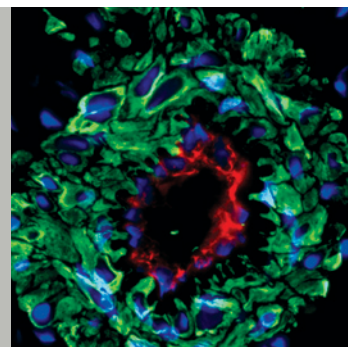
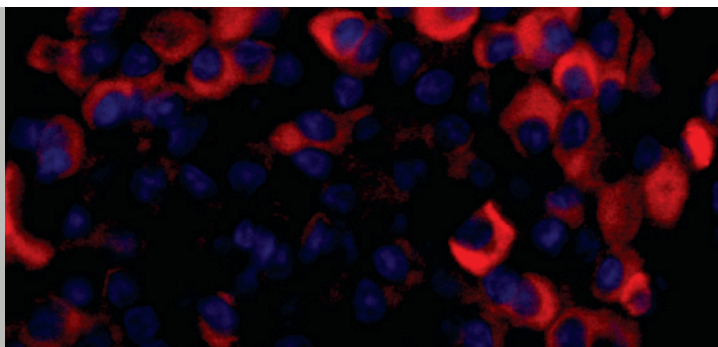
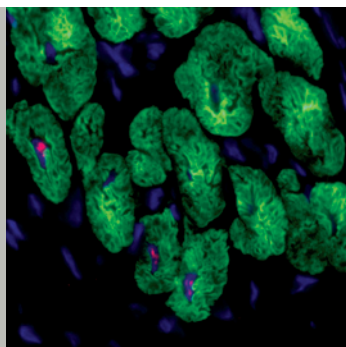


MINERVA FOUNDATION
AND
MINERVA FOUNDATION INSTITUTE
FOR MEDICAL RESEARCH



SCIENTIFIC REPORT 2011





Minerva Foundation Institute for Medical Research
BIOMEDICUM Helsinki 2U
Tukholmankatu 8, 00290 Helsinki
FINLAND

Tel. +358-9-191 25700

Fax +358-9-191 25701

www.helsinki.fi/minerva

Front page image: Ki67+ proliferating cardiomyocytes (left panel), a large group of c-kit+ cardiac progenitor cells (middle panel) and a newly formed artery (right panel) in the infarcted rat hearts (by courtesy of Katariina Immonen and Päivi Lakkisto/Unit of Cardiovascular Research).

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SUMMARY OF RESEARCH AND ACTIVITIES DURING THE YEAR 2011

The Minerva Foundation Institute for Medical Research is a privately owned research institute located at the Biomedicum-Helsinki Research Conglomerate at Meilahti campus. The Institute focuses on creating a fruitful combination of basic biomedical research and clinical investigations relevant for common disease. The objectives of research range from study of fundamental molecular mechanisms in cultured cell models and genetically manipulated animals to investigation of human patient materials. The aim is to generate new knowledge basis and innovations for the development of future diagnostic approaches, preventive measures and treatments for common diseases such as diabetes, cardiovascular diseases and neurodegenerative disorders. The work carried out in the seven research units of the Institute during the year 2011 is summarized in this report.

The funds of Minerva Foundation are directed at maintaining and further developing a research infrastructure that serves in the most effective way the substance activities of the groups, and encourages the scientists to maximise efforts to acquire external research funds. During 2011 the Institute budget was covered 46% by funds from the Minerva Foundation and 54% by external research funds raised by the scientists.

To enhance the research impact and to maximise the gain for the investment in the Institute, it is necessary to constantly develop integration and collaboration within Minerva. Therefore,

the senior researchers gathered in April in a meeting aimed at consolidating the research strategy as well as finding common interests and ideas for new collaborative work. This effort was in September complemented by a seminar boat cruise to Tallinn, in which practically the entire personnel participated. As a result of these integration initiatives, the research units have indeed come up with new collaborative research activities, and also external grants shared by Minerva research units have been received. A major challenge is to achieve in core research areas a critical mass that further boosts the scientific impact of the Institute. As one move in this direction, research of cellular lipid metabolism and cardiovascular diseases has been strengthened by recruitment of a new group led by academy professor Elina Ikonen, who will initiate operation at Minerva premises in 2012. Her group will occupy part of the space liberated as a majority of prof. Dan Lindholm's unit has during the year 2011 moved to Helsinki University Institute of Biomedicine.

Despite the ongoing restructuring of the Institute, the work in the research units in 2011 has been efficient and productive. Minerva scientists published in international peer reviewed journals a total of 35 original articles, with a mean impact factor of 4.434. In addition, one Ph.D. thesis and one M.Sc. thesis were completed.

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 annual review covers the period from January 1, 2011, to December 31, 2011. The board of trustees included the following persons:

Professor Jim Schröder, chair
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Professor Per-Henrik Groop
Professor Carola Grönhagen-Riska
Professor Christer Holmberg
Professor Krister Höckerstedt
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The administration is handled by following groups:

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THE MINERVA FOUNDATION INSTITUTE FOR MEDICAL RESEARCH

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



► **Members of the Unit:**

Ilkka Tikkanen, M.D., Dr.Med.Sci., Docent, head
 Mika Laine, M.D., Dr.Med.Sci., Docent
 Päivi Lakkisto, M.D., Dr.Med.Sci.
 Eeva Palojoiki, M.D., Dr.Med.Sci.
 Hanna Forsten, V.M.D.
 Jere Paavola, M.D.
 Heli Segersvärd, M.D.
 Juuso Siren, M.B.
 Anneliis Ihermann, B.Sc., student
 Katariina Immonen, B.Sc., laboratory technician
 Riikka Kosonen, M.Sc., laboratory technician

Collaborators

Katriina Aalto-Setälä, M.D., Dr.Med.Sci., Docent,
 University of Tampere, Finland
 Barbara Ehrlich, Ph.D., Professor, Yale University
 School of Medicine, New Haven, USA
 Ari Harjula, M.D., Dr.Med.Sci., Professor, Helsinki
 University Hospital, Finland
 Esko Kankuri, M.D., Dr.Med.Sci., Docent, University of
 Helsinki, Finland
 Markku Kupari, M.D., Dr.Med.Sci., Professor, Helsinki
 University Hospital, Finland
 Eero Mervaala, M.D., Dr.Med.Sci., Professor,
 University of Helsinki, Finland
 Kari Pulkki, M.D., Dr.Med.Sci., Professor, University of
 Eastern Finland, Kuopio, Finland
 Ilkka Pörsti, M.D., Dr.Med.Sci., Professor, University of
 Tampere, Finland
 Yibing Qyang, Ph.D., Associate Professor, Yale
 University School of Medicine, New Haven, USA

Funded by the Minerva Foundation, Finska
 Läkaresällskapet, the Liv och Hälsa Foundation, the
 Finnish Foundation for Cardiovascular Research,
 the Paavo Nurmi Foundation, and Hospital District
 of Helsinki and Uusimaa research grants (EVO).

UNIT OF CARDIOVASCULAR RESEARCH

Main research activities

The group studies mechanisms and repair of end-organ damage in cardiovascular and renal diseases. The group has investigated the local expression and effects of components of the renin-angiotensin-aldosterone system, other vasoactive factors and apoptosis in hypertension, heart failure, and in progression of renal damage. In addition, the cardiovascular and renal protective properties of new cardiovascular drugs have been evaluated. Recently, the Unit's research has focused on exploring the regenerative and reparative mechanisms of cardiac injury after myocardial infarction and in the development of heart failure.

Our studies suggested an important role for HO-1 and HO-1-CO pathway in maintaining cellular homeostasis in the post-myocardial infarction (MI) heart. HO-1 induction significantly promoted neovascularization by inducing the expression of VEGF family members and several other angiogenic factors. As a novel finding, HO-1 upregulated the PlGF gene expression resulting in a significant increase in PlGF protein levels. Induction of HO-1-CO pathway may prove a useful tool to protect against pathological myocardial changes and development of heart failure after MI facilitating cardiac recovery.

We studied the effects of angiotensin II blockade on formation of new cardiomyocytes, neovascularization, and post-MI ventricular remodelling. The number of newly formed cardiomyocytes increased clearly after MI in rats. Treatment with a selective angiotensin type 1 receptor antagonist losartan neither stimulated nor prevented cardiomyocyte regeneration. However, angiotensin II blockade increased vascular densities in the infarct border zone and favourably modulated remodelling of the non-infarcted myocardium.

Adverse cardiac remodelling after myocardial injury was also explored in diabetic rats in collaboration with professor Eero Mervaala's research group. The beneficial effects of the calcium sensitizer levosimendan against post-MI heart failure and remodelling was associated with altered expression of several genes of the renin-angiotensin system, glycerolipid metabolism, purine metabolism, cell-cycle pathway and pathways in cancer. These genes may represent novel drug targets for treatment of heart failure and diabetic cardiomyopathy.

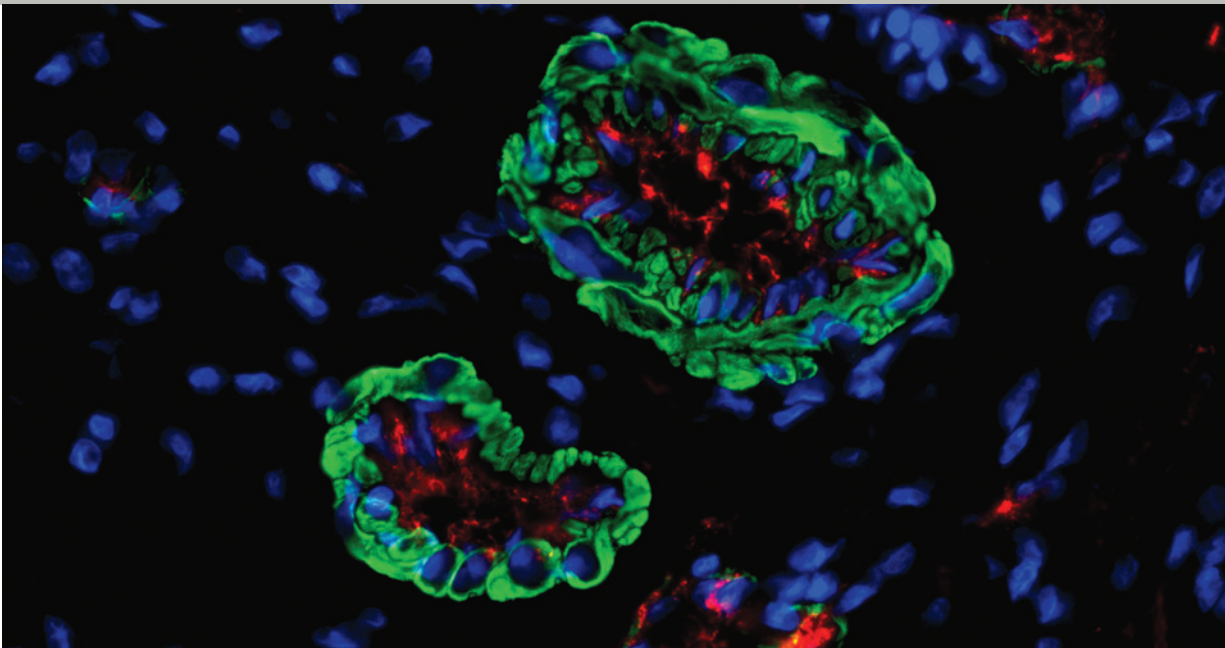


Figure. New blood vessels in the infarct area of post-MI rat heart.

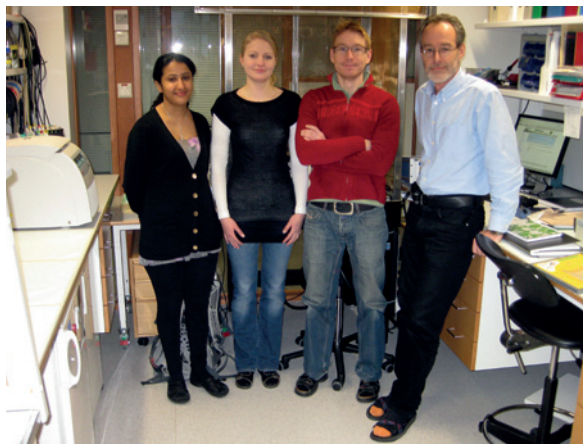
The role of local, paracrine mechanisms of cardiac regeneration was studied in collaboration with Professor Ari Harjula's research group utilizing modified myoblast cell sheet transplantation methodology. The antiapoptotic factor bcl-2 was shown to improve efficacy of myoblast sheet transplantation and promote proangiogenic paracrine signaling. Overexpression of hHGF in myoblast sheets enhanced their angiogenic potential in rat chronic heart failure.

In collaboration with Professor Ehrlich and Associate Professor Qyang, we characterized the physiology of cardiomyocytes derived from human induced pluripotent stem cells (iPSC) and murine embryonic stem cells (mESC). We showed that these induced cardiomyocytes express typical sarcomeric markers, exhibit normal calcium transients, respond to β -adrenergic and electric stimulation, and demonstrate characteristic changes in action potential duration in response to cardioactive drugs. iPSC-derived cardiomyocytes offer an optimal platform for studying disease mechanisms and drug therapies. Our exciting studies with iPSC-derived cardiomyocytes continue in collaboration with Docent Katriina Aalto-Setälä's group in Tampere.

Prognosis of severe systolic heart failure is poor despite effective treatment with currently available drugs. One possible explanation for the progression of cardiac failure may be dysregulation of cardiac reparative mechanisms. The novel findings on cardiac regenerative potential open a completely new avenue for preventing and treating chronic heart failure after myocardial injury. By combining treatments promoting cardiac regeneration with current established therapies for cardiovascular diseases, the results of the treatments could improve significantly in the future.

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- Lakkisto P, Siren J-M, Kytö V, Forsten H, Laine M, Pulkki K, Tikkanen I. Heme oxygenase-1 induction protects the heart and modulates cellular and extracellular remodeling after myocardial infarction in rats. *Exp Biol Med* 2011; 236(12):1437–48
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► Members of the Unit:

Kid Törnquist, Ph.D., Professor, head
 Nina Bergelin, Ph.D.
 Tero Viitanen, Ph.D.
 Veronica Kalhori, M.Sc.

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

Kati Kemppainen, M.Sc.
 Christoffer Löf, M.Sc.
 Pramod Sukumaran, M.Sc.
 Yasir Asghar, M.Phil.
 Ilari Pulli, M.Sc.

The Unit was part of the Centre of Excellence in Cell Stress and Molecular Ageing (2010–2014, Åbo Akademi University).

Collaborators

John Eriksson, Professor, Åbo Akademi University, Turku, Finland
 Lea Sistonen, Professor, Åbo Akademi University, Turku, Finland
 Peter Slotte, Professor, Åbo Akademi University, Turku, Finland
 Johnny Näsman, Ph.D., Docent, Åbo Akademi University, Turku, Finland

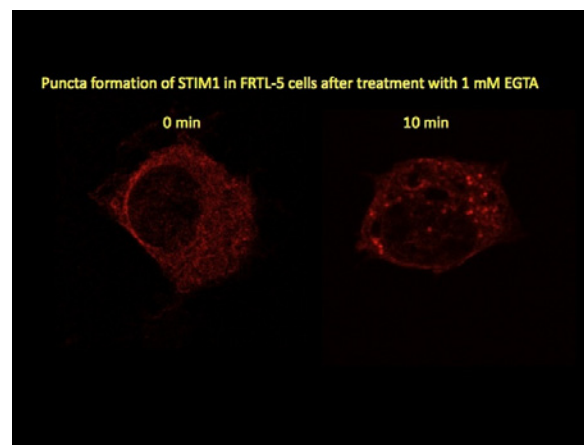
Funded by The Sigrid Juselius Foundation, the Academy of Finland, the Liv och Hälsa Foundation, and Åbo Akademi University. Christoffer Löf was funded by the Turku Doctoral Program of Biomedical Sciences.

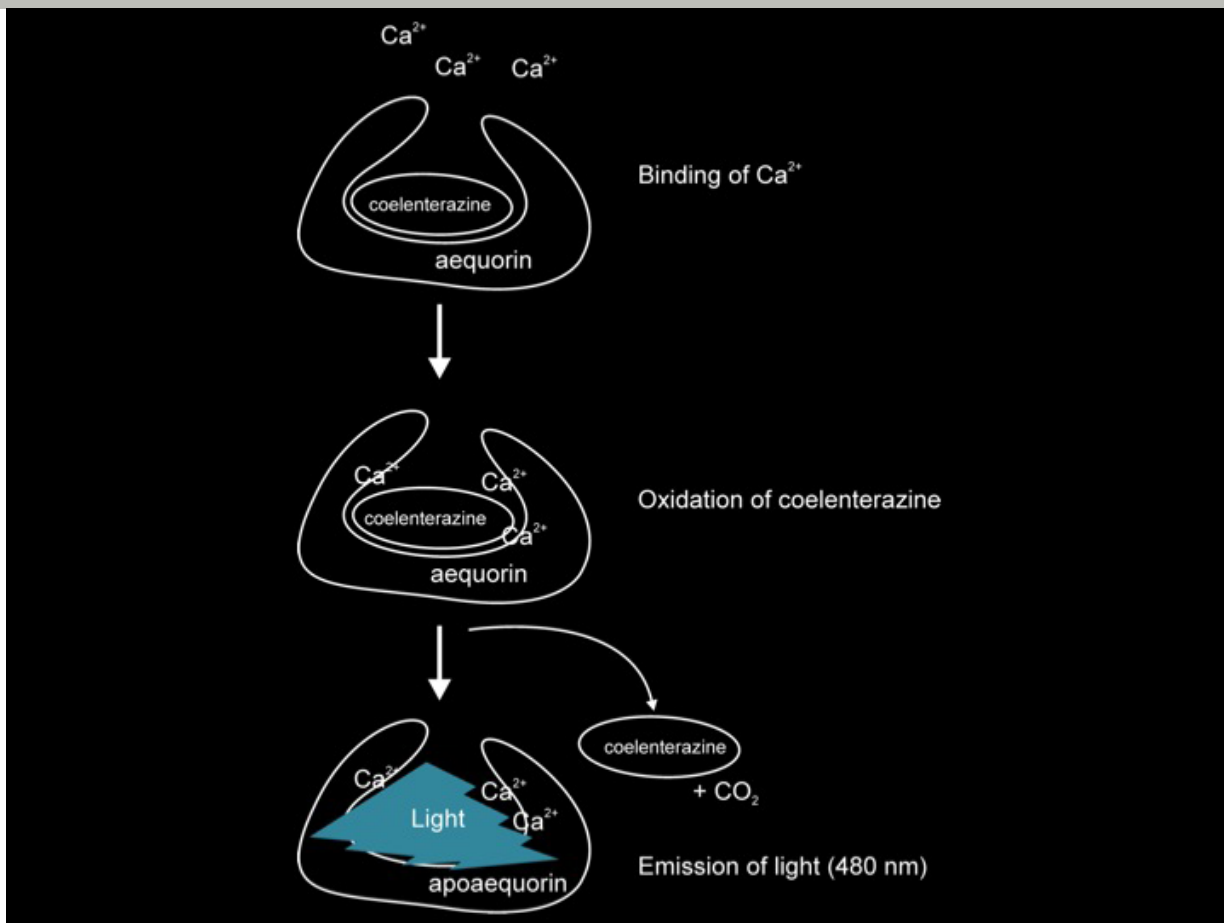
UNIT OF CELLULAR PHYSIOLOGY

Main research activities

The main interests of the Unit are to understand how calcium signaling is regulated in various cell types, and how different lipid metabolites participate in this process. The other main topic is how lipid derivatives, especially metabolites of sphingomyelin, modulate thyroid cancer cell migration and proliferation.

A substantial amount of work has been done to understand calcium signaling in thyroid epithelial cells. In addition to receptor-mediated entry mechanisms, the regulation of store-operated calcium entry is the focus of our investigations. During the last year, a substantial amount of work has been dedicated to investigating the mechanisms by which a member of the canonical transient receptor potential (TRPC) cation channels is regulated. We have shown that rat thyroid FRTL-5 cells express the TRPC2 channel. Very little is known in regard to the physiological significance of this channel, and the first reports are in preparation. In brief, TRPC2 regulates a multitude of signaling mechanisms, and participates in at least the regulation of proliferation, migration and TSH receptor signaling. Furthermore, the importance of TRPC2 in regulating the electrophysiological properties of thyroid cells has been investigated. Of interest is also the importance of stromal interaction molecules (STIM, the endoplasmic reticulum calcium sensors) and its redistribution to endoplasmic reticulum-plasma membrane (ER-PM) junctions and interactions with the calcium channel subunit ORAI1. This





coupling may be of relevance in thyroid cell physiology. Several manuscripts have now been submitted describing these results. The importance of TRPC channels in human thyroid cancer cells is also under investigation. In these cells, the channel expression profile seems quite complex.

Investigations on the importance of sphingosine 1-phosphate (S1P) in regulating thyroid cancer cell migration have continued. The mechanism by which S1P enhances migration of e.g. follicular cancer cells is of major interest. Furthermore, S1P seem to enhance the levels of hypoxia-induced factor 1a (HIF-1a) in thyroid cancer cells, resulting in an enhanced migration. A manuscript describing these effects is now under revision. Furthermore, the importance of S1P in regulating the migration and proliferation of anaplastic thyroid cancer cells has been investigated in more detail.

In addition to investigating calcium channels, we are also interested in the regulation of the HERG potassium channel. Preliminary data suggests that HERG is expressed in both normal and cancer cells, but may regulate proliferation in cancer cells only. More detailed investigations are, however, still needed to evaluate the importance of this channel in thyroid cancer cells.

The Unit has validated the use of organelle-targeted aequorin for the determination of organellar calcium. Presently the method is used for measuring real-time calcium changes in

several cellular compartments, and exciting collaborative projects have emanated using this technique.

Awards, honours and positions of trust

KT is a member of the Board of Directors of the Centre for Biotechnology (University of Turku and Åbo Akademi University), and of the University Collegium (Åbo Akademi University). KT is a deputy member of the Council of the Department of Biosciences (Åbo Akademi University).

Publications

Löf C, Sukumaran P, Viitanen T, Törnquist K: TRPC2 – of mice but not men. *Adv Exp Med Biol* 2011; 704:125–134

Törnquist K: Sphingosine 1-phosphate, sphingosine kinase and autocrine calcium signalling in thyroid cells. *Acta Physiologica (Oxf)*, 2011; in press



► Members of the Unit:

Hannele Yki-Järvinen, M.D., Dr.Med.Sci., F.R.C.P,
Professor, head

Ksenia Sevastianova, M.D., Dr.Med.Sci.

Anna Kotronen, M.D., Dr.Med.Sci., Docent

Linda Mustelin, M.D.

Julia Perttilä, Ph.D.

Leena Juurinen, M.D.

Alexandre Santos, M.D.

Antti Hakkarainen, M.Sc. Techn.

Jenni Hyysalo, medical student

Elina Petäjä, medical student

Susanna Lallukka, medical student

Anne Salo, laboratory technician

Katja Sohlo, laboratory technician

Mia Urjansson, laboratory technician

Contributors

Matej Oresic, Professor, VTT, Finland

Aila Rissanen, Professor, HUCH, Finland

Nina Lundbom, Professor, University of Helsinki,
Finland

Bart Staels, Professor, France

Keith Frayn, Professor, UK

Marju Orho-Melander, Professor, Malmö, Sweden

Ulf Smith, Professor, Stockholm, Sweden

Anu Wartiovaara, Professor, University of Helsinki,
Finland

Olkkonen Vesa, Docent, Minerva Foundation

Institute for Medical Research, Finland

Markku Peltonen, Docent, National Public Health
Institute, Finland

Funded by

EU www.hepadip.org 2005–2010

Sigrid Juselius Foundation

Novo Nordisk Foundation

Academy of Finland

EVO foundation large grant 2011–2013

EVO foundation small grant 2012–2012

the Liv och Hälsa Foundation

UNIT OF ENDOCRINOLOGY

Main research activities

The group has continued its studies addressing diagnosis, pathophysiology and treatment of non-alcoholic fatty liver disease (NAFLD). Regarding diagnosis, we have performed lipidomics and metabolomics analysis of 700 subjects whose liver fat has been quantitated by ¹H-MRS or liver biopsy and found that a combination of 3 markers can do as well in predicting NAFLD in humans as can the NAFLD score (published by our group in Gastroenterology in 2010). A patent application has been submitted and a manuscript is ready for submission. Regarding pathophysiology, we have observed that although LDL cholesterol concentrations are unchanged in subjects with NAFLD, cholesterol metabolism in NAFLD is characterized by increased synthesis and diminished absorption of cholesterol. These changes are associated with liver fat content independent of body weight. The increased cholesterol synthesis could contribute to complications associated with NAFLD such as formation of cholesterol rich gallstones. We have also characterized in detail which coagulation factors are overproduced not just in obesity but independent of obesity in subjects with NAFLD. We found FVIII, FIX, FXI and FXII activities to be increased in human NAFLD and correlate with features of insulin resistance. The relationships between NAFLD and these coagulation factors were independent of age, gender and BMI, suggesting that the fatty liver can contribute to the risk of venous thrombosis. We have also continued studies addressing the physiological function and clinical significance of the rs738409 C→G single nucleotide polymorphism in the patatin-like phospholipase domain-containing 3 (PNPLA3; adiponutrin), which leads to a missense mutation (I148M). The I148M mutation impedes triglyceride hydrolysis in vitro, and its carriers have (now confirmed in 20 independent studies) a 70% increase in liver fat content and 4-fold increased risk of developing severe liver disease. The in vitro studies have been supervised by docent Vesa Olkkonen and performed by Julia Perttilä at Minerva. In clinical studies, we have shown that a low-carbohydrate diet for 7 days is an effective in decreasing liver fat in subjects who are homozygous for the rs738409 PNPLA3 G or C allele. This trial was registered at www.hus.fi as 233775.

Awards, honours and positions of trust

2011 Harold Rifkin Visiting Professorship (H.Y.-J.). Albert Einstein College of Medicine, New York USA

Chairperson (H.Y.-J.), *Finnish Diabetes Research Society*, 1998–2011

Publications

Bunck MC, Cornér A, Eliasson B, Heine RJ, Shaginian RM, Taskinen MR, Smith U, Yki-Järvinen H, Diamant M. Effects of exenatide on measures of β -cell function after 3 years in metformin-treated patients with type 2 diabetes. *Diabetes Care* 2011; 34:2041–2047

Bunck MC, Eliasson B, Cornér A, Heine RJ, Shaginian RM, Taskinen MR, Yki-Järvinen H, Smith U, Diamant M. Exenatide treatment did not affect bone mineral density despite body weight reduction in patients with type 2 diabetes. *Diabetes Obes Metab*. 2011; 13:374–377

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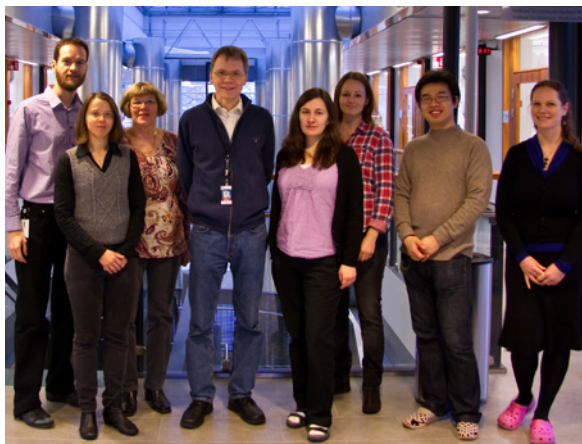
Sevastianova K, Kotronen A, Gastaldelli A, Perttilä J, Hakkarainen A, Lundbom J, Suojanen L, Orho-Melander M, Lundbom N, Ferrannini E, Rissanen A, Olkkonen VM, Yki-Järvinen H. Genetic variation in PNPLA3 (adiponutrin) confers sensitivity to weight loss-induced decrease in liver fat in humans. *Am J Clin Nutr* 2011; 94:104–111

Sevastianova K, Sutinen J, Greco D, Sievers M, Salmenkivi K, Perttilä J, Olkkonen VM, Wågsäter D, Lidell ME, Enerbäck S, Eriksson P, Walker UA, Auvinen P, Ristola M, Yki-Järvinen H. Comparison of dorsocervical with abdominal subcutaneous adipose tissue in patients with and without antiretroviral therapy-associated lipodystrophy. *Diabetes* 2011; 60:1894–900

Theses completed in the group in 2011

The following Ph.D. thesis was accepted at the University of Helsinki this year:

Ksenia Sevastianova: Combination antiretroviral therapy -associated lipodystrophy: Insights into pathogenesis and treatment. November 25, 2011.



► Members of the Unit:

Vesa Olkkonen, Ph.D., Docent, head
 Olivier Béaslas, Ph.D.
 Eija Nissilä, Ph.D.
 Marion Weber-Boyvat, Ph.D.
 Terhi Vihervaara, M.Sc.
 You Zhou, M.Sc.
 Liisa Arala, laboratory technician
 Eeva Lehto, laboratory technician

Collaborators

Elina Ikonen, M.D., Dr.Med.Sci., Professor,
 University of Helsinki, Finland
 Matti Jauhiainen, Ph.D., Docent, National Institute
 for Health and Welfare, Helsinki, Finland
 Gerd Schmitz, Dr.Med.Sci., Professor, University
 Clinic Regensburg, Germany
 Miranda Van Eck, Ph.D., Associate Professor,
 Leiden/Amsterdam Center for Drug Research,
 Leiden, the Netherlands
 Daoguang Yan, M.D., Dr.Med.Sci., Professor, Jinan
 University, Guangzhou, China
 Hannele Yki-Järvinen, M.D., Dr.Med.Sci., Professor,
 Minerva Foundation Institute for Medical
 Research and University of Helsinki, Finland

Funded by the Academy of Finland, the
 European Union 7th Framework Programme,
 the Sigrid Juselius Foundation, the Novo
 Nordisk Foundation, the Finnish Foundation
 for Cardiovascular Research, the Liv och Hälsa
 Foundation, and the Magnus Ehrnrooth
 Foundation.

UNIT OF LIPID SIGNALLING AND HOMEOSTASIS

Main research activities

One major activity of the group focuses on the mechanisms that the cell employs to sense its lipid status and to relay this information to regulate (i) expression of the genes and activity of the enzymes of lipid metabolism as well as (ii) other processes associated with the lipid composition of membranes. Such processes are, for example, intracellular vesicle transport responsible for the trafficking of molecules between subcellular organelles and signal transduction events. Oxysterols are oxidized derivatives of cholesterol that act as potent regulators of cellular lipid metabolism. They also have a multitude of other cellular effects on cell survival, signaling and differentiation processes, and they are enriched in pathological structures such as atherosclerotic lesions, cataracts and gall stones. Moreover, oxysterols are implicated in the pathology neurodegenerative disorders and age onset macular degeneration. We have identified a family of 12 human genes that encode cytoplasmic oxysterol-binding proteins, ORPs. The project aims to elucidate the function of these proteins in the control of cellular lipid metabolism and in the development of atherosclerotic and other diseases involving skewed lipid signaling. We also investigate their wider functional connections with intracellular transport processes and signaling cascades. To reach our goals we employ a spectrum of methods, from molecular cell biology approaches and laboratory animal models to analysis of human specimens.

During 2011, the Unit elucidated together with D. Yan (Guangzhou, China) the functional role of ORP8 in systemic lipid metabolism by employing adenoviral overexpression of the protein in mouse liver and its RNAi-mediated silencing in cultured hepatocytes. The data shows that ORP8 has the capacity to down-regulate serum and hepatic lipid levels via modulation of the activity of sterol regulatory element binding proteins (SREBP), transcription factors acting as master controllers of lipid metabolism. A putative mechanism underlying the effect is the observed interaction of ORP8 with the nuclear pore complex component NUP62 (Zhou et al., 2011). We created an ORP8 knock-out mouse model, which was bred to pure C57B6 Rcc background and used for the analysis of serum lipids/lipoproteins and liver tissue lipids. ORP8 deficiency was shown to result in elevated serum high-density lipoprotein (cholesterol,

phospholipid and apolipoprotein A-I) levels, suggesting a role of ORP8 as a new regulator of plasma HDL (Béaslas et al., in preparation). The role of ORP8 in hepatic insulin signaling was investigated with J. Brüning (Cologne, Germany) – ORP8 was found to be regulated in mouse liver by the micro-RNA miR-143 associated with obesity and to play a role in hepatocyte insulin signaling (Jordan et al., 2011). We also elucidated the function of ORP7, which interacts with a small chaperone protein GATE16/Gabarapl2 – via this interaction ORP7 was found to regulate the stability of the Golgi vesicle transport protein GS28, suggesting a novel mechanism by which oxysterols modify membrane trafficking in the Golgi complex (Zhong et al., 2011).

A collaborative project that focuses on the molecular mechanisms underlying non-alcoholic fatty liver disease (NAFLD) is carried out together with the Unit of Endocrinology led by Prof. Hannele Yki-Järvinen. NAFLD refers to the accumulation of fat within hepatocytes in the form of triglycerides (TG) exceeding 5% to 10% of liver weight. NAFLD affects more than 20% of the population in Western countries. A single nucleotide polymorphism (rs738409, G allele) in the PNPLA3 gene, which encodes the lipid droplet-associated protein adiponutrin, is strongly associated with increased liver fat content, independent of obesity and insulin resistance. The aim of this project is to elucidate the molecular basis of adiponutrin function within hepatocytes and to facilitate the development of new preventive measures and treatments for metabolic syndrome and type 2 diabetes. A postdoctoral fellow, Julia Perttilä, Ph.D., is co-supervised in this activity by H. Yki-Järvinen and V. Olkkonen. Important new results of this project are that PNPLA3 expression in hepatocytes is regulated by glucose via the carbohydrate response element binding protein, ChREBP, and that mutant PNPLA3 carrying the rs738409 G-allele slows down the kinetics of hepatocyte triacylglycerol hydrolysis (Perttilä et al., *Am. J. Physiol.*, in press). Two further studies have been published from the collaborative efforts (Sevastioanova et al., 2011a,b); The results of these are summarized in the section of the Unit of Endocrinology. Moreover, we have during 2011 initiated a new project addressing the functional role of micro-RNAs in fatty liver.

Awards, honours and positions of trust

VO has acted as a Secretary, Treasurer and Council Member of the Scandinavian Society for Atherosclerosis Research, as well as Council Member in the Finnish Atherosclerosis Society.

Publications

- Jordan SD, Krüger M, Willmes DM, Redemann N, Wunderlich FT, Brönneke HS, Merkwirth C, Kashkar H, Olkkonen VM, Böttger T, Braun T, Seibler J, Brüning JC. Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. *Nat Cell Biol.* 2011; 13(4):434–46
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- Zhong W, Zhou Y, Li S, Zhou T, Ma H, Wei K, Li H, Olkkonen VM, Yan D. OSBP-related protein 7 interacts with GATE-16 and negatively regulates GS28 protein stability. *Exp Cell Res.* 2011; 317(16):2353–63
- Zhou T, Li S, Zhong W, Vihervaara T, Béaslas O, Perttilä J, Luo W, Jiang Y, Lehto M, Olkkonen VM, Yan D. OSBP-Related Protein 8 (ORP8) Regulates Plasma and Liver Tissue Lipid Levels and Interacts with the Nucleoporin Nup62. *PLoS One.* 2011; 6(6):e21078. Epub Jun 15



► Members of the Unit:

Heikki Koistinen, M.D., Dr.Med.Sci., Docent, head
 Agnieszka Biala, M.Sc.
 Jaakko Hulkkonen, student
 Stephanie von Kraemer, M.Sc.
 Paulina Skrobuk, M.Sc.
 Anna Zitting, M.B.

Collaborators

Sture Andersson, Professor, Children's Hospital,
 Helsinki University Central Hospital, Finland
 Matti Jauhiainen, Docent, National Institute for
 Health and Welfare, Finland
 Hannele Laivuori, Docent, Haartman Institute,
 University of Helsinki, Finland
 Timo Lakka, Professor, University of Eastern Finland,
 Finland
 Nina Lundbom, Professor, Department of Radiology,
 Helsinki University Central Hospital, Finland
 Kari Teramo, Professor, Women's Hospital, Helsinki
 University Central Hospital, Finland
 Jaakko Tuomilehto, Professor, Department of Public
 Health, University of Helsinki and
 National Institute for Health and Welfare, Finland

Funded by the Minerva Foundation, Ahokas
 Foundation, the Finnish Academy of Science,
 Finska Läkaresällskapet (to Paulina Skrobuk),
 Finnish Diabetes Research Foundation, the Liv och
 Hälsa Foundation, the Novo Nordisk Foundation,
 and the Sigrid Juselius Foundation.

UNIT OF METABOLISM

Main research activities

In 2011, the main research activity of the group has focused on mechanisms whereby fatty acids induce insulin resistance in skeletal muscle. We have been working in vitro both in isolated human skeletal muscle strips, as well as L6 muscle cells and primary human muscle cells. Muscle strips or muscle cells have been exposed to palmitate or products of lipid metabolism, and biochemical analysis on different intracellular metabolic and stress sensing signaling pathways has been ongoing. Two manuscripts relating to this work have been submitted, and we are currently performing additional experiments to complete these studies.

In addition, sample collection for projects "MAMA – The Molecular Mechanisms of Insulin Resistance in Gestational Diabetes (PI docent Heikki Koistinen)" and "FUSION-Finnish United States Investigation on NIDDM Genetics (PI prof. Jaakko Tuomilehto)" has been ongoing actively, and will continue in 2012.



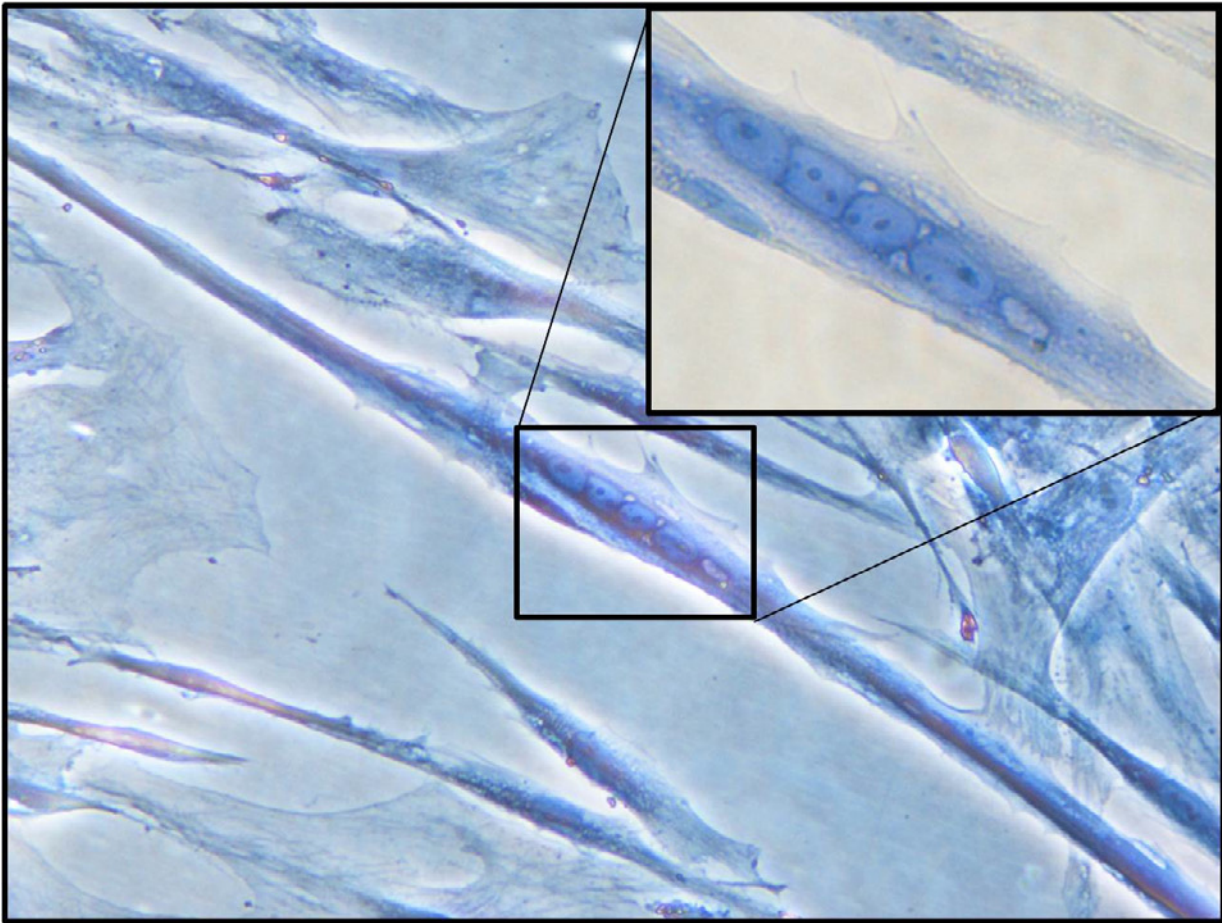


Figure. The formation of multinuclei myotubes during the differentiation of primary human muscle cells.

Awards, honours and positions of trust

HK received a 3-year grant (2012–2014) of 200 000 € from Finnish Medical Foundation.

HK was invited to the board of the Programme for Translational Medicine at Medical Faculty of Helsinki University.





► Members of the Unit:

Dan Lindholm, M.D., Dr.Med.Sci., Professor, head
 Laura Korhonen, M.D., Dr.Med.Sci., Docent
 Céline Bruelle, Ph.D.
 Tuija Mustonen Ph.D.
 Timofey Tselykh, Ph.D.

Alise Hyrskyluoto, M.Sc., FGSN
 Jenny Kivinen, M.Sc.
 Raili Koivuniemi, M.Sc., FGSN
 Johanna Mäkelä, M.Sc.
 Noora Putkonen, M.Sc., HBGS
 Melania Olivieri, M.Sc., Exchange student from Italy
 Hai Do Thi, M.Sc.
 Henrica Karlberg, B.Sc., M.Sc. autumn 2011

Julia Döhla, B.Sc., Transmed student HY
 Heidi Ikonen, summer student
 Malin Ljungren, summer student from KI, Sweden
 Lina Smeds, summer student

Collaborator

Jyrki Kukkonen, Professor, University of Helsinki,
 Finland
 Urmas Arumäe, Docent, Institute of Biotechnology,
 University of Helsinki, Finland
 Natale Belluardo, Professor, University of Palermo,
 Italy
 Noam Zelcer, Ass Professor, University of Amsterdam,
 Holland

The group is a member of Finnish Graduate School
 in Neuroscience (FGSN) and Helsinki Biomedical
 Graduate School (HBGS).

Funded by Academy of Finland, the Sigrid Juselius
 Foundation, the Liv och Hälsa Foundation, Finska
 Läkaresällskapet, Magnus Ehrnrooth and Minerva
 Foundation.

UNIT OF NEUROSCIENCE

Main research activities

We study basic mechanisms underlying neurodegenerative diseases and excitotoxic cell damage in the brain. We focus on protein ubiquitination and mitochondrial and endoplasmic reticulum (ER stress pathways in the pathogenesis for Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) with the aim to combat cell death and degeneration. We are also studying neurotrophic factors, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), and their signaling in regulation of cell-specific events. The group has partly moved to the nearby Institute of Biomedicine, Faculty of Medicine, but more than 3 people are still active at Minerva and other keep a tight link and collaboration with people at Minerva.

Projects

Signaling Mechanisms and Organelle Dysfunctions in the Regulation of Cell Death in Neurodegenerative Diseases

We have studied the role of mitochondria and ER stress in models of Huntingtons disease (HD), PD and ALS. We have generated transgenic mice with overexpression of the peroxisome proliferator-activated receptor-gamma coactivators (PGC-1s) to study neuronal survival and the role of inflammation in brain disease. Dopaminergic neurons in the substantia nigra of PGC1 transgenic mice were resistant to the neurotoxin MPTP as a model for PD. We are currently looking closer into the pathways and proteins mediating this neuroprotection using different methods including gene profiling and studies of human dopaminergic neurons in culture. Apart from this, we are interested in the role of autophagy in brain disorders and have shown that cytoprotective autophagy is activated early, via the mTor pathway, in cells expressing mutant huntingtin protein, but that autophagy is then suppressed causing increased cell death and protein aggregates. The further understanding of this switch in and regulation of autophagy in neurons would be important for enhancing neuronal viability and for treatment of protein aggregates in various diseases.

The Roles of the Ubiquitin-Proteasomal System (UPS) and De-Ubiquitinating Enzymes (DUBs) in Synaptic Signaling and Neurodegeneration

Disturbances in the UPS are linked to neurological disorders but little is known about the roles played by the UPS in synaptic

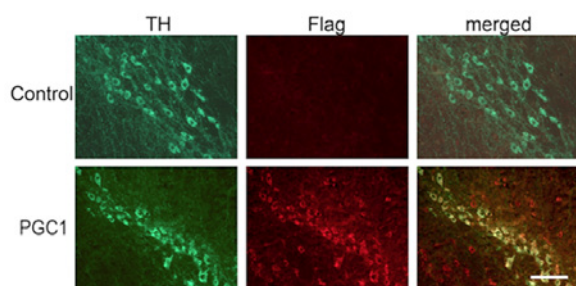


Figure. Immunostaining of substantia nigra in control and PGC1 transgenic mice showing expression of PGC1 (red) in TH-positive dopaminergic neurons (green). Coexpressing neurons are shown as yellow cells in the merged picture (for details see publication Mudo et al in CMLC).

degeneration. Our main interest is to understand how the UPS and various DUBs particularly USP-14 regulate synaptic function and how functional disturbances in synapses are linked to degenerative diseases such as HD.

MIR/IDOL in the Regulation of Membrane Receptors

Receptor ubiquitination by specific E3-ubiquitin ligases has emerged as an important step in the regulation of various membrane proteins. We have previously cloned an E3 ubiquitin-ligase, Mir/Myliip, from the brain. In a recent study showed that Mir also influences the levels of lipoprotein receptors such as LDLR and VLDLR, and the protein was consequently named Idol (Inducible degrader of the LDLR). We are currently studying the role of Mir/Myliip/Idol in the brain with regard to its physiological regulation, its target proteins and interacting molecules, including the MSAP protein that our group cloned previously. The results have potential significance for therapeutics of metabolic disorders and for cell signaling in the brain.

Regulation of Neural Progenitor Cells (NPCs)

NPCs are highly dividing cells with the capacity for self-renewal and can give rise to both neurons and glial cells. We have studied how cell-surface proteins may influence cell proliferation of NPCs, focusing on transmembrane serine protease inhibitors, HAI-1 and HAI-2, that are expressed by NPCs. We are now studying the precise regulation of HAIs in NPCs and how they affect protease activities and signalling pathways in the NPCs. The information gained will add to our understanding about the regulation of NPCs in the brain as well as of growth regulation in brain tumors.

Awards, honours and positions of trust

DL has acted as a Chair of the Panel in Neuroscience at the Swedish Research Council (Vetenskapsrådet Sverige), and as a Member of Scientific Board of Swedish Brain Foundation (Hjärnfonden Sverige). He was elected as a Editorial Board Member for The Journal of Biological Chemistry USA for the period 2011–2016.

Publications

- Di Liberto V, Mäkelä J, Korhonen L, Olivieri M, Tselykh T, Mälikä A, Hai DT, Belluardo N, Lindholm D, Mudò G. Involvement of estrogen receptors in the resveratrol-mediated increase in dopamine transporter in human dopaminergic neurons and in striatum of female mice. *Neuropharmacology* 2011; Epub Oct 24
- Hyrskyluoto A, Reijonen S, Kivinen J, Lindholm D, Korhonen L. GADD34 mediates cytoprotective autophagy in mutant huntingtin expressing cells via the mTOR pathway. 2011; Epub Sep 7
- Jääntti MH, Putula J, Somerharju P, Frohman MA, Kukkonen JP. OX(1) orexin/hypocretin receptor activation of phospholipase D. *Br J Pharmacol* 2011; Epub Jun 30
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- Näreoja K, Kukkonen JP, Rondinelli S, Toivola DM, Meriluoto J, Näsman J. Adrenoceptor activity of muscarinic toxins identified from mamba venoms. *Br J Pharmacol* 2011; 164(2b):538–50
- Putkonen N, Kukkonen JP, Mudo G, Putula J, Belluardo N, Lindholm D, Korhonen L. Involvement of cyclin-dependent kinase-5 in the kainic acid-mediated degeneration of glutamatergic synapses in the rat hippocampus. *Eur J Neurosci* 2011; 34(8):1212–1221
- Putula J, Turunen PM, Jääntti MH, Ekholm ME, Kukkonen JP. Agonist ligand discrimination by the two orexin receptors depends on the expression system. *Neurosci Lett* 2011; 20;494(1):57–60
- Siltanen A, Kitabayashi K, Lakkisto P, Mäkelä J, Pätälä T, Ono M, Tikkanen I, Sawa Y, Kankuri E, Harjula A. hHGF overexpression in myoblast sheets enhances their angiogenic potential in rat chronic heart failure. *PLoS One* 2011; 6(4):e19161

Theses completed in the group in 2011

The following Master's thesis was accepted at Åbo Akademi this year:

Henrica Karlberg: MIR/MSAP i regleringen av lipoproteinreceptorer i makrofager. December 2011.



► **Members of the Unit:**

Jakob Stenman, M.D., Dr.Med.Sci., head
 Kristina Hotakainen, M.D., Dr.Med.Sci.
 Susanna Lintula, Ph.D.
 Lin Feng, M.Sc.
 Ho Huu Tho, M.D.
 Anne Ahmanheimo, bioanalyst
 Laura Mäkelä, laboratory analyst

Collaborators

Clinical collaborators at Helsinki University Central Hospital, Finland:
 Caj Haglund, M.D., Dr.Med.Sci., Docent, Department of Surgery
 Jarmo Salo, Professor, Division of General Thoracic and Esophageal Surgery, Department of Cardiothoracic Surgery
 Jari Räsänen M.D., Dr.Med.Sci., Division of General Thoracic and Esophageal Surgery, Department of Cardiothoracic Surgery
 Heikki Joensuu, Professor, Departement of Oncology
 Antti Rannikko, D.M., Dr.Med.Sci., Docent, Department of Urology
 Helena Isoniemi, D.M., Dr.Med.Sci., Docent, Department of Transplantation and Liver Surgery Clinic
 Marko Lempinen M.D., Dr.Med.Sci., Departement of Transplantation and Liver Surgery Clinic

Funded by Finska Läkaresällskapet, Tekes (the Finnish Funding Agency for Technology and Innovation) in collaboration with Thermo Fischer Scientific, Yhtyneet Medix Laboratoriot, HYKS Instituutti and Expression Analytics Oy, and the Minerva Foundation for Medical Research.

UNIT OF TARGETED GENE-EXPRESSION ANALYSIS

Main research activities

The aim of the research group is to develop new multigene predictor assays for cancer diagnostics with the main focus on colon, prostate and breast cancer. In these disease groups, there are approximately 500,000 new cases yearly in Europe and the US. We are also developing diagnostic gene sets for smaller disease groups such as Barrett's esophagus and Primary Sclerosing Cholangitis, in which there are specific difficulties with currently available diagnostic tests. A specific challenge for assays based on mRNA expression analysis have been formalin-fixed paraffin-embedded (FFPE) tissue samples, in which the RNA molecules are degraded into shorter fragments. Genome-controlled RT-PCR has been used successfully for this purpose. This technique is based on competitive PCR and enables the amplification and quantitative measurement of exceptionally short DNA fragments, making it suitable for analysis of FFPE samples. Using melting curve analysis, wild type and reference PCR amplicons are separately detected and quantified in a closed-tube-assay.

Improved diagnostics for colon cancer: In Dukes B colon cancer, the disease has spread outside the bowel but not into regional lymph nodes at diagnosis. While 80% of cases can be cured by surgery alone, 20% subsequently develop recurrent disease if no additional therapy is administered. Currently adjuvant chemotherapy is therefore given to all Dukes B colon cancer patients, although the vast majority of these patients are not expected to benefit from the treatment. Our aim is to identify gene expression patterns that would reflect aggressive behavior of the tumor associated with an increased risk of disease recurrence. This would allow better targeting of adjuvant chemotherapy to the patients most likely to benefit from it and decrease the morbidity associated with chemotherapy. The study material includes mRNA isolated from archival FFPE samples and corresponding clinical follow-up data from 800 colon carcinoma patients.

Targeting of breast cancer therapy: There are several agents currently available for breast cancer chemotherapy that target specific genes over-expressed in cancer cells. Such drugs include Tamoxifen (targeting the estrogen receptor, ER), trastuzumab (HER2 or erbB2 tyrosine kinase), anthracyclins (TOP2A-

enzyme), lapatinib (erbB1 and erbB2 tyrosine kinases) and bevasizumab (VEGF growth factor, VEGFR-2 ligand). Assays for measuring the protein expression of these target genes with immunohistochemistry or in situ hybridization techniques are already in clinical practice. The aim is to develop multigene predictor assays that include all currently known molecular targets for the drugs being used in breast cancer chemotherapy. This type of assay could potentially provide a more accurate quantitative analysis of multiple target molecules than the currently available assays. The study material, including mRNA isolated from archival FFPE samples and corresponding clinical follow-up data from 2000 breast cancer patients, is currently being assembled.

Evaluation of treatment benefit in prostate cancer: The widespread use of Prostate Specific Antigen (PSA) testing and screening programs have resulted in a dramatic increase in prostate cancer incidence over the past decade. Currently a large number of prostate cancer patients are treated with surgery, radiation therapy, hormone therapy or a combination of these modalities even though only a minor portion of patients are likely to benefit. Our aim is to develop a diagnostic multigene predictor assay that would provide additional prognostic information from a fine needle biopsy taken at the time of diagnosis. This would allow more accurate targeting of treatment modalities to patients most likely to benefit from them. The study material includes mRNA isolated from archival FFPE samples and corresponding clinical follow-up data from 500 patients operated for prostate cancer at the Helsinki University Central Hospital over the time period 1982–1998.

Publications

Orpana A, Ho TH, Stenman. Multiple heat pulses during PCR extension enables amplification of GC-rich sequences and reduces amplification bias. 2012; Epub Jan 5

A new group at Minerva in 2012:

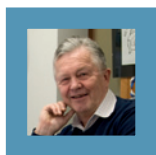
UNIT OF MEMBRANE BIOLOGY



Prof. Ikonen's group is internationally recognized for its pioneering studies in the organization of sterol-sphingolipid rich membrane domains and in the mechanisms of sterol transport in mammalian cells. The group has made seminal observations on the functional role of lipids in human cholesterol-sphingolipid storage diseases and is actively pursuing

the role of cellular lipid imbalance in major European health burdens, including obesity and cancer. The group currently participates in the EU-funded LipidomicNet and coordinates an ESF EuroMembrane network. Ikonen is a member of Biocentrum Helsinki and the coordinator of Helsinki Functional Imaging Center, a national level research infrastructure on multimodal imaging. The group has significant synergy with several research units at Minerva Institute, both on biological questions related to lipid transport and signaling, and on technical approaches to tackle them.

ADDITIONAL RESEARCH



Frej Fyhrquist, M.D., Dr.Med.Sci.,
Dhc, Professor Emeritus
Outi Saijonmaa, Ph.D., Docent
Anders Eriksson, DVM
Emilie Kvist, student

Collaborators

Timo Strandberg, Professor of Geriatrics, University of Oulu, Finland

Funded by

The Liv och Hälsa Foundation, the Paulo Foundation, and the Perklén Foundation.

Main research activities

The team has studied leucocyte telomere length (LTL) using Southern blot in more than 3000 subjects. In the Helsinki Businessman Study, LTL was inversely related to smoking and body mass index during a long follow-up period, from 1964–1973 to 2002–2003, when DNA for telomere measurement was isolated from blood leucocytes of 622 participants. While studies on LTL have generally been cross-sectional, this was the first published longitudinal study on the relation of LTL to life style. In another study comprising the same subjects, high cholesterol in the beginning combined with low cholesterol at the end was related to the shortest LTL and highest mortality, in accord with the “cholesterol paradox”. Also during 2011, the inverse relation of LTL with cardiovascular risk and transient ischemic attack in the LIFE study (1254 subjects) was reported. Moreover, four manuscripts on associations of LTL with 1) alcohol consumption, 2) physical activity, 3) *VD* polymorphisms of the ACE gene, and 4) an invited review on telomeres and cardiovascular aging (Annals of Medicine) were submitted.

Publications

- Fyhrquist F, Silventoinen K, Saijonmaa O, Kontula K, de Faire U, Os I, Dahlöf B. Telomere length and cardiovascular risk in hypertensive patients with left ventricular hypertrophy, the LIFE study. *J Hum Hypertens*. 2011; 25(12):711–8
- Strandberg TE, Saijonmaa O, Tilvis RS, Pitkälä KH, Strandberg AY, Salomaa V, Miettinen TA, Fyhrquist F. Telomere length in old age and cholesterol across the life course. *J Am Geriatr Soc*. 2011; 59(10):1979–81
- Strandberg TE, Saijonmaa O, Tilvis RS, Pitkälä KH, Strandberg AY, Miettinen TA, Fyhrquist F. Association of telomere length in older men with mortality and midlife body mass index and smoking. *J Gerontol A Biol Sci Med Sci*. 2011; 66(7):815–20



Ralph Gräsbeck, M.D., Dr.Med.Sci.,
Dhc, Professor

At the end of 2011 the former Biochemistry Unit consists of only one person, Prof. Ralph Gräsbeck., now 81. His main topics are still the metabolism of vitamin B₁₂ and related diseases and reference values.

Together with Prof. Stephan M Tanner in Columbus, Ohio, he published a long review in *Pediatric Research* on the Imerslund-Gräsbeck syndrome (abbreviated IGS). The review also describes in detail congenital intrinsic factor deficiency (IFD), which is difficult to distinguish from IGS.

Orphanet Encyclopedia (of Rare Diseases) invited Gräsbeck to publish a short article on IFD which appeared in September. Gräsbeck participated in the diagnosis and publishing of a Danish child with this condition.

This child had pains in the site where cyanocobalamin was injected, not an uncommon phenomenon, apparently caused by sensitivity to cyanide. Maybe one metabolic role of vitamin B₁₂ is to catch and remove cyanide, which always occurs in the body.

Gräsbeck is constantly approached by doctors and patients for advice regarding the diagnosis and treatment of such patients. As a special honor he was invited to a Vitamin B₁₂ Symposium in Nancy, France in September 2012 to give a “Distinguished Contribution” lecture covering his entire contribution in the vitamin B₁₂ field.

Gräsbeck has continued to write reviews of antique books in the library of the Finska Läkarsällskapet. He also published his reminiscences of his stay in Norway when the country was occupied by the Germans in 1940. The text appeared in *Svensk Medicinhistorisk Årsbok* (Swedish Yearbook of Medical History).

Publications

- Gräsbeck R, Tanner SM. Congenital intrinsic factor deficiency. Short article (2 pp) in *Orphanet Encyclopedia*, September 2011. <http://www.orpha.net/consor/cgi-bin/index.php>
- Gräsbeck R, Tanner SM. Juvenile selective vitamin B₁₂ malabsorption: 50 years after its description – 10 years of genetic testing. *Pediatr Res*. 2011; 70(3):222–228
- Gräsbeck R. Jørgen Ruud, läkare och nazist. Upplevelser i Norge 1940. (Jørgen Ruud. Physician and Nazi. Adventures in Norway 1940). *Svensk Med-hist Tidskr*. 2010; 14(1):189–194
- Leunbach Lund T, Johansen P, Tanner SM, Gräsbeck R, Hegelstad J. Homozygot mutation in intrinsic factor-genet hos et barn med svær vitamin B₁₂-mangel (Homozygous mutation in the intrinsic factor gene in a child with severe vitamin B₁₂ deficiency). *Ugeskr Laeger* 2011; Epub Feb 21

PUBLICATIONS 2011

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2. **Bunck MC, Cornér A, Eliasson B, Heine RJ, Shaginian RM, Taskinen MR, Smith U, Yki-Järvinen H, Diamant M.** Effects of exenatide on measures of {beta}-cell function after 3 years in metformin-treated patients with type 2 diabetes. *Diabetes Care* 2011; 34:2041–2047
3. **Bunck MC, Eliasson B, Cornér A, Heine RJ, Shaginian RM, Taskinen MR, Yki-Järvinen H, Smith U, Diamant M.** Exenatide treatment did not affect bone mineral density despite body weight reduction in patients with type 2 diabetes. *Diabetes Obes Metab* 2011; 13:374–377
4. **Di Liberto V, Mäkelä J, Korhonen L, Olivieri M, Tselykh T, Mäkiä A, Hai DT, Belluardo N, Lindholm D, Mudò G.** Involvement of estrogen receptors in the resveratrol-mediated increase in dopamine transporter in human dopaminergic neurons and in striatum of female mice. *Neuropharmacology* 2011; Epub Oct 24
5. **Fyhrquist F, Silventoinen K, Saijonmaa O, Kontula K, de Faire U, Os I, Dahlöf B.** Telomere length and cardiovascular risk in hypertensive patients with left ventricular hypertrophy, the LIFE study. *J Hum Hypertens* 2011; 25(12):711–8
6. **Gräsbeck R, Tanner SM.** Congenital intrinsic factor deficiency. Short article (2 pp) in Orphanet Encyclopedia, September 2011. <http://www.orpha.net/consor/cgi-bin/index.php>
7. **Gräsbeck R, Tanner SM.** Juvenile selective vitamin B12 malabsorption: 50 years after its description – 10 years of genetic testing. *Pediatr Res* 2011; 70(3):222–228
8. **Gräsbeck R.** Jørgen Ruud, läkare och nazist. Upplevelser i Norge 1940. (Jørgen Ruud. Physician and Nazi. Adventures in Norway 1940). *Svensk Med-hist Tidsk.* 2010; 14(1):189–194
9. **Hyrskyluoto A, Reijonen S, Kivinen J, Lindholm D, Korhonen L.** GADD34 mediates cytoprotective autophagy in mutant huntingtin expressing cells via the mTOR pathway. *Exp Cell Res* 2011; Epub Sep 7
10. **Jäntti MH, Putula J, Somerharju P, Frohman MA, Kukkonen JP.** OX(1) orexin/hypocretin receptor activation of phospholipase D. *Br J Pharmacol* 2011; Epub Jun 30
11. **Jordan SD, Krüger M, Willmes DM, Redemann N, Wunderlich FT, Brönneke HS, Merkwirth C, Kashkar H, Olkkonen VM, Böttger T, Braun T, Seibler J, Brüning JC.** Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. *Nat Cell Biol* 2011; 13(4):434–46
12. **Kotronen A, Joutsu-Korhonen L, Sevastianova K, Bergholm R, Hakkarainen A, Pietiläinen KH, Lundbom N, Rissanen A, Lassila R, Yki-Järvinen H.** Increased coagulation factor VIII, IX, XI and XII activities in non-alcoholic fatty liver disease. *Liver Int* 2011; 31:176–183
13. **Lakkisto P, Siren J-M, Kytö V, Forsten H, Laine M, Pulkki K, Tikkanen I.** Heme oxygenase-1 induction protects the heart and modulates cellular and extracellular remodeling after myocardial infarction in rats. *Exp Biol Med* 2011; 236(12):1437–48
14. **Lee MY, Sun B, Schliffke S, Yue Z, Ye M, Paavola J, Bozkulak EC, Amos PJ, Ren Y, Ju R, Jung YW, Ge X, Yue L, Ehrlich BE, Qyang Y.** Derivation of functional ventricular cardiomyocytes using endogenous promoter sequence from murine embryonic stem cells. *Stem Cell Res* 2011; Epub Aug 22
15. **Leunbach Lund T, Johansen P, Tanner SM, Gräsbeck R, Hegelstad J.** Homozygous mutation in intrinsic factor gene in a child with severe vitamin B₁₂ deficiency. *Ugeskr Laeger* 2011; Epub Feb 21
16. **Li F, An SL, Zhou Y, Liang ZK, Jiao ZJ, Jing YM, Wan P, Shi XJ, Tan WL.** Milk and Dairy Consumption and Risk of Bladder Cancer: A Meta-analysis. *Urology.* 2011; 78(6):1298–305
17. **Löf C, Viitanen T, Sukumaran P, Törnquist K.** TRPC2: Of Mice But Not Men. *Adv Exp Med Biol* 2011; 704:125–34
18. **Mudò G, Mäkelä J, Liberto VD, Tselykh TV, Olivieri M, Piepponen P, Eriksson O, Mäkiä A, Bonomo A, Kairisalo M, Aguirre JA, Korhonen L, Belluardo N, Lindholm D.** Transgenic expression and activation of PGC-1 α protect dopaminergic neurons in the MPTP mouse model of Parkinson's disease. *Cell Mol Life Sci* 2011; Epub Oct 8
19. **Näreoja K, Kukkonen JP, Rondinelli S, Toivola DM, Meriluoto J, Näsman J.** Adrenoceptor activity of muscarinic toxins identified from mamba venoms. *Br J Pharmacol* 2011; 164(2b):538–50
20. **Okkonen M, Lakkisto P, Korhonen A-M, Parviainen I, Reinikainen M, Varpula T, Pettilä V.** Plasma cell-free DNA in patients needing mechanical ventilation. *Critical Care* 2011; 15(4):R196
21. **Orpana A, Ho TH, Stenman.** Multiple heat pulses during PCR extension enables amplification of GC-rich sequences and reduces amplification bias. *Anal Chem* 2012; Epub Jan 5
22. **Putkonen N, Kukkonen JP, Mudò G, Putula J, Belluardo N, Lindholm D, Korhonen L.** Involvement of cyclin-dependent kinase-5 in the kainic acid-mediated degeneration

- of glutamatergic synapses in the rat hippocampus. *Eur J Neurosci* 2011; 34(8):1212–1221
- 23. Putula J, Turunen PM, Jääntti MH, Ekholm ME, Kukkonen JP.** Agonist ligand discrimination by the two orexin receptors depends on the expression system. *Neurosci Lett* 2011; 494(1):57–60
- 24. Putula J, Turunen PM, Johansson L, Näsman J, Ra R, Korhonen L, Kukkonen JP.** Orexin/hypocretin receptor chimaeras reveal structural features important for orexin peptide distinction. *FEBS Lett* 2011; 585(9):1368–74
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