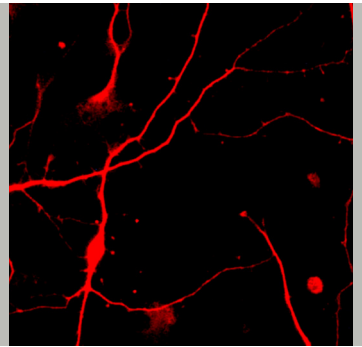
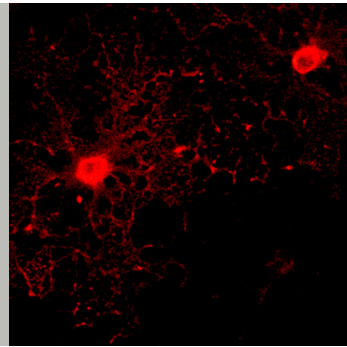
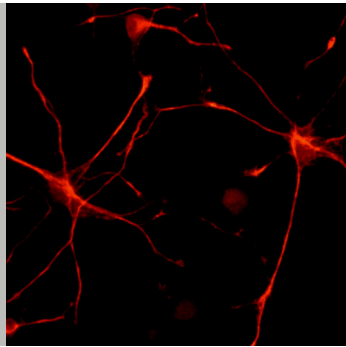
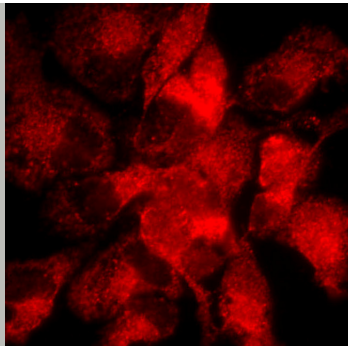


MINERVA FOUNDATION
AND
MINERVA FOUNDATION INSTITUTE
FOR MEDICAL RESEARCH



SCIENTIFIC REPORT 2010





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Front page image: Fluorescent antibody staining of neural progenitor cells cultured as a neurosphere, and differentiated into glial cells and neurons (by courtesy of Raili Koivuniemi/Unit of Neuroscience).

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SUMMARY OF RESEARCH AND ACTIVITIES DURING THE YEAR 2010

The Minerva Foundation Institute for Medical Research is a privately owned research institute that focuses on a fruitful combination of basic biomedical research and clinical investigation relevant to common diseases. The objectives of the research range from studying fundamental molecular mechanisms in cultured cell models and genetically manipulated animals to investigating human patient materials. The aim is to produce new knowledge and innovations for the development of future diagnostic approaches, preventive measures and treatments for conditions such as diabetes, cardiovascular diseases, neurodegenerative disorders and cancer.

The Institute is located in the Biomedicum Research Conglomerate at the Meilahti campus and consists of seven Research Units. During the year 2010, the institute has undergone significant remodeling. The two new groups recruited in 2009 have moved their personnel to the Minerva Institute and got organized into full action during 2010. The group of Dr. Jakob Stenman develops and implements novel polymerase chain reaction-based methodology for tumor profiling. Docent Vesa Olkkonen's group investigates new regulatory mechanisms responsible for maintaining cellular lipid homeostasis. In addition, his group studies the integration of lipid signals with regimes such as membrane trafficking and cell signaling cascades, particularly in relation to cardiovascular diseases and fatty liver/metabolic syndrome.

Along with the growth of the Institute, the administration and principles of function of the Institute have been reviewed, and

new sharpened routines have been implemented. The funds of the Minerva Foundation are directed at maintaining and further developing a research infrastructure to serve the activities of the groups in the most effective way, and also encouraging the scientists to maximize efforts to acquire external research funds. During 2010, 48% of the costs of the Institute were covered by funds from the Minerva Foundation and 52% by external research funds raised by the scientists. In addition, the groups have substantial external funds that are not administered via the Minerva Institute. The administration has been enforced with novel guidelines that meet the demands set by the full-cost funding model employed by the Academy of Finland.

During 2010, a leading figure at the Minerva Institute, Prof. Dan Lindholm, received a professorship in biochemistry and molecular biology at the Medical Faculty of Helsinki University. While he will remain affiliated to the Minerva Institute, he has prepared to move part of his research operation to the Institute of Biomedicine at Helsinki University. This change is accompanied by space becoming available at Minerva during 2011, and discussions on the strategic decisions that this altered situation necessitates have been initiated.

The research at the Minerva Institute in 2010 has been successful and productive. The Research Units published a total of 38 articles, 35 of which appeared in international peer-reviewed journals with a mean impact factor of 4.658. The success of the work carried out is illustrated by the fact that six Ph.D. theses were finalized and defended by Minerva researchers during 2010 – in addition, two M.Sc. theses 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, 2010, to December 31, 2010. The board of trustees included the following persons:

Professor Jim Schröder, chair
Docent Caj Haglund, vice chair
Professor Carl G. Gahmberg
Professor Per-Henrik Groop
Professor Carola Grönghagen-Riska
Professor Christer Holmberg
Professor Krister Höckerstedt
Professor Carl Gustaf Nilsson
Docent Carina Wallgren-Pettersson, secretary
M.Sc. (Econ. & Bus. Adm.) Carl-Magnus Westermarck

The administration is handled by following groups:

Financial Committee

Jim Schröder, chair
Per-Henrik Groop
Carola Grönghagen-Riska
Carl-Magnus Westermarck

Nomination Committee

Jim Schröder, chair
Caj Haglund
Carl G. Gahmberg
Carola Grönghagen-Riska

Scientific Committee

Carl G. Gahmberg, chair
Caj Haglund
Christer Holmberg
Krister Höckerstedt
Carl Gustaf Nilsson
Carina Wallgren-Pettersson

THE MINERVA FOUNDATION INSTITUTE FOR MEDICAL RESEARCH

Board of Directors

Professor Per-Henrik Groop, chair
Docent Heikki Koistinen
Professor Dan Lindholm
Docent Vesa Olkkonen
Docent Jakob Stenman
Docent Ilkka Tikkanen
Professor Kid Törnquist
Professor Hannele Yki-Järvinen
Carita Estlander-Kortman
Tuulikki Nyman, secretary (retired in May 2010)
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. fall 2010
 Eeva Palojoki, M.D., Dr.Med.Sci.
 Tom Bäcklund, M.D.
 Hanna Forsten, V.M.D.
 Tina Grönholm, M.D.
 Jere Paavola, M.D.
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 Juuso Siren, M.B.
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 Riikka Kosonen, M.Sc., laboratory technician
 Jarkko Lakkisto, laboratory technician

Collaborators

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 Kuopio, Finland
 Ilkka Pörsti, M.D., Dr.Med.Sci., Professor, University of Tampere, Finland

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

UNIT OF CARDIOVASCULAR RESEARCH

Main research activities

The group studies the mechanisms and repair of end-organ damage in cardiovascular and renal diseases. The group has investigated the local expression 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.

We were able to show for the first time expression of the novel angiotensin 1-7 Mas receptor in eye tissue in the rat. Angiotensin 1-7 may be involved in the regulation of intraocular pressure, acting as a counter-regulatory component against the vascular and proliferative actions of angiotensin II.

We studied adverse cardiac remodelling after myocardial infarction (MI) in diabetic and non-diabetic rats in collaboration with professor Eero Mervaala's research group. Type 2 diabetes was associated with more pronounced cardiac hypertrophy, interstitial fibrosis and sustained apoptotic cardiomyocyte loss. The results of these studies suggested important roles for Akt-FOXO3a, Sirt1 - p53 and p38 MAPK in the regulation of post-infarct cardiac remodelling in type 2 diabetes.

Heme oxygenase-1 (HO-1), a cytoprotective enzyme also implicated in cell cycle regulation and angiogenesis, was studied in post-MI cardiac remodelling and regenerative process. HO-1 mediates anti-oxidative, anti-apoptotic, anti-inflammatory and anti-proliferative properties. These are based on breakdown of the pro-oxidative heme and production of the biologically active molecules biliverdin/bilirubin and carbon monoxide (CO) (Figure 1).

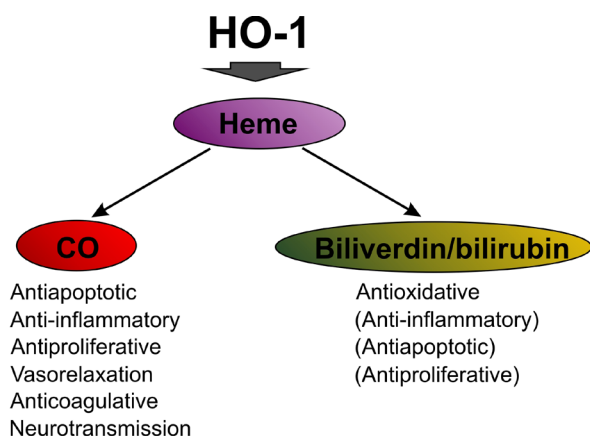


Fig.1. Effects of HO-1 reaction products biliverdin/bilirubin and carbon monoxide.

Our studies showed that HO-1 induction and CO promote cardiac regeneration and neovascularization after experimental MI. Treatment with a CO-donor increased the amount of c-kit-positive cardiac stem/progenitor cells (Figure 2) and stimulated their differentiation into cardiomyocytes and vascular structures in the infarct area. HO-induction increased expression of vascular endothelial growth factor (VEGF) family members and several other angiogenic factors. In addition, HO-induction protected against pathological post-MI cellular and extracellular left ventricular remodelling. The numerous beneficial effects of the HO-1/CO pathway may thus provide a new tool to promote repair of cardiac injury and for the prevention and treatment of post-MI cardiac failure.

We have also studied the role of local, paracrine mechanisms of cardiac regeneration in collaboration with Professor Ari Harjula and his research group using modified myoblast cell sheet transplantation methodology. Transfer of myoblasts over-expressing the anti-apoptotic bcl-2 protein to the infarct area resulted in increased cardiac VEGF expression, promoted neovascularization and improved systolic function of the heart after experimental MI.

Prognosis of severe systolic heart failure has remained poor despite optimal medical therapy. The novel findings on cardiac stem/progenitor cells 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.

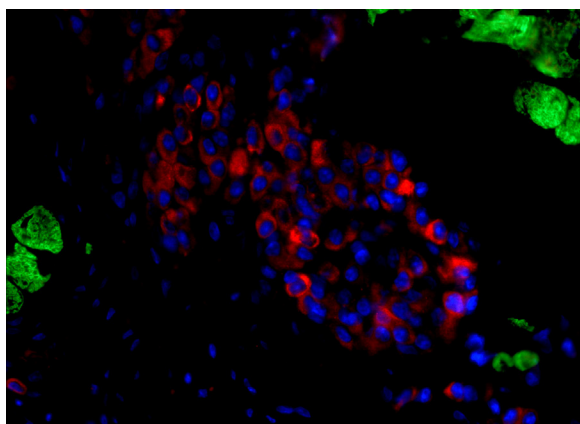


Fig. 2. C-kit-positive progenitor cells (red) and cardiomyocytes (green) in the infarct area of post-MI rat heart.

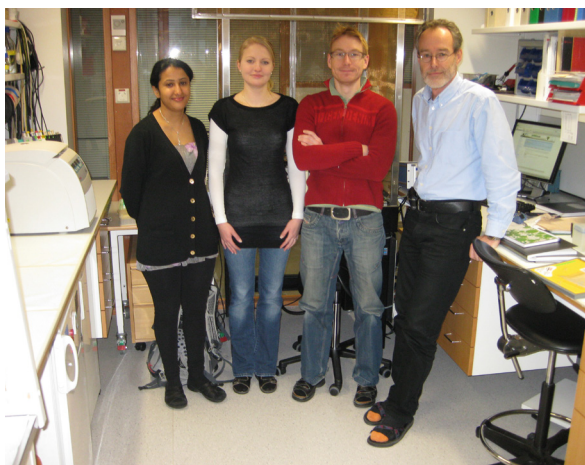
Publications

- Kitabayashi K, Siltanen A, Pätälä T, Mahar MA, Tikkanen I, Koponen J, Ono M, Sawa Y, Kankuri E, Harjula A. Bcl-2 expression enhances myoblast sheet transplantation therapy for acute myocardial infarction. *Cell Transplant* 2010; 19: 573-588
- Lakkisto P, Kytö V, Forsten H, Siren JM, Segersvärd H, Voipio-Pulkki LM, Laine M, Pulkki K, Tikkanen I. Heme oxygenase-1 and carbon monoxide promote neovascularization after myocardial infarction by modulating the expression of Hif-1 α , SDF-1 α and VEGF-B. *Eur J Pharmacol* 2010; 635: 156-164
- Saukkonen K, Lakkisto P, Kaunisto MA, Varpula M, Voipio-Pulkki LM, Varpula T, Pettilä V, Pulkki K. Heme oxygenase 1 polymorphisms and plasma concentrations in critically ill patients. *Shock* 2010; 34: 558-564
- Syväranta S, Helske S, Laine M, Lappalainen J, Kupari M, Mäyränpää MI, Lindstedt KA, Kovanen PT. Vascular endothelial secreting mast cells and myofibroblasts: a novel self-perpetuating angiogenic pathway in aortic valve stenosis. *Arterioscler Thromb Vasc Biol* 2010; 30: 1220-1227
- Vahtola E, Louhelainen M, Forsten H, Merasto S, Raivio J, Kaheinen P, Kytö V, Tikkanen I, Levijoki J, Mervaala E. Sirtuin1-p53, forkhead box O3a, p38 and post-infarct cardiac remodeling in the spontaneously diabetic Goto-Kakizaki rats. *Cardiovasc Diabetol* 2010; 9:5(pp.1-13)

Theses completed in the group in 2010

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

Päivi Lakkisto: Heme oxygenase-1 in cardiovascular diseases. December 17, 2010.



► **Members of the Unit:**

Kid Törnquist, Ph.D., Professor, head
Nina Bergelin, Ph.D. fall 2010
Tero Viitanen, Ph.D. summer 2010
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.
Md. Yasir Asghar, M.Phil.
Ilari Pulli, B.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
Mika Scheinin, Professor, University of Turku, Finland
Petra Sipilä, M.D., Dr.Med.Sci, University of Turku, Finland
Lea Sistonen, Professor, Åbo Akademi University, Turku, Finland
Peter Slotte, Professor, Åbo Akademi University, Turku, Finland

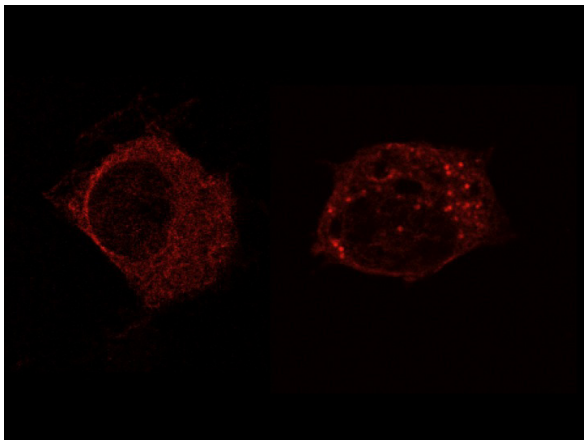
Funded by the Sigrid Juselius Foundation, the Academy of Finland, the Liv och Hälsa Foundation, and Åbo Akademi University. Christoffer Löf was funded by the Turku Graduate School of Biomedical Sciences.

UNIT OF CELLULAR PHYSIOLOGY

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. We are currently also investigating the importance of TRPC channels in human thyroid cancer cells.

Investigations on the importance of S1P in regulating thyroid cancer cell migration have been particularly successful. Our studies show that S1P potently stimulates the migration of some thyroid cancer cell types, whereas the migration of some other tumor cell types is blocked. The receptor profile for S1P is of crucial importance in this phenomenon. Furthermore, in thyroid cancer cells, vascular endothelial growth factor receptor 2 (VEGFR-2) is an important part of the signaling complex activated by S1P. This is emphasized by recent results, which show that blocking VEGFR-2 strongly attenuates the migratory response evoked by S1P. We have recently focused on the interactions between S1P1 and VEGFR-2. We showed that S1P-receptors form complexes with VEGFR-2, and that the S1P1/VEGFR-2 complex associates with PKC α and ERK1/2. Furthermore, the complex evokes bidirectional signalling, as S1P-induced phosphorylation of ERK1/2 is sensitive to VEGFR-2 kinase inhibition, and VEGF-A-induced ERK1/2 phosphorylation is sensitive to Ptx-treatment as well as S1P1 siRNA treatment. Both S1P- and VEGF-A-induced haptotaxis is sensitive to Ptx-treatment and S1P1 siRNA treatment. We also showed that the phosphorylation of ERK1/2, evoked by both VEGF-A and the S1P1 agonist SEW-2871, is inhibited by PKC α and PKC- β 1 siRNA.



We hypothesise that VEGFR-2 forms a signaling complex with S1P1, leading to bidirectional signaling that regulates ERK1/2 phosphorylation and haptotaxis of ML-1 cells. This signaling complex thus appears to be a significant regulator of thyroid cancer cell migration.

In addition, we have investigated further the autocrine signaling evoked by S1P, especially in regard to protecting cells from apoptosis. We found that S1P protected cells from FasL-induced cell death in an NF- κ B-dependent manner. NF- κ B was activated by extracellular S1P via S1P2 receptors and Gi protein signaling. Our study also demonstrates that extracellular S1P stimulates cells to rapidly produce and secrete additional S1P, which can further amplify the NF- κ B activation. Our results suggest the existence of a self-amplifying loop of autocrine S1P with the capacity to enhance cell survival. This mechanism may provide increased understanding of the multifaceted role of S1P in regulating cell fate during normal development and carcinogenesis.

An exciting series of investigations aim at understanding the mechanisms of lipid actions on potassium channels. Our previous investigations have shown that ceramide promotes internalization and ubiquitin-mediated degradation of the HERG potassium channel. We have now characterized how another lipid, diacylglycerol (DAG), modulates HERG. DAG is an important second messenger in cells, and is always produced when agonists activate phospholipase C. In the present report, using human embryonic kidney cells stably expressing HERG, we show that diacylglycerol potently inhibits the HERG current. This is mediated by protein kinase C-induced endocytosis of the channel protein and is dependent on the dynein-dynamin complex. The HERG protein was found only in early endosomes, but not in lysosomes. Thus, diacylglycerol is an important lipid participating in the regulation of HERG surface expression and

function. This observation is important, considering that the HERG channel regulates the repolarization of e.g. cardiac action potentials. And more recently, HERG has an important role in regulating migration and proliferation of cancer cells. In line with this, we are planning further studies on HERG in thyroid cancer cells.

It may also be worth mentioning that 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 mitochondria.

Publications

- Alam CM, Valkonen SH, Ohls S, Törnquist K, Hänninen A: Peritoneal B Lymphocytes Escape Sphingosine-1-phosphate Retention, Migrate to Pancreatic Lymph Nodes and Present Islet-antigen in Non-Obese Diabetic Mice. *Diabetologia* 2010; 53:346-355
- Bergelin N, Löf C, Balthasar S, Kalhori V, Törnquist K: S1P receptor 1 and VEGF receptor 2 form a signalling complex with ERK1/2 and PKC α regulating ML-1 thyroid carcinoma cell migration. *Endocrinology* 2010; 151:2994-3005
- Blom T, Bergelin N, Meinander A, Slotte JP, Eriksson J, Törnquist K: A self-amplifying loop of autocrine sphingosine-1-phosphate enhances NF- κ B-activation and survival. *BMC Cell Biology* 2010; 11:45
- Ekokoski E, Aitio O, Törnquist K, Yli-Kauhaluoma J, Tuominen RK: HIV-1 Tat-peptide inhibits protein kinase C and protein kinase A through substrate competition. *Eur J Pharm Sci* 2010; 40:404-411
- Ramström C, Chapman H, Viitanen T, Fox H, Kivelä J, Soini S, Korhonen L, Lindholm D, Pasternack M, Törnquist K: HERG (KCNH2) K⁺ channel surface expression is modulated by diacylglycerol. *Cell Mol Life Sci* 2010; 67:157-169

Theses completed in the group in 2010

The following Ph.D. theses were accepted at the University of Helsinki and Åbo Akademi University this year:

- Tero Viitanen:** GABAergic signalling in the brain: Inhibition, shunting and excitation. June 23, 2010 (University of Helsinki).
- Nina Bergelin:** Interactions between sphingosine 1-phosphate and vascular endothelial growth factor signalling in thyroid carcinoma cells. September 17, 2010 (Åbo Akademi University).



► **Members of the Unit:**

Hannele Yki-Järvinen, M.D., Dr.Med.Sci., F.R.C.P.
Professor, head
Anna Kotronen, M.D., Dr.Med.Sci.
Janne Makkonen, M.D., Dr.Med.Sci. summer 2010
Julia Perttilä, Ph.D.
Piia Simonen, M.D, Dr.Med.Sci.
Antti Hakkarainen, M.Sc. Techn.
Leena Juurinen, M.D.
Alexandre Santos, M.D.
Ksenia Sevastianova, M.D.
Jenni Hyysalo, medical student
Elina Petäjä, medical student
Kaisa Makkonen, laboratory technician
Katja Sohlo, laboratory technician
Mia Urjansson, laboratory technician

UNIT OF ENDOCRINOLOGY

The group has continued its studies addressing the prevalence, diagnosis and pathophysiology of non-alcoholic fatty liver disease (NAFLD). In the Finnish middle-aged population, the prevalence of NAFLD is 3-fold higher than that of alcoholic fatty liver disease (AFLD). Regarding diagnosis, the presence of the metabolic syndrome and type 2 diabetes, fasting serum (fs) insulin, fs-aspartate aminotransferase (AST), and the AST/alanine aminotransferase ratio were shown to be independent predictors of NAFLD. The NAFLD score had an area under the receiver operating characteristic curve of 0.87 in the estimation and 0.86 in the validation group. The optimal cut-off point of -0.640 predicted increased liver fat content with a sensitivity of 86% and specificity of 71%. The addition of genetic information to the score only improved the accuracy of the prediction by less than 1%. Using the same variables, we developed an equation from which the liver fat percentage of each individual could be estimated.

We also determined whether 3.0-T proton-decoupled phosphorus 31 ((31)P) magnetic resonance (MR) spectroscopy can be used to differentiate between stages of nonalcoholic fatty liver disease (NAFLD) by resolving the components of phosphomonoester (PME) and phosphodiester (PDE) compounds and enabling detection of a greater number of other phosphorus-containing compounds. NADPH, a marker of inflammation and fibrinogenic activity in the liver, was increased in patients with Nonalcoholic steatohepatitis (NASH) and those with cirrhosis. Proton-decoupled (31)P 3.0-T MR spectroscopy thus showed promise in the differentiation of NAFLD stages.

Regarding NAFLD pathophysiology, animal studies suggest that endocannabinoids could contribute to the development of nonalcoholic fatty liver disease (NAFLD). In addition, NAFLD is associated with multiple changes in lipid concentrations in liver biopsies. We performed hepatic venous catheterization studies in combination with [(2)H(2)]palmitate infusion in the fasting state and during a low-dose insulin infusion in 9 subjects with various degrees of hepatic steatosis as determined by liver biopsy. The splanchnic balance of endocannabinoids and individual lipids was determined using ultraperformance liquid chromatography coupled to mass spectrometry. We found the human fatty liver to take up 2-arachidonoylglycerol and over-produce triacylglycerols containing saturated fatty acids, which might reflect increased de novo lipogenesis.

Publications

- Bunck MC, Cornér A, Eliasson B, Heine RJ, Shaginian RM, Wu Y, Yan P, Smith U, Yki-Järvinen H, Diamant M, Taskinen M-R. One-year treatment with exenatide vs. Insulin Glargine: effects on postprandial glycemia, lipid profiles and oxidative stress. *Atherosclerosis* 2010; 212: 223-229
- Bunck MC, Diamant M, Eliasson B, Cornér A, Shaginian RM, Heine RJ, Taskinen M-R, Yki-Järvinen H, Smith U. Exenatide affects circulating cardiovascular risk biomarkers independently of changes in body composition. *Diabetes Care* 2010; 33: 1734-1737
- Kottronen A, Seppänen-Laakso T, Westerbacka J, Kiviluoto T, Arola J, Ruskeepää AL, Yki-Järvinen H, Orešič M. Comparison of Lipid and Fatty Acid Composition of the Liver, Subcutaneous and Intra-abdominal Adipose Tissue, and Serum. *Obesity* 2010; 18: 937-944
- Kottronen A, Yki-Järvinen H, Männistö S, Saarikoski L, Korpi-Hyövälti E, Oksa H, Saltevo J, Saaristo T, Sundvall J, Tuomilehto J, Peltonen M. Non-alcoholic and alcoholic fatty liver disease – two diseases of affluence associated with the metabolic syndrome and type 2 diabetes: the FIN-D2D Survey. *BMC Public Health* 2010; 10: 237-243
- Kottronen A, Yki-Järvinen H, Sevastianova K, Bergholm R, Hakkarainen A, Pietiläinen KH, Juurinen L, Lundbom N, Sørensen TI. Comparison of the Relative Contributions of Intra-Abdominal and Liver Fat to Components of the Metabolic Syndrome. *Obesity*; Epub 2010 Jun 10
- Romeo S, Huang-Doran I, Baroni MG, Kottronen A. Unravelling the pathogenesis of fatty liver disease: patatin-like phospholipase domain-containing 3 protein. *Curr Opin Lipidol* 2010; 21:247-252
- Sevastianova K, Hakkarainen A, Kottronen A, Cornér A, Arkkila P, Westerbacka J, Bergholm R, Lundbom J, Lundbom N, Yki-Järvinen H. Nonalcoholic fatty liver disease: detection of elevated nicotinamide adenine dinucleotide phosphate with in vivo 3.0-T 31P MR spectroscopy with proton decoupling. *Radiology* 2010; 256: 466-473
- Simonen P, Kottronen A, Hallikainen M, Sevastianova K, Makkonen J, Hakkarainen A, Lundbom N, Miettinen TA, Gylling H, Yki-Järvinen H. Cholesterol synthesis is increased and absorption decreased in non-alcoholic fatty liver disease independent of obesity. *J Hepat* 2010; 1:153-159

- Vanni E, Bugianesi E, Kottronen A, De Minicis S, Yki-Järvinen H, Svegliati-Baroni G. From the metabolic syndrome to NAFLD or vice versa? *Dig Liver Dis*. 2010; 42(5):320-30
- Westerbacka J, Kottronen A, Fielding BA, Wahren J, Hodson L, Perttälä J, Seppänen-Laakso T, Suortti T, Arola J, Hultcrantz R, Castillo S, Olkkonen VM, Frayn KN, Orešič M, Yki-Järvinen H. Splanchnic balance of free fatty acids, endocannabinoids, and lipids in subjects with nonalcoholic fatty liver disease. *Gastroenterology* 2010, 6:1961-1971

Theses completed in the group in 2010

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

Janne Makkonen: Adipose tissue inflammation, liver fat and insulin resistance in humans. June 18, 2010.



► Members of the Unit:

Vesa Olkkonen, Ph.D., Docent, head
 Olivier Béaslas, Ph.D.
 Eija Nissilä, Ph.D.
 Terhi Vihervaara, M.Sc.
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 Research and University of Helsinki, Finland

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 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

The main project of the group deals with 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, for instance on cell survival, signaling and differentiation processes, and they are enriched in pathological tissues such as atherosclerotic lesions. We have identified a family of 12 human genes that encode cytoplasmic oxysterol-binding proteins, ORPs (Fig. 1). Our work focuses on the elucidation of the function of these proteins in the control of cellular lipid metabolism and in

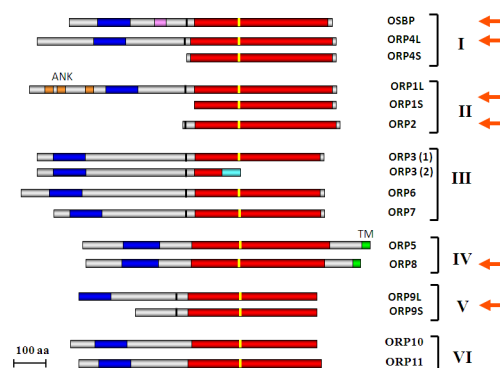


Fig. 1. Schematic presentation of the human ORP protein family. PHD, pleckstrin homology domain; D, dimerization region determined for OSBP; FFAT, two phenylalanines in an acidic tract (endoplasmic reticulum targeting motif); OF, OSBP fingerprint sequence, consensus EQVSHHPP; ORD, OSBP-related ligand binding domain; ANK, ankyrin repeats; TM, trans-membrane segment targeting the endoplasmic reticulum. The ORP subfamilies I-VI are identified by Roman numerals on the right. The red arrows indicate documented in vitro binding of oxysterols, cholesterol or both.



Fig. 2. A molecular model on the structure of ORP1L ligand binding domain. β -strands are depicted in light blue and α -helical segments in red. The top-most α -helices form a lid that closes the ligand binding cavity. A sterol bound within the cavity is displayed in green. A leucine (L561) side chain of the lid that makes contact with the bound sterol is shown in white.

the development of atherosclerotic diseases. We also investigate their wider functional connections, for instance with intracellular transport processes. 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 2010, the Unit elucidated the functional role of ORP family members and their sterol ligand binding (see Fig. 2) in intracellular organelle motility, transport of cargo molecules along the endocytic pathway, intracellular cholesterol transport, and removal of cholesterol from macrophage foam cells (Vihervaara et al. 2010; Jansen et al. 2010). We also characterized a previously poorly known family member, ORP11, which is expressed abundantly in adipose tissue, and suggest a role for it in lipid sensing/transport at the Golgi apparatus-late endosome interface (Zhou et al. 2010). Moreover, the role of macrophage ORP8 in the development of atherosclerotic lesions in LDL receptor knock-out mice has been assessed; the results support our hypothesis that ORP8 acts in this cell type as a pro-atherogenic factor (manuscript in preparation).

A collaborative project that focuses on the molecular mechanisms underlying non-alcoholic fatty liver disease (NAFLD) has been launched 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 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 thereby gain a new level of understanding of the mechanisms of hepatic fat accumulation. The knowledge gained will facilitate the development of new preventive measures and treatments for metabolic syndrome and type 2 diabetes. A postdoctoral fellow, Julia Perttilä, Ph.D., has been co-supervised in this activity by H. Yki-Järvinen and V. Olkkonen. The first report from this collaborative axis within the Minerva Institute has been published and another two studies submitted for publication. The results are summarized in the section of the Unit of Endocrinology.

Awards, honours and positions of trust

Vesa Olkkonen has acted as a Secretary Treasurer and Council Member of the Scandinavian Society for Atherosclerosis Research.

Publications

- Jansen M, Ohsaki Y, Rega LR, Bittman R, Olkkonen VM, Ikonen E. Role of ORPs in sterol transport from plasma membrane to ER and lipid droplets in mammalian cells. *Traffic* 2010; Epub Nov 9
- Westerbacka J, Kotronen A, Fielding BA, Wahren J, Hodson L, Perttilä J, Seppänen-Laakso T, Suortti T, Arola J, Hultcrantz R, Castillo S, Olkkonen VM, Frayn KN, Orešić M, Yki-Järvinen H. Splanchnic balance of free fatty acids, endocannabinoids, and lipids in subjects with nonalcoholic fatty liver disease. *Gastroenterology* 2010; 6:1961-1971
- Vihervaara T, Uronen R-L, Wohlfahrt G, Björkhem I, Ikonen, Olkkonen VM. Sterol binding by OSBP-related protein 1L regulates late endosome motility and function. *Cellular and Molecular Life Sciences* 2010; Epub Aug 6
- Zhou Y, Li S, Mäyränpää MI, Zhong W, Bäck N, Yan D, Olkkonen VM. OSBP-related protein 11 (ORP11) dimerizes with ORP9 and localizes at the Golgi-late endosome interface. *Experimental Cell Research* 2010; 316: 3304-16



► Members of the Unit:

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Collaborators

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 University of Helsinki, Finland
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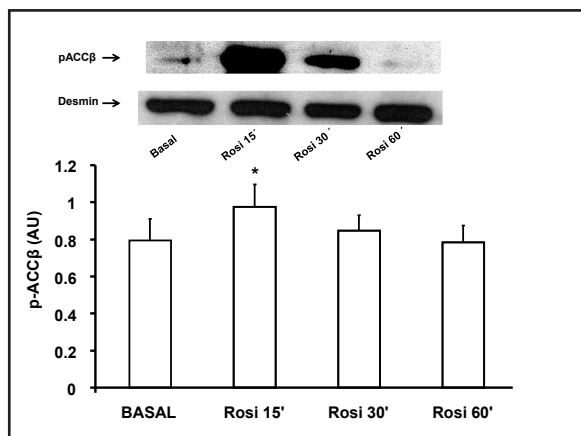
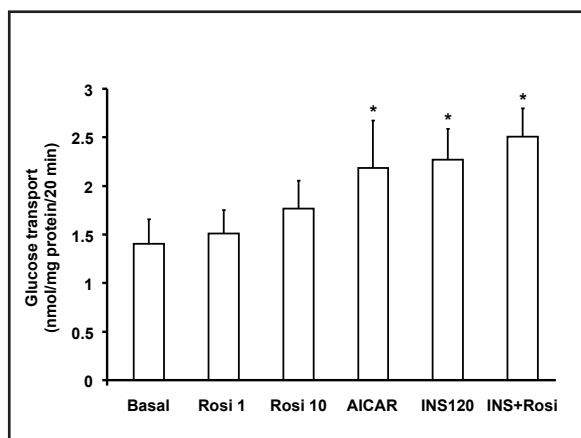
UNIT OF METABOLISM

Main research activities

Thiazolidinediones (TZDs) such as rosiglitazone are widely used as anti-diabetic drugs, and animal studies suggest that TZDs may have direct metabolic actions in skeletal muscle. We examined whether acute exposure to rosiglitazone stimulates the glucose transport rate in isolated skeletal muscle strips from non-diabetic men. We recruited a cohort of 15 non-diabetic men, and obtained open muscle biopsies from the m. vastus lateralis to subsequently isolate small muscle strips. Strips were exposed to rosiglitazone, AICAR (an activator of AMP-activated protein kinase (AMPK)), or insulin. As expected, AICAR and insulin increased the glucose transport rate significantly. Exposure to rosiglitazone transiently increased phosphorylation of ACC β , indicating that exposure to rosiglitazone activated AMPK. However, rosiglitazone did not affect the basal or insulin-stimulated glucose transport rate or phosphorylation of Akt-Ser473 or Akt-Thr308 in isolated muscle strips (Skrobuk et al. 2010).

In a collaborative study with the Karolinska Institutet, the direct effect of a NO donor (Spermine NONOate) on glucose transport was studied in isolated human skeletal muscle. Exposure of isolated human skeletal muscle strips to spermine NONOate significantly increased glucose transport, concomitant with increased cGMP concentration. Analysis of the canonical insulin signaling cascade implicated an insulin-independent signaling mechanism. Accordingly, Spermine NONOate increased AMPK α 1-associated activity (Deshmunkh et al. 2010).





In 2010, the main research activity of the group has focused on mechanisms whereby fatty acids induce insulin resistance in skeletal muscle. In order to facilitate these studies, we have set up and characterized primary human muscle cell lines and currently have cell lines from 16 clinically characterized subjects. These cells will be invaluable for further studies on the mechanisms of how glucose and lipid metabolism is regulated in human skeletal muscle.

In April 2010, two students of the group, Ph.D. student Paulina Skrobuk and Master's student Johannes Kärkkäinen, presented their data as poster presentations at the Keystone meeting on

Diabetes, in Whistler, Canada. Student of genetics Stephanie von Kraemer finished her Master's Thesis entitled "Do MC-acylcarnitines, products of incomplete β -oxidation, affect insulin signaling in L6 muscle cells?" in 2010.

Publications

Deshmukh AS, Long YC, de Castro Barbosa T, Karlsson HKR, Glund S, Zavadski WJ, Gibbs EM, Koistinen HA, Wallberg-Henriksson H, Zierath JR. Nitric oxide increases cGMP levels, AMPK α 1-isoform-specific activity and glucose transport in human skeletal muscle. *Diabetologia* 2010; 53:1142-50

Skrobuk P, Kuoppamaa H, Hiukka A, Koistinen HA: Acute exposure to rosiglitazone does not affect glucose transport in intact human skeletal muscle. *Metabolism, Clinical and Experimental* 2010; 59:224-230

Theses completed in the group in 2010

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

Stephanie von Kraemer: Do acylcarnitines, products of incomplete β -oxidation, affect insulin signaling in L6 muscle cell? November 2010.



► Members of the Unit:

Dan Lindholm, M.D., Dr.Med.Sci., Professor, head
 Laura Korhonen, M.D., Dr.Med.Sci., Docent, Academy
 Researcher Fellow
 Minna Kairisalo, M.Sc., Ph.D. fall 2010
 Tuija Mustonen Ph.D.
 Sami Reijonen, M.Sc., Ph.D. fall 2010
 Timofey Tselykh, Ph.D.
 Alise Hyrskyluoto, M.Sc.
 Jenny Kivinen, M.Sc.
 Raili Koivuniemi, M.Sc.
 Johanna Mäkelä, M.Sc.
 Noora Putkonen, M.Sc.
 Heli Fox, M.Sc.
 Pilvi Jalonen, B.Sc., M.Sc. spring 2010
 Henrica Karlberg, B.Sc.
 Eeva Lehto, laboratory technician

Collaborator

Jyrki Kukkonen, M.D., Professor, University of
 Helsinki, Finland

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

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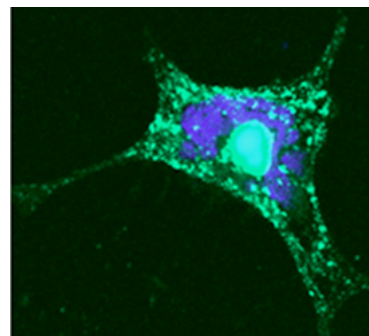
UNIT OF NEUROSCIENCE

We study basic mechanisms underlying neurodegenerative diseases and excitotoxic cell damage in the brain. We focus on protein ubiquitination and cell death pathways in disease pathogenesis with the aim of contributing to better therapies to combat cell death and degeneration. We are also studying neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), and their signaling as well as cellular mechanism that regulate the proliferation, survival, and cell fate determination and differentiation of neuronal precursor cells in the brain. The most actual projects around these general themes are described briefly below.

Ongoing projects

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

We have studied the link between BDNF and the Inhibitor of Apoptosis Proteins (IAPs) in neurons and using transgenic mice over-expressing X chromosome-linked IAP in neurons. We observed that XIAP protects against oxidative stress in a signaling pathway involving NF- κ B. XIAP also regulates BDNF gene expression in a complex web involving cytokines such as Interleukin-6. In addition, we have continued to study the role of mitochondria and endoplasmic reticulum (ER) stress in disease pathogenesis in neurological disorders, such as Huntington's disease (HD), excitotoxicity and amyotrophic lateral sclerosis (ALS). We also study neuroinflammation and the roles played by peroxisome proliferator-activated receptor-gamma coactivators (PGC-1s) and nuclear receptors in the regulation of cell viability and mitochondrial metabolism in neurons. We will study signaling pathways and genes mediating increased neuronal survival using Affymetrix chips and various cell culture methods and in vivo approaches.



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 degeneration. Our main interest is to understand how the UPS and various DUBs regulate synaptic function and how functional disturbances in synapses are linked to neuronal disorders and represent early signs of 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/Myip, from the brain. Mir/Myip affects the neuronal cytoskeleton and responses to PDGF in fibroblasts. A recent study showed that Mir also influences the levels of lipoprotein receptors such as LDLR and VLDL, and the protein was consequently named Idol (Inducible degrader of the LDLR). We are currently studying the role of Mir/Myip/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

Neural progenitor cells (NPCs) are highly dividing cells with the capacity for self-renewal and can give rise to both neurons and glial cells. These cells are expressed in the developing neuroepithelium of the embryonic brain, and then in discrete neurogenic areas in the adult brain. NPCs are regulated by intrinsic and extrinsic factors that influence neurogenesis and brain tissue homeostasis. We have studied proteins that influence cell proliferation of NPCs, focusing on transmembrane proteins such as the HAI-1 and HAI-2 proteins that belong to the Kunitz type of serine protease inhibitors. These proteins have earlier been associated with cancer growth and tumorigenesis, and we found them to be highly expressed in NPCs. We are now studying the precise regulation of HAIs in the brain and their downstream targets and signaling pathways in NPCs. The information gained would add to our understanding about the HAI molecules and ensuing changes in protease activity in the regulation of NPCs and possible other cell types in the brain as well as in brain tumors.

Awards, honours and positions of trust

Dan Lindholm has acted as a Member of Scientific Panel of Swedish Research Council (Vetenskapsrådet Sverige) and as a Member of Scientific Board of Swedish Brain Foundation (Hjärnfonden Sverige).

Publications

- Hong C, Duit S, Jalonen P, Out R, Scheer L, Sorrentino V, Boyadjian R, Rodenburg KW, Foley E, Korhonen L, Lindholm D, Nimpf J, van Berkel TJ, Tontonoz P, Zelcer N. The E3 ubiquitin ligase IDOL induces the degradation of the low density lipoprotein receptor family members VLDLR and ApoER2. *J Biol Chem* 2010; 285(26):19720-6
- Kairisalo M, Bonomo A, Hyrskyluoto A, Mudò G, Belluardo N, Korhonen L, Lindholm D. Resveratrol reduces oxidative stress and cell death and increases mitochondrial antioxidants and XIAP in PC6.3-cells. *Neurosci Lett* 2010; Epub Nov 19
- Lind U, Alm Rosenblad M, Hasselberg Frank L, Falkbring S, Brive L, Laurila JM, Pohjanoksa K, Vuorenää A, Kukkonen JP, Gunnarsson L, Scheinin M, Mårtensson Lindblad LG, Blomberg A. Octopamine receptors from the barnacle balanus improvisus are activated by the alpha2-adrenoceptor agonist medetomidine. *Mol Pharmacol* 2010; 78(2):237-48
- Mäkelä J, Koivuniemi R, Korhonen L, Lindholm D. Interferon-gamma produced by microglia and the neuropeptide PACAP have opposite effects on the viability of neural progenitor cells. *PLoS One* 2010; 14;5(6):e11091
- Reijonen S, Kukkonen JP, Hyrskyluoto A, Kivinen J, Kairisalo M, Takei N, Lindholm D, Korhonen L. Downregulation of NF-kappaB signaling by mutant huntingtin proteins induces oxidative stress and cell death. *Cell Mol Life Sci* 2010; 67(11):1929-41
- Turunen PM, Ekholm ME, Somerharju P, Kukkonen JP. Arachidonic acid release mediated by OX1 orexin receptors. *Brit J Pharmacol* 2010; 159: 212-21
- Turunen PM, Putula J, Kukkonen JP. Filtration assay for arachidonic acid release. *Anal Biochem* 2010; Epub Jul 23

Theses completed in the group in 2010

The following Ph.D. theses were accepted at the University of Helsinki this year:

Minna Kairisalo: Oxidative stress and XIAP signalling in neuronal cells. October 15, 2010.

Sami Reijonen: Endoplasmic reticulum stress and cell death mechanisms in the cell model of Huntington's disease. October 8, 2010.

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

Pilvi Jalonen: Design and validation of RT-QPCR reveal a BDNF-induced upregulation of Myip/Idol in hippocampal neurons. June 2010.



► **Members of the Unit:**

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 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:

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Jari Räsänen M.D., Dr.Med.Sci., Division of General Thoracic and Esophageal Surgery, Department of Cardiothoracic Surgery

Heikki Joensuu, Professor, Department 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., Department 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

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 that are 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

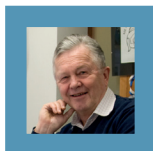
with immunohistochemistry or in situ hybridization techniques are already in clinical practice. The aim is to develop multigene predictor assays that include all currently known molecular targets for the drugs being used in breast cancer chemotherapy. This type of assay could potentially provide a more 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.



ADDITIONAL RESEARCH



Frej Fyhrquist, M.D., Dr.Med.Sci.,

Dhc, Professor Emeritus
Outi Saijonmaa, Ph.D., Docent
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Emilie Kvist, student
Anna Nyman, student

Collaborators

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Ilkka Immonen, Docent, Department of Ophthalmology, Helsinki University Central Hospital, Finland

Funded by

Finska Läkaresällskapet, Understödsföreningen Liv och Hälsa, the Paulo Foundation, and the Perklén Foundation.

Frej Fyhrquist, a former Head of the Institute, has together with his collaborators studied leucocyte telomere length using Southern blot in more than 2400 subjects. His group has showed that short leucocyte telomeres are associated with nephropathy in patients with diabetes type 1 (Fyhrquist et al. 2010). They observed an association of leucocyte telomere length in older men with mortality and midlife body mass index and smoking. The subjects participated in the Helsinki Businessman Study (collaboration with Professor T. Strandberg, University of Oulu). They failed to observe an association of leucocyte telomere length with age-related macular degeneration (collaboration with docent Ilkka Immonen, Helsinki University Central Hospital). Further, they found a novel association of leucocyte telomere length with the D-allele of the ACE I/D polymorphism in 1274 patients with hypertension and left ventricular hypertrophy, possibly linking angiotensin 2 to telomere attrition.

Publications

- Eriksson A, Hansson K, Häggström J, Järvinen AK, Lord P. Pulmonary blood volume in mitral regurgitation in cavalier King Charles spaniels. *J Vet Intern Med* 2010; 24(6):1393-9
- Fyhrquist F, Tiitu A, Saijonmaa O, Forsblom C, Groop P-H. Telomere length and progression of nephropathy in Diabetes type 1 patients. *J Intern Med* 2010; 267:278-286
- Nordestgaard BG, Kontula K, Benn M, Dahlöf B, de Faire U, Edelman JM, Eliasson E, Fyhrquist F, Hille DA, Ibsen H, Lyle PA, Berg K, Sandberg M, Sethi AA, Wong PH, Os I. Effect of ACE insertion/deletion and 12 other polymorphisms on clinical outcomes and response to treatment in the LIFE study. *Pharmacogenetics and Genomics* 2010; 20(2):77-85

Reviews:

Dalman D, Fyhrquist F, Nilsson P. Telomerer, hjärtkärnlåldrande och livsstil (Review in Swedish). *Läkartidningen* (Sweden) 2010; 107:3053-3055



Ralph Gräsbeck, M.D., Dr.Med.Sci.,

Dhc, Professor Emeritus

In July Ralph Gräsbeck, M.D., D.Msc, Dhc, professor emeritus celebrated his 80th birthday. He is one of the founders of the Institute. He still works at the Institute daily, but no longer performs experimental work and his former Biochemistry Group is now reduced to one person. This is due to several reasons, inter alia that he no longer lectures or is in contact with students, his former junior collaborators are reaching retirement age, young medical doctors are not as interested in research as before, and only minute grants have been given for his research. His main topic, vitamin B12, no longer attracts much attention though it is generally accepted that about 12 per cent of the aged population in the Western world suffers from cobalamin deficiency. The endocytosis of the B12-intrinsic factor complex has now been elucidated and its mechanism shown to be common to many substrates and not restricted to vitamin B12 alone. Instead of performing experiments, Gräsbeck has collaborated by e-mail with a large number of doctors and patients in diagnosing and treating enigmatic cases of vitamin B12 deficiency, written invited reviews on the Imerslund-Gräsbeck syndrome (IGS) and criticized via a letter-to-the-editor a report on complications following treatment with cyanocobalamin. One long review on IGS is being finalized together with Dr. Stephan Tanner in Columbus, Ohio, specialist on the mutations in IGS and congenital intrinsic factor deficiency. A report on a Danish infant with congenital cobalamin deficiency will appear in early 2011.

Upon request, Gräsbeck has provided advice concerning the establishment of reference values and written summaries of books in the antique library of the Finska Läkaresällskapet (Finnish Medical Society). He was awarded a congress travel grant by the Finnish Society (Academy) of Sciences and Letters. The same academy gave him a small grant for the study of the history of medicine.

Publications

- Gräsbeck R. Correspondence on "Involuntary movements during vitamin B12 treatment": Was cyanocobalamin perhaps responsible. *J Child Neurol* 2010; 25:794-795
- Gräsbeck R. Ralphs och Olgas sjukdom. Imerslund-Gräsbecks syndrom ("IGS"). *Klin kemi i Norden* 2010; 223:20-32
- Ahlfors UG, Gräsbeck R, Lindfors O, Wallgren EI. Det historiska biblioteket. In the book J Edgren (ed.) *Finska Läkaresällskapet - 175 år*. Otava, Keuru, 2010, pp 86-95

PUBLICATIONS 2010

- Ahlfors UG, Gräsbeck R, Lindfors O, Wallgren EI. Det historiska biblioteket. In the book J Edgren (ed.) Finska Läkaresällskapet - 175 år. Otava, Keuru, 2010, pp 86-95
- Alam C, Valkonen S, Ohls S, Törnquist K, Hänninen A: Peritoneal B Lymphocytes Escape Sphingosine-1-phosphate Retention, Migrate to Pancreatic Lymph Nodes and Present Islet-antigen in Non-Obese Diabetic Mice. *Diabetologia* 2010; 53:346-355
- Bergelin N, Löf C, Balthasar S, Kalhori V, Törnquist K: S1P receptor 1 and VEGF receptor 2 form a signalling complex with ERK1/2 and PKC α regulating ML-1 thyroid carcinoma cell migration. *Endocrinology* 2010; 151:2994-3005
- Blom T, Bergelin N, Meinander A, Slotte JP, Eriksson J, Törnquist K: A self-amplifying loop of autocrine sphingosine-1-phosphate enhances NF- κ B-activation and survival. *BMC Cell Biology* 2010; 11:45
- Bunck MC, Cornér A, Eliasson B, Heine RJ, Shaginian RM, Wu Y, Yan P, Smith U, Yki-Järvinen H, Diamant M, Taskinen M-R. One-year treatment with exenatide vs. Insulin Glargine: effects on postprandial glycemia, lipid profiles and oxidative stress. *Atherosclerosis* 2010; 212: 223-229
- Bunck MC, Diamant M, Eliasson B, Cornér A, Shaginian RM, Heine RJ, Taskinen M-R, Yki-Järvinen H, Smith U. Exenatide affects circulating cardiovascular risk biomarkers independently of changes in body composition. *Diabetes Care* 2010; 33: 1734-1737
- Dalman D, Fyhrquist F, Nilsson P. Telomerer, hjärtkärlåldrande och livsstil (Review in swedish) . *Läkartidningen* (Sweden) 2010; 107:3053-3055
- Deshmukh AS, Long YC, de Castro Barbosa T, Karlsson HKR, Glund S, Zavadski WJ, Gibbs EM, Koistinen HA, Wallberg-Henriksson H, Zierath JR. Nitric oxide increases cGMP levels, AMPK α 1-isoform-specific activity and glucose transport in human skeletal muscle. *Diabetologia* 2010; 53:1142-50
- Ekokoski E, Aitio O, Törnquist K, Yli-Kauhaluoma J, Tuominen RK: HIV-1 Tat-peptide inhibits protein kinase C and protein kinase A through substrate competition. *Eur J Pharm Sci* 2010; 40:404-411
- Eriksson A, Hansson K, Häggström J, Järvinen AK, Lord P. Pulmonary blood volume in mitral regurgitation in cavalier King Charles spaniels. *J Vet Intern Med* 2010; 24(6):1393-9
- Gräsbeck R. Correspondence on "Involuntary movements during vitamin B12 treatment": Was cyanocobalamin perhaps responsible. *J Child Neurol* 2010; 25:794-795
- Gräsbeck R. Ralphs och Olgas sjukdom. Imerslund-Gräsbecks syndrom ("IGS"). *Klin kemi i Norden* 2010; 223:20-32
- Hong C, Duit S, Jalonen P, Out R, Scheer L, Sorrentino V, Boyadjian R, Rodenburg KW, Foley E, Korhonen L, Lindholm D, Nimpf J, van Berkel TJ, Tontonoz P, Zelcer N. The E3 ubiquitin ligase IDOL induces the degradation of the low density lipoprotein receptor family members VLDLR and ApoER2. *J Biol Chem* 2010; 25;285(26):19720-6
- Jansen M, Ohsaki Y, Rega LR, Bittman R, Olkkonen VM, Ikonen E. Role of ORPs in sterol transport from plasma membrane to ER and lipid droplets in mammalian cells. *Traffic* 2010; Epub Nov 9
- Kairisalo M, Bonomo A, Hyrskyluoto A, Mudò G, Belluardo N, Korhonen L, Lindholm D. Resveratrol reduces oxidative stress and cell death and increases mitochondrial antioxidants and XIAP in PC6.3-cells. *Neurosci Lett* 2010; Epub Nov 19
- Kitabayashi K, Siltanen A, Pätälä T, Mahar MA, Tikkanen I, Koponen J, Ono M, Sawa Y, Kankuri E, Harjula A. Bcl-2 expression enhances myoblast sheet transplantation therapy for acute myocardial infarction. *Cell Transplant* 2010; 19: 573-588
- Kotronen A, Seppänen-Laakso T, Westerbacka J, Kiviluoto T, Arola J, Ruskeepää AL, Yki-Järvinen H, Orešič M. Comparison of Lipid and Fatty Acid Composition of the Liver, Subcutaneous and Intra-abdominal Adipose Tissue, and Serum. *Obesity* 2010; 18: 937-944
- Kotronen A, Yki-Järvinen H, Männistö S, Saarikoski L, Korpi-Hyövälti E, Oksa H, Saltevo J, Saaristo T, Sundvall J, Tuomilehto J, Peltonen M. Non-alcoholic and alcoholic fatty liver disease – two diseases of affluence associated with the metabolic syndrome and type 2 diabetes: the FIN-D2D Survey. *BMC Public Health* 2010; 10: 237-243
- Kotronen A, Yki-Järvinen H, Sevastianova K, Bergholm R, Hakkaraian A, Pietiläinen KH, Juurinen L, Lundbom N, Sørensen TI. Comparison of the Relative Contributions of Intra-Abdominal and Liver Fat to Components of the Metabolic Syndrome. *Obesity* 2010; Epub Jun 10
- Lakkisto P, Kytö V, Forsten H, Siren JM, Segersvärd H, Voipio-Pulkki LM, Laine M, Pulkki K, Tikkanen I. Heme oxygenase-1 and carbon monoxide promote neovascularization after myocardial infarction by modulating the expression of Hif-1 α , SDF-1 α and VEGF-B. *Eur J Pharmacol* 2010; 635: 156-164
- Lind U, Alm Rosenblad M, Hasselberg Frank L, Falkbring S, Brive L, Laurila JM, Pohjanoksa K, Vuorenpää A, Kukkonen JP, Gunnarsson L, Scheinin M, Mårtensson Lindblad LG, Blomberg A. Octopamine receptors from the barnacle balanus improvisus are activated by the α 2-adrenoceptor agonist medetomidine. *Mol Pharmacol* 2010; 78(2):237-48
- Mäkelä J, Koivuniemi R, Korhonen L, Lindholm D. Interferon-gamma produced by microglia and the neuropeptide PACAP

- have opposite effects on the viability of neural progenitor cells. *PLoS One* 2010; 14:5(6):e11091
- Nordestgaard BG, Kontula K, Benn M, Dahlöf B, de Faire U, Edelman JM, Eliasson E, Fyhrquist F, Hille DA, Ibsen H, Lyle PA, Berg K, Sandberg M, Sethi AA, Wong PH, Os I. Effect of ACE insertion/deletion and 12 other polymorphisms on clinical outcomes and response to treatment in the LIFE study. *Pharmacogenetics and Genomics* 2010; 20(2):77-85
- Reijonen S, Kukkonen JP, Hyrskyluoto A, Kivinen J, Kairisalo M, Takei N, Lindholm D, Korhonen L. Downregulation of NF-kappaB signaling by mutant huntingtin proteins induces oxidative stress and cell death. *Cell Mol Life Sci* 2010; 67(11):1929-41
- Romeo S, Huang-Doran I, Baroni MG, Kotronen A. Unravelling the pathogenesis of fatty liver disease: patatin-like phospholipase domain-containing 3 protein. *Curr Opin Lipidol* 2010; 21:247-252
- Saukkonen K, Lakkisto P, Kaunisto MA, Varpula M, Voipio-Pulkki LM, Varpula T, Pettilä V, Pulkki K. Heme oxygenase 1 polymorphisms and plasma concentrations in critically ill patients. *Shock* 2010; 34: 558-564
- Sevastianova K, Hakkarainen A, Kotronen A, Cornér A, Arkkila P, Westerbacka J, Bergholm R, Lundbom J, Lundbom N, Yki-Järvinen H. Nonalcoholic fatty liver disease: detection of elevated nicotinamide adenine dinucleotide phosphate with in vivo 3.0-T 31P MR spectroscopy with proton decoupling. *Radiology* 2010; 256: 466-473
- Simonen P, Kotronen A, Hallikainen M, Sevastianova K, Makkonen J, Hakkarainen A, Lundbom N, Miettinen TA, Gylling H, Yki-Järvinen H. Cholesterol synthesis is increased and absorption decreased in non-alcoholic fatty liver disease independent of obesity. *J Hepatol* 2010; 1:153-159
- Skrobuk P, Kuoppamaa H, Hiukka A, Koistinen HA: Acute exposure to rosiglitazone does not affect glucose transport in intact human skeletal muscle. *Metabolism, Clinical and Experimental* 2010; 59:224-230
- Syväranta S, Helske S, Laine M, Lappalainen J, Kupari M, Mäyränpää MI, Lindstedt KA, Kovanen PT. Vascular endothelial secreting mast cells and myofibroblasts: a novel self-perpetuating angiogenic pathway in aortic valve stenosis. *Arterioscler Thromb Vasc Biol* 2010; 30: 1220-1227
- Turunen PM, Ekholm ME, Somerharju P, Kukkonen JP. Arachidonic acid release mediated by OX1 orexin receptors. *Brit J Pharmacol* 2010; 159: 212-21
- Turunen PM, Putula J, Kukkonen JP. Filtration assay for arachidonic acid release. *Anal Biochem* 2010; Epub Jul 23
- Vahtola E, Louhelainen M, Forsten H, Merasto S, Raivio J, Kaheinen P, Kytö V, Tikkanen I, Levijoki J, Mervaala E. Sirtuin1-p53, forkhead box O3a, p38 and post-infarct cardiac remodeling in the spontaneously diabetic Goto-Kakizaki rats. *Cardiovasc Diabetol* 2010; 9:5(pp.1-13)
- Vanni E, Bugianesi E, Kotronen A, De Minicis S, Yki-Järvinen H, Svegliati-Baroni G. From the metabolic syndrome to NAFLD or vice versa? *Dig Liver Dis.* 2010; 42(5):320-30
- Vihervaara T, Uronen R-L, Wohlfahrt G, Björkhem I, Ikonen, Olkkonen VM. Sterol binding by OSBP-related protein 1L regulates late endosome motility and function. *Cellular and Molecular Life Sciences* 2010; Epub Aug 6
- Westerbacka J, Kotronen A, Fielding BA, Wahren J, Hodson L, Perttälä J, Seppänen-Laakso T, Suortti T, Arola J, Hultcrantz R, Castillo S, Olkkonen VM, Frayn KN, Orešić M, Yki-Järvinen H. Splanchnic balance of free fatty acids, endocannabinoids, and lipids in subjects with nonalcoholic fatty liver disease. *Gastroenterology* 2010; 6:1961-1971
- Zhou Y, Li S, Mäyränpää MI, Zhong W, Bäck N, Yan D, Olkkonen VM. OSBP-related protein 11 (ORP11) dimerizes with ORP9 and localizes at the Golgi-late endosome interface. *Experimental Cell Research* 2010; 316: 3304-16

