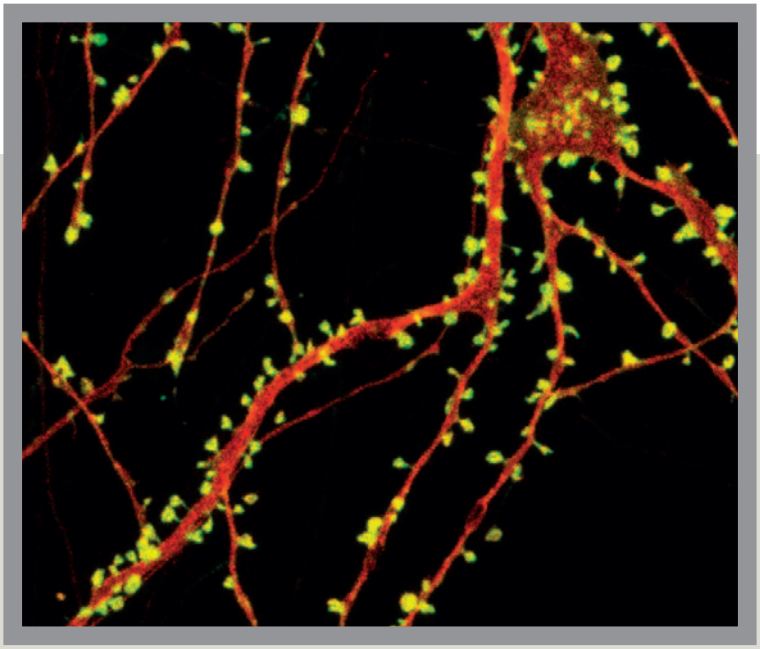
MINERVA FOUNDATION
&
MINERVA FOUNDATION INSTITUTE FOR MEDICAL RESEARCH



Scientific Report 2015

Front page image: Confocal microscope image of cultured primary neurons expressing inactive Rif GTPase (red). Actin cytoskeleton staining (green) visualizes the dendritic spines, small protrusions along the neuronal dendrites (courtesy of Pirta Hotulainen/Cellular neuroscience).

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Research groups

SUMMARY OF RESEARCH AND ACTIVITIES DURING THE YEAR 2015

Minerva Foundation Institute for Medical Research is a privately owned research institute located at Biomedicum, Academic Medical Center Helsinki. The Institute, the history of which dates back to 1959, combines basic biomedical research with clinical investigation relevant for common diseases.

The overarching aims of the Minerva Foundation Institute are to generate a new knowledge base as well as innovations for the development of future diagnostic approaches, preventive measures and treatments for common diseases such as diabetes and cardiovascular diseases, as well as neurodegenerative and neuropsychiatric disorders. The study objectives rooted in fundamental molecular mechanisms of disease are addressed at Minerva Foundation Institute by approaches ranging from cultured cells and genetically manipulated animal models to investigation of human patients. The research undertaken in the groups of the Institute during the year 2015 is outlined in this report.

The resources of Minerva Foundation are directed at maintaining and further developing a research infrastructure that serves, in the most effective way, the work in the research groups. The groups are responsible for acquiring external funds to cover the costs of special reagents, stipendium support of doctoral students, and salaries of personnel. In 2015, external funds raised by the research groups covered 47% of the total Institute budget. To enhance and expedite research at the Institute, in 2015 the Foundation decided to recruit a new group leader. The open recruitment process was carried out during May-September 2015 and resulted in Docent Pirta Hotulainen and her group of four scientists joining the Minerva Institute.

To promote the exchange of information and seed new collaborations, the Institute organized four meetings of the Minerva Discussion Forum. This small-scale forum with invited lecturers is aimed at creating new contacts with scientists working close to the areas of emphasis of the Institute. In addition to building external collaboration, the integration and spirit of the Institute were promoted by weekly internal meetings and a recreational event organized at the Seurasaari outdoor museum in June.

Despite the present limitations in external funding, research progress at the Institute thrived in 2015. Minerva scientists published a total of 35 articles in international peer-reviewed journals, with a median impact factor of 4.85. In addition, two doctoral theses were finalized and defended during the year.

EVENTS AT MINERVA 2015

Seminars and Symposia

MINERVA DISCUSSION FORUM, BIOMEDICUM HELSINKI:

Mika Kivimäki, University Collage London, UK: *Mendelian randomization - a relevant approach for diabetes and cardiovascular disease research?* January 9, 2015.

Onni Niemelä, Etelä-Pohjanmaan sairaanhoitopiiri, Lääketieteellisen tutkimuksen yksikkö: *Biomarkers of alcohol consumption and related liver disease.* January 30, 2015.

Ville Hietakangas, Institute of Biotechnology, HY: *Drosophila model for sugar sensing and metabolism.* February 6, 2015.

Jukka Kallijärvi, Folkhälsan Research Center: *Studies of animal models of the mitochondrial hepatopathy GRACILE syndrome.* April 17, 2015.

Docent Lectures

Jakob Stenman: *HDR-brakyterapia lasten alempien virtsateiden syövän hoidossa.* February 11, 2015. Lastenklinikka. (Targeted gene-expression analyses)

You Zhou: *Medical Statistics.* April 13, 2015. Biomedicum Helsinki. (Endocrinology)

Doctoral Dissertations

Tho Ho Huu: Development of amplification-based technologies for enrichment of nucleic acids with difficult sequences or low-abundance point mutations. February 13, 2015. (Targeted gene-expression analyses)

Veronica Kalhori: Spingosine-1-phosphate-evoked invasion of follicular thyroid cancer cells:
Evidence for the involvement of HIF-1α, MMP_2 and MMP_9. September 11, 2015. (Cellular physiology)

ADMINISTRATION

THE MINERVA FOUNDATION

The main purpose of the Foundation is to promote research in medicine and biosciences by maintaining the Minerva Foundation Institute for Medical Research. This scientific review covers the period from January 1 – December 31, 2015. During this period, the board of trustees included the following persons:

Professor Jim Schröder, chair
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Docent Patrik Finne
Professor Per-Henrik Groop
Professor Carola Grönhagen-Riska
M.Sc. (Econ. & Bus. Adm.) Thomas Ramsay
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M.Sc. (Econ. & Bus. Adm.) Carl-Magnus Westermarck

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THE MINERVA FOUNDATION INSTITUTE FOR MEDICAL RESEARCH

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Professor Kid Törnquist
Professor Hannele Yki-Järvinen
Carita Estlander-Kortman
Cia Olsson, secretary

Group Members

Ilkka Tikkanen, M.D., Dr.Med.Sci., Docent, Head
Mika Laine, M.D., Dr.Med.Sci., Docent
Päivi Lakkisto, M.D., Dr.Med.Sci., Docent
Jere Paavola, M.D., Dr.Med.Sci
Heli Segersvärd, M.D.
Juuso Siren, M.D.
Mikko Hänninen, M.B.
Karri Kalervo, M.B.
Suneeta Narumanchi, M.Sc.
Katariina Immonen, B.Sc., Laboratory Technician
Riikka Kosonen, M.Sc., Laboratory Technician
Sanni Perttunen, B.Sc., Laboratory Technician

External funding

The Aarne Koskelo Foundation
The Finnish Foundation for Cardiovascular Research
The Finnish Foundation for Laboratory Medicine
The Finska Läkaresällskapet
The Liv och Hälsa Foundation
Research Funding of the Helsinki-Uusimaa Hospital District
(government special state subsidiary for health sciences)

CARDIOVASCULAR RESEARCH

Main research activities

Our research group studies mechanisms and repair of end organ damage in cardiovascular and renal diseases. Specifically, we are investigating the local expression and effects of components of the renin-angiotensin-aldosterone system and other vasoactive factors, as well as apoptosis in hypertension, heart failure, and progression of renal damage. In addition, the cardiovascular and renal protective properties of new cardiovascular drugs are evaluated in our group. During recent years, the group's research has focused on exploring the regenerative and reparative mechanisms of cardiac injury after myocardial infarction and heart failure development to identify new, potential targets for novel cardiovascular medicines.

We have shown that heme oxygenase-1 (HO-1) and carbon monoxide (CO) have potential roles in cardiac recovery and repair after myocardial injury. Our novel findings demonstrated that treatment with a specific CO-donor, CORM-3, improves both structural and functional cardiac recovery after myocardial infarction (MI) in rats. These effects were associated with increased expression of angiogenic factors and altered expression of several miRNA molecules involved in myocardial homeostasis, remodeling, and repair. Thus, induction of the HO-1-CO pathway may prove to be a useful tool to facilitate cardiac recovery after myocardial injury and protect against development of heart failure after MI.

During 2015, we continued our collaboration with Docent Hannele Laivuori, University of Helsinki, to explore the association of HO-1 gene (HMOX1) polymorphisms with an increased risk of preeclampsia. Our findings have shown that the long allele of a guanine-thymine microsatellite repeat seems to predispose to a late-onset, less severe form of preeclampsia, supporting the role of HO-1 in the pathogenesis of preeclampsia. Based on these findings and the demonstrated role of HO-1 as a cardiovascular protective factor, we started a collaboration with Professor Per-Henrik Groop and the FinnDiane Study Group, University of Helsinki,

Helsinki University Hospital (HUCH), and Folkhälsan Institute of Genetics, to study the possible involvement of HO-1 and HO-1 gene (HMOX1) polymorphisms in the development of cardiovascular and renal complications in type 1 diabetes.

A study on role of HO-1 in cardiac arrest patients was completed in collaboration with Professor Ville Pettilä, HUCH, utilizing patient material obtained from a 12-month prospective observational multicenter study (FINNRESUSCI) from 21 Finnish intensive care units. In this study, we showed that higher plasma HO-1 levels are associated with a longer time to return spontaneous circulation as well as poor long-term outcome.

During 2015, our group has participated in the COST Action BM1005, European Network on Gasotransmitters, in which Päivi Lakkisto served as a member of the Management Committee.

Molecular mechanisms of cardiac regeneration and repair have been studied in several collaborative projects with Professors Eero Mervaala and Heikki Ruskoaho and their research groups at the University of Helsinki, and with Professor Risto Kerkelä and coworkers at the University of Oulu. In these studies, the role and effects of selected miRNAs, antagomirs and transcription factors as well as the role of the HO-1-CO pathway in the development of cardiac structural and functional changes after myocardial injury/overload were studied utilizing the cardiac cryoinfarction model in adult zebrafish and a drug-induced cardiac hypertrophy/failure model in embryonic zebrafish. In addition, rat neonatal and zebrafish cardiomyocyte cell culture models were used to examine molecular and cellular pathways related to cardiac regeneration and repair. Furthermore, the role and function of septin7b in the zebrafish heart was studied in collaboration with Dr. Surjya Dash and Professor Sanna Lehtonen, University of Helsinki. With easy delivery of drugs and methodology for gene silencing, these models provide a robust platform for studying the development of cardiac hypertrophy and failure, as well as factors affecting recovery of cardiac function from heart failure.

Lastly, we have also been working in several collaborative clinical studies that continued in 2015. Specifically, with Docent Veli-Pekka Harjola, HUCH, and Dr. Yvan Devaux, Centre de Recherche Public de la Santé, Luxembourg, we have analyzed changes in potential miRNAs associated with the outcome of cardiogenic shock patients (CardShock project). The effects of drug treatment on the progression of disease in aortic stenosis patients, notably on factors and signal cascades involved in formation of fibrosis, have been investigated in collaboration with Professor Markku Kupari, Helsinki University Hospital (ROCKAS study). In collaboration with Docent Ville Pettilä, HUCH, potential new biomarkers of acute kidney injury (FINNAKI study) have been examined. Ilkka Tikkanen has participated in the international collaboration elucidating the blood pressure lowering and vascular effects of the sodium glucose co-transporter 2 (SGLT2) inhibitor empagliflozin, a novel glucose lowering drug with cardiovascular protective properties, in the treatment of type 2 diabetes.

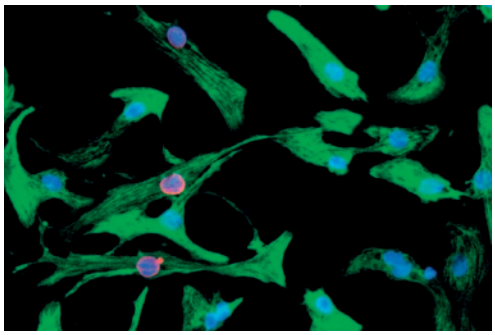


Figure. Brdu+ cultured neonatal rat cardiomyocytes.

Publications 2015

Chen Y, **Paavola J**, Stegaev V, Stark H, Chazot PL, Wen JG, Konttinen YT. Activation of histamine H3 receptor decreased cytoplasmic Ca2+ imaging during electrical stimulation in the skeletal myotubes. *Eur J Pharmacol.* 2015; 5;754:173-8.

Chilton R, **Tikkanen I**, Cannon CP, Crowe S, Woerle HJ, Broedl UC, Johansen OE. Effects of empagliflozin on blood pressure and markers of arterial stiffness and vascular resistance in patients with type 2 diabetes. *Diabetes Obes Metab.* 2015; 17:1180-1193.

Haltia O, Törmänen S, Eräranta A, Jokihaara J, Nordhausen K, Rysä J, Ruskoaho H, **Tikkanen I**, Mustonen J, Pörsti I. Vasopeptidase inhibition corrects the structure and function of the small arteries in experimental renal insufficiency. *J Vasc Res.* 2015; 9;52(2):94-102.

Lakkisto P. Hemioksygenaasi-1 – mahdollinen uusi merkkiaine. *KLIIN LAB* 2/2015.

Penttinen K, Swan H, Vanninen S, **Paavola J**, Lahtinen AM, Kontula K, Aalto-Setälä K. Antiarrhythmic effects of dantrolene in patients with catecholaminergic polymorphic ventricular tachycardia and replication of the responses using iPSC models. *PLoS One.* 2015; 10:e0125366.

Purhonen AK, Juutilainen A, Vänskä M, Lehtikangas M, **Lakkisto P**, Hämäläinen S, Koivula I, Jantunen E, Pulkki K. Human plasma cell-free DNA as a predictor of infectious complications of neutropenic fever in hematological patients. *Infect Dis* (Lond). 2015; 47(4):255-9.

Metsärinne K, **Tikkanen I**. Behandling och uppföljning av primär hypertension. *Finska Läkaresällskapets Handlingar.* 1/2015, Årg 175.

Group members

Pirta Hotulainen, Ph.D., Docent, Head
Enni Bertling, Ph.D.
Rimante Minkeviciene, Ph.D.
Amr Abou Elezz, M.Sc.
Iryna Hlushchenko, M.Sc.

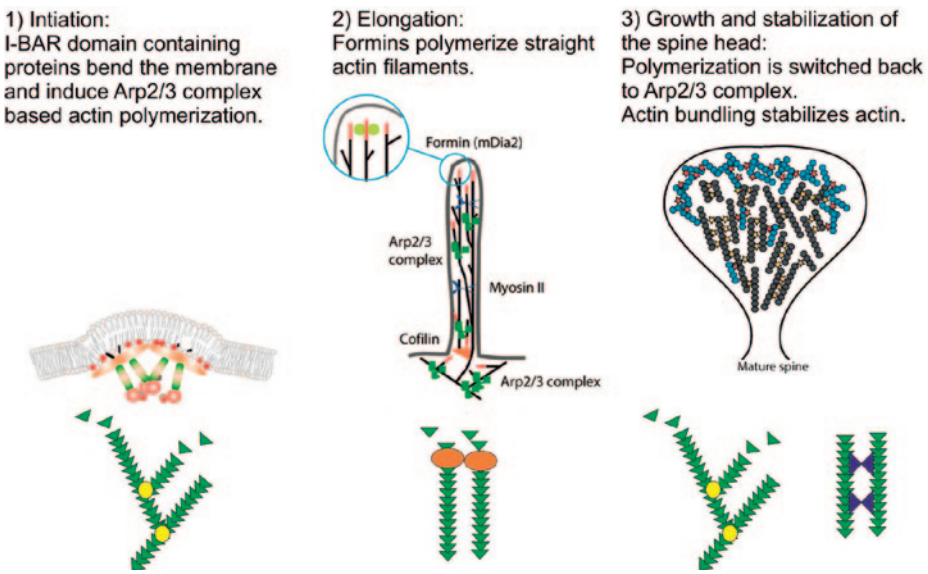
A new group at Minerva 2015:

CELLULAR NEUROSCIENCE

Pirta Hotulainen, Adjunct Professor, was awarded a group leader post at the Minerva Institute beginning October 1, 2015. Her research group moved to Minerva from the Neuroscience Center, University of Helsinki, at the end of the year. The group comprises the principal investigator, two postdoctoral fellows and two doctoral students. Research in the group focuses on regulation of the actin cytoskeleton in neurons, particularly its disruption in neuropsychiatric diseases.

A typical neuron has a cell body, dendrites and an axon. The site of initiation of an action potential is termed the axon initial segment (AIS). The signal sent by an axon is transmitted to dendrites through synapses. On dendrites, most excitatory synapses are located in small protrusions called dendritic spines. Precise control of the dendritic spine morphology and density, as well as the length and location of the AIS, is critical for normal brain function. Accordingly, both aberrant spine morphology and non-functional AIS are linked to many neurological diseases.

The actin cytoskeleton is a structural element underlying proper morphology of dendritic spines and structure of the AIS. Although it is evident that many actin-regulating genes are involved in schizophrenia and autism, we do not yet understand how mutations affect the proteins´ or brain function in these diseases. The current view is that in schizophrenia, spine pruning, starting by adolescence, is too effective and leads to decreased spine density. In contrast, in autism there are too many spines that are not pruned.



In order to understand which actin binding proteins are important for proper neuronal function and which proteins might be the best drug targets, we need a comprehensive picture of the actin regulation in neurons. Thus, the Cellular Neuroscience group aims at a broad understanding of the regulation of the actin cytoskeleton in dendritic spines and the axon initial segment. This focus includes characterization of disease-linked mutations, how these mutations affect protein function and dendritic spine density and morphology. To date, the group has elucidated the molecular mechanisms underlying dendritic spine initiation (Saarikangas *et al.*, 2015), dendritic filopodia elongation (Hotulainen *et al.*, 2009), spine head growth (Hotulainen *et al.*, 2009) and spine head maintenance (Koskinen *et al.*, 2014). Importantly, the new insights gained in these studies can be used to find and develop new targets for therapeutic agents for neuropsychiatric diseases.

Selected publications

Saarikangas J, Kourdougli N, Senju Y, Chazal G, Segerstråle M, **Minkeviciene R**, **Kuurne J**, Mattila PK, Garrett L, Hölter SM, Becker L, Racz I, Hans W, Klopstock T, Wurst W, Zimmer A, Fuchs H, Gailus-Durner V, Hrabě de Angelis M, von Ossowski L, Taira T, Lappalainen P, Rivera C, **Hotulainen P**. MIM-induced membrane bending promotes dendritic spine initiation. *Dev Cell*. 2015; 22;33:644-59.

Koskinen M, **Bertling E**, Hotulainen R, Tanhuanpää K, **Hotulainen P**. Myosin IIb controls actin dynamics underlying the dendritic spine maturation. *Mol Cell Neurosci*. 2014; 61:56-64.

Hotulainen P, Llano O, Smirnov S, Tanhuanpää K, Faix J, Rivera C, Lappalainen P. Defining mechanisms of actin polymerization and depolymerization during dendritic spine morphogenesis. *J Cell Biol*. 2009; 20;185:323-39.

CELLULAR PHYSIOLOGY

Group members

Kid Törnquist, Ph.D., Professor, Head
Veronica Kalhori, M.Sc., Ph.D. fall 2015

In addition, the following researchers were working at Åbo Akademi University:

Muhammad Yasir Asghar, Phil.lic.
Basit Butt, M.D.
Kati Kemppainen, M.Sc.
Melissa Magnusson, M.Sc.
Ilari Pulli, M.Sc.
Taru Lassila, B.Sc.

External funding

The Liv och Hälsa Foundation
The Sigrid Juselius Foundation
Svenska Kulturfonden
Magnus Ehrnrooth Foundation

Main research activities

The research group investigates calcium and lipid signaling in cancer cells. Specifically, the group studies three main areas of calcium and lipid signaling in thyroid and other human tumor-derived cancer cells: 1) the importance of the canonical transient receptor potential (TRP) C-family of ion channels, as well as the actions of the sphingomyelin metabolite sphingosine 1-phosphate (S1P), 2) the interactions between TRPC-channels and S1P, and 3) the receptors important for the regulation of cell migration in different cell types.

In normal, as well as cancerous, human thyroid cells, several members of the TRPC ion channel family are expressed. Since some members may have redundant effects, and gene expression silencing of one channel may give results difficult to interpret or be without an effect, it is challenging to pinpoint the exact functions of the individual receptors. Our previous work has shown that these channels are of significance in regulating thyroid physiology. Current efforts are aimed to understand the importance of TRPC channels in human thyroid cancer cells, especially on invasion and migration. These studies have shown that that members of the TRPC channel family, in particular TRPC1, are important regulators of the expression of receptors crucial for migration of cancer cells. For example, gene expression knockdown of TRPC1 potently hampered both the proliferation and migration of thyroid cancer cells. Of special significance was the observation that TRPC1-evoked calcium entry regulated the expression of the $S1P_3$ receptor, a receptor important in regulating expression of both vascular endothelial growth factor (VEGF) receptor 2 and hypoxia-inducible factor 1-α. In addition, $S1P_3$ participates in the regulation of both matrix-metalloproteinase

2 and 9. These receptors and factors are crucial for cancer cell invasion and migration. TRPC channels are permeable to both sodium and calcium ions, and it is already known that regulation of cell migration is dependent on the entry of calcium ions and not the depolarization obtained through sodium ions entry. Thus, modulating TRPC1 channel expression and activity, in particular calcium entry, has a significant impact on thyroid cancer invasion, migration and proliferation.

In some cell types, S1P can inhibit migration by activating the $S1P_2$ receptor. In collaboration with professor John Eriksson's group we described a novel mechanism by which S1P, by activating $S1P_2$ and its downstream effector Rho-associated kinase, induced serine phosphorylation of vimentin and a reorganization of the vimentin filaments. This phosphorylation resulted in a dramatic inhibition of cell migration. Our observation thus described a novel mechanism by which S1P can block migration of cells.

We have also started to use the chicken embryo chorioallantoic membrane (CAM) model to investigate the proliferation and migration of thyroid cancer cells. This *in ovo* model appears to be a suitable and inexpensive model, and the first investigations will be finished in the coming year. This model can also be used to investigate the angiogenic properties of thyroid cancer cells, since these cells secrete angiogenic factors such as VEGF.

Several other proteins participate in regulating cellular calcium homeostasis. Present studies are now aimed at investigating the importance of Orai channels and the stromal interacting molecules (STIM), in particular STIM-1, on thyroid physiology. Orai1 and STIM-1 are of importance in regulating store-operated calcium entry, the calcium entry process activated when intracellular calcium stores are depleted due to agonist-induced signaling.

Ongoing work in our group also aims to understand compartmentalized calcium signaling in different types of cells. Since compartmentalized calcium signaling results in ion hot spots in cells it may affect specialized signaling pathways or organelles. Of special interest are calcium signals in the caveolae, endoplasmic reticulum, mitochondria and endosomes. Together with our collaborators, we have developed novel tools to enhance these ongoing studies.

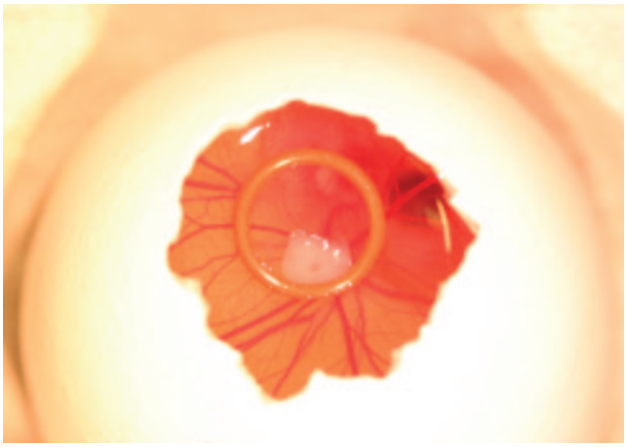


Figure. Follicular thyroid cancer cells growing on the chorioallantoic membrane of a fertilized chicken egg. The cells, embedded in Matrigel, are kept in place by the plastic ring.

Publications 2015

Asghar MY, Magnusson M, Kemppainen K, Sukumaran P, Lof C, Pulli I, **Kalhori V**, **Tornquist K**. Transient receptor potential canonical 1 (TRPC1) channels as regulators of sphingolipid- and VEGF receptor expression: implications for thyroid cancer cell migration and proliferation. *J Biol Chem.* 2015; 26;290(26):16116-31.

Hyder CL, Kemppainen K, Isoniemi KO, Imanishi SY, Goto H, Inagaki M, Fazeli E, Eriksson JE, **Törnquist K**. Sphingolipids inhibit vimentin-dependent cell migration. *J Cell Sci.* 2015; 1;128(11):2057-69.

Kalhori V, Törnquist K. MMP2 and MMP9 participate in S1P-induced invasion o f follicular ML-1 thyroid cancer cells. *Mol Cell Endocrinol.* 2015; 15;404:113-22.

Pulli I, **Blom T**, Löf C, Magnusson M, Rimessi A, Pinton P, **Törnquist K**. A novel chimeric aequorin fused with caveolin-1 reveals a sphingosine kinase 1 -regulated Ca2+ microdomain in the caveolar compartment. *Biochim Biophys Acta.* 2015; 1853(9):2173-82.

Thesis completed in the group in 2015

The following doctoral thesis was accepted at Åbo Akademi University (Turku) this year:

Veronica Kalhori: Sphingosine-1-phosphate –evoked invasion of follicular thyroid cancer cells: evidence for the involvement of HIF-1α, MMP2 and MMP9. September 11, 2015.

Group members

Hannele Yki-Järvinen, M.D., Dr.Med.Sci., F.R.C.P, Professor, Head
Nidhina Haridas, Ph.D.
You Zhou, Ph.D.
Bian Hua, M.D.
Susanna Lallukka, M.D.
Gemma Llaurado, M.D.
Panu Luukkonen, M.D.
Elina Petäjä, M.D.
Sanja Sädevirta, M.D.
Jenni Hyysalo, Medical Student
Jarkko Soronen, M.Sc.
Antti Hakkarainen, M.Sc. Techn.
Aila Karioja-Kallio, Laboratory Technician
Anne Salo, Laboratory Technician
Mia Urjansson, Laboratory Technician

External funding

The Academy of Finland
EU/EFPIA Innovative Medicines Initiative Joint Undertaking
(EMIF grant number 115372)
EU H2020 Elucidating Pathways of Steatohepatitis
(EPoS, grant number 634413)
The Novo Nordisk Foundation
Research Funding of Helsinki-Uusimaa Hospital District (EVO)
The Sigrid Jusélius Foundation
Orionin Tutkimussäätiö
The Paulo Foundation
Suomen Lääketieteen Säätiö
Biomedicum Helsinki Foundation

ENDOCRINOLOGY

Main research activities

During 2015, we continued our studies addressing the pathogenesis and diagnosis of *non-alcoholic fatty liver disease* (NAFLD) in humans. Specifically, we used two models of acquired NAFLD in our studies. One model is associated with the I148M gene variant in patatin-like phospholipase domain containing 3 protein (PNPLA3) ('PNPLA3 NAFLD') and the other with the E167K variant in transmembrane 6 superfamily member 2 (TM6SF2) (TM6SF2 NAFLD'). While neither of these models increases the risk of non-alcoholic steatohepatitis (NASH) and severe liver injury, they are associated with insulin resistance, an increased risk of type 2 diabetes, or cardiovascular disease.

In our study, we genotyped 300 Finnish subjects for risk alleles of *TM6SF2* (E167K, rs58542926 and *PNPLA3* (I148M, rs738409). Liver measurements of these subjects were also collected using proton magnetic resonance spectroscopy ^{1}H-MRS and circulating lipids by ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS). We compared the plasma lipidome between E167K carriers (TM6SF2$^{EK/KK}$) and non-carriers (TM6SF2^{EE}), and among three groups of NAFLD: i) carriers of E167K but not I148M in PNPLA3 ('TM6SF2 NAFLD'), ii) carriers of the I148M but not E167K ('PNPLA3 NAFLD') and iii) non-carriers of either risk allele ('Non-risk NAFLD'). Hepatic and adipose tissue insulin sensitivities were measured using the euglycemic hyperinsulinemic clamp technique combined with infusion of [3-^{3}H]glucose in 111 subjects. We found liver fat content was 34% higher in TM6SF2$^{EK/KK}$ than TM6SF2^{EE}. Insulin sensitivities of glucose production and lipolysis were significantly higher at any given LFAT in the TM6SF2$^{EK/KK}$ than in the TM6SF2^{EE} group. Comparison of three NAFLD groups with similar LFATs showed that both the 'TM6SF2 NAFLD' and 'PNPLA3 NAFLD' had significantly lower triglyceride levels and were characterized by lower levels of most common TAGs compared to the 'non-risk NAFLD' group. Based on these results, we concluded that the

E167K variant of TM6SF2 is associated with a distinct subtype of NAFLD characterized by preserved insulin sensitivity of lipolysis and hepatic glucose production and lack of hypertriglyceridemia despite clearly increased LFAT content.

Jenni Hyysalo is currently preparing her thesis, a study comparing the serum lipidome in 'PNPLA3 NAFLD' and 'Obese/Metabolic NAFLD' as well as a study examining the impact of genetic variation in apoCIII on NAFLD. The thesis includes the first population-based study addressing the prevalence of NASH based on a score validated using 300 liver biopsies. Jenni will finish her studies for the M.D. in Tartu (scheduled for June 2016) and then defend her thesis at the University of Helsinki.

A Spanish endocrinologist, Dr. Gemma Llaurado completed part of her PhD training at Minerva during 2015. In her work at Minerva, Gemma addressed NAFLD in type 1 diabetes and showed that NAFLD is less common in patients who lack the portal-peripheral insulin gradient than in age-gender and weight-matched controls. In addition, she determined that this result is associated with enhanced hepatic insulin sensitivity when measured directly using the euglycemic insulin clamp technique combined with [3-^{3}H]glucose. She also showed that the lipolysis is more suppressed in type 1 diabetic patients. These data support the idea that portal hyperinsulinemia, in the face of enhanced peripheral FFA flux, contributes to NAFLD. Importantly, the enhanced hepatic sensitivity to insulin in the type 1 diabetic patients may increase the risk of hypoglycemia.

A Chinese endocrinologist, Dr. Bia Huan spent one year in our research group at Minerva to study factors regulating liver volume in humans. Bia established an equation to predict liver volume in humans, which allows accurate quantification of the fat content of the entire liver when the % liver fat is measured using proton magnetic resonance spectroscopy.

Hannele Yki-Järvinen has edited the first ever textbook section and written a chapter in the International Textbook of Diabetes Mellitus, 4th edition. Thus, NAFLD is indeed an essential feature of the pathogenesis of metabolic syndrome and type 2 diabetes, and a condition that the diabetologist needs to consider as liver disease now can be classified as a complication of metabolic syndrome and type 2 diabetes.

Publications

Bian H, Hakkarainen A, **Zhou Y**, Lundbom N, **Olkkonen VM**, **Yki-Järvinen H**. Impact of non-alcoholic fatty liver disease on liver volume in humans. *Hepatol Res.* 2015; 45(2):210-9.

Haridas PA, **Soronen J**, **Sädevirta S**, **Mysore R**, Quagliarini F, Pasternack A, Metso J, **Perttilä J**, Leivonen M, Smas CM, Fischer-Posovszky P, Wabitsch M, Ehnholm C, Ritvos O, Jauhiainen M, **Olkkonen VM**, **Yki-Järvinen H**. Regulation of angiopoietin-like proteins (ANGPTLs) 3 and 8 by insulin. *J Clin Endocrinol Metab.* 2015; 100(10):E1299-E1307.

Llauradó G, Sevastianova K, **Sädevirta S**, Hakkarainen A, Lundbom N, Orho-Melander M, Groop PH, Forsblom C, **Yki-Järvinen H**. Liver fat content and hepatic insulin sensitivity in overweight patients with type 1 diabetes. *J Clin Endocrinol Metab.* 2015; 100(2):607-16.

Männistö VT, Simonen M, **Hyysalo J**, Soininen P, Kangas AJ, Kaminska D, Matte AK, Venesmaa S, Käkelä P, Kärjä V, Arola J, Gylling H, Cederberg H, Kuusisto J, Laakso M, **Yki-Järvinen H**, Ala-Korpela M, Pihlajamäki J. Ketone body production is differentially altered in steatosis and non-alcoholic steatohepatitis in obese humans. *Liver Int.* 2015; 35(7):1853-61.

Yki-Järvinen H. Chapter 19. Pathogenesis of nonalcoholic fatty liver disease (NAFLD). International Textbook of Diabetes Mellitus, Fourth Edition. Edited by Ralph A. DeFronzo, K. George M. M. Alberti, Ele Ferrannini, and Paul Zimmet. © 2015 JohnWiley & Sons, Ltd. Published 2015 by JohnWiley & Sons, Ltd.

Yki-Järvinen H. Nutritional modulation of non-alcoholic fatty liver disease and insulin resistance. *Nutrients.* 2015; 7:9127-9138.

Yki-Järvinen H, Bergenstal RM, Bolli GB, Ziemen M, Wardecki M, Muehlen-Bartmer I, Maroccia M, Riddle MC. Glycaemic control and hypoglycaemia with new insulin glargine 300 U/mL versus glargine 100 U/mL in people with type 2 diabetes using basal insulin and oral antihyperglycaemic drugs (EDITION 2 randomised 12-month trial including 6-month extension). *Diabetes Obes Metab.* 2015; 17(12):1142-9.

Yki-Järvinen H, **Luukkonen PK**. Heterogeneity of NAFLD. *Liver Int.* 2015; 35(12):2498-500.

Zhou Y, **Laurado G**, Oresic M, Hyötyläinen T, Orho-Melander M, **Yki-Järvinen H**. Circulating triacylglycerol signatures and insulin sensitivity in NAFLD associated with the E167K variant in TM6SF2. *J Hepatol.* 2015; 62(3):657-63.

Docent Lecture

The title of docent was granted at the University of Helsinki this year:
You Zhou: *Medical Statistics.* April 13, 2015. Biomedicum Helsinki.

LIPID SIGNALING AND HOMEOSTASIS

Group members

Vesa Olkkonen, Ph.D., Professor, Head
Marion Weber-Boyvat, Ph.D.
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Annukka Kivelä, Ph.D.
Henriikka Kentala, M.Sc.
Raghavendra Mysore, M.Sc.
Hanna Ruhanen, M.Sc.
Saundarya Shah, B.Sc.
Eeva Jääskeläinen, Laboratory Technician

External funding

The Academy of Finland
The Finnish Foundation for Cardiovascular Research
The Liv och Hälsa Foundation
The Magnus Ehrnrooth Foundation
The Sigrid Jusélius Foundation

Main research activities

The group aims to elucidate how cells maintain specific lipid compositions of organelles and sense their internal lipid status to control a number of vital cellular processes, including signal transduction and vesicle transport. A novel concept, that of membrane contact sites (MCSs), zones of close contact between the limiting membranes of intracellular organelles, has moved into the focus of biomedical research. Such contacts provide high capacity and specificity platforms for the inter-organelle transport of small molecules and signals. In this context, the cytoplasmic oxysterol-binding protein (OSBP) homologs, ORPs, identified by the group are of primary interest. A major project in the group aims to unravel the function of these proteins and other new MCS protein components, as well as the roles of MCSs in diseases involving aberrant lipid transport or signaling.

In 2015, major activity in the group was directed at understanding the role of ORPs in cell signaling at MCSs (Fig. 1) and the impact of their binding to sterols. We showed that ORP3, in association with its endoplasmic reticulum (ER) anchor, the VAMP-associated protein A (VAPA) at ER-plasma membrane contact sites, regulates the activity of the small GTPase R-Ras, with downstream effects on the activity of Akt/protein kinase B and β1-integrins (Weber-Boyvat, M. *et al.*, 2015b). Moreover, the cellular sterol status was shown to modify the subcellular distribution of several ORP-VAPA complexes and the structure of the organelles with which these complexes associate (Kentala, H. *et al.,* 2015). A collaborative study with F. van Kuppeveld (Utrecht University, the Netherlands) revealed an inhibitory potential of compounds targeting OSBP/ORPs

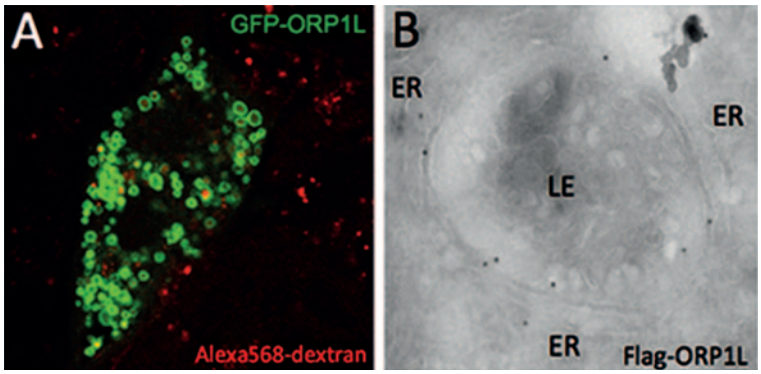

Figure. ORP1L at contact sites between late endosomes (LE) and endoplasmic reticulum (ER). (A) HuH7 hepatoma cell expressing GFP-ORP1L mutant Q459P (green), after overnight internalization of the endocytosed marker Alexa568-dextran (red). The green rings represent GFP-ORP1L Q459P which labels LE encircled by ER membranes (LE-ER contact sites). (B) Immuno electron micrograph showing a LE encircled by ER membranes. The gold particles mark localization of FLAG-ORP1L. Courtesy of Juho Pirhonen (A) and Nils Bäck (B).

against enterovirus replication (Strating, J.R. *et al.,* 2015). Ongoing work initiated in 2015 elucidates the coordinated regulation of MCSs and the exocytotic vesicle transport machinery, the function of ORP4L, a family member involved in the control of cellular Ca^{2+} homeostasis, energy metabolism, proliferation and viability, as well as the components and function of MCSs in endothelial cells which play key roles in cardiovascular diseases.

A second major activity in the group in 2015 focused on elucidating the role of novel molecular machineries in the development of non-alcoholic fatty liver disease (NAFLD), insulin resistance, type 2 diabetes, and their cardiovascular sequelae. Specifically, we finalized a study addressing the regulation of Angiopoietin-like proteins (ANGPTL) 3 and 8 by insulin *in vivo* and *in vitro* (Haridas, P.A. *et al.* 2015). This work suggested that ANGPTL8, which is highly induced by insulin in adipose tissue but poorly secreted, has an intracellular function in adipocyte lipid metabolism. Ongoing work aims to characterize this function and the mechanisms of ANGPTL8 regulation by insulin. Two studies addressing the role of microRNAs in NAFLD liver (Soronen, J. *et al.*, in press) and in visceral adipose tissue of obese subjects (Mysore, R. *et al.*, in press) were also finalized.

Publications

Haridas PA, **Soronen J**, **Sädevirta S**, **Mysore R**, Quagliarini F, Pasternack A, Metso J, **Perttilä J**, Leivonen M, Smas CM, Fischer-Posovszky P, Wabitsch M, Ehnholm C, Ritvos O, Jauhiainen M, **Olkkonen VM**, **Yki-Järvinen H**. Regulation of angiopoietin-like proteins (ANGPTLs) 3 and 8 by insulin. *J Clin Endocrinol Metab*. 2015; 100(10):E1299-E1307.

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Olkkonen VM, Gylling H, **Ikonen E**. Kasvisterolit, kolesterolin esiasteet ja oksisterolit: pienet määrät, suuret vaikutukset. *Duodecim*, 2015; 131(3):235-41.

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MEMBRANE BIOLOGY

Group members

Elina Ikonen, M.D. Dr.Med.Sci., Academy Professor (Director), Head
Tomas Blom, Ph.D., Academy Research Fellow, Team Leader
Ivonne Brock, Ph.D.
Andrea Dichlberger, Ph.D. (team of T. Blom)
Maarit Hölttä-Vuori, Ph.D.
Kristiina Kanerva, Ph.D.
Shiqian Li, Ph.D.
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Simon Pfisterer, Ph.D.
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Juho Pirhonen, Medical Student
Veijo Salo, MD, Ph.D. Student
Lauri Vanharanta, Medical Student
Kecheng Zhou, Ph.D. Student (team of T. Blom)
Pipsa Kaipainen, Laboratory Technician
Anna Uro, Laboratory Technician

External funding

The Academy of Finland: Centre of Excellence in Biomembrane Research (prolipids.helsinki.fi), Academy Professorship
The Finnish Foundation for Cardiovascular Research
The Sigrid Jusélius Foundation
Biocentrum Helsinki, Biocenter Finland
University of Helsinki, Research Excellence and Infrastructure Funding

Main research activities

Cholesterol and sphingolipids can promote the formation of lipid rafts - liquid-ordered domains that regulate functions of membrane associated proteins - in cell membranes. We aim to understand how raft lipids move in cells. Defects in these mechanisms lead to severe human diseases of cholesterol and sphingolipid storage and associated protein dysfunctions. We also develop, in collaborative efforts, novel tools to analyze cholesterol and sphingolipid transport and metabolism in cells. In 2015, we reviewed the fluorescent cholesterol and sphingolipid analogs developed and validated with Prof. Robert Bittman (Ikonen and Blom, 2015).

Ceramide is a building block of all sphingolipids and a key regulator of cell death. It is produced in lysosomes during sphingolipid breakdown and can be used for sphingolipid re-synthesis outside lysosomes. Whether ceramide can be exported from lysosomes, and if so, by which mechanism, has so far been unknown. In 2015, we reported that LAPTM4B, a lysosomal membrane protein of previously unknown function, facilitates ceramide export from lysosomes (Blom *et al.*, 2015). LAPTM4B silencing led to lysosomal ceramide accumulation, while LAPTM4B overexpression rescued lysosomal ceramide overload in the lysosomes of Farber's disease (acid ceramidase-deficient) human patient cells. Furthermore, we provided evidence for a specific interaction between LAPTM4B and ceramide.

LAPTM4B is overexpressed in several cancers and often associated with chemotherapy resistance, and the mechanisms involved in the ac-

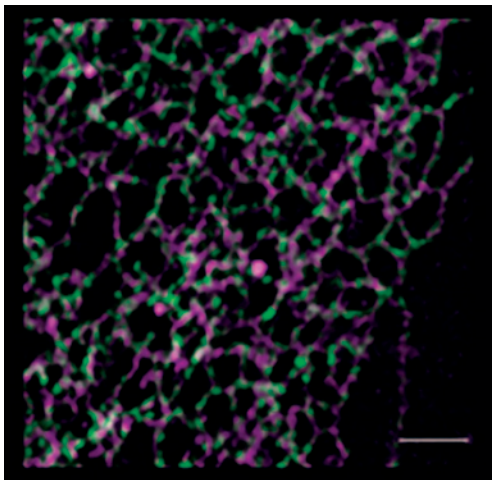

Figure. Lipid droplets forming from the endoplasmic reticulum. A fluorescently labeled fatty acid was added to lipid starved human epithelial A431 cells and the cells were imaged live 5 minutes after fatty acid addition. Magenta shows the fluorescent fatty acid in the endoplasmic reticulum and in forming lipid droplets, green indicates an endoplasmic reticulum marker protein. Scale bar, 2 µm. Courtesy of Veijo Salo (Membrane biology group).

quired resistance are not well understood. To this end, we showed that the ceramide export function of LAPTM4B and its effects on cell death pathways may explain how LAPTM4B affects chemotherapy responses (Blom *et al.*, 2015).

In addition to our studies on the cellular roles of ceramide, we have also examined cholesterol-binding proteins and the role of altered cholesterol balance in cancer. Several cholesterol-interacting membrane proteins have been characterized. One of them, StARD3, is localized in late endosomal compartments. Interestingly, the *StARD3* gene is part of the *HER2* amplicon, a chromosomal region commonly amplified in breast cancers. We showed that as a result of this chromosomal amplification, the StARD3 protein is also overexpressed in a subset of breast cancers (Vassilev *at el.,* 2015). Moreover, we showed that StARD3 overexpression has major effects on the cholesterol balance of breast cancer cells. This imbalance, in turn, can promote cancer progression. Specifically, increased plasma membrane cholesterol content upon StARD3 overexpression altered lipid raft-dependent functions, such as cell adhesion (Vassilev *et al.*, 2015).

Publications 2015

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Pfisterer SG, **Ikonen E**. Lipid transport takes the 'omics' highway. *Curr Opin Lipidol.* 2015 Aug;26(4):348-9.

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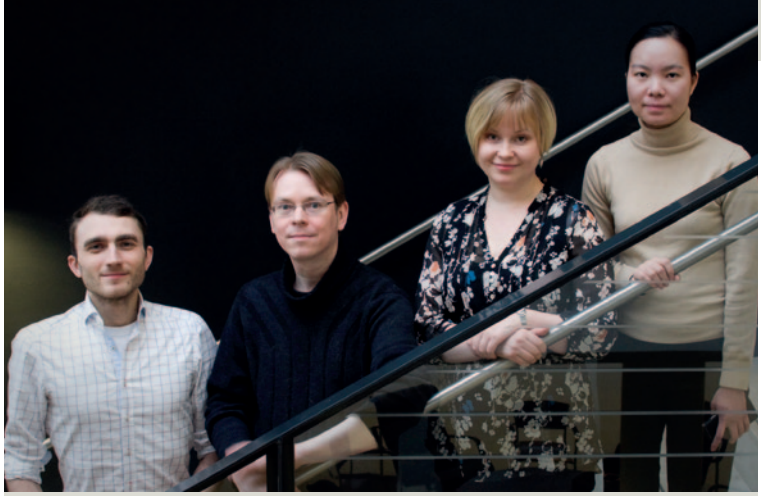

METABOLISM

Main research activities

We collaborate closely with the FUSION-study (Finland-United States Investigation of NIDDM Genetics, head principal investigator (PI) Prof. Michael Boehnke, University of Michigan, Ann Arbor, USA), with the head of the group, Docent Heikki Koistinen being one of the FUSION PIs. As part of ongoing genetic analyses of the FUSION- and METSIM- (Metabolic Syndrome in Men, PI, Prof. Markku Laakso, University of Eastern Finland) studies, we aim to find several gene variants associated with insulin resistance and type 2 diabetes (T2D) risk. Since previous GWA- studies have mainly pinpointed genes affecting insulin secretion as T2D risk genes, such a finding would be a major advance in the field.

One example of an insulin resistance-associated gene, identified in a large international collaboration that also included subjects from the FUSION- and METSIM cohorts, is a missense variant of AKT2 (unpublished data). AKT, which is also known as protein kinase B, is an important effector in insulin signaling and has several isoforms. In a previous collaborative study with Professor Juleen Zierath at Karolinska Institute in Sweden, we identified the AKT2 isoform as indispensable for insulin action on glucose metabolism in human myotubes. Specifically, in muscle cells where AKT2 was silenced by siRNA, insulin action on glucose uptake and glycogen synthesis was completely abolished (Bouzakri, K. *et al.* 2006). The novel AKT2 variant is specific for Finns (MAF = ~1%) and very rare in non-Finnish Europeans. It is associated with higher fasting insulin concentrations, and it predisposes patients to T2D. There are several carriers of this AKT2 allele in the METSIM-cohort. In collaboration with Professors Markku Laakso and Pirjo Nuutila at the University of Turku and Turku PET Centre, we have started a project to study the effect

Group members

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Selina Mäkinen, M.Sc.
Petro Kyrylenko, Medical Student

External funding

The Academy of Finland
Research Funding of Helsinki-Uusimaa Hospital District (EVO)
University of Helsinki

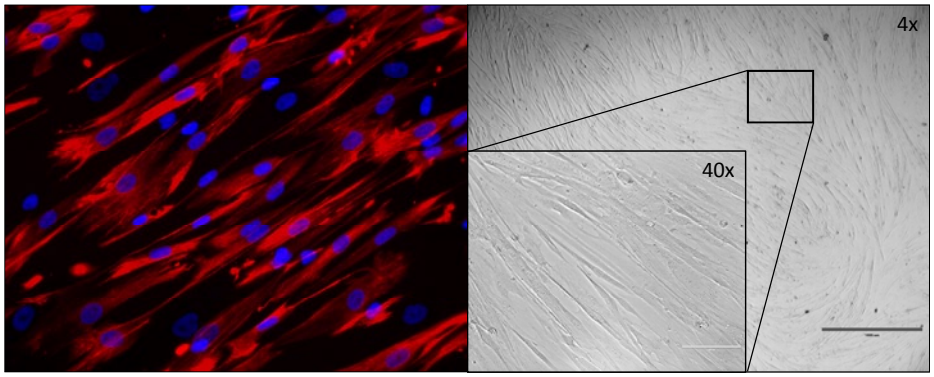


Figure. Fluorescence image of primary human myoblasts stained with desmin (left). Brightfield image of differentiated, multinucleated primary human myotubes (right).

of this AKT2 variant on the pathogenesis of insulin resistance. Tissue specific (e.g. skeletal muscle, liver, adipose tissue) glucose uptake has been analysed *in vivo* during the euglycemic hyperinsulinemic clamp with [^{18}F]-FDG-PET at Turku PET Centre. Furthermore, given the particular relevance of AKT2 in glucose metabolism in skeletal muscle, we have created primary muscle cell cultures from carriers of the AKT2 variant and wild type controls for detailed *in vitro* studies that are currently ongoing at Minerva. These data will complement the *in vivo* investigations and provide further mechanistic evidence of the pathophysiological significance of the Finnish AKT2 variant.

In our other research activities, we have participated in a collaboration where >350 000 individuals from 102 cohorts were analysed for contiguous homozygous segments (runs of homozygosity, ROH), which reflect distant parental relatedness. The effect of ROH on several traits of public health importance was determined. Significant associations were found between ROH and height, FEV1, general cognitive ability and educational attainment. Increased homozygosity was associated with decreased trait value in each case. These data indicate that increased cognitive function and height have been positively selected during evolution of mankind (Joshi, P.K. *et al.* Nature 2015).

In other collaborative efforts, fine mapping of 39 established T2D loci in >27 000 cases and 57 000 controls provided further insight into how integrative analysis of genetic and genomic information can reveal molecular mechanisms whereby genetic variants impact the disease (Gaulton, K.J. *et al.* 2015). Body mass index (BMI) and body shape change with advancing age, with substantial differences between men and women. To study the genetic regulation of this phenomenon, a meta-analysis of 114 studies (>300 000 people) was performed. We identified 15 loci which regulate BMI in an age-dependent manner, and 44 loci which regulate body shape in a sex-dependent manner (Winkler, T.W. *et al.* 2015).

There is an emerging connection between bone, glucose metabolism and insulin sensitivity. We studied markers of bone metabolism in response to acute hyperinsulinemia in men and women. Concentrations of bone formation (PINP, total osteocalcin (OC), the uncarboxylated form of OC (ucOC)) and resorption markers (CTX, TRAcP5b) were analysed. CTX levels decreased significantly during a 4-h insulin infusion, suggesting that insulin decreases bone resorption. The ratio of uncarboxylated-to-total OC was also affected by insulin. Thus, insulin has direct effects on bone metabolism in humans (Ivaska, K.K. *et al.* 2015).

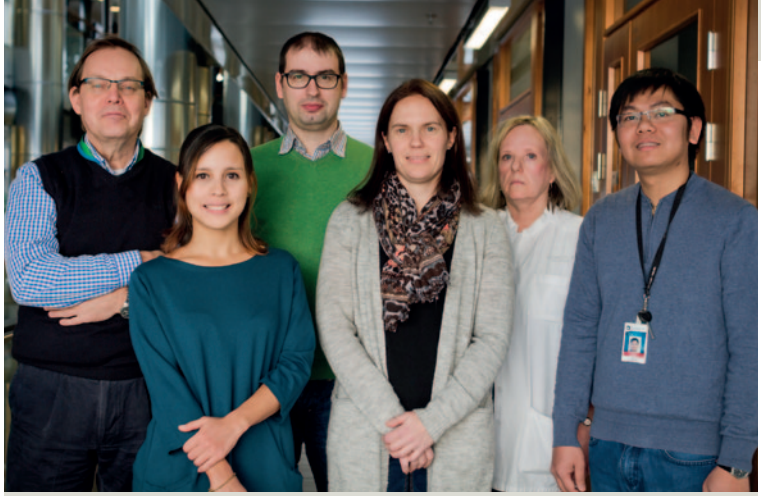
Publications 2015

Gaulton KJ, ..., **Koistinen HA**, ..., et al. Genetic fine mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. *Nat Genet.* 2015; 47:1415-1425.

Ivaska KK, Heliövaara MK, Ebeling P, Bucci M, Huovinen V, Väänänen HK, Nuutila P, **Koistinen HA**. The effects of acute hyperinsulinemia on bone metabolism. *Endocr Connect.* 2015; 4:155-162.

Joshi PK, ..., **Koistinen HA**, ..., et al. Directional dominance on stature and cognition in diverse human populations. *Nature.* 2015; 523:459-462.

Winkler TW, ..., **Koistinen HA**, ..., et al. The influence of age and sex on genetic associations with adult body size and shape: a large-scale genome-wide interaction study. PLoS *Genet.* 2015; 11:e1005378. doi: 10.1371/journal.pgen.1005378.



NEURONAL SIGNALING

Group members

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Kristiina Söderholm, Laboratory Technician

External funding

The Academy of Finland
The Finska Läkaresällskapet
The Liv och Hälsa Foundation
The Magnus Ehrnrooth Foundation
The Novo Nordisk Fonden
The Parkinson Foundation Finland

Main research activities

We study basic mechanisms underlying neurodegenerative diseases and nerve cell damage in the brain. Our specific areas of interest are the roles of mitochondrial and endoplasmic reticulum (ER) stress pathways as well as autophagy in the pathogenesis and models of brain diseases. During 2015, we were invited to write a review, recently published (Lindholm *et al.*, 2015), on the significance of these pathways and their putative roles, particularly in human Parkinson´s disease (PD). In addition to PD, we study cell lipid metabolism and how it is influenced by neurotrophic factors, such as nerve growth factor (NGF), which we previously determined to regulate lipoprotein receptors in neurons. We employ various proteomic and gene expression methods, cell culture and animal models of disease, as well as genetically modified mice. Although the majority of the group is housed in Department of Biochemistry and Developmental Biology, Faculty of Medicine, the group is actively engaged in research at Minerva.

1. Role of PGC-1 in neuroprotection and control of mitochondrial functions in neurons

Peroxisome proliferator activated receptor-gamma (PPARγ) coactivator 1-alpha (PGC-1) is a master regulator of mitochondrial and oxidative stress in cells. Previously, using transgenic mice overexpressing PGC1 in the brain, we showed that dopaminergic neurons in the substantia nigra are resistant to the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (Mudo *et al.*, 2012). PGC-1 acts by binding PPARγ in cell nuclei to regulate expression of various genes. We analyzed the effects

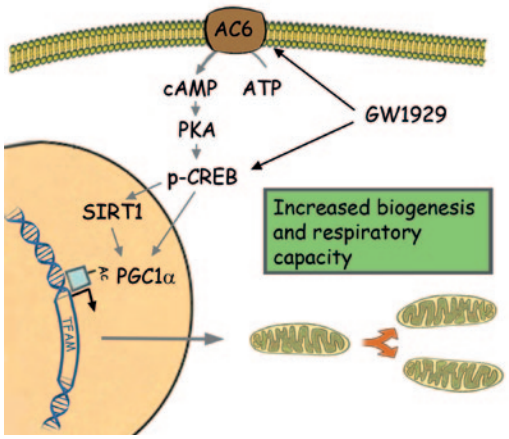
of chemical compounds and drugs that are known to influence PPARγ signaling. These drugs are usually employed for treatment of metabolic disorders such as type-2 diabetes, and we are studying whether they may also have beneficial effects in brain disorders (Patrone *et al.*, 2014). For many of these studies we are testing mitochondrial functions in living neurons in real-time (Seahorse XF Analyzer).

We have recently obtained evidence that a PPARγ agonist exerts a protective function, in cultured human dopaminergic cells, by increasing PGC1, as well as mitochondrial function (Mäkelä *et al.*, 2015). We further described a novel mechanism for this PPARγ agonist by showing that it acts partly by stimulating the adenylate cyclase-6 (AD6) -protein-kinase A-cyclic AMP signaling pathway in neurons (Mäkelä *et al.*, 2015, see Figure 1 attached). Next, we aim to examine the role of AD-6 and other adenylate cyclases in human dopaminergic neurons to reveal whether these can be considered significant drug targets in the future in human PD.

2. Role of NGF in regulation of lipoprotein uptake in neurons

Neurotrophins, such as nerve growth factor (NGF), are important regulators of neuronal development, differentiation and maintenance of neurons in the embryonic and adult brain. Brain tissue is rich in various lipids and lipoproteins that constitute various membranes of neurons and glial cells. Recent studies have shown that lipid and cholesterol metabolism influence viability of neurons during development and in degenerative diseases, such as PD, Huntington´s disease (HD) and Alzheimer´s disease (AD). However, the mechanisms by which lipoprotein levels are regulated in neurons are not fully understood.

Using primary cultured septal neurons and phaeochromocytoma PC6.3 cells expressing receptors for the neurotrophins, we were able to show that NGF increases low-density lipoprotein receptor (LDLR) levels via the NGF receptor, TrkA (Do *et al.*, 2015). Pro-NGF, acting via the p75 neurotrophin receptor (p75NTR), also increased LDLRs protein in these cells similar to that observed using simvastatin, a cholesterol-lowering drug commonly used in the clinic. Detailed analysis of the mechanism, showed that enhanced transcription of the *LDLR gene* was



involved in the effect elicited by the neurotrophins and by statin. Furthermore, NGF and pro-NGF both increased lipoprotein uptake by neuronal cells (Do *et al.*, 2015). Addition of serum-derived lipoprotein particles in conjunction with NGF or simvastatin enhanced neurite outgrowth. These results show that NGF and simvastatin are able to stimulate lipoprotein uptake by neurons with a positive effect on neurite outgrowth. Functionally, the increases in LDLRs and lipoprotein particles in neurons by neurotrophic factors could play a functional role in neuroregeneration after brain injuries. Our aim is to continue this line of investigation in the future.

Publications 2015

Do HT, Bruelle C, Pham DD, Jauhiainen M, Eriksson O, **Korhonen LT**, **Lindholm D**. Nerve growth factor (NGF) and pro-NGF increase low-density lipoprotein (LDL) receptors in neuronal cells partly by different mechanisms: role of LDL in neurite outgrowth. *J. Neurochem.* 2015 Oct 20. [Epub ahead of print]

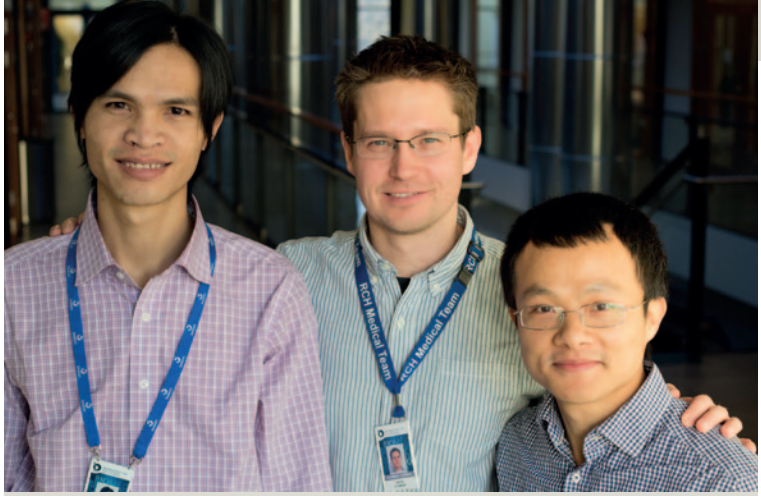
Mäkelä J, **Tselykh TV**, Kukkonen JP, Eriksson O, **Korhonen LT**, **Lindholm D**. Peroxisome proliferator-activated receptor-γ (PPARγ) agonist is neuroprotective and stimulates PGC-1α expression and CREB phosphorylation in human dopaminergic neurons. *Neuropharmacol.* 2015 Nov 26. [Epub ahead of print]

Lindholm D, **Mäkelä J**, Di Liberto V, Mudò G, Belluardo N, Eriksson O, Saarma M. Current disease modifying approaches to treat Parkinson's disease. *Cell. Mol. Life Sci.* 2015 Nov 30. [Epub ahead of print]

Thesis completed in the group in 2015

The following Master´s thesis was accepted at the University of Helsinki this year:

Annetta Redmann: Role of FGF21 in neuroinflammation. May 2015.



TARGETED GENE-EXPRESSION ANALYSIS

Group members

Jakob Stenman, M.D., Dr.Med.Sci., Docent, Head
Ho Huu Tho, M.D., Dr.Med.Sci. spring 2015
Kien Dang, M.D.

External funding

The Finska Läkaresällskapet
The Liv och Hälsa Foundation

Main research activities

The aim of the research group is to develop novel diagnostic assays based on PCR and Next Generation Sequencing. Technical development is focused on applications where there are specific difficulties in utilizing conventional PCR, as well as on improvements in sequencing pre-amplification.

We have developed a novel technique (ExBP-RT) for ultra-sensitive detection of expressed mutations. This technique provides a means for early detection of malignant transformation in pre-malignant conditions as well as for disease monitoring from plasma samples. (Ho, TH *el al*, Nucl Acids Res. 2015). During the past year we have applied the ExBP-RT technique to study the occurrence of expressed KRAS and BRAF mutations in endoscopic biopsy samples, as a means for detection of early signs of malignant transformation in paediatric patients with inflammatory bowel disease, as well as in adult patients treated for oesophageal atresia in their early childhood. Our results indicate that tissue expression of mutations associated with malignant transformation can occur years, or even decades before clinical manifestation and could provide

valuable markers for selection of patients at risk for follow-up programmes. Another ongoing study focuses on prognostic molecular events in colorectal cancer. We have investigated RNA and protein expression of several potential markers and ExBP-RT has been successfully utilized to detect KRAS and BRAF mutations in a large material of formalin-fixed, paraffin-embedded (FFPE) surgical specimens. We are currenly working in collaboration with our previous PhD-student, Dr. Tho Ho, now situated at Vietnam Military Medical University (VMMU) to develop assays based on ExBP-RT for detection of other known Ras mutations.

During the past year, we have continued development of a novel PCR amplification technology called Heat Pulse Extension PCR (HPE-PCR). This technique can overcome difficulties in amplifying long GC rich and repetitive sequences by pulsatile temperature cycling during the extension step of PCR. We have previously demonstrated the utility of this technique by amplifying repetitive expansions in Fragile X syndrome as well as in Type I Myotonic Dystrophy. The technique has been adopted into routine clinical use at HUSLAB of the Helsinki University Central Hospital. Currently, we are focusing on improving the reaction efficiency further in order to enable amplification of longer repeat expansions that are present in Amyotrophic Lateral Sclerosis (ALS). The HPE-PCR technique has proven effective for reduction of GC-bias in sequencing pre-amplification. This improves the sequencing coverage of poorly amplifying regions. European and US patents have been granted for the HPE-PCR technology and collaboration with industrial partners has been established.

Publications 2015

Söderholm S, Anastasina M, Islam MM, Tynell J, Poranen MM, Bamford DH, **Stenman J**, Julkunen I, Šaulienė I, De Brabander JK, Matikainen S, Nyman TA, Saelens X, Kainov D. Immuno-modulating properties of saliphenylhalamide, SNS-032, obatoclax, and gemcitabine. *Antiviral Res.* 2015 Dec 29. [Epub ahead of print]

Docent Lecture

The title of docent was granted at the University of Helsinki this year:

Jakob Stenman: *HDR-brakyterapia lasten alempien virtsateiden syövän hoidossa*. February 11, 2015. Lastenklinikka.

Thesis completed in the group in 2015

The following doctoral thesis was accepted at the University of Helsinki this year:

Tho Ho Huu: Development of amplification-based technologies for enrichment of nucleic acids with difficult sequences or low-abundance point mutations. February 13, 2015.

TELOMERE RESEARCH

Group members

Frej Fyhrquist, M.D., Dr.Med.Sci., Professor emeritus
Outi Saijonmaa, Ph.D., Docent
Anders Eriksson-Palojärvi, D.V.M.
Anna Nyman, M.Sc.

External funding

The Finska Läkaresällskapet

Main research activities

To date, the team has studied leukocyte telomere length (LTL) in more than 5 000 subjects. During the years 2012-2014, we found associations of LTL with 1) alcohol consumption, 2) physical activity, 3) body mass index, and 4) insertion/deletion (I/D) polymorphism of the angiotensin-I converting enzyme (ACE) gene. In cooperation with Professor Ilkka Pörsti, University of Tampere, we have measured LTL in 522 healthy Finnish subjects participating in a study on haemodynamics. Preliminary results show the known relation of LTL to age and gender, but no association of heart rate and or number of other variables with LTL. The results will be analyzed and published in 2016.

A study focusing on telomeres and cardiovascular health in the young, was started in 2012 in collaboration with Professor A. Jula (Institute of Health and Welfare, Turku) as a sub-study of the STRIP project (Sepelvaltimotaudin Riskitekijöiden Interventio Projekti) comprising >500 subjects, each, in the intervention and control groups, with follow-up covering early childhood to maturity. This longitudinal study has been ongoing during 2013–2015, and results will be analyzed and submitted for publication during 2016. Preliminary results suggest that healthy life style intervention is associated with slower telomere attrition compared with controls.

Publications 2015

Fyhrquist F, Nilsson PM. Chapter 20: Telomere biology and vascular aging. *Early Vascular Aging (EVA), NEW DIRECTIONS IN CARDIOVASCULAR PROTECTION.* Edited by Peter M. Nilsson, Michael H. Olsen and Stéphane Laurent. © 2015 Elsevier Inc.

Fyhrquist F, **Saijonmaa O**. Telomeerit ja ikääntymiseen liittyvät sairaudet. *Erikoislääkäri* 2/2015, 25vsk.

PUBLICATIONS 2015

Original articles

1. Asghar MY, Magnusson M, Kemppainen K, Sukumaran P, Lof C, Pulli I, **Kalhori V**, **Tornquist K**. Transient receptor potential canonical 1 (TRPC1) channels as regulators of sphingolipid- and VEGF receptor expression: implications for thyroid cancer cell migration and proliferation. *J Biol Chem*. 2015; 26;290(26):16116-31.
2. **Bian H**, Hakkarainen A, **Zhou Y**, Lundbom N, **Olkkonen VM**, **Yki-Järvinen H**. Impact of non-alcoholic fatty liver disease on liver volume in humans. *Hepatol Res*. 2015; 45(2):210-9.
3. **Blom T**, **Li S**, **Dichlberger A**, Bäck N, Kim YA, Loizides-Mangold U, Riezman H, Bittman R, **Ikonen E**. LAPTM4B facilitates late endosomal ceramide export to control cell death pathways. *Nat Chem Biol*. 2015; 11(10):799-806.
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5. Chilton R, **Tikkanen I**, Cannon CP, Crowe S, Woerle HJ, Broedl UC, Johansen OE. Effects of empagliflozin on blood pressure and markers of arterial stiffness and vascular resistance in patients with type 2 diabetes. *Diabetes Obes Metab*. 2015; 17:1180-1193.
6. **Do HT**, **Bruelle C**, **Pham DD**, Jauhiainen M, Eriksson O, **Korhonen LT**, **Lindholm D**. Nerve growth factor (NGF) and pro-NGF increase low-density lipoprotein (LDL) receptors in neuronal cells partly by different mechanisms: role of LDL in neurite outgrowth. *J Neurochem*. 2015 Oct 20. [Epub ahead of print]
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Reviews

1. **Blom T**, **Li S**, **Dichlberger A**, Bäck N, Kim YA, Loizides-Mangold U, Riezman H, Bittman R, **Ikonen E**. Lipidikuljettajaproteiinin metsästys johti syövän lääkeresistenssin jäljille. *Duodecim*, 2015; 131, 20, s. 1900.
2. **Fyhrquist F**, **Saijonmaa O**. Telomeerit ja ikääntymiseen liittyvät sairaudet. *Erikoislääkäri* 2/2015, 25vsk.
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Other publications

1. **Fyhrquist F**, Nilsson PM. Chapter 20: Telomere biology and vascular aging. *Early Vascular Aging (EVA), NEW DIRECTIONS IN CARDIOVASCULAR PROTECTION.* Edited by Peter M. Nilsson, Michael H. Olsen and Stéphane Laurent. © 2015 Elsevier Inc.
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3. **Yki-Järvinen H**. Chapter 19. Pathogenesis of nonalcoholic fatty liver disease (NAFLD). International Textbook of Diabetes Mellitus, Fourth Edition. Edited by Ralph A. DeFronzo, K. George M. M. Alberti, Ele Ferrannini, and Paul Zimmet. © 2015 JohnWiley & Sons, Ltd. Published 2015 by JohnWiley & Sons, Ltd.


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