: Preeclampsia is still one of the major pregnancy pathologies and affects 5% of all pregnant women worldwide. This syndrome is associated with elevated blood pressure and proteinuria of the pregnant woman and long-term morbidity of mother and child. The placenta and the release of placental factors is key to the development of preeclampsia. Aponecrotic release of subcellular fragments from the placental trophoblast induces an inflammatory response of the mother and thus preeclampsia. The main focus of this study is to identify the pathways and mechanisms within the placental syncytiotrophoblast that lead to the release of aponecrotic fragments into the maternal circulation and how they interfere with the maternal inflammatory system. Placenta-specific biomarkers (proteins and nucleic acids) are released from the syncytiotrophoblast and can be used as predictors to develop preeclampsia. Their expression patterns will be used as a surrogate for the proper development of the trophoblast. Experiments will be carried out using placental villous explants (tissue cultures), isolated primary cells from the human placenta (first trimester and term) as well as trophoblast-derived cell lines for transfection/silencing experiments. The student will be trained in placental biology and will receive insights in the turnover of placental trophoblast and its effect on maternal endothelial cell function. In addition, the student will get background knowledge on current concepts of the etiologies of preeclampsia and where demands for future research exist.

Hypothesis: Cell biological changes of the placental trophoblast result in aponecrosis and the release of protein/nucleic acid factors that may have a predictive value for preeclampsia.

Experimental approaches:

- Isolation and stratification of placental factors via differential centrifugation
- Sources for isolation: maternal blood, *ex-vivo* placental perfusion, explant cultures of placental villi and isolated trophoblasts (all from healthy and diseased pregnancies)
- Determination of RNA/protein levels of markers in placentas from IUGR and preeclampsia cases and age-matched healthy controls
- Determination of markers (RNA/protein) on the single cell level in the placenta
- Determination of markers in maternal tissues (endothelium, smooth muscle cells, etc.) from pregnant and non-pregnant women, healthy and diseased pregnant women
- Determination of new nucleic acid markers in serum of pregnant women (in cooperation with Prof. Sensen, TU Graz)
- *In vitro* effects of circulating factors on the placental trophoblast (isolated primary cells and villous explants)
- *In vitro* effects of circulating factors on the maternal endothelium (isolated primary cells)

Collaborations within DP-iDP:

- Martin Gauster: Effect of placental factors on maternal platelets (in vitro assays)
- Akos Heinemann: Effect of placental factors on maternal leukocytes (in vitro assays)

- Christian Wadsack: Short-term effects of circulating factors on the placental trophoblast (placental perfusion system) and isolation via differential centrifugation
- Gernot Desoye: Crosstalk of preeclampsia factors with factors of maternal diabetes (in vitro assays)
- Christine Moissl-Eichinger: Relation between the gut microbiome and factors in preeclampsia (microbiome analysis)
- Daniela Ulrich and Karoline Mayer-Pickel: Relation between circulating factors and clinical parameters (analysis of clinical parameters)

Know-how and infrastructure of the research group:

- PI works in the field for more than 20 years (h-index: 50; citations: >9000; Researchgate)
- Fully equipped cell culture, biochemistry, molecular biology and histology labs
- Hypoxic workstation with four chambers, allowing parallel cultures using four different oxygen concentrations
- Time lapse microscopy under adaptable oxygen concentrations
- Laser scanning microscopy
- Stereological workstation with systematic random sampling equipment
- Laser microdissection workstation
- Various fluorescence microscopes
- Transmission and scanning electron microscopes

References:

1. Huppertz B. Placental origins of preeclampsia: challenging the current hypothesis. Hypertens 2008;51:970-5.
2. Huppertz B, Meiri H, Gizurarson S, Osol G, Sammar M. Placental protein 13 (PP13): a new biological target shifting individualized risk assessment to personalized drug design combating pre-eclampsia. Hum Reprod Update 2013;19:391-405.
3. Huppertz B. Maternal-fetal interactions, predictive markers for preeclampsia, and programming. J Reprod Immunol 2015;108:26-32.

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Programming function of fetal endothelial progenitor cells by maternal metabolism

Summary

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

Supervisor:	Prof. Dr. Ursula Hiden
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: Metabolic derangements such as diabetes affect cellular functions, and endothelial dysfunction is a well-known consequence of obesity and diabetes. I am interested in the effect of maternal metabolic derangements in pregnancy, such as gestational diabetes mellitus (GDM), on development and function of fetal and placental endothelium.

Accumulating evidence demonstrates that the intrauterine environment determines fetal development and susceptibility to diseases later in life, a process referred to as programming. For instance, maternal metabolic derangements increase the offspring's risk for metabolic and cardiovascular diseases in later life (1). This has raised interest in the process of vascular development and its programming by adverse maternal environments. Changes in DNA methylation are the main mechanism underlying the permanent effect of programming (2). Elucidating the mechanisms and functional consequences of programming endothelial function *in utero* by maternal environment has become a particular focus of my research.

Background: Endothelial colony forming cells (ECFCs) are circulating endothelial progenitor cells that repair endothelial damage and participate in angiogenesis. Due to major angiogenic and vascular remodelling processes during fetal development and during the perinatal period, ECFCs are highly abundant in cord blood of newborns. We and others have identified functional changes of fetal ECFCs isolated form cord blood, when mothers had GDM (Leopold et al., unpublished, 3). These included reduced proliferation, migration and wound healing. Not only metabolic environment determines endothelial function. Endothelial function also differs between males and females, and, besides hormones, genetics plays a role. Indeed, we have demonstrated sex specific transcriptome in placental endothelial cells (4), and have identified sex dependent functional changes of placental endothelial cells after exposure to maternal GDM (Cvitic et al., unpublished).

This PhD project aims at investigating the role of DNA-methylation and fetal sex in the programming of ECFC function by GDM.

Hypothesis: Intrauterine environment associated with GDM causes sex dependent, functional defects in fetal ECFCs via DNA methylation.

Experimental approaches: ECFCs will be isolated from cord blood after normal and GDM pregnancy. Two approaches will be followed in parallel: On one hand, genome wide methylation analysis (Illumina Infinium Human Methylation450 BeadChips), whole genome gene expression analysis (Affymetrix GeneChip Human 1.0 ST arrays), and subsequent pathway analysis (Ingenuity Pathway Analysis) will identify genes and functional pathways that are programmed by intrauterine environment of GDM in cord blood ECFCs from male vs female newborn. On the other hand, investigating various aspects of ECFC function will determine and identify functional effects of GDM on these cells, and will be related to the pathway analyses. Functional assays will include a panel of assays well established in our lab, such as the spheroid sprouting assay and fibrin assay to measure angiogenesis, electrical impedance sensing and transwell assay to analyze monolayer barrier function. Further functional assays, such as the adhesion of ECFCs to an injured endothelial monolayer *in vitro*, will be established by the student. The role of key genes in these processes, as determined by methylation and gene expression analyses, will be investigated using siRNA silencing.

Collaborations within DP-iDP:

- M. Gauster will train the students in physiology and morphology of the placenta.

- A. Heinemann will support students in adhesion assay.

Know-how and infrastructure of the research group: We are located at the Department of Obstetrics and Gynecology and thus, have direct access to fetal tissues that can be obtained after delivery, such as placenta and cord blood. From these we can isolate different types of endothelial cells, and we have long standing expertise in endothelial cell culture and biology, and the effect of metabolic derangement on them. Moreover, we have established collaborations with experts in genome methylation analysis and bioinformatics.

References:

1. Barker DJ. The origins of the developmental origins theory. *J Intern Med.* 2007;261(5):412-7.
2. Reynolds RM, Jacobsen GH, Drake AJ. What is the evidence in humans that DNA methylation changes link events in utero and later life disease? *Clin Endocrinol (Oxf).* 2013;78(6):814-22.
3. Ingram DA, Lien IZ, Mead LE, Estes M, Prater DN, Derr-Yellin E, DiMeglio LA, Haneline LS. In vitro hyperglycemia or a diabetic intrauterine environment reduces neonatal endothelial colony-forming cell numbers and function. *Diabetes*. 2008;57(3):724-31.
4. Cvitic S, Longtine MS, Hackl H, Wagner K, Nelson MD, Desoye G, Hiden U. The human placental sexome differs between trophoblast epithelium and villous vessel endothelium. PLoS One. 2013; 8(10):e79233.

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Immune mediators in the placenta of hypertensive, diabetic and/or obese women

Summary

Akos Heinemann, Otto Loewi Research Center, Pharmacology, Medical University of Graz

Supervisor:	Prof. Dr. Akos Heinemann
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: Accumulation of leukocytes in tissues is a key feature of inflammation and a major determinant of tissue damage. Pregnancy is an immunological challenge for mother and fetus, and numerous immune cells take part in the development of the decidua. Particularly macrophages are thought to be crucial in maintaining an immune-tolerant environment in the semi-allogeneic setting of trophoblast invasion (1).

The main focus of our laboratory is to define the mechanisms that govern the trafficking of leukocytes from bone marrow, where they are generated, to the inflammatory site and their subsequent activation in tissue, where they may become harmful. Chemoattractants, their receptors, and adhesion molecules both on the leukocytes and the endothelial side, play crucial roles in the multi-step process of leukocyte infiltration by facilitating leukocyte locomotion and activation, and are thus considered as promising therapeutic targets in various inflammatory conditions. Conversely, several endogenous mediators exist that down-regulate the responsiveness of leukocytes and might hence exert potent anti-inflammatory effects. When supplemented pharmaceutically, these mediators might likewise open novel therapeutic avenues. Among others we have elucidated the opposing roles of two cyclooxygenase (COX) products, prostaglandin (PG) E_2 and D_2 in leukocyte trafficking in human and animal models, and have characterized their receptors at the molecular and pharmacological level. While we have shown that its receptor EP4 is a negative regulator of eosinophil and neutrophil trafficking, and endothelial, platelet and macrophage activation (2, 3), we have also revealed a novel role for PGD_2 and its receptors DP1 and DP2 as potent activators of eosinophils, basophils and macrophages (4, 5).

Background: It has been suggested previously that PGE_2 and PGD_2 might play distinct roles in pathological conditions of pregnancy, but how these prostaglandins contribute to the regulation of leukocyte function in the developing decidua and, even more, how responses to PGE_2 and PGD_2 of immune cells in the placenta are altered in different gestational pathologies has not been addressed in detail. Currently we are elucidating the expression patterns and levels of enzymes involved in prostaglandin synthesis (COX isoforms, PGE and PGD synthases) and receptors for PGE_2 and PGD_2 in placental tissue.

Hypothesis: We hypothesize that an imbalance of anti-inflammatory PGE_2 effects and pro-inflammatory PGD_2 actions might contribute to complications in pregnancy, such as hypertension, preeclampsia or preterm labor. We will characterize these alterations on the cellular (e.g. endothelial cells, macrophages, innate lymphoid cells) and tissue level (i.e. placenta). The studies will provide insights in the regulation of leukocyte and endothelial function, in particular trafficking and activation, and how this process impacts on inflammation and tissue damage in the placenta.

Experimental approaches: The used methodologies comprise isolation and culturing of cells from fetal and maternal blood and placental tissues, characterization of cells by flow cytometry, quantitation and modulation of the biosynthesis of mediators and signaling molecules with RNA techniques including siRNA gene knock-down, immunoprecipitation and Western blot, and laser-scanning microscopy. The student will also be trained to characterize and quantitate specific lipid subclasses by mass spectroscopy (GC-MS, LC-MS).

Collaborations within DP-iDP:

- G. Desoye will teach the student how to isolate endothelial cells and macrophages from the placenta and/or umbilical cord, introduce them to the biology of angiogenesis and help with assays of 2-D network formation and tube formation.
- G. Marsche will supervise the studies addressing lipid metabolism of placental macrophages.

- C. Wadsack will provide clinically well-defined placenta samples and help the student with ex vivo placenta perfusion assays.
- In studies conducted by M. van Poppel the students will learn how to measure and statistically analyze cytokine plasma profiles in normal and pathological pregnancies.

Know-how and infrastructure of the research group: The laboratory of Akos Heinemann has a long-standing expertise in the field of leukocyte biology and pharmacology, but also in hemodynamic regulation and vascular biology. Studies are routinely carried out both with primary cells isolated from humans and cells from animal sources, complemented with cell lines for transfection/silencing experiments. The group comprises two post-doctoral fellows, three technicians and five PhD students. Various techniques are being used for a detailed analysis of leukocyte and endothelial cell function, and the group has considerable experience with *in vivo* models of leukocyte trafficking and inflammation. All the required equipment is available at the institute, including animal and cell culture facilities, radionuclide laboratory, flow cytometry, real-time PCR systems, fluorescence plate reader, tissue processing and fluorescence microscopy, and a microscopy system to study cell-to-cell interaction and thrombus formation under flow conditions. The group has also access to video-tracking of leukocyte locomotion and laser-scanning microscopy at the CMR.

References:

1. Svensson-Arvelund, J; Ernerudh, J. The role of macrophages in promoting and maintaining homeostasis at the fetal-maternal interface. Am J Reprod Immunol. 2015; 74(2): 100-9.
2. Konya, V; Marsche, G; Schuligoi, R; Heinemann, A E-type prostanoid receptor 4 (EP4) in disease and therapy. Pharmacol Ther. 2013; 138(3):485-502
3. Konya, V; Maric, J; Jandl, K; Luschnig, P; Aringer, I; Lanz, I; Platzer, W; Theiler, A; Bärnthaler, T; Frei, R; Marsche, G; Marsh, LM; Olschewski, A; Lippe, IT; Heinemann, A; Schuligoi, R; EP4 receptor prevents acute lung injury in mice by enhancing the microvascular barrier function. Brit J Pharmacol. 2015. 172(18): 4454-4468.
4. Schuligoi, R; Sturm, E; Luschnig, P; Konya, V; Philipose, S; Sedej, M; Waldhoer, M; Peskar, BA; Heinemann, A CRTH2 and D-type prostanoid receptor antagonists as novel therapeutic agents for inflammatory diseases. Pharmacology. 2010; 85(6): 372-382.
5. Jandl, K; Stacher, E; Bálint, Z; Sturm, EM; Maric, J; Peinhaupt, M; Luschnig, P; Aringer, I; Fauland, A; Konya, V; Dahlen, SE; Wheelock, CE; Kratky, D; Olschewski, A; Marsche, G; Schuligoi, R; Heinemann, A. Activated prostaglandin D2 receptors on macrophages enhance neutrophil recruitment into the lung. J Allergy Clin Immunol. 2016; 137(3):833-843

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The molecular organization of mitochondria – plasma membrane interaction and its importance for health and disease

Summary

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

Supervisor:	Prof. Dr. Wolfgang Graier
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: Mitochondrial functions by far exceed that of serving as the cells most powerful energy supplier. During the last years intriguing evidence accumulated that pointed to an essential involvement of mitochondria in cellular signaling, metabolism and multiple cell functions. Notably, these organelles seek to communicate with nearly all cell membranes, including the endoplasmic reticulum, the nuclear envelop, lysosomes, lipid droplets and the plasma membrane. These interactions are often established by specific, transient protein boundaries that physically stabilize the inter-organelle interface and, thus, facilitate the exchange of ions, small molecule substrates, proteins, (phopsho)lipids, and other (signaling) molecules. While the junction between mitochondria and the endoplasmic reticulum got very much attention recently and has been highlighted in terms of its contribution to cancer metabolism[1,2], the interaction of mitochondria with the plasma membrane has been only merely studied so far. However, there are strong functional findings that emphasize the important role of mitochondria-plasma membrane junction in the maintenance of ion channel activity[3] and the activity of cytosolic enzymes[4]. Notably, most of these interactions build on the transfer of Ca^{2+} of which the mitochondrial sequestration is highly regulated and is subject of sophisticated modulatory mechanisms that adapt this function along with the energy demand of the organelle/cell[5,6].

Hypothesis and Objectives: Our data provides evidence for a dynamic interaction between the mitochondria and the plasma membrane, protein bridging and the exchange of Ca^{2+}, substrates and (phospho)lipids. Notably, these transient junctions appear to depend on the cellular energetic status and are highly affected during aging and cancerogenesis, thus, yielding a metabolic switch supporting cancer growth or manifestation of age-related cellular dysfunction. Accordingly we hypothesize that the formations of transient mitochondria-plasma membrane junctions play an important role in cellular homeostasis and the generation of disease or organelle dysfunction. This project will investigate 1st, the molecular basis of mitochondria-plasma membrane interaction, 2nd, actual processes of ion/substrate/protein/lipid transfer, and, 3rd, their contribution and consequences for cell/organelle functions in health, cancerogenesis and aging.

Methodology: The PhD candidate will utilize all kinds of molecular biology techniques (cloning, mutagenesis, real time PCR, CRISPR/Cas9, si/shRNA, morpholinos, etc.), standard biochemistry techniques like Western blotting, and highly sophisticated (super-resolution SIM) fluorescence (FRET, FRAP) microscopy and electrophysiology. The laboratory is specialized for single-cell and sub-cellular analyzes of signaling pathways, ion movements, protein-protein interactions, and metabolism. While most of the experiments will be conducted in isolated and cultured single cells, completing studies will be performed in *Caenorhabditis elegans*.

References:

1. Lovy, A., Foskett, J. K. & Cárdenas, C. InsP3R, the calcium whisperer: Maintaining mitochondrial function in cancer. *Mol Cell Oncol* 3, e1185563 (2016).
2. Madreiter-Sokolowski, C.T. , Gottschalk, B., Parichatikanond, W., Eroglu, E., Klec, C., Waldeck-Weiermair, M., Malli, R. & Graier, W.F. Resveratrol specifically kills cancer cells by a devastating increase in the Ca^{2+} coupling between the greatly tethered endoplasmic reticulum and mitochondria. *Cell Physiol Biochem* 39, 1404–1420 (2016).

3. Malli, R., Frieden, M., Osibow, K. & Graier, W. F. Mitochondria efficiently buffer subplasmalemmal Ca^{2+} elevation during agonist stimulation. *J Biol Chem* 278, 10807–10815 (2003).
4. Charoensin, S., Eroglu, E., Opelt, M., Bischof, H., Madreiter-Sokolowski, C.T., Kirsch, A., Depaoli, M.R., Frank, S., Schrammel, A., Mayer, B., Waldeck-Weiermair, M., Graier, W.F. & Malli, R. Intact mitochondrial Ca^{2+} uniport is essential for agonist-induced activation of endothelial nitric oxide synthase (eNOS). *Free Radic Biol Med* 102, 248–259 (2017).
5. Madreiter-Sokolowski, C. T., Klec, C., Parichatikanond, W., , Stryeck, S., Gottschalk, B., Pulido, S., Rost, R., Eroglu, E., Hofmann, N.A., Bondarenko, A.I., Madl, T., Waldeck-Weiermair, M., Malli, R. & Graier, W.F. PRMT1-mediated methylation of MICU1 determines the UCP2/3 dependency of mitochondrial Ca^{2+} uptake in immortalized cells. *Nat Comms* 7, 12897 (2016).
6. Trenker, M., Malli, R., Fertschai, I., Levak-Frank, S. & Graier, W. F. Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca^{2+} uniport. *Nat. Cell Biol.* 9, 445–452 (2007).

Maternal platelet activation and placental endocrine activity

Summary

Martin Gauster, Cell Biology, Gottfried Schatz Research Center, Histology and Embryology, Medical University of Graz

Supervisor:	PD Dr. Martin Gauster
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests:

- Cytokines and chemokines in placenta (patho-)physiology
- Maternal-fetal cross-talk in early pregnancy
- Trophoblast differentiation

Background: Maternal platelets were shown to adhere to uteroplacental arteries, which underwent remodeling by invaded fetal trophoblast cells [1]. We have recently shown maternal platelets on villous explant cultures from human first trimester placenta, indicating that adherence of maternal platelets to the villous surface is a common process even in very early stages of gestation [2]. Moreover, we detected maternal platelets on trophoblast columns of anchoring placental villi and adjacent lining of the intervillous space. Activation of adhering maternal platelets at openings of uteroplacental arteries and anchoring villi represents an underappreciated source of inflammatory chemokines and cytokines at the maternal-fetal interface. A localized inflammatory insult or an initial mechanical injury to placental villi have been suggested as initial trigger of perivillous fibrinoid deposition [3], which has been shown to be increased in some pregnancy pathologies. Accordingly, our preliminary immunohistochemistry analysis showed abundant platelet staining at the villous surface of placental tissue from early-onset preeclampsia, compared to age-matched control. Thus, it is tempting to speculate that platelet-derived factors released from adhering maternal platelets perfuse the intervillous space and thereby contribute to an inflammatory microenvironment in the placenta. An inflammatory intervillous microenvironment is considered to affect villous trophoblast differentiation and function [4,5]. Our preliminary data from trophoblast and placental explant culture showed significantly decreased expression and release of the pregnancy hormone human chorionic gonadotropin in response to incubation with human platelet lysates, suggesting an adverse effect of platelet-derived factors on trophoblast differentiation and placental endocrine activity.

Hypothesis: On the basis of our preliminary data, the hypothesis will be tested whether platelet activation and subsequent release of platelet-derived factors impair hormone synthesis and secretion from villous trophoblasts.

Experimental approaches: To address this hypothesis, adherence and activation of platelets will be tested on different trophoblast subtypes and cell models using platelet adhesion and degranulation assays. Synthesis and release of pregnancy maintaining hormones (chorionic gonadotropin, placental lactogen, progesterone, and estrogens) as well as other key players in metabolism and blood pressure (leptin, angiotensinogen, endothelin) will be analyzed in trophoblast and placental explant culture in response to platelet-derived factors. Finally, effects of antiplatelet drug therapy, such as combined aspirin and P2Y inhibitor treatment, will be tested in above mentioned models.

Collaborations within DP-iDP:

- Prof. Desoye will provide primary trophoblasts isolated from human first trimester and term placenta
- Prof. Heinemann and Prof. Marsche will assist with platelet adhesion- and aggregation assays

Know-how and infrastructure of the research group: Dr.#Gauster's research laboratory is equipped for standard molecular biological and biochemical protocols such as western blotting, qPCR, DELFIA and ELISA. Moreover, the department provides expert knowledge in cell biological and histological techniques. Dr.#Gauster has access to state-of-the-art microscopes (e.g. inverse fluorescence microscope, confocal laser scanning microscope, electron micro-

scope) and efficient software tools to obtain stereology based quantities. Available cell culture devices exceed basic equipment (e.g. hypoxia workstation and live-cell imaging). Tissue specimens will be fixed and embedded routinely by an in-house paraffin infiltration processor and immunohistochemical staining of tissue sections will be performed by a staining robot (autostainer).

References:

1. Sato Y, Fujiwara H, Zeng BX, Higuchi T, Yoshioka S, Fujii S,. Platelet-derived soluble factors induce human extravillous trophoblast migration and differentiation: platelets are a possible regulator of trophoblast infiltration into maternal spiral arteries. Blood 2005; 106:428-435.
2. Blaschitz A, Siwetz M, Schlenke P, Gauster M,. Adhering maternal platelets can contribute to the cytokine and chemokine cocktail released by human first trimester villous placenta. Placenta 2015; 36:1333-1336.
3. Benirschke K, Kaufmann P, Baergen RN. Pathology of the Human Placenta 2006 pp 42-49. Springer: New York, NY.
4. Gauster M, Moser G, Orendi K, Huppertz B,. Factors involved in regulating trophoblast fusion: potential role in the development of preeclampsia. Placenta 2009; 30 Suppl A:S49-54.
5. Siwetz M, Blaschitz A, El-Heliebi A, Hiden U, Desoye G, Huppertz B, Gauster M,. TNF-alpha alters the inflammatory secretion profile of human first trimester placenta. Lab.Invest. 2016.

Stress-response pathways in human placenta in the first trimester of pregnancy

Summary

Gernot Desoye, Department of Obstetrics and Gynecology, Medical University of Graz

Supervisor:	Prof. Dr. Gernot Desoye
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Research interests: The long-term interest has been to understand the effect of maternal changes (endocrine, metabolic, inflammatory) associated with diabetes mellitus and/or obesity on placental function, and what these may entail for fetal growth and development. In particular we have focused on excessive fetal and neonatal adiposity as main phenotype. Our recent concepts have concentrated on the early pregnancy period, in which trajectories of placental growth and development are established, which track throughout pregnancy. Placental size may then determine early nutrient supply to the fetus and, hence, contribute to fetal overgrowth. Thus, it is imperative to identify pro-inflammatory alterations in the mother and to understand, how these influence first trimester placental growth, which is mostly dictated by growth of the trophoblast, the cell type bathing in maternal blood.

Background: Ongoing work has established that a) the trophoblast response to the intrauterine environment depends on the specific period, i.e. early (weeks 5-8) or late (weeks 9-12), in the first trimester of pregnancy, and b) consequences of the environmental perturbations associated with maternal inflammation lead to changes in trophoblast cell cycle and apoptosis. The stressors and their signaling pathways that are involved, as well as their variation with the pregnancy period in the first trimester remain to be determined and the key determinants of these variations (e.g. oxygen tension, concentrations of metabolites such as palmitate, glucose and cytokines etc) need to be identified.

Hypothesis: Multiple maternal stressors that are influenced by maternal obesity induce trophoblast responses that converge in the activation of the ASK1/2-JNK stress response pathway.

Experimental approaches: The trophoblast layer of first trimester placentas will be micro-dissected, the activation status of the ASK1/2-JNK signaling pathway determined and its dependency on a) gestational age (weeks 5-12) and b) maternal inflammatory state analysed. Concentration measurements of potential stressors in the maternal serum (e.g. TNFα, IL1α/β, IL6, IL8, IL10, glucose, oxidized lipoproteins, sFas-L, palmitate) as well as signaling mediators in the tissue (e.g. TNFR, TLR4, NOX2, Fas, TRAFs) will identify targets for manipulation (pharmacologic inhibitors, siRNA) and subsequent functional studies (cell cycle, apoptosis). DNA-methylation analyses will determine potential alterations in methylation of the targets associated with maternal inflammation to establish candidates that may track throughout pregnancy.

Collaborations within DP-iDP:

- Martin Gauster: Explant studies and immunohistochemistry
- Akos Heinemann: Cytokine measurements by multiplexing
- Gunther Marsche: Quantification of oxidized lipoproteins

Know-how and infrastructure of the research group: The laboratory has a long-standing expertise in the field of placenta under conditions of low-grade inflammation such as diabetes mellitus and maternal overweight and obesity. Studies are routinely carried out with human primary cells isolated from both placental surfaces (trophoblast, endothelial cells, tissue-resident macrophages), a first trimester trophoblast cell line that was recently generated here, as well as with placental explants. The present thesis will capitalize on the large bank of placental tissue from the first trimester of pregnancy (weeks 5-12) combined with maternal serum taken at the time of pregnancy termination. This will allow comprehensive phenotyping of the pregnancies (including placental volume and crown rump length) and identification of confounders and mediators of placental changes by maternal low grade-inflammation.

Various techniques are established for the detailed analysis of placental structure and function with a focus on trophoblast and villus core, such as (semi-)quantitative immunohistochemistry, proliferation/cell cycle/apoptosis assays, signalling pathway analysis and pyrosequencing. For experiments in low oxygen tension a hypoxia work bench is available. All equipment, which is not available at the Department's research laboratory, such as laser capture microdissection, RNASeq and multiplexing are available either at the collaboration partners or at the core facilities of the clinical research center.

References:

1. Nishida T, Hattori K, Watanabe K. The regulatory and signalling mechanisms of the ASK family. Adv Biol Regul. 2017; 66:2-22.
2. Engin A. The Pathogenesis of Obesity-Associated Adipose Tissue Inflammation. Adv Exp Med Biol. 2017; 960:221-245.
3. Fröhlich JD, Huppertz B, Abuja PM, König J, Desoye G. Oxygen modulates the response of first-trimester trophoblasts to hyperglycemia. Am J Pathol. 2012;180:153-64.
4. Gauster M, Majali-Martinez A, Maninger S, Gutschi E, Greimel PH, Ivanisevic M, Djelmis J, Desoye G, Hiden U. Maternal Type 1 diabetes activates stress response in early placenta. Placenta. 2017; 50:110-116.
5. Desoye G, van Poppel M. The feto-placental dialogue and diabesity. Best Pract Res Clin Obstet Gynaecol. 2015;29:15-23.

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FUSION Technology - Computational approaches at the interface of multi-omics, imaging and clinical data for next-generation biomarker discovery

Summary

Marc Brehme & Thomas Pieber, CBmed GmbH

Supervisors:	Prof. Dr. Thomas Pieber Dr. Marc Brehme
Availability:	This position has been occupied.
Offered by:	CBmed
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: In light of the complexity of biological systems, multiple data layers have to be considered through and interdisciplinary approach, data integration and computational modeling in order to better understand the pathophysiological underpinnigs of disease at a systems-level in a personalized, and quantitative way. In its pursuit towards assisting the predictive, preventive, personalized and participatory medicine(P4-medicine) of the 21st century (Flores et al., 2013), CBmed combines cutting-edge multi-omics and imaging Core Lab technologies with clinical patient data towards personalized prognostic and diagnostic disease biomarkers discovery (Rotroff and Motsinger-Reif, 2016). In a show-case, project 2.51, CBmed is establishing a 'FUSION Technology' framework, integrating six Core Lab technologies in a systematic and quality-controlled framework for the analysis of pairs of FFPE tissue and serum samples from retrospective (n=60) and prospective (n=10) colon cancer grade II patient cohorts, split into relapsed vs. relapse-free individuals with the goal to identify predictive relapse risk markers as a rationale for post-operative chemotherapy (CTx), which is hitherto of unclear preventive benefit, while the decision for administration of adjuvant CTx in stage II colon cancer is serious. While survival rates vary significantly due to sporadic patient relapse (Meyerhardt and Mayer, 2005), adjuvant CTx of all patients represents an "overtreatment" with unnecessary exposure to toxicities and reduced quality of life opposed to barely measurable benefit (Benson et al., 2004; Lewis et al., 2016), urging the need for patient-specific biomarkers.

Hypothesis and Objectives: Continuing and building on available data and IT-infrastructure set forth in FUSION Technology show-case pilot project 2.51, this PhD opportunity will be integral to the secondgeneration project 2.52, which will be at the core of CBmed's interdisciplinary multi-omics, imaging and clinical data-driven biomarker discovery, with multiple intersection points to CBmed research activities in cancer, metabolism, immunity, and inflammation to neurodegenerative diseases. The hypothesis governing this approach is that the discovery, validation, and clinical translation of robust and patient-specific clinical biomarkers in complex diseases requires and benefits from the consideration and understanding of the complexity of the human biological system (Nielsen, 2012). This is tackled at CBmed by state-of-the-art omics technologies such as MALDI-TOF proteomics, LC-MS metabolomics or next-generation sequencing (NGS/RNA-seq) based variant analysis or transcriptome profiling, in combination with quantitative digital imaging-based pathology, *in vivo* clinical imaging (PET-MRI/CT) and clinical data informatics. The discovery of patient-specific disease biomarkers from the resulting high-dimensional, heterogeneous and complex datasets requires advanced computational modeling and data science expertise that demand the dedication of a committed PhD student at the interface of CBmed with the Medical Universities of Graz (MUG) and Vienna (MUW), and industry, an interdisciplinary opportunity.

Methodology: Using retrospective biobank-derived patient samples and/or biospecimens obtained in prospective clinical studies, several omics- and imaging technologies will be available to generate personalized omics-level molecular and imaging data, that are to be considered in computational models of disease (Hadizadeh Esfahani et al., 2018) in order to assist in the discovery of complex, patient-specific, predictive biomarkers (Brehme et al., 2016). Eight Core Labs, Genetics & Genomics (NGS), Digital Pathology, Immunology, Metabolomics, Proteomics, Clinical MALDI, In vivo Imaging, and Clinical Data / Digital Biomarkers represent the core of CBmed's biomarker capabilities. Their integrative combination represents the data input to the FUSION approach, considering the synergistic integration of

omics and imaging methodologies, to enable discovery of patient-specific, predictive biomarkers. The challenges to be addressed are **i)** the combination and management of diverse technologies and resulting datasets in a versatile data-processing framework (FUSION), **ii)** the expansion and flexible adaptation of FUSION to interface with diverse samples types, model systems and tissues sources, **iii)** the generalization of FUSION to robustly tackle diverse biomarker discovery challenges across diseases, **iv)** the interface with clinical data resources. The core challenge will be the top-level integration of molecular omics-level data with clinical sample and model system phenotypes (Brehme et al., 2014), and associated clinical or phenotypic data, to assist validation and translation in the clinic. Statistical, mathematical and graphtheoretic interactome network analysis methods (Brehme et al., 2009; Brehme et al., 2014; Rolland et al., 2014), from data processing and normalization to clustering, differential expression analyses, linear and hybrid modeling to network modeling and machine learning approaches are relevant. Strong command of programming languages such as R or Python are crucial alongside knowledge in algebraic methods, including mathematical modeling tools such as MatLAB. This is the opportunity to work at the interface of data science and medicine with the potential for translational impact in cooperation with clinics and industry.

References (*selection*):

1. Benson, A.B., 3rd, Schrag, D., Somerfield, M.R., Cohen, A.M., Figueredo, A.T., Flynn, P.J., Krzyzanowska, M.K., Maroun, J., McAllister, P., Van Cutsem, E., Brouwers, M., Charette, M., and Haller, D.G. (2004). American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol *22*, 3408-3419.
2. **Brehme, M.**, Hantschel, O., Colinge, J., Kaupe, I., Planyavsky, M., Kocher, T., Mechtler, K., Bennett, K.L., and Superti-Furga, G. (2009). Charting the molecular network of the drug target Bcr-Abl. Proc Natl Acad Sci U S A *106*, 7414-7419.
3. **Brehme, M.**, Koschmieder, S., Montazeri, M., Copland, M., Oehler, V.G., Radich, J.P., Brümmendorf, T.H., and Schuppert, A. (2016). Combined Population Dynamics and Entropy Modelling Supports Patient Stratification in Chronic Myeloid Leukemia. Scientific Reports *6*, 24057.
4. **Brehme, M.**, Voisine, C., Rolland, T., Wachi, S., Soper, J.H., Zhu, Y., Orton, K., Villella, A., Garza, D., Vidal, M., Ge, H., and Morimoto, R.I. (2014). A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. Cell Rep *9*, 1135-1150.
5. Flores, M., Glusman, G., Brogaard, K., Price, N.D., and Hood, L. (2013). P4 medicine: how systems medicine will transform the healthcare sector and society. Per Med *10*, 565-576.
6. Hadizadeh Esfahani, A., Sverchkova, A., Saez-Rodriguez, J., Schuppert, A.A., and **Brehme, M.** (2018). A systematic atlas of chaperome deregulation topologies across the human cancer landscape. PLoS Comput Biol *14*, e1005890.
7. Lewis, C., Xun, P., and He, K. (2016). Effects of adjuvant chemotherapy on recurrence, survival, and quality of life in stage II colon cancer patients: a 24-month follow-up. Support Care Cancer *24*, 1463-1471.
8. Meyerhardt, J.A., and Mayer, R.J. (2005). Systemic therapy for colorectal cancer. N Engl J Med *352*, 476-487.
9. Nielsen, J. (2012). Translational and systems medicine. Journal of Internal Medicine *271*, 108-110.
10. Rolland, T., Taşan, M., Charloteaux, B., Pevzner, Samuel J., Zhong, Q., Sahni, N., Yi, S., Lemmens, I., Fontanillo, C., Mosca, R., Kamburov, A., Ghiassian, Susan D., Yang, X., Ghamsari, L., Balcha, D., Begg, Bridget E., Braun, P., **Brehme, M.**, Broly, Martin P., Carvunis, A.-R., Convery-Zupan, D., Corominas, R., Coulombe-Huntington, J., Dann, E., Dreze, M., Dricot, A., Fan, C., Franzosa, E., Gebreab, F., Gutierrez, Bryan J., Hardy, Madeleine F., Jin, M., Kang, S., Kiros, R., Lin, Guan N., Luck, K., MacWilliams, A., Menche, J., Murray, Ryan R., Palagi, A., Poulin, Matthew M., Rambout, X., Rasla, J., Reichert, P., Romero, V., Ruyssinck, E., Sahalie, Julie M., Scholz, A., Shah, Akash A., Sharma, A., Shen, Y., Spirohn, K., Tam, S., Tejeda, Alexander O., Trigg, Shelly A., Twizere, J.-C., Vega, K., Walsh, J., Cusick, Michael E., Xia, Y., Barabási, A.-L., Iakoucheva, Lilia M., Aloy, P., De Las Rivas, J., Tavernier, J., Calderwood, Michael A., Hill, David E., Hao, T., Roth, Frederick P., and Vidal, M. (2014). A Proteome-Scale Map of the Human Interactome Network. Cell *159*, 1212-1226.

11. Rotroff, D.M., and Motsinger-Reif, A.A. (2016). Embracing Integrative Multiomics Approaches. Int J Genomics *2016*, 1715985.

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Posttranslational control of lipid mobilization

Summary

Ruth Birner-Gruenberger, Diagnostic & Research Institute of Pathology, Medical University of Graz

Supervisor:	Prof. Dr. Ruth Birner-Gruenberger
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 05, 2018 00:00 and April 02, 2018 23:59 (Europe/Zurich)

Description

Background: In mammals energy is stored mainly in the form of fat and mobilized during periods of starvation and increased energy demand by a process termed lipolysis. The main sites for long and short term intracellular fat storage are lipid droplets (LDs) in adipose tissue and the liver, respectively. LDs are coated with proteins with roles in signaling, cytoskeletal organization, intracellular trafficking and lipid metabolism including intracellular lipases, such as adipose triglyceride lipase (ATGL), and their regulators, e.g. perilipins and comparative gene identification 58 protein (CGI-58). ATGL catalyzes the initial and rate limiting step of fat mobilization which is dependent upon activation of ATGL by CGI-58. The release of fatty acids from stored fat by lipases is tightly controlled by several hormones to meet energy demands while avoiding toxicity. Molecular mechanisms of this regulation are very complex and poorly understood. We found different protein species of lipases and CGI-58 in our studies, identified that CGI-58 is phosphorylated in adipose tissue and that its phosphorylation is required for full activation of lipolysis in fat cells (1,2).

Hypothesis and Objectives: We will elucidate the function and mechanism of phosphorylation of CGI-58 protein, including its effect on fat mobilization and on its interaction with other proteins, and the interplay of different regulatory sites of CGI-58 protein. To obtain the bigger picture we will reveal the phosphoprotein network involved in stimulation of lipolysis. This approach will identify processes in fat cells important for their function in providing fuel or heat under starvation or cold exposure. It will also allow us to reveal the ongoing molecular processes over time and enable us to identify enzymes responsible for phosphorylation of key proteins involved in lipolysis, coregulated metabolic pathways and organelle interaction.

Methodology: Cell culture, confocal fluorescence microscopy, overexpression, knock out/down, site specific mutations, phosphoproteomics, lipidomics, biochemical assays

References:

1. Sahu-Osen, A et al. CGI-58/ABHD5 is phosphorylated on Ser239 by protein kinase A: control of subcellular localization. J Lipid Res. 2015; 56(1):109-121
2. Birner-Gruenberger, R et al. The lipolytic proteome of mouse adipose tissue. Mol Cell Proteomics. 2005; 4(11): 1710-1717.

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Machine learning based microbiome analysis

Summary

Gregor Gorkiewicz, Institute of Pathology, Medical University of Graz

Supervisor:	Prof. Dr. Gregor Gorkiewicz
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 02, 2017 00:00 and September 17, 2017 23:59 (Europe/Zurich)

Description

Background:

The human microbiome is an integral part of the human body contributing significantly to health and disease. Comparative analyses of next-generation sequencing derived microbiome datasets (16S rRNA genes, metagenomes, metatranscriptomes), originating from healthy and diseased individuals, enables identification of candidate microbes associated with the respective health states [1]. Such “microbial signatures” could potentially serve as diagnostic markers and could be exploited to develop microbiome-based therapies (i.e. “beneficial or probiotic bacteria”) in future.

Hypothesis and Objectives:

Our group has performed several clinical studies, including therapeutic interventions, in the context of the gastrointestinal microbiota and disease (e.g. fecal microbiota transplantation (FMT) in ulcerative colitis [2]). By applying classical multivariate statistics and bioinformatics tools developed for comparative microbiome analysis we were able to discern specific microbial signatures associated with disease and/or cure. Nevertheless, robust biostatistical classification of microbiome data is hampered by several significant challenges (e.g. high degree of interindividual variation, noise, etc.) and complex nature of data (including metadata). Prior research has demonstrated the feasibility of applying machine learning methods to classification of microbiomic data, which expands the “instrumentarium” available for robust microbiome data classification [3].

Methodology:

The PhD candidate will work as a key person within the OMICS network at MUG with a focus on microbiome data. The canditade will handle various microbiome datasets (16S rRNA gene, metagenomes) orginating from different clinical studies and conditions. Machine learning approaches should be implemented in the existing microbiome analysis IT framework and novel measures developed. Datasets will be analyzed to discern robust microbiome signatures specific for diseases and/or interventions. Such identification will enable specific biomolecular analysis of the respective candidate taxa and the development of robust diagnostic signatures in future.

References:

1. Zmora N, Zeevi D, Korem T, Segal E, Elinav E. Taking it Personally: Personalized Utilization of the Human Microbiome in Health and Disease. Cell Host Microbe. 2016;19(1):12-20. doi: 10.1016/j.chom.2015.12.016.
2. Kump PK, Gröchenig HP, Lackner S, Trajanoski S, Reicht G, Hoffmann KM, Deutschmann A, Wenzl HH, Petritsch W, Krejs GJ, Gorkiewicz G, Högenauer C. Alteration of intestinal dysbiosis by fecal microbiota transplantation does not induce remission in patients with chronic active ulcerative colitis. Inflamm Bowel Dis. 2013;19(10):2155-65. doi: 10.1097/MIB.0b013e31829ea325.

3. Statnikov A, Henaff M, Narendra V, Konganti K, Li Z, Yang L, Pei Z, Blaser MJ, Aliferis CF, Alekseyenko AV. A comprehensive evaluation of multicategory classification methods for microbiomic data. Microbiome. 2013;1(1):11. doi: 10.1186/2049-2618-1-11.

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Liquid biopsies for early detection of cancer

Summary

Ellen Heitzer, Institute of Human Genetics, Medical University of Graz

Supervisor:	Prof. Dr. Ellen Heitzer
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 02, 2017 00:00 and September 17, 2017 23:59 (Europe/Zurich)

Description

Background:

The analysis of ctDNA (cell-free circulating tumor DNA) is a very promising tool and might revolutionize cancer care with respect to early detection, identification of minimal residual disease, assessment of treatment response, and monitoring tumor evolution (1-3). The utility of ctDNA as a reliable biomarker to early detect cancer recurrence, to predict tumor burden and treatment response, as well as to identify resistance mechanisms and the emergence of novel actionable targets has been proven in numerous studies (summarized in (4-7)). Moreover, the assessment of ctDNA levels can be used as prognostic marker. It has been shown that patients having higher ctDNA levels at certain time points, i.e. prior to therapy initiation, prior or after tumor resection had significantly shorter PFS (progression-free survival) and/or OS. In contrast to the above mentioned clinical applications with bearing on established and late stage disease, there are little is a paucity of valid studies published that prove the applicability of ctDNA as a diagnostic biomarker enabling early detection of cancer. The early detection of cancer is a desirable objective as it allows the initiation of effective therapies against tumor cells which have accumulated fewer oncogenic events. The major challenges in the analysis of plasma DNA is the differentiation of circulating DNA derived from the tumor from non-tumor circulating DNA. According to a comprehensive study from Bettegowda et al. only 47% of patients with stage I cancers of any type had detectable ctDNA using conventional high resolution mutational analysis. Similar to this study several other studies attempted to quantify the presence of tumor-specific alterations. Patient- specific mutations from tumor tissue were evaluated and studies have picked up cancer- associated DNA alterations that are common in cancer, such as point mutations, SCNAs, rearrangements or promoter methylation in cfDNA. These approaches appeared powerful and achieved a good sensitivity, however prior knowledge about the tumor associated alterations is necessary. For early cancer screening no information about the tumor is available and therefore independent qualitative and quantitative parameters are needed to identify a tumor. Even though the detection of cancer-specific mutations offers a genotypic means to distinguish tumoral from non-tumoral plasma DNA, a major problem is that every cancer has a unique fingerprint and therefore there is no universal marker that can be used for cancer screening. Exact knowledge about the biology of cfDNA/ctDNA might reveal other useful parameters, which can be used in the early detection setting and might dramatically increase sensitivity/specificity for detecting early stage disease. However, compared to the number of studies addressing the clinical applicability of ctDNA, data regarding the actual origin, the kinetics, and the mechanisms of release and clearance are limited and often contradictory. The aim of the proposed thesis is to close this gap.

Hypothesis and Objectives:

The main objective of the proposed thesis is to further elucidate ctDNA biology in order to eventually use liquid biopsies as a cancer screening tool. Additionally, we intend to improve existing analysis methods and to develop advanced bioinformatics tools that enable detection of cancer at its earliest stage. The pairing of clinical and genomic data by the use of sophisticated bioinformatics approaches and databases, will eventually improve the rate of early diagnosis for cancer patients.

Through using novel applications of machine learning methods, including neural networks, to further characterise the cfDNA landscape in the blood and compare the cfDNA profile of healthy people to disease, we aim to extracted several untargeted parameters from whole genome sequencing data sets from plasma DNA (8). It is well known that the sensitivity and specificity of diagnostic tests can often be improved if multiple parameters are measured. Since

additional parameters such as size and origin of cell-free DNA might improve the algorithm, and at the same time shed light into the biology of cfDNA, we intend to establish and validate pre-analytical and analytical protocols in order to comprehensively assess these parameters. Moreover, we want to find out at which tumor size, ctDNA can be detected in the circulation.
The student will optimize and develop our analysis algorithms starting from library preparation to bioinformatic analyses. Therefore, experience with Liunux-based NGS data analysis is mandatory; programming skills are advantageous. An experience with lab work is an advantage, but no requirement.

Specific aims are:

1. Assessment of available blood collection systems and library preparation protocols to establish standard operating procedures for ctDNA analysis
2. Assessment of DNA fragment length in healthy individuals and cancer patients
3. Analysis of genomewide methylation patterns of plasma DNA in order to trace back the tissues-of-origin
4. Testing of established protocols/parameter on patient samples

Methodology:

The PhD candidate will employ next generation sequencing based methodologies, such as whole genome sequencing, bisulfite sequencing, in order to characterize fragment size, tissue of origin and nucleosome occupancy patterns in plasma DNA samples from in healthy individuals and cancer patients. Moreover, the candidate will make use of bioinformatic approaches such as read depth analysis, pathway analysis, or machine learning technologies.

References:

1. Heitzer E, Auer M, Hoffmann EM, Pichler M, Gasch C, Ulz P, et al. Establishment of tumor- specific copy number alterations from plasma DNA of patients with cancer. International journal of cancer 2013;133:346-56.
2. Mohan S, Heitzer E, Ulz P, Lafer I, Lax S, Auer M, et al. Changes in colorectal carcinoma genomes under anti-egfr therapy identified by whole-genome plasma DNA sequencing. PLoS genetics 2014;10:e1004271.
3. Ulz P, Belic J, Graf R, Auer M, Lafer I, Fischereder K, et al. Whole-genome plasma sequencing reveals focal amplifications as a driving force in metastatic prostate cancer. Nature communications 2016;7:12008.
4. Heitzer E, Ulz P, Geigl JB. Circulating tumor DNA as a liquid biopsy for cancer. Clinical chemistry 2015;61:112-23.
5. Siravegna G, Bardelli A. Blood circulating tumor DNA for non-invasive genotyping of colon cancer patients. Molecular oncology 2016;10:475-80.
6. Diaz LA, Jr., Bardelli A. Liquid biopsies: Genotyping circulating tumor DNA. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2014;32:579-86.
7. Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: Monitoring cancer-genetics in the blood. Nature reviews Clinical oncology 2013;10:472-84.
8. Ulz P, Thallinger GG, Auer M, Graf R, Kashofer K, Jahn SW, et al. Inferring expressed genes by whole-genome sequencing of plasma DNA. Nature genetics 2016;48:1273-8.

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Functional characterization of tumor-associated neutrophils (TANs) in lung cancer progression and metastasis

Summary

Akos Heinemann, Institute of Experimental and Clinical Pharmacology, Medical University of Graz

Supervisor:	Prof. Dr. Akos Heinemann
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 02, 2017 00:00 and September 17, 2017 23:59 (Europe/Zurich)

Description

Background:

The accumulation of genetic alterations and the loss of normal cellular regulatory processes will lead to the development of cancer, a heterogeneous disease characterized by histologic subtypes and mutational landscapes. More recently the field has realized that a large proportion of cells within the tumor microenvironment (TME) are non-cancerous cells, including fibroblasts and immune cells leading to the development of immune-based therapies. Lung cancer is the leading cause of cancer deaths worldwide and kills more patients each year than does breast, colon, prostate and pancreas cancer, combined. Non-small cell lung cancer (NSCLC), comprises mainly of lung adenocarcinoma (L-ADCA) and lung squamous cell carcinoma (L-SCCA), representing ~80% of all lung cancer cases. Although surgical intervention for early stage NSCLC can be curative, traditional chemo- and radiotherapy, when required, are of limited effectiveness. Given the limited treatment options it is not surprising that initial success of immune therapies for NSCLC has created enthusiasm for these novel therapeutics. Unfortunately, just ~20% of NSCLC patients benefit from novel therapies and underlying mechanisms for treatment failure are mostly unknown. Immune checkpoint inhibitor therapy, e.g anti-PD1/anti-PDL1 antibodies, likely fails for one of two fundamental reasons: (1) an antigen-driven immune response is not present or (2) an antigen-driven immune response is present, but one or more immune suppressive factors reside within the tumour microenvironment (TME) that function to derail an otherwise effective immune response. This highlights the need to identify additional immune suppressive factors located within the TME that when targeted, would improve the efficacy of immune checkpoint blockade.

Hypothesis and Objectives:

Several recent studies have shown the important role of neutrophils in cancer and our hypothesis that tumor-associated neutrophils (TANs) act as immune suppressive entity in the TME is largely based on two recent studies. (1) *Gentles* and colleagues showed that the neutrophil gene signature predicts mortality better than any other immune cell signature in a cohort of >18000 patients encompassing 25 different cancer types (Gentles et al., 2015). (2) Our group demonstrated in a cohort of 73 NSCLC patients that neutrophils are the most prevalent immune cell type present in NSCLC and have identified that neutrophils constrain antigen-driven immune responses in tumor, but not in non-adjacent lung tissue, strongly suggesting that this is a tumor-specific phenomenon (Kargl et al., 2017).

The purpose of this study is to (1) identify lymphocyte suppressive TAN subpopulations present in the TME in primary tumor and metastasis and (2) to elucidate the mechanism by which they get recruited into the TME, (3) inhibit lymphocyte function and limit immune checkpoint inhibitor efficacy. Further, we will analyze (4) the role of TAN subpopulations on tumor cells in the TME.

Methodology:

The PhD candidate will characterize TANs using flow cytometry, next-generation sequencing and multiplex fluorescence microscopy. Functional analysis of neutrophils will be investigated in assays of shape change, chemotaxis and Ca^{2+} signaling and levels of neutrophil specific enzymes will be assessed by ELISA and western blots. Lymphocyte/neutrophil and tumor cell/neutrophil interactions will be studied using co-culture systems. In this study, we will make use of established human non-small cell lung cancer cell lines, tumor tissue from consented patients and mouse models.

References:

Busch SE, Hanke ML, Kargl J, Metz HE, MacPherson D, Houghton AM. Lung Cancer Subtypes Generate Unique Immune Responses. *J Immunol.* 197(11):4493-4503(2016)

Coffelt SB, Wellenstein MD, deVisser KE. Neutrophils in cancer: neutral no more. *Nat Rev Cancer.;*16(7):431-46(2016)

Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, Nair VS, Xu Y, Khuong A, Hoang A, Hoang CD, Diehn M, West RB, Plevritis SK, Alizadeh AA. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nature Medicine.*;21, 938-945(2015)

Kargl J, Busch SE, Yang GHY, Kim KH, Hanke ML, Metz HE, Hubbard JJ, Madtes DK, McIntosh MW, Houghton AM. Neutrophils and Tregs Constrain Antigen-Driven Immune Responses in NSCLC. *Nature Commun*.8, 14381 doi: 10.1038/ncomms14281(2017)

Co-supervision and Cooperations:

Julia Kargl, Institute of Experimental and Clinical Pharmacology, Meduni Graz

Horst Olschewski, Division of Pulmonology, Meduni Graz

Grazyna Kwapiszewska & Andrea Olschewski, LBI for Lung Vascular Research, Graz

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PoCOsteo (Point-of-care in-office device for identifying individuals at high risk of osteoporosis and osteoporotic fracture)

Summary

Hans Peter Dimai, Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of Graz

Supervisor:	Prof. Dr. Hans Peter Dimai
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 02, 2017 00:00 and September 17, 2017 23:59 (Europe/Zurich)

Description

Background:

As a consequence of the ageing population, osteoporosis has become a major health priority in many countries. The significance of osteoporosis lies in the fractures which occur after only a low, or sometimes even without any trauma, typically at skeletal sites such as the thoracic and the lumbar spine, the hip, the proximal humerus and the distal forearm. All osteoporotic fractures are associated with significant morbidity, and both hip and vertebral fractures are also associated with excess mortality[1]. Although useful non-invasive tools, aside from bone-mineral density (BMD) measurement, have been developed recently to estimate an individual's absolute fracture probability for a future period of up to ten years, only little of such information can be obtained from blood biochemical markers[2,3]. Furthermore, and irrespective of the fact that currently available biochemical markers may provide information on treatment response and also to some extent on an individual's future fracture risk, such markers are not available for routine use in health care facilites other than highly specialized institutions such as research facilities or tertiary hospitals.

Objectives:

The overall objective of the PoCOsteo project is the development, clinical validation and preparation for commercialisation of a whole-blood point-of-care tool for metabolic bone diesases, particularly osteoporosis. T o realize this objective, two Technology-Readyness-Level 3 (TRL3) devices for individual proteomic and genomic electrochemical sensors will form the base of the further development. These sensors have been developed / are being developed by two project partners; a) University of Gent (Belgium), who developed and characterised an electrochemical proteomic sensor for two types of bone-turnover markers, i.e. proteins reflecting bone formation and/or bone loss, and b) Universitat Rovira I Virgili (Spain), who is highly specialised in genomic electrochemical sensors which involve cost effective Printed Circuit Board (PCB) based DNA electrodes[4-7]. The sensors will be further optimised, combined in a single portable microfluidic cartridge and integrated into a complete and independent whole-blood point-of-care bone health assessment tool. The tool will then be clinically validated in two large tertiary hospitals (one in Austria, one in the Iran), involving a validation cohort of ~1,500 patients at the Austrian study center. The aim is to reach TRL6 / TRL7 for the combined microfluidic cartridges and the complete tool. Furthermore, depending on the proteomic and/or genomic sensors available at year three of the project, a fracture risk assessment model will be developed within the framework of the validation cohort recruited in the course of the first 18 months of the project. The risk model will be based on the incidence of vertebral fractures, hip fractures, proximal humeral fractures, and distal forearm fractures occurring during a pre-specified period of the project.

Methods:

The PhD candidate will be carrying out the following activities: a) recruitment of patients eligible to be integrated into the validation cohort (n~1.500), b) supervision of (pre-analytical) sample processing under involvement of the Biobank Graz, one of Europe's largest biobanks c) history taking in patients assigned to the validation cohort, d) data aquisition and data management e) assessment of the patients' absolute fracture probability by using online fracture risk assessment tools, including BMD as obtained by the current gold-standard method dual-x-ray absorptiometry (DXA), f) supervision (and also performing) of BTM (and if available at that time, also genomic) measurement in

whole blood samples, using the first available prototypes of the microfluidic cartridge containing point-of-care bone health assessment tool, g) validating BTM results against “gold-standard” methods such as ELISA/ECLIA a.o., and if available at that time, validating results of genomic measurements against high throughput DNA sequencing h) assessment of vertebral fractures (based on follow-up radiographs) and non-veretebral fractures (based on interviews / questionnaires), and i) development of a fracture risk assessment model/tool (depending on the proteomic and/or genomic sensors developed and available so far).

References:

1. Cooper C, Cole ZA, Holroyd CR, Earl SC, Harvey NC, Dennison EM, Melton LJ, Cummings SR, Kanis JA; IOF CSA Working Group on Fracture Epidemiology. Secular trends in the incidence of hip and other osteoporotic fractures. Osteoporos Int. 2011 May;22(5):1277-88. doi: 10.1007/s00198-011-1601-6.
2. Marques A, Ferreira RJ, Santos E, Loza E, Carmona L, da Silva JA. The accuracy of osteoporotic fracture risk prediction tools: a systematic review and meta-analysis. Ann Rheum Dis. 2015 Nov;74(11):1958-67. doi: 10.1136/annrheumdis-2015- 207907. Epub 2015 Aug 6. Review.
3. Dimai HP. Use of dual-energy X-ray absorptiometry (DXA) for diagnosis and fracture risk assessment; WHO-criteria, T- and Z-score, and reference databases. Bone. 2016 Dec 29. pii: S8756-3282(16)30386-6. doi: 10.1016/j.bone.2016.12.016. [Epub ahead of print]
4. Khashayar P, Amoabediny Gh, Larijani B, Hosseini M, Verplancke R, Schaubroek D, De Keersmaecker M, Adriaens A, Vanfleteren J. Characterization of gold nanoparticle layer deposited on gold electrode by various techniques for improved sensing abilities. Biointerface Research in Applied Chemistry. 2016; 6(4): 1380-90
5. Khashayar P, Amoabediny G, Larijani B, Vanfleteren J. Bone biosensors: knowing the present and predicting the future. Journal of Micromechanics and Microengineering 2016; 26(2)
6. Salvo P, Henry OY, Dhaenens K, Acero Sanchez JL, Gielen A, Werne Solnestam B, Lundeberg J, O'Sullivan CK, Vanfleteren J.Fabrication and functionalization of PCB gold electrodes suitable for DNA-based electrochemical sensing. Biomed Mater Eng. 2014;24(4):1705-14.
7. Sánchez JL, Henry OY, Joda H, Solnestam BW, Kvastad L, Johansson E, Akan P, Lundeberg J, Lladach N, Ramakrishnan D, Riley I, O'Sullivan CK. Multiplex PCB-based electrochemical detection of cancer biomarkers using MLPA-barcode approach. Biosens Bioelectron. 2016 Aug 15;82:224-32

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Structural and Functional Studies of RGG-mediated Nuclear Import

Summary

Tobias Madl, Institute of Molecular Biology and Biochemistry, Medical University of Graz

Supervisor:	Prof. Dr. Tobias Madl
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 02, 2017 00:00 and September 17, 2017 23:59 (Europe/Zurich)

Description

Background:

Motifs rich in arginine and glycine residues were recognized several decades ago to play functional roles in RNA-binding and were termed RG/RGG motifs[1, 2]. More than 1000 proteins harbor the intrinsically disordered RG/RGG motif, and these proteins play essential roles in a plethora of physiological processes such as transcription, pre-mRNA splicing, DNA damage signaling and mRNA translation[2], and very recently in neuroprotection[3]. We have shown that the RG/RGG-motif of FUS is involved in transportin-1 – mediated nuclear import, and that transportin-1 can bind both, the known proline-tyrosine nuclear localization signal (PY-NLS) and the RG/RGG-motif of FUS simultaneously[4, 5]. Arginine methylation of the RG/RGG motif in combination with mutations found in Amyotrophic Lateral Sclerosis (ALS) patients decrease the affinity of FUS for transportin-1 and reduce the efficiency of nuclear import. The resulting cytosolic inclusions are the hallmark of FUS-associated ALS disease. Despite these findings, the underlying molecular details including the implications for other RG/RGG proteins remain unknown.

Hypothesis and Objectives:

Based on our recent studies and supported by our preliminary data, we hypothesize that a yet unknown RG/RGG binding site is present in transportin-1 and that this site is essential for nuclear import of RG/RGG proteins. We propose that that arginine methylation of RG/RGG sites regulates nuclear import of the large class of RG/RGG proteins and that disease mutations found in cancer and neurodegeneration modulate these interactions. We propose to use the proteins FUS and CIRP as model systems to reveal the structural and functional mechanisms of nuclear import of RG/RGG-containing proteins by:

Aim 1) studying structure & function of RG/RGG recognition in the CIRP/FUS – transportin-1 complexes

Aim 2) studying regulation of the CIRP/FUS - transportin-1 interaction by arginine methylation, disease mutations, RanGTP, and RNA

This might set the base for the discovery of new potential druggable targets in the future for the treatment of a plethora of diseases with different phenotypes, though caused by the same molecular disease mechanisms (i.e. misregulation of RG/RGG-mediated nuclear import).

Methodology:

The PhD candidate will make use of our recent methodological achievements for studying structure of large protein complexes by combining solution Nuclear Magnetic Resonance (NMR) spectroscopy, and molecular modeling[6-11], and extend it with complementary approaches such as Mass Spectrometry (MS).

References:

1. Kiledjian, M. & Dreyfuss, G. Primary structure and binding activity of the hnRNP U protein: binding RNA through RGG box. *The EMBO journal* **11**, 2655-2664 (1992).

2. Thandapani, P., O'Connor, T.R., Bailey, T.L. & Richard, S. Defining the RGG/RG motif. *Molecular cell* **50**, 613-623 (2013).

3. Peretti, D. et al. RBM3 mediates structural plasticity and protective effects of cooling in neurodegeneration. *Nature* (2015).

4. Dormann, D. et al. Arginine methylation next to the PY-NLS modulates Transportin binding and nuclear import of FUS. *The EMBO journal* **31**, 4258-4275 (2012).

5. Suarez-Calvet, M. et al. Monomethylated and unmethylated FUS exhibit increased binding to Transportin and distinguish FTLD-FUS from ALS-FUS. *Acta Neuropathol* **131**, 587-604 (2016).

6. Gobl, C., Madl, T., Simon, B. & Sattler, M. NMR approaches for structural analysis of multidomain proteins and complexes in solution. *Progress in nuclear magnetic resonance spectroscopy* **80C**, 26-63 (2014).

7. Huang, J.R. et al. Transient Electrostatic Interactions Dominate the Conformational Equilibrium Sampled by Multidomain Splicing Factor U2AF65: A Combined NMR and SAXS Study. *Journal of the American Chemical Society* **136**, 7068-7076 (2014).

8. Karagoz, G.E. et al. Hsp90-Tau complex reveals molecular basis for specificity in chaperone action. *Cell* **156**, 963-974 (2014).

9. Lorenz, O.R. et al. Modulation of the Hsp90 chaperone cycle by a stringent client protein. *Molecular cell* **53**, 941-953 (2014).

10. Madl, T., Gabel, F. & Sattler, M. NMR and small-angle scattering-based structural analysis of protein complexes in solution. *Journal of structural biology* **173**, 472-482 (2011).

11. Muller, R. et al. High-resolution structures of the IgM Fc domains reveal principles of its hexamer formation. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 10183-10188 (2013).

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Optical control of neuronal signaling

Summary

Rainer Schindl, Institute for Biophysics, Medical University of Graz

Supervisors:	Prof. Dr. Roland Malli PD Dr. Rainer Schindl
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 02, 2017 00:00 and September 17, 2017 23:59 (Europe/Zurich)

Description

Background:

Optical techniques present a powerful tool to precisely control signaling of excitable cells for neuroscience application. Electrical responses of neurons can be shaped by light to provide a powerful tool for the treatment of neurological and psychiatric diseases (Jiang et al., Nature Mat., Shapiro et al., Nature Comm). Recently, we have generated and used organic semiconductors that can be stimulated by laser light pulses (Sytnyk et al. Nat. Comm, 2017). These semiconductors have the size of single cells with thin needles in nanometer dimensions. These needles present perfect contact sites with the plasma membrane of a living single cell. For our experiments, we selected single HEK and RBL cells that were in direct contact to the semiconductor structures. Both cell lines are commonly known as non-excitable ones. A laser light beam was then focused on the semiconductor structure in order to stimulate the attached cell. Induced electrical signals were then recorded with the patch clamp technique. Hence, the developed organic semiconductors present a powerful and novel tool to stimulate cells by light.

Hypothesis and Objectives:

Optical control of retinal neurons

Macular degeneration, one of the most common progressive retinal diseases, can lead to blindness by reducing the visual spot. While specific light dependent cells get lost, the neuronal cells that signal electro-chemical responses to the brain still remain. Hence a long term aim of this PhD thesis is to further develop the semiconductor signaling for these retinal ganglion cells and various other neuronal cells. A first task will be to extract retinal ganglion cells from either rats or mice. The PhD student will also learn as a second task how to measure and visualize ganglion as well as neuron cells in contact with the semiconductor structures. Therefore, the student will perform high resolution fluorescence microscopy and scanning electron microscopy experiments on the cell – semiconductor contact sites. A third task is to perform electro-physiological recordings of single ganglion and neuron cells. These cells signal with action potentials, a transient depolarization to send the activation signal to the axon. In the semiconductor approach the light pulse will generate a similar depolarization of the ganglion or neuronal cell. A fourth task is to control the action potential signaling and read-out with the patch-clamp technique. This PhD thesis will provide a novel and fundamental insight into optical control of retinal neuronal signaling.

Methodology:

The PhD student requires motivation to learn different techniques and likes to be involved in intense collaboration with leading laboratories in this multifaceted research field. Experiments will be performed at the institute for biophysics and with collaboration partners within the Medical University of Graz. Semiconductors will be provided by an international collaboration partner and the PhD student requires basic knowledge in physical and chemical principles. Isolation of neuronal cells and cell culture will be important to achieve live cell recordings in contact with semiconductor structures. The PhD student will also perform fluorescence and electron microscopy as well as whole-cell electrophysiological recordings. Prospective students should be able to plan experiments independently.

References:

Cellular interfaces with hydrogen-bonded organic semiconductor hierarchical nanocrystals. Sytnyk M, Jakešová M, Litviňuková M, Mashkov O, Kriegner D, Stangl J, Nebesářová J, Fecher FW, Schöfberger W, Sariciftci NS, **Schindl R**, Heiss W, Głowacki ED. Nat Commun. 2017 Jul 21;8(1):91. doi: 10.1038/s41467-017-00135-0

Heterogeneous silicon mesostructures for lipid-supported bioelectric interfaces. Jiang Y, Carvalho-de-Souza JL, Wong RC, Luo Z, Isheim D, Zuo X, Nicholls AW, Jung IW, Yue J, Liu DJ, Wang Y, De Andrade V, Xiao X, Navrazhnykh L, Weiss DE, Wu X, Seidman DN, Bezanilla F, Tian B. Nat Mater. 2016 Sep;15(9):1023-30. doi: 10.1038/nmat4673.

Infrared light excites cells by changing their electrical capacitance. Shapiro MG, Homma K, Villarreal S, Richter CP, Bezanilla F. Nat Commun. 2012 Mar 13;3:736. doi: 10.1038/ncomms1742.

Calcium signaling and gene regulation

Summary

Rainer Schindl, Institute for Biophysics, Medical University of Graz

Supervisors:	Prof. Dr. Roland Malli PD Dr. Rainer Schindl
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 02, 2017 00:00 and September 17, 2017 23:59 (Europe/Zurich)

Description

Background:

An increase in cellular calcium concentration in an important mechanism to stimulate various cell processes. These calcium signals can be fast, in microsecond range to stimulate release of vesicles in a neuronal cell or can last over hours to stimulate gene regulation (Berridge, Biochemical Society Transactions). In addition, calcium signals form repetitive spikes and waves or can be localized to micro-domains in a single living cell. The store-operated Ca^{2+} channel complex is a ubiquitously expressed system to generate long lasting Ca2+ signals at junctions of the plasma-membrane and the endoplasmic reticulum. The slow Ca2+ influx activates transcription factors like the nuclear factor of activated T-cells (Frischauf et al., Science Signaling). How enhanced cellular calcium levels can generate such diverse signaling modes is still not well understood. Moreover dysfunctional Ca^{2+} signaling is suggested as a cofactor for cancer cell development.

Hypothesis and Objective:

Calcium signaling complexes to stimulate gene regulation

The PhD will study the role of calcium ion channels and Ca2+ dependent gene regulation for physiological and patho-physiological processes (Frischauf et al., Science Signaling). The investigated Ca^{2+} channels will include the store-operated Ca^{2+} channel complex, STIM1 and Orai1 but also the recently discovered endoplasmic reticulum Ca^{2+} load activated channel TMCO1 (Wang et al.). Specifically, this PhD project will investigate how physiological calcium signaling in single immune and muscle cells are regulated. These live cell recordings will aim to understand micro-domain signaling and ion-channel protein network to activate transcriptional programs. Experiments will be conducted in close collaboration with international laboratories. This will include structural biologists to collaborate on atomic structure of transcription factors. Oncologists to investigate the impact of Ca^{2+} for cancer cell development. As well as computer simulation experts to model the Ca^{2+} signaling processes. Pathological calcium signaling will focus on immune deficiency, myopathy and cancer.

Methodology:

The PhD student requires the spirit to learn different techniques and likes to be involved in intense collaboration with leading laboratories in this multifaceted research field. At the institute of biophysics the PhD student will perform live cell techniques including electrophysiological patch-clamp recordings and fluorescence imaging. Calcium proteins will be genetically engineered by using molecular biology techniques and biochemical techniques such as cysteine scanning methods.

References:

Calcium signalling remodelling and disease. Michael J. Berridge. Biochemical Society TransactionsApr 01, 2012,40(2)297-309;DOI: 10.1042/BST20110766

A calcium-accumulating region, CAR, in the channel Orai1 enhances Ca(2+) permeation and SOCE-induced gene transcription. Frischauf I, Zayats V, Deix M, Hochreiter A, Polo IJ, Muik M, Lackner B, Svobodová B, Pammer T, Litviňuková M, Sridhar AA, Derler I, Bogeski I, Romanin C, Ettrich RH, Schindl R. Sci Signal. 2015 Dec 22;8(408):ra131. doi: 10.1126/scisignal.aab1901.

TMCO1 Is an ER Ca(2+) Load-Activated Ca(2+) Channel. Wang QC, Zheng Q, Tan H, Zhang B, Li X, Yang Y, Yu J, Liu Y, Chai H, Wang X, Sun Z, Wang JQ, Zhu S, Wang F, Yang M, Guo C, Wang H, Zheng Q, Li Y, Chen Q, Zhou A, Tang TS. Cell. 2016 Jun 2;165(6):1454-1466. doi: 10.1016/j.cell.2016.04.051

The crosstalk between inflammation, endothelium and extracellular matrix

Summary

Grazyna Kwapiszewska-Marsh, LBI for Lung Vascular Research, Institute of Physiology, Medical University of Graz

Supervisor:	PD Dr. Grazyna Kwapiszewska-Marsh
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 02, 2017 00:00 and September 17, 2017 23:59 (Europe/Zurich)

Description

Background:

Diverse molecular pathways active in pulmonary hypertension converge to generate a pathophenotype that exhibits many features common to cancer cells. This includes abnormal endothelial cell apoptosis and proliferation, increased migration and proliferation of smooth muscle cells (SMC). Abnormal infiltration of inflamatory cells drives extracellular matrix (ECM) turnover (1, 2) and vasculopathy. By using expression profiling and bioinformatics approaches we recently have shown that the ECM pathway was the most prominently regulated during vascular remodeling (3).

Hypothesis and Objectives:

Our recent findings pointed towards several so far unstudied collagens, metalloproteases, as well as their processing enzymes as important new mediators in the pathophysiology of pulmonary hypertension (4). Moreover, we have found that plethora of inflammatory cells are associated disease pathogenesis. Therefore, we hypothesize that this inflammatory component is a potent source of ECM modifiying enzymes, which promotes vascular remodeling. The PhD student will investigate the crosstalk between inflammatory cells and the mechanisms responsible for the altered vessel microenvironment, exemplary: 1) which processing enzymes are produced by identified inflammatory cells, 2) which ECM components are substrates for identified degrading enzymes, 3) the role of ECM products on endothelial and smooth muscle cell function in vitro and in vivo.

Methodology:

The PhD candidate will learn to isolate primary residual and inflammatory cells from the tissue. He/she will perform real-time PCR, western blot, flow cytometry analysis, ELISA, zymography, immunohisto- and immunofluorescence labelling followed by microscopy. Functional in vitro read-outs will comprise: proliferation, migration, apotosis, and resistance measurements. Next generation sequencing and CRISPR-Cas9 will be applied to define the role of new players. In vivo studies will use preclinical pulmonary hypertension models together with haemodynamic and vascular remodeling analysis.

References:

1. Humbert, M., Morrell, N.W., Archer, S.L., Stenmark, K.R., MacLean, M.R., Lang, I.M., Christman, B.W., Weir, E.K., Eickelberg, O., Voelkel, N.F., et al. 2004. Cellular and molecular pathobiology of pulmonary arterial hypertension. J Am Coll Cardiol 43:13S-24S.
2. Jeffery, T.K., and Wanstall, J.C. 2001. Pulmonary vascular remodeling: a target for therapeutic intervention in pulmonary hypertension. Pharmacol Ther 92:1-20.
3. Hoffmann J, Wilhelm J, Marsh LM, Ghanim B, Klepetko W, Kovacs G, Olschewski H, Olschewski A, Kwapiszewska G. Distinct differences in gene expression patterns in pulmonary arteries of patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis with pulmonary hypertension. Am J Respir Crit Care Med. 2014 Jul 1;190(1):98-111.

4. Hoffmann J, Marsh LM, Pieper M, Stacher E, Ghanim B, Kovacs G, König P, Wilkens H, Haitchi HM, Hoefler G, Klepetko W, Olschewski H, Olschewski A, Kwapiszewska G. Compartment-specific expression of collagens and their processing enzymes in intrapulmonary arteries of IPAH patients. Am J Physiol Lung Cell Mol Physiol. 2015 May 15;308(10):L1002-13. doi: 10.1152/ajplung.00383.2014.

Hormones and their link to microbiome and immune system

Summary

Barbara Obermayer-Pietsch, Division of Endocrinology and Diabetology, Endocrine Lab Platform, Department Internal Medicine, Medical University of Graz

Supervisor:	Prof. Dr. Barbara Obermayer-Pietsch
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 02, 2017 00:00 and September 17, 2017 23:59 (Europe/Zurich)

Description

Background:

Hormonal and metabolic changes are known to be associated in women with polycystic ovary syndrome (PCOS), affecting up to 20% of women worldwide and adding to the incidence of diabetes, obesity, fertility problems, depression and related long-term problems [1]. We recently published the first evidence of an interaction of stool microbiome with hormonal and metabolic features in women with PCOS diagnosed according to the Rotterdam criteria, with potential links to gut endotoxinemia and immunological changes [2].

A significant reduction in phylogenetic diversity and the number of observed operational taxonomic units (OTUs), accompanied by characteristic phylogenetic microbiome profile shifts between samples from PCOS women and controls has been demonstrated based on lower relative abundance of certain bacterial taxa. We have shown evidence for the hypothesis that diet-induced gut bacterial dysbiosis and subsequent gut barrier dysfunction and endotoxemia may drive the chronic inflammation and subsequent insulin resistance and androgen hypersecretion associated with PCOS [2]. Intestinal epithelial barrier damage, potential changes in phytoestrogen metabolism and (auto)immunological and genetic profiles in PCOS women may promote insulin resistance and lipid storage through an up-regulation of pro-inflammatory and auto-immune signaling. As this model is not only important for women, but also for men with metabolic syndrome and/or diabetes and/or gonadal dysfunction, this hypothesis might have a significant impact on our knowledge about both hormonal as well as metabolic dysregulations via (auto)immunological and microbiomal links in a large proportion of women and men.

Hypothesis and Objectives:

Based on our recent results that hormonal secretion and metabolic changes are associated and/or modulated by certain microbiomal and immunological profiles, we want to add novel insights to the unclear etiology and treatment options of PCOS and related clinical problems and generate new diagnostic and potential therapy concepts based on our human and animal pilot studies.

Furthermore, associations between host genetics and certain members of the gut microbiome will be investigated, since some bacteria predisposing to a healthy or unhealthy metabolic state may be heritable, thus explaining familiar components not only in PCOS.

Probiotic intervention might decrease gut permeability in systemic and functional tests as well as systemic inflammation, and increase gut microbiome diversity analysed by NGS techniques. Thus, intervention studies will define the potential impact of the gut microbiome on glucose, lipid, and hormone metabolism via and the translocation of bacterial products across the intestinal barrier by the investigation of gut barrier integrity, endotoxemia, and inflammation. In addition, recent approaches in the clarification of autoimmune changes of endocrine regulation will be conducted in our established large PCOS-, pregnancy-, and cardiovascular cohorts in context with these findings.

Methodology:

The PhD candidate will be involved in the planning and performance of clinical studies and biobanking. Besides insights into enzyme-linked and radioimmunological assays for hormonal and metabolic measurements, hormone and phytoestrogen metabolites will be assessed by HPLC-MS. Microbiome analyses based on MiSeq sequencing after DNA extraction from samples will be processed using open-source software according to published protocols

with established adaptations and bioinformatic tools. Real-time quantitative PCR will be used for the confirmation of relative abundances of bacteria, whole blood gene expression and immunological changes in PCOS patients and controls as well as functional tests and flow cytometry. Studies will be complemented by cell culture models of hormonal and immunological interaction.

References:

1. Lindheim L, Bashir M, Münzker J, Trummer C, Zachhuber V, Pieber TR, Gorkiewicz G, Obermayer-Pietsch B. The Salivary Microbiome in Polycystic Ovary Syndrome (PCOS) and Its Association with Disease-Related Parameters: A Pilot Study. *Front Microbiol. 2016;7:1270.*
2. Lindheim L, Bashir M, Münzker J, Trummer C, Zachhuber V, Leber B, Horvath A, Pieber TR, Gorkiewicz G, Stadlbauer V, Obermayer-Pietsch B. Alterations in Gut Microbiome Composition and Barrier Function Are Associated with Reproductive and Metabolic Defects in Women with Polycystic Ovary Syndrome (PCOS): A Pilot Study. *PLoS One. 2017 Jan 3;12(1):e0168390.*
3. Mattar R, de Campos Mazo DF, Carrilho FJ. Lactose intolerance: diagnosis, genetic, and clinical factors. *Clin Exp Gastroenterol. 2012;5: 113–21.*

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Investigating the potassium homeostasis of the nucleus

Summary

Roland Malli, Institute of Molecular Biology and Biochemistry, Medical University of Graz

Supervisor:	Prof. Dr. Roland Malli
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 02, 2017 00:00 and September 17, 2017 23:59 (Europe/Zurich)

Description

Background:

We recently developed a series of novel genetically-encoded potassium ion (K^+) indicators, which enable real-time monitoring of K^+ in single cells and subcellular compartments (patent pending, manuscript submitted). Our data point to high K^+ levels and spatial subcellular K^+ dynamics (e.g. within the nucleus) of intact living cells. However, the regulatory mechanisms and the role of K^+ signals within the nucleus in physiology and pathology remains largely elusive. Just few reports from the 1970ties [1] and 1980ties [2] speculate about increased nuclear K^+ levels and their putative impact on DNA stability and gene expression. Within this thesis project the mechanisms responsible for controlling the nuclear K^+ homeostasis should be investigated. Moreover, K^+ fluctuations within the nucleus in response to defined stresses and stimuli should be explored using high resolution fluorescence microscopy. Finally, DNA stability, DNA-protein interactions, and changes in gene expression should be correlated with defined nuclear K^+ alterations.

Hypothesis and Objectives:

We hypothesise that K^+ fluctuations within the nucleus are fundamental to dynamically control DNA metabolism and gene expression in health and diseases. With the usage of the novel K^+ probes in combination with genetic manipulations of the expression levels of K^+ channels, exchangers and transporters of the nuclear envelope the nuclear K^+ homeostasis will be characterized. Consequences of K^+ varaiations within the nucleus on DNA integrity and gene expression will be analyzed.

Methodology:

In addition to classical cell culture, biochemistry- (WB) and molecular biology techniques (PCR, siRNA library screens) the Ph.D. candidate will work with genetically encoded tools and probes as demonstrated in references [3,4] and use state-of-the-art fluorescence imaging techniques [3,4] to visualize subcellular K^+ signals in real-time on the level of individual cells.

References:

1. Dick, D. A. The distribution of sodium, potassium and chloride in the nucleus and cytoplasm of Bufo bufo oocytes measured by electron microprobe analysis. The Journal of physiology 284, 37–53 (1978).
2. Paine, P. L., Pearson, T. W., Tluczek, L. J. M. & Horowitz, S. B. Nuclear sodium and potassium. Nature 291, 258–261 (1981).
3. Eroglu, E., Gottschalk, B., Charoensin, S., Blass, S., Bischof, H., Rost, R., Madreiter-Sokolowski, C.T., Pelzmann, B., Bernhart, E., Sattler, W., Hallström, S., Malinski, T., Waldeck-Weiermair, M., Graier, W.F. & Malli, R. Development of novel FP-based probes for live-cell imaging of nitric oxide dynamics. Nat. Commun. 7: 10623, 2016

4. Madreiter-Sokolowski, C.T., Klec, C., Parichatikanond, W., , Stryeck, S., Gottschalk, B., Pulido, S., Rost, R., Eroglu, E., Hofmann, N.A., Bondarenko, A.I., Madl, T., Waldeck-Weiermair, M., Malli, R. & Graier, W.F. PRMT1-mediated methylation of MICU1 determines the UCP2/3-dependency of mitochondrial Ca^{2+} uptake in immortalized cells. Nat. Commun. 7:12897, 2016

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The molecular organization of mitochondria – plasma membrane interaction and its importance for health and disease

Summary

Wolfgang Graier, Institute of Molecular Biology and Biochemistry, Medical University of Graz and NIKON-Center of Excellence

Supervisor:	Prof. Dr. Wolfgang Graier
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 02, 2017 00:00 and September 17, 2017 23:59 (Europe/Zurich)

Description

Background:

Mitochondrial functions by far exceed that of serving as the cells most powerful energy supplier. During the last years intriguing evidence accumulated that pointed to an essential involvement of mitochondria in cellular signaling, metabolism and multiple cell functions. Notably, these organelles seek to communicate with nearly all cell membranes, including the endoplasmic reticulum, the nuclear envelop, lysosomes, lipid droplets and the plasma membrane. These interactions are often established by specific, transient protein boundaries that physically stabilize the inter-organelle interface and, thus, facilitate the exchange of ions, small molecule substrates, proteins, (phopsho)lipids, and other (signaling) molecules. While the junction between mitochondria and the endoplasmic reticulum got very much attention recently and has been highlighted in terms of its contribution to cancer metabolism [1,2], the interaction of mitochondria with the plasma membrane has been only merely studied so far. However, there are strong functional findings that emphasize the important role of mitochondria-plasma membrane junction in the maintenance of ion channel activity [3] and the activity of cytosolic enzymes [4]. Notably, most of these interactions build on the transfer of Ca^{2+} of which the mitochondrial sequestration is highly regulated and is subject of sophisticated modulatory mechanisms that adapt this function along with the energy demand of the organelle/cell [5,6].

Hypothesis and Objectives:

Our data provides evidence for a dynamic interaction between the mitochondria and the plasma membrane, protein bridging and the exchange of Ca^{2+}, substrates and (phospho)lipids. Notably, these transient junctions appear to depend on the cellular energetic status and are highly affected during aging and cancerogenesis, thus, yielding a metabolic switch supporting cancer growth or manifestation of age-related cellular dysfunction. Accordingly we hypothesize that the formations of transient mitochondria-plasma membrane junctions play an important role in cellular homeostasis and the generation of disease or organelle dysfunction. This project will investigate 1st, the molecular basis of mitochondria-plasma membrane interaction, 2nd, actual processes of ion/substrate/ protein/lipid transfer, and, 3rd, their contribution and consequences for cell/organelle functions in health, cancerogenesis and aging.

Methodology:

The PhD candidate will utilize all kinds of molecular biology techniques (cloning, mutagenesis, real time PCR, CRISPR/Cas9, si/shRNA, morpholinos, etc.), standard biochemistry techniques like Western blotting, and highly sophisticated (super-resolution SIM) fluorescence (FRET, FRAP) microscopy and electrophysiology. The laboratory is specialized for single-cell and sub-cellular analyzes of signaling pathways, ion movements, protein-protein interactions, and metabolism. While most of the experiments will be conducted in isolated and cultured single cells, completing studies will be performed in *Caenorhabditis* elegans.

References:

1. Lovy, A., Foskett, J. K. & Cárdenas, C. InsP3R, the calcium whisperer: Maintaining mitochondrial function in cancer. *Mol Cell Oncol* **3,** e1185563 (2016).

2. Madreiter-Sokolowski, C.T. , Gottschalk, B., Parichatikanond, W., Eroglu, E., Klec, C., Waldeck-Weiermair, M., Malli, R. & Graier, W.F. Resveratrol specifically kills cancer cells by a devastating increase in the Ca^{2+} coupling between the greatly tethered endoplasmic reticulum and mitochondria. *Cell Physiol Biochem* **39,** 1404–1420 (2016).

3. Malli, R., Frieden, M., Osibow, K. & Graier, W. F. Mitochondria efficiently buffer subplasmalemmal Ca^{2+} elevation during agonist stimulation. *J Biol Chem* **278,** 10807–10815 (2003).

4. Charoensin, S., Eroglu, E., Opelt, M., Bischof, H., Madreiter-Sokolowski, C.T., Kirsch, A., Depaoli, M.R., Frank, S., Schrammel, A., Mayer, B., Waldeck-Weiermair, M., Graier, W.F. & Malli, R. Intact mitochondrial Ca^{2+} uniport is essential for agonist-induced activation of endothelial nitric oxide synthase (eNOS). *Free Radic Biol Med* **102,** 248–259 (2017).

5. Madreiter-Sokolowski, C. T., Klec, C., Parichatikanond, W., , Stryeck, S., Gottschalk, B., Pulido, S., Rost, R., Eroglu, E., Hofmann, N.A., Bondarenko, A.I., Madl, T., Waldeck-Weiermair, M., Malli, R. & Graier, W.F. PRMT1-mediated methylation of MICU1 determines the UCP2/3 dependency of mitochondrial Ca^{2+} uptake in immortalized cells. *Nat Comms* **7,** 12897 (2016).

6. Trenker, M., Malli, R., Fertschai, I., Levak-Frank, S. & Graier, W. F. Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca^{2+} uniport. *Nat. Cell Biol.* **9,** 445–452 (2007).

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New lipid mediators in gastrointestinal and respiratory diseases

Summary

Akos Heinemann & Rudolf Schicho, Institute of Experimental and Clinical Pharmacology, Medical University of Graz

Supervisors:	Prof. Dr. Akos Heinemann Prof. Dr. Rudolf Schicho
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

Bioactive lipids, such as eicosanoids derive from cell membranes and are generated via cellular activation or through lipid metabolism. They take part in fundamental biological processes, such as proliferation, apoptosis, migration, lymphocyte activation and trafficking, all of them important features of inflammation. Recent research has highlighted that they act as signaling molecules and transmitters in inflammatory processes. Among these lipids, prostaglandins, leukotrienes, and their metabolites, originate from arachidonic acid and represent one of the best investigated biologically active lipids that regulate inflammation through amplification or reduction.

Hypothesis and Objectives:

Lipolytic enzymes that produce arachidonic acid are therefore of high interest as drug targets. Using mice in which specific lipolytic enzymes have been knocked out, the role of new arachidonic acid-derived lipid mediators from will be investigated using *in vivo* models of gastrointestinal inflammation and cancer and in respiratory diseases such as asthma.

Methodology:

The content of lipids, inflammation markers, and the pathological status will be determined in colon and lung tissue of mice and human samples. Primary cell culture from colon and airway epithelium from wild-type and knock-out mice will be used to determine specific pathways associated with these lipid transmitters. Many of the lipids signal via GPCRs and could have an essential impact on homeostatic systems, such as the endocannabinoid system. The PhD candidate will quantify receptor expression by real-time PCR, Western blot, fluorescence microscopy and flow cytometry. Functional responses of monocytes, macrophages, monocyte-derived macrophages and neutrophils will be investigated in assays of shape change, integrin up-regulation, chemotaxis and Ca^{2+} signaling. Levels of PGD_2 and its metabolites will be assessed by HPLC-MS analysis.

References:

1. Kohnz RA, Nomura DK. Chemical approaches to therapeutically target the metabolism and signaling of the endocannabinoid 2-AG and eicosanoids. Chem Soc Rev. 2014 Oct 7;43(19):6859-69. doi: 10.1039/c4cs00047a.
2. Sturm, E. M., Radnai, B., Jandl, K., Stancic, A., Parzmair, G. P., Hogenauer, C., Kump, P., Wenzl, H., Petritsch, W., Pieber, T. R., Schuligoi, R., Marsche, G., Ferreiros, N., Heinemann, A. and Schicho, R., Opposing Roles of Prostaglandin D2 Receptors in Ulcerative Colitis. *J Immunol* 2014.

3. Frei RB, Luschnig P, Parzmair GP, Peinhaupt M, Schranz S, Fauland A, Wheelock CE, Heinemann A, Sturm EM. Cannabinoid receptor 2 augments eosinophil responsiveness and aggravates allergen-induced pulmonary inflammation in mice. Allergy. 2016 Jul;71(7):944-56. doi: 10.1111/all.12858.

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Role of PCK2 in the metabolic adaptation of lung cancer cells

Summary

Horst Olschewski & Katharina Leithner, Division of Pulmonology, Department of Internal Medicine, Medical University of Graz

Supervisors:	Dr. Katharina Leithner Prof. Dr. Horst Olschewski
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

The metabolism of cancer cells is re-programmed to support cell growth and survival. To this end, rapidly growing cancers, including lung cancer, utilize large amounts of glucose [1-3]. In cancer cells glucose is metabolized primarily by glycolysis [1-4]. Glycolytic intermediates are used for the generation of building blocks and NADPH (reduced nicotinamide adenine dinucleotide phosphate) required for cell growth and survival [1-4]. However, due to the aberrant vascular network and due to the high demand, the supply with oxygen and nutrients, like glucose, is often inadequate [5,6]. The mechanisms of adaptation of cancer cells to glucose depletion are poorly understood.

Recently, our group described a novel metabolic salvage pathway in glucose-depleted lung cancer cells, involving the key gluconeogenesis enzyme PEPCK (mitochondrial isoform, PCK2) [7]. PEPCK activity is present at significant levels in the liver, but also in the kidney and in brown and white adipose tissue. We and others found that the mitochondrial isoform of PEPCK, PCK2, is overexpressed in different non-hepatic cancers [7-9]. Silencing of PCK2 enhanced apoptosis and cell death under glucose deprivation in different cancer cell lines [7-9] and has recently been shown to reduce lung cancer xenograft growth in a subcutaneous model *in vivo* [9]. The metabolic function of PCK2 and the mechanisms of enhanced survival of cancer cells expressing PCK2 in nutrient-deprived cancer cells remain unclear.

Hypothesis and Objectives:

Our recent data provide evidence that PCK2 expression is associated with endoplasmic reticulum (ER) stress under glucose deprivation in a panel of lung cancer cell lines. We hypothesize that the PCK2 pathway provides biosynthetic intermediates that are required for the resolution of ER stress in glucose-deprived lung cancer cells. In order to elucidate the role of the ER stress response in the pro-survival effect of PCK2, we will assess the impact of PCK2 knockdown on ER stress, both, *in vitro* and in a subcutaneous *in vivo* lung cancer xenograft model using inducible expression of PCK2shRNA. We will identify the signaling nodes regulating PCK2 expression and their relation to ER stress. Moreover, we will analyze, which type of cell death (apoptosis, necroptosis, necrosis) is induced under conditions of PCK2 silencing. The underlying mechanisms will be studied, focusing on the different branches of the unfolded protein response that are activated as an adaptive response in cells following ER stress. Additionally, mitochondrial respiratory function will be assessed under glucose deprivation in the presence or absence of PCK2 knockdown. The study will help to elucidate basic mechanisms of cancer cell survival in a nutrient-limited microenvironment and thus might help to identify potential novel approaches to lung cancer therapy.

Methodology:

The PhD candidate will apply 2D and 3D cell culture methods and a subcutaneous *in vivo* lung cancer model. He or she will perform protein and mRNA expression analyses and use pharmacological, siRNA-mediated and shRNA-mediated inhibition of PCK2, ER stress regulators and cell death mediators. Apoptosis and survival will be assessed using detection of caspase-3-cleavage using FACS and propidium iodide, respectively. Colony forming capability of cells will be studied using standard methods, and proliferation will be asssed using the EdU and MTS assays. Mitochondrial respiration will be measured using the Seahorse Analyzer.

References:

1. Vander Heiden MG, Cantley LC, Thompson CB: Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009, 324(5930):1029-1033.

2. Schulze A, Harris AL: How cancer metabolism is tuned for proliferation and vulnerable to disruption. *Nature* 2012, 491(7424):364-373.

3. Cairns RA, Harris IS, Mak TW: Regulation of cancer cell metabolism. *Nat Rev Cancer* 2011, 11(2):85-95.

4. DeBerardinis RJ, Thompson CB: Cellular metabolism and disease: what do metabolic outliers teach us? *Cell* 2012, 148(6):1132-1144.

5. Vaupel P: Hypoxia and aggressive tumor phenotype: implications for therapy and prognosis. *Oncologist* 2008, 13 Suppl 3(1083-7159):21-26.

6. Cantor JR, Sabatini DM: Cancer cell metabolism: one hallmark, many faces. *Cancer Discov* 2012, 2(10):881-898.

7. Leithner K, Hrzenjak A, Trotzmuller M, Moustafa T, Kofeler HC, Wohlkoenig C, Stacher E, Lindenmann J, Harris AL, Olschewski A, Olschewski H: PCK2 activation mediates an adaptive response to glucose depletion in lung cancer. *Oncogene* 2015, 34(8):1044-1050.

8. Mendez-Lucas A, Hyrossova P, Novellasdemunt L, Vinals F, Perales JC: Mitochondrial Phosphoenolpyruvate Carboxykinase (PEPCK-M) Is a Pro-survival, Endoplasmic Reticulum (ER) Stress Response Gene Involved in Tumor Cell Adaptation to Nutrient Availability. *J Biol Chem* 2014, 289(32):22090-22102.

9. Vincent EE, Sergushichev A, Griss T, Gingras MC, Samborska B, Ntimbane T, Coelho PP, Blagih J, Raissi TC, Choiniere L, Bridon G, Loginicheva E, Flynn BR, Thomas EC, Tavare JM, Avizonis D, Pause A, Elder DJ, Artyomov MN, Jones RG: Mitochondrial Phosphoenolpyruvate Carboxykinase Regulates Metabolic Adaptation and Enables Glucose-Independent Tumor Growth. *Mol Cell* 2015, 60(2):195-207.

Nutritional control of p53 activity and its role in metabolic flexibility

Summary

Andreas Prokesch, Institute of Cell Biology, Histology and Embryology, Medical University of Graz

Supervisor:	PD Dr. Andreas Prokesch
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

The tumor suppressor p53 is a transcription factor activated in cancerous cells by a variety of stress signals such as DNA damage, oncogene activation, nutrient deprivation, and hypoxia. Once activated, the p53 pathway has a wide range of downstream effects among which cell death, cell cycle arrest, autophagy, and regulation of cellular metabolism are most prominent (1;2). While many functional aspects for the p53-mediated cellular stress response during tumorigenesis (and therefore in rapidly dividing cells) are by now well established, much less is known about the role of p53 in non-transformed, terminally differentiated cells. Targeting p53 has enormous potential in cancer therapy since it is mutated in more than 50% of all human tumors (3). More recently, data have emerged that place the p53 pathway in the center of the control of cancer metabolism showing that it is essential to re-program glucose and lipid homeostasis (4;5). These functions of p53 in energy metabolism are largely consistent with its role as a tumor suppressor, although some reports suggest pro-Warburg-effects of p53. Importantly, most studies focused on the metabolic effects of p53-ablation during malignant transformation of cells. At present, little research has been conducted on the role of p53 in post-mitotic cell types, the p53-mediated control mechanisms of substrate allocation in healthy tissues, and its consequences for systemic energy homeostasis and metabolic flexibility (the capacity of an organism to adapt utilization of nutrients to their availability).

In a recent publication (Prokesch et al., FASEB J, 2016 (6)) we showed that acute knock-out of p53 in mouse liver disturbs glycogen storage and leads to lipid accumulation in hepatocytes. Furthermore we showed that the p53 protein accumulates in hepatocytes under starvation. Accordingly, in the acute liver knock-out model amino acid and glucose homeostasis is disrupted. These and preliminary data in other tissues suggest that, beyond its role as tumor suppressor, p53 plays a role as metabolic regulator in post-mitotic cells and tissues.

Hypothesis and Objectives:

With our preliminary data as foundation we recently acquired an FWF-DACH grant (lead applicant Andreas Prokesch). This project is a collaboration with the Charite in Berlin (Prof. Michael Schupp) and the German Institute for Nutritional Research (Prof. Tim Schulz). In this consortium we will utilize novel tissue-specific, inducible knock-out mouse models to investigate the role of p53 in the metabolism of liver, white adipose tissue, brown adipose tissue, and skeletal muscle.

The PhD student working in Graz will be focused on (i) elucidating the upstream mechanisms of starvation-induced p53 stabilization (Aim 1 of the proposal) and (ii) work on the liver- and white adipose tissue (WAT)-specific aspects of the project (aims 2 and 3 of the proposal). For the upstream mechanism we hypothesize regulation by the cellular energy sensor AMPK. This was already shown in cultured cells (Prokesch et al., FASEB J, 2016 (6)) and will be investigated in vivo by the PhD student. Another mechanism we will investigate is specific MDM2 (the canonical endogenous p53 inhibitor) degradation under starvation. As proposed p53 downstream mechanisms in WAT and liver we recently obtained data, suggesting a regulation of autophagy (and potentially lipophagy) by p53 under starvation. Therefore, the PhD student will employ autophagy-related assays to the used model systems.

Methodology:

In addition to classical cell culture work (protein overexpression, Crispr/cas9, RNAi, proliferation assays) followed by downstream analyses such as western blot, qPCR, cell cycle analyses, the student will perform immunoprecip-

itation assays followed by mass spectrometry (collaboration with Ruth Birner-Grünberger) to elucidate p53 interaction partner dynamics upon starvation. In the mouse models the student will perform metabolic phenotyping (e.g. metabolic cages (collaboration with Dagmar Kratky), insulin and glucose tolerance tests) and will prepare RNA-seq libraries (collaboration with Andrew Pospisilik, MPI, Freiburg) and samples for untargeted proteomics (collaboration with Christoph Magnes, Joanneum Research) from selected tissues. Primary cells derived from these mouse models will be used to measure mRNA and protein expression, oxygen consumption (Seahorse, collaboration with Wolfgang Graier), ROS production, and autophagy (LC3B lipidation, fluorescence microscopy, and electron microscopy).

References:

1. Vousden, K. H., Prives, C. (2009) Blinded by the Light: The Growing Complexity of p53. *Cell* **137,** 413-431
2. Junttila, M. R., Evan, G. I. (2009) p53--a Jack of all trades but master of none. *Nat.Rev.Cancer* **9,** 821-829
3. Muller, P. A., Vousden, K. H. (2014) Mutant p53 in cancer: new functions and therapeutic opportunities. *Cancer Cell* **25,** 304-317
4. Berkers, C. R., Maddocks, O. D., Cheung, E. C., Mor, I., Vousden, K. H. (2013) Metabolic regulation by p53 family members. *Cell Metab* **18,** 617-633
5. Vousden, K. H., Ryan, K. M. (2009) p53 and metabolism. *Nat.Rev.Cancer* **9,** 691-700
6. Prokesch, A., Graef, F. A., Madl, T., Kahlhofer, J., Heidenreich, S., Schumann, A., Moyschewitz, E., Pristoynik, P., Blaschitz, A., Knauer, M., Muenzner, M., Bogner-Strauss, J. G., Dohr, G., Schulz, T. J., Schupp, M. (2016) Liver p53 is stabilized upon starvation and required for amino acid catabolism and gluconeogenesis. *FASEB J.*

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The role of adhering maternal platelets on villous trophoblast

Summary

Martin Gauster, Institute of Cell Biology, Histology and Embryology, Medical University of Graz

Supervisor:	PD Dr. Martin Gauster
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

Previous immunostaining studies in early human placental tissues by Sato et al. detected platelets within maternal uterine blood vessels that contained so-called endovascular trophoblasts, which detach from placental villi and invade as extravillous trophoblasts (EVTs) into the maternal decidua [1]. In uterine arteries, which transport maternal blood into the intervillous space, platelets attached to the surface of endovascular trophoblasts or to vessel walls that were infiltrated by perivascular trophoblasts. In the same study the authors demonstrated that CD41+ platelets adhered to CD146+ EVTs and that most of the platelets expressed P-selectin on the cell surface, showing that they had been activated. Moreover, co-culture with platelets enhanced invasion of EVTs without affecting their secretion of matrix metalloproteinase-2 (MMP-2) or MMP-9. However, morphological observations suggested that platelet-derived soluble factors induced EVT differentiation toward the endovascular phenotype [1]. Indeed, platelet-derived factors such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF), which are released upon platelet activation, enhanced trophoblast invasion [2,3].

In contrast to EVT, the surface of human syncytiotrophoblast in principle is not considered to induce aggregation of maternal platelets, whereas adherence of the latter to perivillous fibrinoid is a regular finding in normal term placenta. Accordingly, maternal platelet aggregates were described on villous surface of dually perfused human term placenta cotyledons. We have recently shown maternal platelets on villous explant cultures from human first trimester placenta, indicating that adherence of maternal platelets to villous surface is a common process even in very early stages of gestation [4]. ELISA analysis of placental explant homogenates and corresponding conditioned culture media revealed considerable levels of well-described platelet-derived factors chemokine (C-C motif) ligand 5 (CCL5) and chemokine (C-X-C motif) ligand 4 (CXCL4). Since neither CCL5 nor CXCL4 was detected in the villous trophoblast layer, adhering platelets seemed to be the only source for both chemokines detected in conditioned culture media [4].

Hypothesis and Objectives:

This project will test the hypothesis whether or not maternal platelets adhere to perivillous fibrinoid, in areas where initial mechanical injury to the villous trophoblastlayer had occurred. While this may happen very early in human pregnancy, the relative proportion of adhering maternal platelets may increase over gestation and could correlate with the degree of perivillous fibrinoid deposition. Adherence of platelets may be followed by degranulation and release of granule-stored factors, which in turn could act not only on villous cytotrophoblast and syncytiotrophoblast, but may also activate passing maternal peripheral blood mononuclear cells (PBMCs). In pregnancies complicated by preeclampsia, the incidence of perivillous fibrinoid and adhering maternal platelets may be increased. Enhanced release of platelet-derived factors and activation of maternal PBMCs may overall contribute to a proinflammatory microenvironment in the intervillous space.

In order to test this hypothesis, the project will analyze how early in human pregnancy adhering maternal platelets can be detected on placental villous surface. Moreover, the relative proportion of adhering maternal platelets and a correlation between perivillous fibrinoid and maternal adhering platelets will be analyzed at different stages of pregnancy. Furthermore, the extent of factors released from adhering platelets will be analyzed in early and term villous placenta under basal conditions as well as after platelet agonist and antagonist treatment. Additionally, effects of platelet-derived factors on villous trophoblast viability, proliferation, syncytialization (cell fusion) and endocrine function will be studied. A translational work package of the project will determine the relative proportion of adhering maternal platelets and the release of platelet-derived factors in early-onset preeclampsia placenta.

Methodology:

The PhD candidate will perform immunohistochemistry with subsequent software based image segmentation and quantitative morphometrical analysis of stained histological structures. Moreover, the student will perform cell- and tissue (explant) culture and will analyze expression and released inflammatory mediators by qPCR and ELISA.

References:

[1] Sato Y, Fujiwara H, Zeng BX, Higuchi T, Yoshioka S, Fujii S,. Platelet-derived soluble factors induce human extravillous trophoblast migration and differentiation: platelets are a possible regulator of trophoblast infiltration into maternal spiral arteries. Blood 2005; 106:428-435.

[2] Bass KE, Morrish D, Roth I, Bhardwaj D, Taylor R, Zhou Y, Fisher SJ,. Human cytotrophoblast invasion is up-regulated by epidermal growth factor: evidence that paracrine factors modify this process. Dev.Biol. 1994; 164:550-561.

[3] Lash GE, Warren AY, Underwood S, Baker PN,. Vascular endothelial growth factor is a chemoattractant for trophoblast cells. Placenta 2003; 24:549-556.

[4] Blaschitz A, Siwetz M, Schlenke P, Gauster M,. Adhering maternal platelets can contribute to the cytokine and chemokine cocktail released by human first trimester villous placenta. Placenta 2015; 36:1333-1336.

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The role of micro-RNAs in the development of RAS-induced chronic myelomonocytic leukemia

Summary

Armin Zebisch, Division of Hematology, Medical University of Graz

Supervisor:	Prof. Dr. Armin Zebisch
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

Chronic myelomonocytic leukemia (CMML) is a clonal disorder of hematopoietic stem and progenitor cells (HSPCs). HSPCs of CMML patients are characterized by increased proliferation on the one hand and by increased differentiation into the myelomonocytic lineage on the other hand. Unfortunately, the prognosis of CMML patients is still dismal, with many cases rapidly transforming into acute myeloid leukemia (AML) and with effective therapeutic options still missing [1].

The RAS proto-oncogenes relay growth-promoting signals from the cell surface to a variety of intracellular signalling cascades. Somatic mutations within two RAS isoforms, NRAS and KRAS, are frequently detected in CMML and induce a CMML-like disorder in a murine in-vivo model. Unfortunately, however, therapeutic approaches directly targeting RAS have failed, which is most likely due to the complexity of RAS activation on the one hand and the fact that different RAS isoforms can induce distinct signalling profiles even resulting in unique disease phenotypes on the other hand. Therefore, scientific attention has recently turned toward inhibition of RAS effectors instead [1].

Micro-RNAs (miRNAs) are small, non-coding RNAs and play a key role in regulation of gene expression. They bind to the 3'UTR of their target genes, thereby causing either inhibition of translation or mRNA decay. Aberrant expression of miRNAs can be caused by the deregulation of oncogenes and tumor-suppressors, respectively, and has been described in a wide range of human malignancies. Furthermore, aberrantly expressed miRNAs have been shown to play a central role in mediating malignant transformation and emerged as attractive targets for cancer therapy [2].

Hypothesis:

We hypothesize that oncogenic RAS mutations induce aberrant expression profiles of specific miRNAs. Furthermore, we hypothesize that deregulation of these miRNAs in the hematopoietic system is involved in the development of CMML.

Project objectives and methodology:

In preliminary experiments, we isolated HSPCs from a transgenic mouse model, where somatic induction of mutant KRAS (Mx1-Cre KRASG12D) causes a CMML-like disease [1, 3]. By analyzing HSPCs of KRAS mutated animals with miRNA arrays, and by comparing the results to their wild-type littermates, we were able to identify a specific, KRAS induced miRNA expression profile.

In the first part of the thesis, the student will validate the aberrant expression of selected miRNA candidates by quantitative real-time PCR (qPCR). In a next step, the student will isolate HSPCs from a transgenic mouse model, where CMML is caused by somatic induction of mutant NRAS (Mx1-Cre NRASG12D) [1, 3]. By qPCR based expression analysis, the student will be able to identify miRNAs, which are equally regulated by mutations in both NRAS and KRAS, and which therefore display interesting candidates for novel CMML therapies targeting RAS mutated cases. Consequently, qPCR expression analysis of these candidates will be extended to primary CMML patient specimens with and without mutations in RAS [4, 5].

In a second part of the thesis, the functional relevance of qPCR validated miRNAs for mediating the CMML hallmarks proliferation and myelomonocytic differentiation, respectively, will be tested. Therefore, miRNA overexpression, achieved by lentiviral transduction of miRNA mimics, as well as miRNA knockdown, achieved by lentiviral transduction of miRNA inhibitors, will be performed in human and murine HSPCs, as well as in a series of leukemic cell

lines carrying either mutant or wildtype RAS. Subsequently, these cells will be subjected to growth, apoptosis/cell-cycle and proliferation assays on the one hand, as well as to classical granulocyte macrophage colony-stimulating factor (GM-SCF) induced myelomonocytic differentiation assays on the other hand [4, 5]. These experiments will enable identification of RAS-regulated miRNAs, that are truly involved in causing CMML like features in HSPC, and which therefore display the most promising candidates for therapeutic interventions.

In a final part of the thesis, the student will study functionally relevant miRNAs in more detail and will aim to delineate specifically regulated miRNA-target genes, which are ultimately needed to mediate the aberrant HSPC functions during CMML development. Therefore, mRNA arrays and qPCR based validation will be employed as described above to validate the most promising candidate genes. In-silico target prediction tools as well as in-vitro target validation assays will be used to select candidates [5], which are indeed regulated by the respective miRNAs. Finally, the student will aim to rescue the biologic effects caused by aberrant miRNA expression by restoring the expression levels of their putative target genes by using the functional assays described above.

References:

1. Cytogenetic and molecular abnormalities in chronic myelomonocytic leukemia. Patnaik MM, et al. Tefferi A. **Blood Cancer J** 2016 Feb 5; 6: e393.
2. Therapeutic Resistance in Acute Myeloid Leukemia: The Role of Non-Coding RNAs. Zebisch A, et al. **Int J Mol Sci** 2016 Dec 10;17(12). pii: E2080.
3. Hematopoiesis and leukemogenesis in mice expressing oncogenic NrasG12D from the endogenous locus. Li Q, et al. **Blood** 2011 Feb 10;117(6):2022-32.
4. Increased Expression of miR-23a Mediates a Loss of Expression in the RAF Kinase Inhibitor Protein RKIP. Hatzl S, et al. **Cancer Res**. 2016 Jun 15;76(12):3644-54.
5. Frequent loss of RAF kinase inhibitor protein expression in acute myeloid leukemia. Zebisch A, et al. **Leukemia**. 2012 Aug;26(8):1842-9.

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The role of Archaea in the human body

Summary

Christine Moissl-Eichinger, Department of Internal Medicine, Medical University of Graz

Supervisor:	Prof. Dr. Christine Moissl-Eichinger
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

Trillions (10^{14}) of microbes live in and on the human body, forming the human microbial community. These complex communities contain taxa from all three domains of life (bacteria, eukaryotes and archaea) as well as viruses.

Scientists have shown that microorganisms associated with the human body play an important role in health maintenance (Morgan et al., 2013). Microbes help in energy harvest and storage, and have a variety of metabolic functions such as fermentation and absorbing undigested carbohydrates; they interact with the immune system and support the development of normal immune functions (Flint et al., 2012; Round and Mazmanian, 2009).

A special group of microorganisms, the archaea, have been poorly studied in relation to the human body. Archaea have been identified in the gastro-intestinal tract, the oral cavity and on the human skin (Dridi et al., 2011; Probst et al., 2013). The dominant archaea in the gastro-intestinal tract are the methanogens, representing almost 10% of all anaerobic microorganisms (Dridi et al., 2009). Methanogens are part of the commensal microbiota and form stable colonization within the human body and are often in syntrophic relationships with other bacteria. These microbes reduce the metabolic products resulted during fermentation leading to an increased efficiency of bacterial fermentation. Methanogens produce methane under anaerobic conditions by using bacterial fermentation products such as hydrogen, carbon dioxide, methanol, acetate and methylamines (Bang and Schmitz, 2015). These microbes are thought to be “key stone” species influencing the community composition and function, by keeping the hydrogen partial pressure at low levels. Much less is known on the human skin archaea, which belong to the thaumarchaeal phylum, and could be involved in skin surface ammonia turnover (Probst et al., 2013), or associated halophilic archaea (Khelaifia et al., 2016), that were even found in human milk samples (Jimenez et al., 2015).

Hypothesis and Objectives:

Hypotheses: *Archaea are normal components of the human microbiota. They are closely interacting with a number of different Bacteria. Archaea play a metabolic key role and their metabolic products can influence the host.*

Objectives:

- Visualization and quantification of Archaea in gut, oral and skin samples
- Detection of Archaea-colocalized and potential syntrophic bacteria
- Cultivation of human-associated Archaea
- Functional analysis of human-associated Archaea in the host system and in cultures

Methodology:

Visualization and quantification will be based on quantitative PCR, 16S rRNA gene sequencing, immunostaining techniques, fluorescence in situ hybridization. Detection of colocalized Bacteria will be based on immuno-capturing, FACS (fluorescence activated cell sorting) and sequencing. Cultivation will be done using our anoxic cultivation facilities (glove box and gas station). Functional analyses will/can be based on stable isotope probing, neuroimaging (in collaboration), transcriptomics and methane detection (gas chromatography).

References:

Bang, C., and Schmitz, R. A. (2015). Archaea associated with human surfaces: Not to be underestimated. *FEMS Microbiol. Rev.* 39, 631–648. doi:10.1093/femsre/fuv010.

Dridi, B., Raoult, D., and Drancourt, M. (2011). Archaea as emerging organisms in complex human microbiomes. *Anaerobe* 17, 56–63. doi:10.1016/j.anaerobe.2011.03.001.

Dridi, B., Henry, M., El Khéchine, A., Raoult, D., and Drancourt, M. (2009). High prevalence of Methanobrevibacter smithii and Methanosphaera stadtmanae detected in the human gut using an improved DNA detection protocol. *PLoS One* 4, e7063. doi:10.1371/journal.pone.0007063.

Flint, H. J., Scott, K. P., Louis, P., and Duncan, S. H. (2012). The role of the gut microbiota in nutrition and health. *Nat. Rev. Gastroenterol. Hepatol.* 9, 577–89. doi:10.1038/nrgastro.2012.156.

Jimenez, E. et al., (2015). Metagenomic analysis of milk of healthy and mastitis-suffering women. Journal of human lactation 31, 406-415.

Khelaifia, S., and Raoult, D. (2016). Haloferax massiliensis sp. nov. the first human-associated halophilic archaea. New Microbes and New Infections 12, 96–98.

Morgan, X. C., Segata, N., and Huttenhower, C. (2013). Biodiversity and functional genomics in the human microbiome. *Trends Genet.* 29, 51–58. doi:10.1016/j.tig.2012.09.005.

Probst, A. J., Auerbach, A. K., and Moissl-Eichinger, C. (2013). Archaea on Human Skin. *PLoS One* 8, e65388. doi:10.1371/journal.pone.0065388.

Round, J. L., and Mazmanian, S. K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* 9, 313–23. doi:10.1038/nri2515.

The Mitochondrial Unfolded Protein Response (mitoUPR) and its Implication on the Metabolic Flexibility of Cancer Cells

Summary

Roland Malli, Institute of Molecular Biology and Biochemistry, Medical University of Graz

Supervisor:	Prof. Dr. Roland Malli
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

An accumulation of unfolded proteins within mitochondria is known to trigger a complex signaling cascade. As a consequence of this mitochondrial unfolded protein response (mitoUPR) the nuclear transcription activity is affected in order to restore homeostasis [1]. This can be achived by upregulating the expression of mitochondrial chaperons, proteases, components of the mitochondrial protein import machinery, and proteins involved in ROS metabolism. If the restoration of homeostasis fails, mitophagy is induced to clear the cell from dysfunctional mitochondria. Moreover, mitoUPR significantely contributes to mitohormetic responses that might counteract cellular aging [2]. However, little is known under which conditions unfolded proteins accumulate within mitochondria and whether or not the mitoUPR is determinant for the metabolic flexibility of cells.

Hypothesis and Objectives:

We hypothesise that mitohormesis and mitoUPR are fundamental for mitochondria to preserver their capability to sense and respond to alterations of the substrate availability in order to meet the energy demand of a cell. This metabolic flexibility might be of high relevance for cancer cells to maintain high proliferation rates and cell growth. The main objectives of this project are: 1st to define stimuli and stresses that initiate the mitoUPR, 2nd to understand the molecular pathways and signaling molecules of the mitoUPR machinery, and 3rd to further investigate respective conseqeunces of the mitoUPR for the energy metabolism of (cancer) cells. Eventually, the putative role of mitoUPR for tumorigenesis and cancer cell survival should be investigated to come up with novel strategies for the treatment of cancer.

Methodology:

In addition to classical cell culture, biochemistry- (WB) and molecular biology techniques (PCR) the Ph.D. candidate will develop novel genetically encoded tools and probes [3,4] and use state-of-the-art fluorescence imaging techniques [3,4] to visualize mitochondrial signaling events and metabolic activities in real-time on the level of individual cells.

References:

1. Mark W. Pellegrino, Amrita M. Nargund, and Cole M. Haynes, Signaling the Mitochondrial Unfolded Protein Response *Biochim Biophys Acta., February ; 1833(2), 2013*
2. Jeanho Yun and Toren Finke, Mitohormesis, Cell Metabolism 19, May 6, 757-766, 2014
3. Eroglu, E., Gottschalk, B., Charoensin, S., Blass, S., Bischof, H., Rost, R., Madreiter-Sokolowski, C.T., Pelzmann, B., Bernhart, E., Sattler, W., Hallström, S., Malinski, T., Waldeck-Weiermair, M., Graier, W.F. & Malli, R. Development of novel FP-based probes for live-cell imaging of nitric oxide dynamics. Nat. Commun. 7: 10623, 2016

4. Madreiter-Sokolowski, C.T., Klec, C., Parichatikanond, W., , Stryeck, S., Gottschalk, B., Pulido, S., Rost, R., Eroglu, E., Hofmann, N.A., Bondarenko, A.I., Madl, T., Waldeck-Weiermair, M., Malli, R. & Graier, W.F. PRMT1-mediated methylation of MICU1 determines the UCP2/3-dependency of mitochondrial Ca^{2+} uptake in immortalized cells. Nat. Commun. 7:12897, 2016

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Deciphering the nucleosome code from the peripheral blood to non-invasively establish cancer associated gene expression patterns

Summary

Ellen Heitzer, Institute of Human Genetics, Medical University of Graz

Supervisor:	Prof. Dr. Ellen Heitzer
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

The analysis of ctDNA (cell-free circulating tumor DNA) is a very promising tool and might revolutionize cancer care with respect to early detection, identification of minimal residual disease, assessment of treatment response, and monitoring tumor evolution. ctDNA analyses, often referred to as “liquid biopsy” offers what tissue biopsies cannot - a continuous monitoring of tumor-specific changes during the entire course of the disease (1). Due to technological improvements, efforts for the establishment of pre-analytical and analytical benchmark, and the inclusion of ctDNA analyses in clinical trial, an actual clinical implementation has come within easy reach. Compared to the number of studies addressing the clinical applicability of ctDNA, data regarding the actual origin, the kinetics, and the mechanisms of release and clearance are limited and often contradictory. Cell-free circulating DNA (cfDNA) is not only found in cancer patients but also in healthy individuals, although elevated levels have been reported in cancer patients (1, 2), suggesting that the release of DNA is in principle a physiological process. cfDNA in blood is double-stranded (3) and forms a specific ladder pattern known from apoptotic cells ranging from 60 to 1,000 bp (4). Several groups have shown that plasma DNA molecules showed a predictable fragmentation pattern reminiscent of nuclease-cleaved nucleosomes, which is typically observed from apoptotic cells (5, 6). In a recent study it was shown that cfDNA is the detritus of cell death and that nucleosome phasing is reflected in the fragmentation pattern of cfDNA and furthermore, that the fragmentation patterns of cfDNA might contain evidence of the epigenetic landscape of their tissue(s)-of-origin (7). These and other data suggest that cfDNA patterning reflects a general picture of gene expression and that mapping and mining of cfDNA fragment ends may aid in the development of novel biomarkers reflecting pathological changes in chromatin marks independent of genotypic differences between contributing cell types (7, 8). Our group investigated whether plasma DNA is able to reflect expression-specific nucleosome occupancy at promoters. Furthermore, we assessed if plasma DNA possesses the sensitivity and accuracy to predict whether genes are expressed or not, and furthermore, we determined if blood samples from patients with cancer are informative for expressed cancer driver genes. Our data suggests that read depth analyses of plasma DNA can reveal functional data such as the expression status of genes due to the nucleosome occupancy pattern at promoter regions and, furthermore, that even the expression status of cancer-related genes can be deduced from the blood of patients with cancer (9) .

Hypothesis and Objectives:

In our proof-of-principle study we developed an analysis pipeline, which appears to be robust and to provide accurate predictions about the expression status of genes from plasma read-depth analysis (9). We have shown that plasma DNA read-depth analyses allow the identification of instrumental cancer driver genes in high-level focal amplifications. Nevertheless, further improvements in bioinformatics analyses might propel the power of none-invasive gene expression prediction from peripheral blood. Hence, we want to scrutinize the decisive parameters and their combinations to develop optimal algorithms for gene expression analyses based on plasma DNA nucleosome occupancy patterns at promoter regions. Moreover, methodological aspects with respect to library preparation should to be optimized. Using the optimized protocols and algorithms we intend to identify and characterize cancer driver genes in high-level focal amplifications, since the stability/ consistency of gene expression patterns in focal amplifications is yet unknown and has not been followed over time.

The student will optimize and develop our analysis algorithms starting from library preparation to bioinformatic analyses. Therefore, knowledge of Linux and programming skills are beneficial. Moreover, clinical samples will be processed in order to investigate gene expression patterns of cancer driver genes over time and possibly identify new mechanisms of resistance against targeted therapies.

Specific aims are:

1. To further optimize library preparation and the gene expression prediction algorithms.
2. To identify cancer driver genes in high-amplitude focal amplifications.
3. To gain insight into the consistency of gene expression patterns within a focal amplification over time.

Methodology:

The PhD candidate will employ next generation sequencing based methodologies, such as whole genome sequencing, bisulfite sequencing, and RNA-Seq in order to characterize gene expression patterns in plasma DNA samples from cancer patients. Moreover, the candidate will make use of bioinformatic approaches such as read depth analysis, pathway analysis, or machine learning technologies.

References:

1. Heitzer E, Ulz P, Geigl JB. Circulating tumor DNA as a liquid biopsy for cancer. Clinical chemistry 2015;61:112-23.
2. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Science translational medicine 2014;6:224ra24.
3. Anker P, Stroun M, Maurice PA. Spontaneous release of DNA by human blood lymphocytes as shown in an in vitro system. Cancer Res 1975;35:2375-82.
4. Nagata S, Nagase H, Kawane K, Mukae N, Fukuyama H. Degradation of chromosomal DNA during apoptosis. Cell death and differentiation 2003;10:108-16.
5. Heitzer E, Auer M, Hoffmann EM, Pichler M, Gasch C, Ulz P, et al. Establishment of tumor-specific copy number alterations from plasma DNA of patients with cancer. International journal of cancer Journal international du cancer 2013;133:346-56.
6. Lo YM, Chan KC, Sun H, Chen EZ, Jiang P, Lun FM, et al. Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. Science translational medicine 2010;2:61ra91.
7. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. Cell-free DNA comprises an in vivo nucleosome footprint that informs its tissues-of-origin. Cell 2016;164:57-68.
8. Ivanov M, Baranova A, Butler T, Spellman P, Mileyko V. Non-random fragmentation patterns in circulating cell-free DNA reflect epigenetic regulation. BMC genomics 2015;16 Suppl 13:S1.
9. Ulz P, Thallinger GG, Auer M, Graf R, Kashofer K, Jahn SW, et al. Inferring expressed genes by whole-genome sequencing of plasma DNA. Nature genetics 2016;48:1273-8.

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The Role of the FGF23-Klotho Axis in Uremic Media Calcification

Summary

Philipp Eller, Institute of Internal Medicine, Intensive Care Unit, Medical University of Graz

Supervisor:	Prof. Dr. Philipp Eller
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

The massive burden of cardiovascular disease in chronic kidney disease and diabetes mellitus is strongly associated with extensive media calcification, reduced vascular compliance, left ventricular hypertrophy, and sudden cardiac death. Media sclerosis and media calcification are regulated by a complex interaction of systemic and local triggers of vascular calcification such as hyperphosphatemia and hyperglycemia, but also critically dependent on diverse physiological protectors from vascular calcification such as fetuin A or vitamin K [1-4]. These triggers and protectors modulate the phenotype of vascular smooth muscle cells, which they are not terminally differentiated cells. In this manner they can eventually react to stress, inflammation or injury by transdifferentiating from contractile to proliferative and/or osteoblastic phenotypes. FGF23 has also been implicated to play a key role in the development of uremic media calcification, since it is upregulated by phosphate intake and leads to hyperphosphaturia by binding to Klotho on tubular epithelial cells. Increased FGF23 levels have been detected in chronic kidney disease (CKD) patients. They increase with the CKD stage and correlate significantly with vessel calcification and cardiovascular mortality.

Hypothesis and Objectives:

We postulate that the FGF23-Klotho axis plays a central role in the phenotypic modulation of vascular smooth muscle cells. Preliminary data from our lab indicate that FGF23 signalling is not only essential for phosphorus hemostasis, but also an important trigger for inflammation in chronic kidney disease. The main objective of this project is to analyse the diverse effects of the FGF23 signalling and to interfere with its various receptors in order to prevent chronic inflammation and concomitant media calcification.

Methodology:

The PhD candidate will learn how to induce and to evaluate an *in vivo* murine model of uremic media calcification using histology, molecular biology, mass spectrometry and vascular wire myography, respectively [1-3]. The FGF23-Klotho Axis will be evaluated in vivo by using blocking antibodies. The PhD student will furthermore perform primary cell culture experiments and investigate the role of FGF23 –Klotho in these cells by performing knock-down experiments. Ultimately we aim to modulate the vascular smooth muscle cell behaviour and thus prevent/treat media sclerosis and media calcification that are associated with heavy burden of morbidity and mortality in patients suffering from diabetes mellitus or end-stage renal disease.

References:

1. Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. Eller K, Kirsch A, Wolf AM, Sopper S, Tagwerker A, Stanzl U, Wolf D, Patsch W, Rosenkranz AR, Eller P. Diabetes. 2011 60(11):2954-62.
2. Regulatory T cells improve nephrocalcinosis but not dystrophic cardiac calcinosis in DBA/2 mice. Kirsch AH, Smaczny N, Riegelbauer V, Sedej S, Hofmeister A, Stojakovic T, Goessler W, Brodmann M, Pilger E, Rosenkranz AR, Eller K, Eller P. Am J Pathol. 2013 183(2):382-90.

3. Heterogeneous susceptibility for uremic media calcification and concomitant inflammation within the arterial tree. Kirsch AH, Kirsch A, Artinger K, Schabhüttl C, Goessler W, Klymiuk I, Gülly C, Fritz G, Frank S, Wimmer R, Brodmann M, Anders HJ, Pramstaller P, Rosenkranz AR, Eller K, Eller P. Nephrol Dial Transpl 2015 30(12):1995-2005.
4. Fibroblast growth factor 23 directly targets hepatocytes to promote inflammation in chronic kidney disease. Singh S, Grabner A, Yanucil C, Schramm K, Czaya B, Krick S, Czaja MJ, Bartz R, Abraham R, Di Marco GS, Brand M, Wolf M, Faul C. Kidney Int. 2016 Nov;90(5):985-996.

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Novel methodological and biomarker driven approaches to cardiovascular risk prediction

Summary

Harald Sourij, Division of Endocrinology and Metabolism, Medical University of Graz

Supervisor:	Prof. Dr. Harald Sourij
Availability:	This position has been occupied.
Offered by:	CBmed
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

Cardiovascular disease (CVD) encompasses a group of disorders of the heart and blood vessels, including coronary heart, cerebrovascular, peripheral artery disease or deep vein thrombosis and pulmonary embolism (1). Although the mortality rates of CVD decreased substantially over the last decade, still almost a half of all deaths are attributable to CVD (2).
As a tool for risk estimation, various risk scores are used, such as the Framingham Risk Score, the United Kingdom Prospective Diabetes Study Risk Engine, the New Zealand Cardiovascular Risk Score, to name just a few of them. However, all these scores use established metabolic diseases, liver diseases and classical risk factor (e.g. smoking, hypertension, lipids) and it is known that they are able to explain only part of the risk of a particular person. (3)
Moreover, all current CVD risk models have one major inherent limitation, being that the predicted risk is based on certain values of various risk factors at a particular time point only, irrespective of previous values, trend over time, treatment adaptions et cetera. From pathophysiologic point of view atherosclerosis and hence CVD is a chronic progressive disease and exposure time to various CVD risk factors plays a fundamental role in this concept.
Early detection and better risk assessment with biomarkers for cardiovascular disease and embedding them into improved risk prediction models may help to identify subjects at increased risk for cardiovascular disease, events and death.

Hypothesis and Objectives:

My research group is currently involved in a series of patient registries including in depth clinical phenotyping and biobanking which will be used for cardiovascular biomarker identification. Recently we have investigated several potential biomarker candidates such as the global arginine bioavailabitly ratio, serum free light chain levels or trimethylamin-N-oxidase levels, which will be investigated further. The PhD student will (i) validate current candidate biomarkers in other patient cohorts, (ii) determine an analyse novel CV biomarker candidates in the Graz Diabetes Registry for Biomarker Research and the BioPersMed cohort. In addition a strong focus will be on the development of novel risk prediction models taking longitudinal risk factor data into account (by using data from large scale trials). This work will be done in joint collaboration with leading statisticians in this field.

Methodology:

Biomarker identification will require the PhD student to work with ELISAs, flow cytometry and to assist in metabolomics analyses. The student will work on systemic reviews and acquire advance statistical skills in biomarker identification using clinical, lab and metabolomics data and by developing risk prediction models.

References:

1. WHO International. Definition of cardiovascular diseases. 2014; 2014

2. Nichols M, Townsend N, Scarborough P, Rayner M. Trends in age-specific coronary heart disease mortality in the european union over three decades: 1980-2009. *Eur Heart J*. 2013;34:3017-3027

3. Magnus P, Beaglehole R. The real contribution of the major risk factors to the coronary epidemics: time to end the “only- 50%” myth. Arch Intern Med 2001; 161:

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Novel biomarker delineating pathophysiology in human type 1 diabetes

Summary

Thomas Pieber, CBmed Center for Biomarker Research in Medicine, Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of Graz

Supervisor:	Prof. Dr. Thomas Pieber
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

Multiple causes are thought to be involved in the development and pathophysiology of type 1 diabetes (T1D). Prenatal genetic factors such as distinct human leukocyte antigen (HLA) variants, are suspected to play a role in the pathophysiology of T1D. Apart from genetic susceptibility, environmental factors such as increased hygiene, altered diet, increased obesity and less breastfeeding seem to play a role in this autoimmune process. But also altered lifestyle, causing less endogenous vitamin D production by decreased exposure to sunlight are suspected to have an impact on T1D development. Another hypothesis suggests enteroviral infections to trigger the onset of T1D. A combination of all these factors probably leads to the onset of auto-reactivity, including production of auto-antibodies by B-cells, activation of self-reactive T-cell clones and decreased immune regulation. The subsequent destruction of insulin-producing pancreatic β-cells finally leads to insulin deficiency and the clinical onset of T1D.

In recent years, alterations in the microflora, immune system and epithelial barrier function of the gastrointestinal tract are put forward as key components in T1D progression. The impact of the intestinal microflora on immune homeostasis in the gut but also at systemic sites has gained tremendous interest in the last few years. Vitamin D deficiency in early life accelerates T1D in diabetes-prone mice, while high-dose vitamin D inhibits disease development in mice and improves suppressor function of regulatory T cells in patients with T1D.

The intestinal microbiota has been shown to interact with and modulate host metabolism, nervous system and cells of the immune system. Immune cells of the duodenum act as a first defense line for foodborne pathogens, together with immune cells of the stomach. Hence, studying this interface between host and the intestinal microbiota is subject of intense research.

Hypothese and Objectives:

Studying whether vitamin D combined with the GLP1 analogue liraglutide can alter the course of T1D by modifying the gut microflora/metabolites, modulating the intestinal and peripheral immune system, and/or changing the epithelial barrier function is of great importance in our understanding of disease progression but also of mechanisms by which vitamin D and liraglutide can affect disease progression. Longitudinal analyses and mechanistic studies can identify the basis for gut microbiome and immune system modulation of T1D and identify biomarkers and promising therapeutics to prevent disease, delay or even reverse diabetes onset. In this regards, we hypothesize that administration of regular high doses of vitamin D combined with a short-course of liraglutide might be a promising approach to restore immune homeostasis in T1D. This project aims to discover disease related biomarkers in subjects exposed to combination therapy of vitamin D together with insulinotropic agents such as glucagon-like peptide 1 (GLP-1) agonists in the early course of the disease. Keyplayer in the immune system will be characterised by immuno-phenotyping and functional assays will be used for the discovery of new biomarkers. We hypothesize that vitamin D could serve as one possible agent in the design of immunomodulatory combination therapies for T1D. GLP-1-targeted therapies, known to stimulate insulin secretion, may also affect inflammatory and immune pathways involved in T1D.

Milestone first year:

Identification of biomarkers for gut barrier function and immune Response (e.g. Treg function). Validation of identified biomarkers.

Milestone second year:

Intervention in newly diagnosed type 1 diabetic patients in a RCT or case control study

Milestone third year:

Data Analysis, publication, finalization of thesis

Timeline:

Month 1-3: Literature Analysis

Month 4-6: Clinicial learning: pathophysiology of type 1 diabetes, state of the art therapy (intensified Insulin therapy, Patient education).

M 7-12: Screening, selection and Validation of gut barrier function test; Validation of Treg function test.

M 13-30: Development of clinical protocol and CRF, statistical Analysis plan, recruitment, and clinical strudy Performance.

M 31-36: Data Analysis, publication

Methodology:

Clinical Trial Performance according GCP Gut barieer function test Flow cytometry and cell culture Statistical Analysis of clinical trials

References

1. Prietl, B. et al. High-dose cholecalciferol supplementation significantly increases peripheral CD4(+) Tregs in healthy adults without negatively affecting the frequency of other immune cells. Eur. J. Nutr. 53, 751–759 (2014).

2. Treiber, G. et al. Cholecalciferol supplementation improves suppressive capacity of regulatory T-cells in young patients with new-onset type 1 diabetes mellitus — A randomized clinical trial. Clin. Immunol. 161, 217–224 (2015).

3. Tauschmann, M. et al. Distribution of CD4pos-, CD8pos- and Regulatory T Cells in the Upper and Lower Gastrointestinal Tract in Healthy Young Subjects. PlosOne 8, e80362 (2013).

4. Bashir, M. et al. Effects of high doses of vitamin D3 on mucosa-associated gut microbiome vary between regions of the human gastrointestinal tract. Eur. J. Nutr. (2015).

5. Miyara, M. et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity 30, 899–911 (2009).

6. Elkord, E., Abd, M., Samid, A. & Chaudhary, B. Helios , and not FoxP3 , is the marker of activated tregs expressing GARP / LAP. Oncotarget 6, 20026–20036 (2015).

7. Spanier, J. A., Nashold, F. E., Mayne, C. G., Nelson, C. D. & Hayes, C. E. Vitamin D and estrogen synergy in Vdr -expressing CD4 + T cells is essential to induce Helios + FoxP3 + T cells and prevent autoimmune demyelinating disease. J. Neuroimmunol. 286, 48–58 (2015).

8. Antonioli, L., Pacher, P., Vizi, E. S. & Haskó, G. CD39 and CD73 in immunity and inflammation. Trends Mol. Med. 19, 355–67 (2013).

9. Ohta, A. & Sitkovsky, M. Extracellular adenosine-mediated modulation of regulatory T cells. Front. Immunol. 5, 1–21 (2014).

10. Schmidt, A., Oberle, N. & Krammer, P. H. Molecular mechanisms of treg-mediated T cell suppression. Front. Immunol. 3, 1–37 (2012).

11. Mandapathil, M., Lang, S., Gorelik, E. & Whiteside, T. L. Isolation of functional human regulatory T cells (Treg) from the peripheral blood based on the CD39 expression. J. Immunol. Methods 346, 55–63 (2009).

12. Swainson, L. A., Mold, J. E., Bajpai, U. D. & Mccune, J. M. Expression of the Autoimmune Susceptibility Gene FcRL3 on Human Regulatory T Cells Is Associated with Dysfunction and High Levels of Programmed Cell Death-1. (2015). doi:10.4049/jimmunol.0903943

13. Dhuban, K. Bin et al. Coexpression of TIGIT and FCRL3 Identifies Helios + Human Memory Regulatory T Cells. J. Immunol. 194, 3687–3696 (2015).

14. Kochi, Y. et al. FCRL3, an autoimmune susceptibility gene, has inhibitory potential on B-cell receptor-mediated signaling. J. Immunol. 183, 5502–5510 (2009).

15. Bajpai, U. D. et al. A functional variant in FCRL3 is associated with higher FcRL3 expression on T cell subsets and rheumatoid arthritis disease activity. Arthritis Rheum 64, 2451–2459 (2012).

16. Harms, R. Z., Lorenzo, K. M., Corley, K. P. & Cabrera, M. S. Altered CD161 bright CD8 + Mucosal Associated Invariant T (MAIT) -Like Cell Dynamics and Increased Differentiation States among Juvenile Type 1 Diabetics. PLoS One 10, e0117335 (2015).

17. Maggi, L. et al. CD161 is a marker of all human IL-17-producing T-cell subsets and is induced by RORC. Eur. J. Immunol. 40, 2174–2181 (2010).

18. Mikulkova, Z. et al. Numerical defects in CD8+CD28- T-suppressor lymphocyte population in patients with type 1 diabetes mellitus and multiple sclerosis. Cell. Immunol. 262, 75–79 (2010).

19. Filaci, G. et al. CD8+ CD28- T regulatory lymphocytes inhibiting T cell proliferative and cytotoxic functions infiltrate human cancers. J. Immunol. 179, 4323–4334 (2007).

20. Gagliani, N. et al. Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. Nat. Med. 19, 739–746 (2014).

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Phenotype-related information extraction from routine data for biomarker research

Summary

Stefan Schulz, Institute for Medical Informatics, Statistics and Dokumentation, Medical Universität of Graz

Supervisor:	Prof. Dr. Stefan Schulz
Availability:	This position has been occupied.
Offered by:	CBmed
Application deadline:	Applications are accepted between February 01, 2017 00:00 and March 19, 2017 23:59 (Europe/Zurich)

Description

Background:

One grand challenge in biomarker research is to complete the picture of information about potential biomarkers using a broad range of clinical data sources. This project addresses the reuse of documented information on clinical phenotypes, past diseases, findings, procedures, lifestyle data, drugs, family history, etc. As most of this routine data resides in free-text narratives such as findings reports and low-structured patient summaries in electronic health record systems, a semantic data extraction platform for the optimized retrieval of biobank samples and a customizable toolkit for content retrieval is currently being built. An important use case is the optimized retrieval of biobank samples, such as needed for biomarker research, which requires information about the clinical context of the patient from which the samples had been taken. This data requires a thoughtful selection of important features like diseases, signs, symptoms, therapies, clinical evolution and laboratory parameters, altogether put into a temporal context, for the best ranking on a cohort search engine.

Hypothesis and Objectives:

The hypothesis is that there is a common core of information needs for biomarker research that can only be addressed by either labour-intensive manual reworking of routine data or by machine processing of the EHR. The objective is to lay the foundations for the development of a customizable toolkit for content extraction. Existing approaches like i2b2 will be capitalized on, as well as existing terminologies and data models (SNOMED CT, clinical models).

Principal objectives are to develop, customize and assess components of a processing pipeline that takes raw clinical texts as they are and enriches them by semantic metadata. The specific tasks are manifold: identification of short forms (abbreviations, acronyms), correction of misspellings, identification of attribute – number – unit triples, identification of temporal contexts, identification of epistemic contexts (diagnostic (un)certainty, intentions), negations. Main purpose of these processing steps are to accurately map textual content to clinical terminologies (SNOMED CT, LOINC, ICD) and pre-defined information models, which could affect, for example, how a patient is ranked on a cohort search engine.

Methodology:

Diverse computational linguistics methods will be used (rule-based, machine learning-based), together with different information retrieval approaches. The student should therefore have a background in computer science, with a focus on computational linguistics and text mining. Familiarity with the medical domain, as well as with medical ontologies and terminologies is desirable.

References:

1. Patterson O., Igo S., and Hurdle J. F. Automatic acquisition of sublanguage semantic schema: Towards the word sense disambiguation of clinical narratives. In AMIA Annual Symposium Proceedings, volume 2010, pages 612–616. American Medical Informatics Association, 2010.
2. Joachims T. Text categorization with support vector machines: Learning with many relevant features. In European Conference on Machine Learning (ECML), pages 137–142, Berlin, 1998. Springer.
3. Baharudin B., Lee L. H., and Khan K. A review of machine learning algorithms for text-documents classification. Journal of Advances in Information Technology, 1(1): 4–20, 2010.

4. Chiticariu L., Li Y., and Reiss F. R. Rule-based information extraction is dead! Long live rule-based information extraction systems! In EMNLP, pages 827–832, 2013.
5. Chapman W. W., Chu D., and Dowling J. N. ConText: an algorithm for identifying contextual features from clinical text. In Proceedings of the Workshop on BioNLP 2007: Biological, Translational, and Clinical Language Processing, pages 81–88. Association for Computational Linguistics, 2007.
6. Meystre S. M., Savova G., Kipper-Schuler K., and Hurdle J. Extracting information from textual documents in the electronic health record: A review of recent research. Yearbook of Medical Informatics, 35:128–144, 2008.
7. Patterson O., Forbush T., Saini S., Moser S., and DuVall S. Classifying the indication for colonoscopy procedures: A comparison of NLP approaches in a diverse national healthcare system. Studies in Health Technology and Informatics, 216:614–618, 2015.
8. Kreuzthaler M., Daumke P., and Schulz S. Semantic retrieval and navigation in clinical document collections. EHealth2015–Health Informatics Meets EHealth: Innovative Health Perspectives: Personalized Health, 212:9–14, 2015.
9. Savova G. K., Masanz J. J., Ogren P. V., Zheng J., Sohn S., Kipper-Schuler K. C., and Chute C. G. Mayo clinical text analysis and knowledge extraction system (cTAKES): architecture, component evaluation and applications. Journal of the American Medical Informatics Association, 17(5):507–513, 2010.
10. Friedman C., Hripcsak G., DuMouchel W., Johnson S. B., and Clayton P. D. Natural language processing in an operational clinical information system. Natural Language Engineering, 1(01):83–108, 1995.

Detection and prognostic value of circulating tumor cells and circulating tumor DNA in patients with intracranial and intraspinal tumors

Summary

Johannes Haybaeck, Division of Neuropathology, Institute of Pathology, Medical University of Graz

Supervisor:	Prof. Dr. Johannes Haybäck
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2016 00:00 and September 30, 2016 23:59 (Europe/Zurich)

Description

Background: 39000 people develop cancer in Austria every year, whereof 627 patients were diagnosed with brain cancer in 2012 (http://www.statistik.at/web_de/statistiken/menschen_und_gesellschaft/gesundheit/krebserkrankungen/index.html). Despite big progress in tumor imaging for diagnostic usage, there are still no reliable biomarkers for tumor diagnostics and monitoring. In the past decades, a variety of genetic aberrations was found in tumors in comparison to healthy cells. Hence, those alterations could be used as potential biomarkers in the future. One promising approach in this regard are analyzes of circulating tumor cells (CTCs). CTCs are cells emerging from a primary tumor circulating in the body. Since tissue biopsy is not an optimal diagnostic tool, detection of those cells in the blood opens a promising field in cancer treatment. Additional to CTCs, circulating tumor DNA (ctDNA) can be found in the blood of cancer patients [1]. Comparison of ctDNA sequences with the “normal” sequence of the patient’s genome opens up new perspectives for minimal invasive cancer diagnosis and especially for personalized treatment [2–4].

Hypothesis and Objectives: The focus of the thesis will be the identification of novel tumor markers in ctDNA and/or CTCs and the confirmation of these markers in the respective tumor tissue.

Collaborators: Collaborator at the Medical University of Graz will be the Department of Neurosurgery. Furthermore the project is going to be collaboration with Prof. Dr. Pantel and his team from the Department of Tumor Biology and the Department of Neurosurgery of the Medical University of Hamburg.

Methodology: Within this thesis blood, cerebrospinal fluid (CSF) and tumor tissue of patients suffering from intracranial or intraspinal tumors will be collected.

Methods for blood and CSF analyzes will be (tumor) cell enrichment via Ficoll®, CTCs identification by immunocytochemistry followed by whole genome amplification, next generation sequencing and sequence variant validation.

Tumor tissue will be then analyzed for aberrations and biomarkers found in CTCs and/or ctDNA. For evaluation in tumor tissue next generation sequencing, qRT-PCR and immunoblot assays will be performed. Furthermore, immunohistochemistry will be an important method.

We expect the student to learn how to plan experiments, work precisely in the laboratory and perform standard methods from molecular and cell biology.

Required experience: Our group is looking for a highly motivated candidate with strong interest in tumor cell biology, who develops own research ideas and has a master’s degree in molecular biology. The candidate should have a high potential to think independently and a deep interest in molecular biological questions and enjoy a challenging project.

References:

[1] Westphal M, Lamszus K. Circulating biomarkers for gliomas. Nat Rev Neurol. 2015;11(10):556-66

[2] Macarthur KM, Kao GD, Chandrasekaran S, Alonso-Basanta M, Chapman C, Lustig RA, Wileyto EP, Hahn SM, Dorsey JF. Detection of brain tumor cells in the peripheral blood by a telomerase promoter-based assay. Cancer Res. 2014;74(8):2152-9

[3] Müller C, Holtschmidt J, Auer M, Heitzer E, Lamszus K, Schulte A, Matschke J, Langer-Freitag S, Gasch C, Stoupiec M, Mauermann O, Peine S, Glatzel M, Speicher MR, Geigl JB, Westphal M, Pantel K, Riethdorf S. Hematogenous dissemination of glioblastoma multiforme. Sci Transl Med. 2014;6(247):247ra101

[4] Sullivan JP, Nahed BV, Madden MW, Oliveira SM, Springer S, Bhere D, Chi AS, Wakimoto H, Rothenberg SM, Sequist LV, Kapur R, Shah K, Iafrate AJ, Curry WT, Loeffler JS, Batchelor TT, Louis DN, Toner M, Maheswaran S, Haber DA. Brain tumor cells in circulation are enriched for mesenchymal gene expression. Cancer Discov. 2014;4(11):1299-309

Investigating the role of N-acetyltransferase 8-like (NAT8L) in whole body energy metabolism and the consequences for the heart

Summary

Juliane Bogner-Strauss, Institute of Biochemistry, Graz University of Technology

Supervisor:	Prof. Dr. Juliane Bogner-Strauss
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2016 00:00 and September 30, 2016 23:59 (Europe/Zurich)

Description

Background:

Imbalances in cellular energy homeostasis can lead to diseases such as type 2 diabetes, cardiovascular disease and cancer. The heart, which has to contract incessantly, is very sensitive to nutritional changes and requires optimal energy to fuel adjustment [1]. N-acetylaspartate (NAA) is one of the most abundant metabolites in brain [2] with yet unknown function. NAA is synthesized by mitochondrial N-acetyltransferase 8-like (NAT8L) and catabolized by cytoplasmic aspartoacylase (ASPA), building the so called NAA pathway [3–7]. Several studies linked NAA to neuronal osmoregulation, lipid synthesis and energy metabolism [8, 9]. We were the first to show that the NAA pathway functionally exists in (brown) adipose tissue [5]. Further, we demonstrated that manipulation of the NAA pathway impacts brown adipocyte lipid turnover (lipogenesis and lipolysis), brown marker gene expression, and cellular respiration *in vitro* [5] and *in vivo*, thereby impacting whole body energy metabolism (unpublished).

Hypothesis and Objectives: Until now we have discovered, that Nat8l-knockout mice show reduced body weight, reduced blood triglycerides, mild hypoglycemia and improved glucose tolerance. Most importantly for this work, we discovered that Nat8l-knockout mice die earlier than their littermates. They die especially in the time after weaning, where there is a nutritional change from high-fat containing mother's milk to chow diet. The observation that Nat8l-knockout mice have increased heart weight upon cold exposure and trends to hypertension and increased heart rate made us hypothesize that the sudden death of these mice is linked to the heart. In this project, we seek to identify the molecular mechanism of how Nat8l influences whole body energy metabolism and how this impacts the heart.

Methodology: This project focuses on Nat8l and its influences on whole body energy metabolism. Candidates who are interested in this project should have basic experience in cell culture and molecular biology techniques, such as Western blot and real-time PCR. Since this project deals with the characterization of Nat8l-knockout mice, we are looking for candidates who are willing to work with laboratory animals and preferentially already have certain experience with mouse handling.

Research interest: https://www.tugraz.at/projekte/cellism/home/

Your experience: Ideally, you have a thorough background in cell culture, molecular biology techniques (cloning, RT-PCR, WB), and preliminary experience in mouse handling.

References:

[1] Kolwicz SC, Purohit S, Tian R. Cardiac Metabolism and its Interactions With Contraction, Growth, and Survival of Cardiomyocytes. Circ Res. 2013;113(5):603–16

[2] Blüml S. In vivo quantitation of cerebral metabolite concentrations using natural abundance 13C MRS at 1.5 T. J Magn Reson. 1999;136(2):219–25

[3] Wiame E, Tyteca D, Pierrot N, Collard F, Amyere M, Noel G, Desmedt J, Nassogne MC, Vikkula M, Octave JN, Vincent MF, Courtoy PJ, Boltshauser E, van Schaftingen E. Molecular identification of aspartate N-acetyltransferase and its mutation in hypoacetylaspartia. Biochem J. 2010;425(1):127–36

[4] Ariyannur PS, Moffett JR, Manickam P, Pattabiraman N, Arun P, Nitta A, Nabeshima T, Madhavarao CN, Namboodiri AM. Methamphetamine-induced neuronal protein NAT8L is the NAA biosynthetic enzyme: implications for specialized acetyl coenzyme A metabolism in the CNS. Brain Res. 2010;1335:1–13

[5] Pessentheiner AR, Pelzmann HJ, Walenta E, Schweiger M, Groschner LN, Graier WF, u. a. NAT8L (N-Acetyltransferase 8-Like) Accelerates Lipid Turnover and Increases Energy Expenditure in Brown Adipocytes. J Biol Chem. 2013;288(50):36040–51

[6] Kaul R, Casanova J, Johnson AB, Tang P, Matalon R. Purification, characterization, and localization of aspartoacylase from bovine brain. J Neurochem. 1991;56(1):129–35

[7] Prokesch A, Pelzmann HJ, Pessentheiner AR, Huber K, Madreiter-Sokolowski CT, Drougard A, Schittmayer M, Kolb D, Magnes C, Trausinger G, Graier WF, Birner-Gruenberger R, Pospisilik JA, Bogner-Strauss JG. N-acetylaspartate catabolism determines cytosolic acetyl-CoA levels and histone acetylation in brown adipocytes. Sci Rep. 2016;6:23723

[8] Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AMA. N-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. Prog Neurobiol. 2007;81(2):89–131

[9] Moffett JR, Arun P, Ariyannur PS, Namboodiri AMA. N-Acetylaspartate reductions in brain injury: impact on post-injury neuroenergetics, lipid synthesis, and protein acetylation. Front Neuroenergetics. 2013;5:11

Role of Perilipin 5 phosphorylation in the development of liver disease

Summary

Guenter Haemmerle, Institute of Molecular Biosciences, University of Graz

Supervisor:	Prof. Dr. Günter Hämmerle
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2016 00:00 and September 30, 2016 23:59 (Europe/Zurich)

Description

Role of Perilipin 5 phosphorylation in the development of liver disease

Background: The prevalence of non-alcoholic fatty liver disease is steadily increasing and correlates with the prevalence of obesity and typ 2 diabetes. However, the underlying molecular mechanisms await further clarification.

Aims/Hypothesis: Lipid droplets (LDs) are enriched with proteins from the perilipin family including the presence of Perilipin 5 (Plin5) on LDs derived from liver and muscle tissue. We could previously show that Plin5-enriched LDs are resistant towards adipose triglyceride lipase (ATGL)-mediated lipolysis [1]. This lipolytic barrier could be resolved upon Protein Kinase A-mediated Plin5 phosphorylation [2]. Notably, mice overexpressing Plin5 in the liver develop hepatic steatosis but are protected from lipotoxicity paralleled by increased hepatic insulin sensitivity [3,4]. This project aims to unravel the role of PKA-mediated Plin5 phosphorylation in the regulation of hepatic lipid and energy metabolism.

Methodology: Generating recombinant adenovirus expressing wildtype and mutant Plin5 (harboring an amino acid exchanged in the proposed PKA phosphorylation side). Mice will be infected with recombinant adenovirus which will lead to increased hepatic expression of wildtype and mutant Plin5, respectively. The impact of increased Plin5 expression on liver and whole body TG homeostasis and energy metabolism will be examined. Cultivation and transfection of mammalian cell lines and applying tracer isotope techniques. Mouse handling and phenotypic characterization (collecting blood samples and organs, enzymatic assays, tissue uptake studies, RNA isolation and qRT-PCR).

References:

[1] Pollak NM1, Schweiger M, Jaeger D, Kolb D, Kumari M, Schreiber R, Kolleritsch S, Markolin P, Grabner GF, Heier C, Zierler KA, Rülicke T, Zimmermann R, Lass A, Zechner R, Haemmerle G. Cardiac-specific overexpression of perilipin 5 provokes severe cardiac steatosis via the formation of a lipolytic barrier. J. Lipid Res. 2013;54:1092–102

[2] Pollak NM, Jaeger D, Kolleritsch S, Zimmermann R, Zechner R, Lass A, Haemmerle G. The interplay of protein kinase A and perilipin 5 regulates cardiac lipolysis. J. Biol. Chem. 2015;290:1295–306

[3] Wang C, Zhao Y, Gao X, Li L, Yuan Y, Liu F, Zhang L, Wu J, Hu P, Zhang X, Gu Y, Xu Y, Wang Z, Li Z, Zhang H, Ye J. Perilipin 5 improves hepatic lipotoxicity by inhibiting lipolysis. Hepatology. 2015;61(3):870-82

[4] Trevino MB, Mazur-Hart D, Machida Y, King T, Nadler J, Galkina EV, Poddar A, Dutta S, Imai Y. Liver Perilipin 5 Expression Worsens Hepatosteatosis But Not Insulin Resistance in High Fat-Fed Mice. Mol. Endocrinol. 2015;29:1414–25

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Role of lipases in mitochondrial dynamics

Summary

Guenter Haemmerle, Institute of Molecular Biosciences, University of Graz

Supervisor:	Prof. Dr. Günter Hämmerle
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2016 00:00 and September 30, 2016 23:59 (Europe/Zurich)

Description

Role of lipases in mitochondrial dynamics

Background: Changes in mitochondrial fatty acid oxidation (FAO) can be involved in the development of heart and liver disorders including cardiomyopathy and non-alcoholic fatty liver disease. However, the impact of changes in cellular triglyceride (TG) catabolism on mitochondrial dynamics, i.e. fusion or fission, is less established. Furthermore, damaged mitochondria have to be recognised an eliminated in a dynamic process designated as mitophagy. It has been shown that increased cellular levels of reactive oxygen species (ROS) are involved in mitochondrial damage and mitohphagy. We could demonstrate that defective lipolysis enhances ROS generation and mitochondrial damage which awaits further clarification [1].

Aims/Hypothesis: Mice lacking adipose triglyceride lipase (ATGL) or its co-activator comparative gene identification-58 (CGI-58) exclusively in the liver show marked hepatic steatosis [2-5]. However, the impact of ATGL- or CGI-58 deficiency on mitochondrial dynamics is less established. This project aims to unravel the specific role of ATGL- or CGI-58 in hepatic lipolysis linked to mitochondrial dynamism using cell culture and mutant mouse models.

Methodology: Cloning of candidate genes in expression vectors and generation of recombinant adenovirus. Cultivation and transfection of mammalian cell lines and applying tracer isotope techniques. Mouse handling and phenotypic characterization (collecting blood samples and organs, enzymatic assays, tissue uptake studies, RNA isolation and qRT-PCR).

References:

[1] Haemmerle G, Moustafa T, Woelkart G, Büttner S, Schmidt A, van de Weijer T, Hesselink M, Jaeger D, Kienesberger PC, Zierler K, Schreiber R, Eichmann T, Kolb D, Kotzbeck P, Schweiger M, Kumari M, Eder S, Schoiswohl G, Wongsiriroj N, Pollak NM, Radner FP, Preiss-Landl K, Kolbe T, Rülicke T, Pieske B, Trauner M, Lass A, Zimmermann R, Hoefler G, Cinti S, Kershaw EE, Schrauwen P, Madeo F, Mayer B, Zechner R. ATGL-mediated fat catabolism regulates cardiac mitochondrial function via PPAR-α and PGC-1. Nat Med. 2011;17(9):1076-85

[2] Ong KT, Mashek MT, Bu SY, Greenberg AS, Mashek DG. Adipose triglyceride lipase is a major hepatic lipase that regulates triacylglycerol turnover and fatty acid signaling and partitioning. Hepatology. 2011;53(1):116-26

[3] Wu JW, Wang SP, Alvarez F, Casavant S, Gauthier N, Abed L, Soni KG, Yang G, Mitchell GA. Deficiency of liver adipose triglyceride lipase in mice causes progressive hepatic steatosis. Hepatology. 2011;54(1):122-32

[4] Zierler KA, Zechner R, Haemmerle G. Comparative gene identification-58/α/β hydrolase domain 5: more than just an adipose triglyceride lipase activator? Curr Opin Lipidol. 2014;25(2):102-9

[5] Guo F, Ma Y, Kadegowda AK, Betters JL, Xie P, Liu G, Liu X, Miao H, Ou J, Su X, Zheng Z, Xue B, Shi H, Yu L. Deficiency of Liver Comparative Gene Identification-58 (CGI-58) Causes Steatohepatitis and Fibrosis in Mice. J Lipid Res. 2013;54(8):2109-20

Investigating the role of mitochondrial aspartate-glutamate carrier in cancer

Summary

Juliane Bogner-Strauss, Institute of Biochemistry, Graz University of Technology

Supervisor:	Prof. Dr. Juliane Bogner-Strauss
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2016 00:00 and September 30, 2016 23:59 (Europe/Zurich)

Description

Investigating the role of mitochondrial aspartate-glutamate carrier in cancer

Background:

Cancer cells have altered nutrient metabolism in order to keep up with increased energetic and biosynthetic needs of constant proliferation. For instance, proliferating cells in culture often choose to catabolize glucose via aerobic glycolysis and use carbons from glutamine as major energy source in tricarboxylic (TCA) cycle. This allows them to utilize glucose-derived carbons for synthesizing amino acids, nucleotides and membrane lipids required for proliferation [1, 2, 3].

Mitochondrial aspartate-glutamate carrier (Aralar, AGC1), most highly expressed in neurons and muscle, is one of the crucial mediators between glycolysis and TCA cycle [4, 5, 6]. It allows the oxidation of glycolysis-produced NADH by taking part in malate-aspartate shuttle (MAS). By its function, it could also assist energy and biomass production via maintaining mitochondrial glutamate oxidation as well as boosting cytosolic aspartate pool which is essential for *de novo* synthesis of nucleotides.

Hypothesis and Objectives: Until now, we have discovered that AGC1 is highly expressed in several cancers including breast carcinoma, pancreas adenocarcionma, ovarian carcinoma, glioblastoma, leimyosarcoma, and pheochromacytoma. AGC1 knockdown in syngenic cell lines reduced the proliferation (*in vitro*) and tumor growth (*in vivo*) however increased the metastatic and cachectic phenotype. In this project, we seek to identify how AGC1 is involved in cancer progression, how it is regulated and which advantages and vulnarabilities it brings to the cancer cell metabolism which could be targeted for therapy in the future.

Methodology: This project is focused on cellular metabolism of cancer. Candidates who are interested in this project should have certain experience in cell culture and basic molecular biology techniques such as western blot and real-time PCR. Throughout the project, additional techniques such as cloning and stable knockdown or overexpression of required genes in cancer cell lines, NMR and LC-MS metabolomics, metabolite labeling, ability to handle laboratory animals for monitoring the tumor growth, metastasis and cachexia and immunohistochemistry etc. will be used frequently.

Research interest: https://www.tugraz.at/projekte/cellism/home/

Your experience: Ideally, you have a thorough background in cell culture, molecular biology techniqes (cloning, qPCR, WB), and preliminary experience in mouse handling.

References:

[1] Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol. 2011;27:441-64

[2] Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer. 2004;4(11):891-9

[3] Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324(5930):1029-33

[4] del Arco A, Satrustegui J. Molecular cloning of Aralar, a new member of the mitochondrial carrier superfamily that binds calcium and is present in human muscle and brain. J Biol Chem. 1998;273(36):23327-34

[5] Begum L, Jalil MA, Kobayashi K, Iijima M, Li MX, Yasuda T, Horiuchi M, del Arco A, Satrústegui J, Saheki T. Expression of three mitochondrial solute carriers, citrin, aralar1 and ornithine transporter, in relation to urea cycle in mice. Biochim Biophys Acta. 2002;1574(3):283-92

[6] del Arco A, Morcillo J, Martínez-Morales JR, Galián C, Martos V, Bovolenta P, Satrústegui J. Expression of the aspartate/glutamate mitochondrial carriers aralar1 and citrin during development and in adult rat tissues. Eur J Biochem. 2002;269(13):3313-20

The role of JAK pathway and its inhibition in cutaneous T cell lymphoma (CTCL)

Summary

Peter Wolf, Research Unit Photodermatology, Department of Dermatology and Venerology, Medical University of Graz

Supervisor:	Prof. Dr. Peter Wolf
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between January 25, 2016 00:00 and March 16, 2016 23:59 (Europe/Zurich)

Description

The role of JAK pathway and its inhibition in cutaneous T cell lymphoma (CTCL)

Background: Mycosis fungoides (MF) is the most common cutaneous T-cell lymphoma (CTCL). CD4+ T-cells with malignant phenotype populate the skin causing lesions that may last over years before they progress and result in systemic involvement of other organs. The lesions are often treated with UVB phototherapy or (oral) psoralen and UV-A light irradiation (PUVA) in early stages of the disease [1]. Several elements of the JAK/STAT signaling pathway have been described to contribute to the pro-inflammatoryBackground: Mycosis fungoides (MF) is the most common cutaneous T-cell lymphoma (CTCL). CD4+ T-cells with malignant phenotype populate the skin causing lesions that may last over years before they progress and result in systemic involvement of other organs. The lesions are often treated with UVB phototherapy or (oral) psoralen and UV-A light irradiation (PUVA) in early stages of the disease [1]. Several elements of the JAK/STAT signaling pathway have been described to contribute to the pro-inflammatory environment in the skin and proliferation and survival of malignant cells. *In vitro* assays demonstrate that STAT3 is constantly activated in CTCL cell lines triggering the production of IL-21 [2], activation of BCL-2, and expression of survivin. In the proposed project, we will investigate the role of JAK pathway and its inhibition in cutaneous T cell lymphoma by administering the JAK1/JAK3 and/or STAT3 inhibitors. Such drugs have significant efficacy in targeting inflammatory responses and have been studied in autoimmunity diseases like rheumatoid arthritis and psoriasis due to their capacity of blocking cytokine signaling of the common gamma chain. environment in the skin and proliferation and survival of malignant cells. *In vitro* assays demonstrate that STAT3 is constantly activated in CTCL cell lines triggering the production of IL-21 [2], activation of BCL-2, and expression of survivin. In the proposed project, we will investigate the role of JAK pathway and its inhibition in cutaneous T cell lymphoma by administering the JAK1/JAK3 and/or STAT3 inhibitors. Such drugs have significant efficacy in targeting inflammatory responses and have been studied in autoimmunity diseases like rheumatoid arthritis and psoriasis due to their capacity of blocking cytokine signaling of the common gamma chain.

Hypothesis and Objectives: We propose three key objectives in the project: First, the identification of JAK1 and JAK2 positive cells in lesional skin of MF patients by TCR sequencing and immunohistochemistry. Second, the establishment of the efficacy of JAK and STAT3 inhibitors in induction of apoptosis in CTCL cell lines and the suitability to combine it with UVB phototherapy or PUVA. Third, the evaluation of the effect of inhibitors such as tofacitinib in an animal model of cutaneous T cell lymphoma by looking at the impact on malignant T-cells and anti-tumor immune response by a variety of methods. Taken together, the project aims to contribute to the understanding of the pathophysiology of MF and ultimately to the improvement of therapies to treat cutaneous T-cell lymphoma by targeting oncogenes in the JAK/STAT pathway.

Methodology: The PhD student will learn to investigate skin samples of archived materials from patients in a clinical study. The techniques that the student will acquire include flow cytometry, real-time PCR, Western blot, multiplex ELISA, immune-bead magnetic cell sorting, and immunohistochemistry/-fluorescence microscopy, FACS and immune function assay (T cell co-proliferation assays). The role of JAK1, JAK2, STAT3, STAT5, STAT4, IRF4, IL-21, IL-9, IL-15 among others, will be addressed, also by double color immunohistochemistry and immunofluorescence in order to identify a specific T-cell by its TCR-beta chain with an antibody and co-stained with the previously mentioned markers.

In addition, the student will gain experience with animal model using C57BL/6 or BoiA mice and the murine T-cell lymphoma cell line EL-4 that has been successfully established in our laboratory by a current PhD student [3, 4]. In addition, the student will acquire photobiologic methods such as UV light treatment and dosimetry (used in control experiments).

References:

[1] Wackernagel A, Hofer A, Legat F, Kerl H, Wolf P. Efficacy of 8-methoxypsoralen vs. 5-methoxypsoralen plus ultraviolet A therapy in patients with mycosis fungoides Br J Dermatol. 2006;154(3):519-23

[2] van der Fits L, Out-Luiting JJ, van Leeuwen MA, Samsom JN, Willemze R, Tensen CP, Vermeer MH. Autocrine IL-21 stimulation is involved in the maintenance of constitutive STAT3 activation in Sezary syndrome. J Invest Dermatol. 2012;132(2):440-7

[3] Vieyra-Garcia PA; Mayer G; Pressl H; Reginato E; Schweintzger NA; Bambach I; Hofer A; Gruber-Wackernagel A; Cerroni L; Wolf P. Increased frequency of regulatory T cells with impaired suppressive capacity after PUVA in cutaneous T-cell lymphoma. J Invest Dermatol. 2014;134:S21

[4] Vieyra-Garcia PA, Wei T, Naym DG, Fredholm S, Fink-Puches R, Cerroni L, Odum N, O'Malley JT, Gniadecki R, Wolf P. STAT3/5-Dependent IL9 Overexpression Contributes to neoplastic cell survival in mycosis fungoides. Clin Cancer Res. 2016;22(13):3328-39

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Lysophosphatidic acid as effector of microglia metabolism

Summary

Wolfgang Sattler, Institute of Molecular Biology and Biochemistry, Center of Molecular Medicine, Medical University of Graz

Supervisor:	Prof. Dr. Wolfgang Sattler
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2016 00:00 and September 30, 2016 23:59 (Europe/Zurich)

Description

Lysophosphatidic acid as effector of microglia metabolism

Background: Microglia modulate neurodegeneration/-regeneration under neuroinflammatory conditions [3]. Microglia are endowed with specific receptor sets that detect subtle alterations in the CNS micromilieu. The inflammatory mediator lysophosphatidic acid (LPA) signals via six G protein-coupled receptors termed LPA_{1-6}. Quantitative proteome studies by our group demonstrated that LPA upregulates the expression of glycolytic enzymes in microglia [4]. During her ongoing thesis project within MOLIN, Joanna Plastira discovered that LPA (C18:1) induces microglia migration and polarization towards a M1-like phenotype.

Hypothesis and Objectives: Based on Joanna's work work we will test the hypothesis that LPA-mediated signaling cascades determine cell fate decisions and effector functions of microglia via modulation of metabolic pathway activity. This will extend findings from peripheral T-lymphocytes [5] to the endogenous brain defense and immune system. The student will characterize LPA-induced metabolic signatures of microglia and study the underlying signaling events leading to metabolic reprogramming. The student will also investigate whether specific LPA-induced metabolic programs are associated with distinct cytokine secretion patterns.

Methodology: The murine BV-2 microglia cell line will be used to establish necessary experimental techniques; thereafter the student will switch to primary mouse microglia. LPA-induced transcriptional alterations in genes relevant for glucose and fatty acid utilization will be quantitated by qPCR arrays and compared to LPS- (M1) and IL-4- (M2) polarized microglia. Glucose uptake by LPA-, LPS- and IL-4-primed microglia will be quantitated with fluorescently labeled 2-NBD-deoxyglucose. Transcriptional regulators of the metabolic response (e.g. p53, c-Myc, HIF, SREBPs) will be analyzed by qPCR and Western blotting. qPCR, Western blotting, and immunofluorescence microscopy will be utilized to study nutrient transporter expression in untreated and treated (LPA, compared to LPS and IL-4) cells. Extracellular acidification rate (approximating glycolytic flux via LDH) and mitochondrial oxygen consumption rate (indicating mitochondrial function) will be determined with a Seahorse XF24 analyzer. NADP/NADPH production (as readout for pentose phosphate pathway induction) will be quantitated with a colorimetric test. Glutamine uptake will be analyzed with [^{14}C]-labeled glutamine. A combination of TLC/GC will clarify whether de novo fatty acid synthesis is coupled to microglia phenotype switching [6]. The student will be able to perform metabolite profiling ('metabolomics') within the consortium. Proteome profiler cytokine arrays and ELISAs will be used to monitor changes in cytokine secretion in response to LPA, LPS (M1) and IL-4 (M2) stimulation. The outcome of these experiments will reveal whether the LPA synthesis/signaling axis could be exploited to pharmacologically modulate the immune response of microglia.

References

[1] Lüscher TF, Landmesser U, von Eckardstein A, Fogelman AM. High-density lipoprotein: vascular protective effects, dysfunction, and potential as therapeutic target. Circ Res. 2014;114(1):171-82

[2] Martin MG, Pfrieger F, Dotti CG. Cholesterol in brain disease: sometimes determinant and frequently implicated. EMBO Rep. 2014;15(10):1036-52

[3] Nayak D, Roth TL, McGavern DB. Microglia Development and Function. Annu Rev Immunol. 2014;32:367-402

[4] Bernhart E, Kollroser M, Rechberger G, Reicher H, Heinemann A, Schratl P, Hallström S, Wintersperger A, Nusshold C, DeVaney T, Zorn-Pauly K, Malli R, Graier W, Malle E, Sattler W. Lysophosphatidic acid receptor activation affects the C13NJ microglia cell line proteome leading to alterations in glycolysis, motility, and cytoskeletal architecture. Proteomics. 2010;10(1):141-58

[5] Ganeshan K, Chawla A. Metabolic regulation of immune responses. Annu Rev Immunol. 2014;32:609-34

[6] Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, Sandouk A, Hesse C, Castro CN, Bähre H, Tschirner SK, Gorinski N, Gohmert M, Mayer CT, Huehn J, Ponimaskin E, Abraham WR, Müller R, Lochner M, Sparwasser T. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. Nat Med. 2014;20(11):1327-33

The role of bile acids in lipid and energy metabolism in health and disease

Summary

Peter Fickert, Laboratory of Experimental and Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz

Supervisor:	Prof. Dr. Peter Fickert
Availability:	This position has been occupied.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 01, 2016 00:00 and September 30, 2016 23:59 (Europe/Zurich)

Description

The role of bile acids in lipid and energy metabolism in health and disease

Research interest: The group of P. Fickert investigates the molecular mechanisms of bile acid (BA) signaling under physiological (e.g. lipid metabolism) and pathophysiological conditions as a critical regulator of liver diseases, such as cholestasis. In addition, certain BAs, such as the side chain-shortened *nor*-ursodeoxycholic acid (norUDCA) are also used in treatment of these disorders. This resulted in several key publications in this area of research and international patents on the clinical use of norUDCA as novel treatment for cholestatic liver diseases (Patent WO2006119803) and atherosclerosis (Patent 07 113 107.2).

Background: BAs previously seen as simple fat emulsifiers are now known to represent a major molecular regulator of lipid, glucose, and energy homeostasis, therefore currently denominated as hormones. The enterohepatic circulation of bile acids from the liver to intestine and back to the liver, describe best their primary place of action, such as control of microbiota diversity and intestine barrier function, and conversion of cholesterol, promoting their biosynthesis and biliary excretion [1, 2]. Our recent data demonstrate that certain BAs, including norUDCA counteract aberrant cellular proliferation, ER-stress and inflammation, and exert different effects on mTORC1 activation, ribosome biogenesis, and autophagy. Using microarray technology and metabolic profiling, our lab was additionally able to uncover profound changes in systemic and hepatic cholesterol, phospholipid, triglyceride and fatty acid metabolism [3]. Intriguingly, it seems that hepatic lipid metabolism is of prime importance in the pathogenesis of various liver diseases, and that “metabolic reprogramming” is essential to exert beneficial effects by BAs such as norUDCA.

Hypothesis and Objectives: The thesis will focus on the characterization of molecular and metabolic pathways through which BAs impact on hepatic and intestinal lipid and energy metabolism in health and disease.

Methodology: Within this research project the candidate will use both *in vivo* and *in vitro* models to determine the impact of BAs on steroid/lipid (patho)-physiology. In the context of the enterohepatic circulation, the student will investigate the impact of different BAs on intestine and liver metabolism using numerous mouse models, e.g. lacking the nuclear BA-receptor FXR or the G protein-coupled bile acid receptor 1 (GPBAR1). This will be further attained using a setting of different dietary manipulation e.g. high fat diet. Part of the project will also include the analysis of the microbiota environment with regard to BA-metabolism. The student will work with liver and intestinal cell lines and will perform knock-down and/or overexpression experiments to elucidate molecular mechanisms *in vitro*.

Consequently, the student should learn to plan experiments independently, develop own research ideas, and perform standard techniques of molecular cell biology (e.g. cloning of cDNA, Q-PCR), cell culture work, cell biology (immunohistochemistry and immunofluorescence microscopy) and protein-protein interactions by immunoprecipitation.

Laboratory environment: The DK-MCD student will work in a young and motivated laboratory of currently 6 scientifically experienced group members and 3 technicians. The laboratory is located at the Center of Medical Research (ZMF), which provides all infrastructures for this project.

Required experience: We are looking for a highly motivated PhD student, with a great passion for science, holding a master in Biology (Biochemistry/Molecular Biology/Cell Biology or similar education).

Further information: https://forschung.medunigraz.at/fodok/suchen.person_uebersicht?sprache_in=en&ansicht_in=&menue_id_in=101&id_in=80264

References:

[1] Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. Nat Rev Drug Discov. 2008;7(8):678-93

[2] Fiorucci S, Distrutti E. Bile Acid-Activated Receptors, Intestinal Microbiota, and the Treatment of Metabolic Disorders. Trends Mol Med. 2015;21(11):702-14

[3] Moustafa T, Fickert P, Magnes C, Guelly C, Thueringer A, Frank S, Kratky D, Sattler W, Reicher H, Sinner F, Gumhold J, Silbert D, Fauler G, Höfler G, Lass A, Zechner R, Trauner M. Alterations in lipid metabolism mediate inflammation, fibrosis, and proliferation in a mouse model of chronic cholestatic liver injury. Gastroenterology. 2012;142(1):140-151