

MINERVA FOUNDATION
AND
MINERVA FOUNDATION INSTITUTE
FOR MEDICAL RESEARCH



ANNUAL REPORT 2009





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SUMMARY OF RESEARCH AND ACTIVITIES DURING THE YEAR 2009

The Minerva Foundation Institute for Medical Research is a privately owned biomedical institute currently consisting of seven research groups. The Minerva Institute has recently moved to a new location in Biomedicum 2 with ultramodern facilities and new laboratories spaces, as part of the larger Biomedicum Helsinki conglomerate of research laboratories. This move has further contributed to the development of the Institute and created an inspiring scientific environment.

The year 2009 has been an exceptional one at Minerva. In April 2009, the Minerva Foundation Institute celebrated its 50th anniversary by hosting a scientific symposium at Biomedicum Helsinki. Distinguished scientists were invited to talk about themes of current interest related to the research performed at Minerva. The symposium was a success, with the participation of an enthusiastic audience consisting of students, scientists and local researchers as well as of invited guests and Minerva employees. The meeting was followed by dinner at Kalastajatorppa, a renowned restaurant in central Helsinki, and a friendly get-together party with a gathering of former and current Minerva staff. The celebration and all its events were highly appreciated by the participants.

Apart from celebration, 2009 was also a year for strenuous and hard work to improve the Institute and its capabilities to support ingenious and creative science. To further strengthen the activities at Minerva, two new groups were recruited to the Institute during this year. These were the group of Dr. Jacob Stenman, working on novel methods to analyze gene expression in medically relevant diseases, and that of Docent Vesa Olkkonen, working on regulatory mechanisms and protein networks involved in maintaining lipid balance in cells, particularly in relation to changes

in cardiovascular diseases and fatty liver/metabolic syndromes. These two groups will establish themselves rapidly and be fully active in the coming year. We are pleased to welcome them both to Minerva and wish them all the best and success in their scientific endeavor.

With the growth of research and activities at Minerva, the administration at the Institute has also been reviewed and made more efficient by including monthly meetings among the Board of Directors. In addition, professional administrators, chosen as representatives for the Finnish Academy, paid a visit to Minerva in December 2009, and a report of this gathering will follow later with valuable suggestions and advice to further improve the administrative measures and routines applied at the Minerva Institute. All this will make the Institute ready to update its activities and meet new challenges in the future.

The research at Minerva is focused on the study of basic cellular and molecular mechanisms underlying important human diseases, including cardiovascular diseases, diabetes and neurological degenerative disorders. The objectives range from studies of single cells and molecules to analyses of experimental animals and human patient material. In this Annual Report, the different groups at Minerva present themselves and their research activities, including recent achievements and a list of publications.

Additionally, a total of three Master's theses (Pro Gradu thesis) were completed at Minerva during this year.

Generous funding has been obtained from The Minerva Foundation, The Sigrid Jusélius Foundation, Medicinska Understödsföreningen Liv och Hälsa, The Finnish Academy and several other sources.

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, 2009, to December 31, 2009. The board of trustees included the following persons:

Professor Jim Schröder, chair
Docent Caj Haglund, vice chair
Professor Carl G. Gahmberg
Docent Per-Henrik Groop
Professor Carola Grönhagen-Riska
Professor Christer Holmberg
Professor Krister Höckerstedt, secretary
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THE MINERVA FOUNDATION INSTITUTE FOR MEDICAL RESEARCH

Board of directors:

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Professor Kid Törnquist
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Professor Hannele Yki-Järvinen
Docent Ilkka Tikkanen
FM Tuulikki Nyman, secretary
DK Carita Estlander-Kortman, financial manager



► **Members of the Unit:**

Dan Lindholm, M.D., D.Msc., Professor, head
 Laura Korhonen, M.D., D.Msc., Docent,
 Academy Researcher Fellow
 Annika Mälkiä, Ph.D, Postdoctoral Fellow
 Heli Fox, M.Sc., Ph.D. student
 Alise Hyrskyluoto, M.Sc., Ph.D. student (FGSN)
 Pilvi Jalonen, student
 Minna Kairisalo, M.Sc. (Pharm), Ph.D. student
 Jenny Kivinen, M.Sc., Ph.D. student
 Raili Koivuniemi, M.Sc., Ph.D. student (FGSN)
 Eeva Lehto, laboratory assistant
 Johanna Mäkelä, M.Sc., biochemist
 Noora Putkonen, M.Sc., Ph.D. student (HBGS)
 Sami Reijonen, M.Sc., Ph.D. student

Collaborator: Jyrki Kukkonen Ph.D., Professor,
 University of Helsinki

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

Funded by the Academy of Finland, the
 Sigrid Jusélius Foundation, Medicinska
 Understödsföreningen Liv och Hälsa, Finska
 Läkaresällskapet, Arvo and Lea Ylppö
 Foundation and by the Minerva Foundation.

UNIT OF NEUROSCIENCE

We study basic mechanisms underlying neurodegenerative diseases and excitotoxic damage by focusing on cell death pathways and proteins in disease pathogenesis. Our aim is to contribute to developing better therapies to combat cell death and degeneration. In this context, we study the organelle dysfunction and protein aggregation that occur during the course of many neurological diseases. Neurotrophic factor signaling and the regulation of neuronal stem cells by growth factors are further interests of the group.

Ongoing projects:

Regulation of Neural Progenitor cells

Neural progenitor cells (NPCs) are highly dividing cells with the capacity for self-renewal, which 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 are interested in proteins that influence the proliferation and stemness of NPCs and have focused on membrane-bound proteases and their inhibitors HAI-1 and HA-2 (see Fig 1), as well as on different ion channels expressed by the NPCs. In our recent study (Sippel et al., see below), we provided evidence to show that the cell death regulator Bruce is expressed in NPCs and affects the process of cytokinesis and the cells' response to stress hormones. Apart from NPCs from rodents, we also study brain cancer stem cells obtained from malignant human gliomas to reveal mechanisms of their cell proliferation and survival.

Cell death control in neurons and in different disease models

In this project, we study cellular signaling and proteins influencing neuronal viability in brain development and in various diseases. Using transgenic mice, we observed that X-linked Apoptosis Protein (XAIP) is able to influence NF-kB

signaling in neurons, reducing oxidative stress and elevating brain-derived neurotrophic factor (BDNF) levels in vivo and in vitro (Kairisalo et al., see below). The precise mechanism by which XIAP affects neuronal signaling is currently under investigation. We have recently become interested in the role of transcription, particularly that of nuclear co-activators such as PGC1, in the regulation of neuronal survival and metabolism.

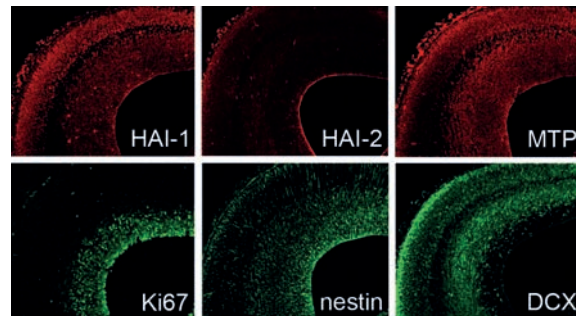
Previous studies in our group have disclosed the importance of endoplasmic reticulum (ER) stress in various neurological diseases, including Huntington's disease (Reijonen et al., *Exp Cell Res*, 2008). We have continued these studies and shown that a decline in NF- κ B activation is a contributing factor in disease pathogenesis and is linked to ER stress and protein aggregation (Reijonen et al., unpublished). In addition, the roles of the proteasome and autophagy, acting either as friend or foe in ER stress and in neuronal diseases, are currently being addressed.

Role of ubiquitin ligases and of MIR/Myip in neuronal metabolism.

Protein degradation is an important cellular event that is disturbed in many diseases. We are interested in ubiquitin ligases and ubiquitin-specific proteases that regulate protein stability in cells, including neurons. We have previously identified the ligase MIR/Myip that interacts with the myosin regulatory light chain and influences neurite outgrowth and cell motility. This protein, also known as Idol, was recently shown to influence the ubiquitination and stability of LDL receptors in the liver and in macrophages (for discussion see Lindholm et al., 2009). We are currently studying the regulation and targets of Myip/Idol in neuronal cells and in brain metabolism in more detail.

Publications

- Kairisalo M, Korhonen L, Sepp M, Pruunsild P, Kukkonen JP, Timmusk T, Blomgren K, Lindholm D. NF- κ B-dependent regulation of Brain-derived Neurotrophic factor in hippocampal neurons by X-linked Inhibitor of Apoptosis Protein. *Eur J Neurosci* 2009; 30: 958-966
- Lindholm D, Bornhauser BC, Korhonen L. Myip makes an Idol turn into regulation of LDL receptor. *Cell Mol Life Sci* 2009; 66: 3399-3402



Expression of the membrane-bound serine protease Matriptase (MTP) and its inhibitors HAI-1 and HAI-2 in developing neuroepithelium as shown by immunohistochemistry

- Ramström C, Chapman H, Viitanen T, Afrasiabi E, Fox H, Kivelä J, Soini S, Korhonen L, Lindholm D, Pasternack M, Törnquist K. Regulation of HERG (KCNH2) Potassium channel surface expression by Diacylglycerol. *Cell Mol Life Sci*; Epub 2009 Oct 27
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- Wootz H, Enjin A, Wallén-Mackenzie Å, Lindholm D, Kullander K. Reduced VGLUT2 expression increases motor neuron viability in ALS-Sod1G93A mice. *Neurobiol Dis*; Epub 2009 Sep 19

Reviews:

- Korhonen L, Putkonen N, Lindholm D. Proteasome role in neurodegeneration, In: *Handbook of the Neuroscience of Aging* 2009 (Editors Patrick R. Hof and Charles V. Mobbs): Pp 155-160. Invited Book chapter
- Trokovic R, och Lindholm D. Inducerade pluripotenta stamceller - nya löften och möjligheter för forskning och terapi. *Finska Läkaresällskapets Handlingar* 2009; 169: 50-55. Review
- Lindholm D. Idé och ork i forskningen. *Finska Läkaresällskapets Handlingar* 2009; 169: 4-5. Ledare

Theses completed in the group in 2009:

- The following Pro Gradu theses were accepted at the University of Helsinki this year:
- Ulrika Furustrand:** Rollen av Cystatin B och Metyltransferase vid celledödsreglering.
- Jenny Kivinen:** Activity-Dependent Regulation of Ubiquitin-Specific Protease 14 (Usp14) in Rat Hippocampal Neurons.
- Maria Sippel:** Glukokortikoider reglerar BRUCE och cellproliferationen i embryonala neurala stamceller.



► **Members of the Unit:**

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 Mika Laine, M.D., D.Msc., Docent
 Eeva Palojoki, M.D., D.Msc.
 Tom Bäcklund, M.D., postgraduate student
 Hanna Forsten, V.M.D., Ph.D. student
 Tina Grönholm, M.D., postgraduate student
 Päivi Lakkisto, M.D., postgraduate student
 Jere Paavola, M.D., postgraduate student
 Heli Segersvärd, Cand. Med., graduate student
 Juha-Matti Siren, Med. Stud., graduate student
 Katariina Immonen, B.Sc., laboratory technician
 Riikka Kosonen, M.Sc., laboratory technician
 Jarkko Lakkisto, laboratory technician

Collaborators:

Ari Harjula, M.D., D.Msc., Professor (Helsinki University Hospital), Esko Kankuri, M.D., D.Msc., Docent (University of Helsinki), Markku Kupari, M.D., D.Msc., Professor (Helsinki University Hospital), Eero Mervaala, M.D., D.Msc., Professor (University of Helsinki), Kari Pulkki, M.D., D.Msc., Professor (University of Kuopio), Ilkka Pörsti, M.D., D.Msc., Professor (University of Tampere)

Funded by The Minerva Foundation, Sigrid Jusélius Foundation, Helsinki University Research grants (EVO) and Finnish Foundation for Cardiovascular Research

UNIT OF CARDIOVASCULAR RESEARCH

Main research activities

The group studies mechanisms and repair of end-organ damage in cardiovascular and renal diseases. In this context, the local expression of components of the renin-angiotensin-aldosterone system, other vasoactive factors, and apoptosis has been studied 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.

Recent studies have shown that the heart contains resident stem/progenitor cells which are able to activate, proliferate and form new cardiac cells after myocardial damage. In agreement with these findings, we were able to demonstrate that neo-formation of cardiomyocytes and vascular structures increases after experimental myocardial infarction (MI) and that this regenerative process can be modulated by therapeutic interventions.

The role of the heme oxygenase-1-carbon monoxide (HO-1/CO) axis, an interesting novel pathway related to cardioprotection and cardiac regeneration after myocardial injury, was studied in the post MI cardiac remodelling and regenerative process. HO-1 acts as an endogenous protec-

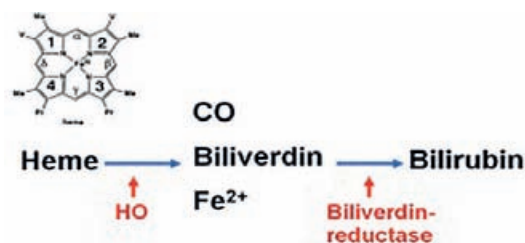


Fig.1. Heme oxygenase-1 and biologically active molecules produced from heme.

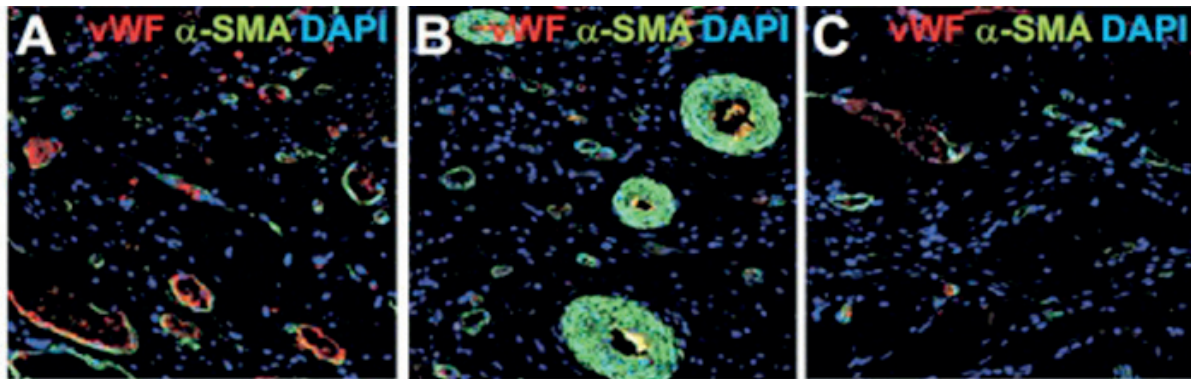


Fig. 2. Generation of new vascular structures after myocardial infarction in the rat either by induction of HO-1 (panel A) or by treatment with a CO-donor (panel B). Panel C: untreated control group. vWF: von Willebrand factor, α-SMA, alpha smooth muscle actin.

tive mechanism in various stress situations, mediating anti-apoptotic, anti-inflammatory, and anti-proliferative effects based on the break-down of pro-oxidative heme and generation of biologically active molecules, biliverdin, CO and iron (Fig. 1). In addition, HO-1 and CO are involved in the regulation of angiogenesis.

Our studies showed for the first time that induction of HO-1 promotes cardiac regeneration and angiogenesis after experimental MI. Part of the protective effects of HO-1 appeared to be mediated through the generation of CO. Treatment with a CO-donor significantly increased the amount of c-kit⁺-positive cardiac stem cells and stimulated their differentiation into cardiomyocytes and vascular structures in the infarct area (Fig. 2). Increased expression of hypoxia inducible factor-1 alpha (Hif-1 alpha), stromal cell derived factor-1 (SDF-1) and vascular endothelial growth factor (VEGF-B) were found in the infarct areas of CO-donor pre-treated hearts. This suggests that these factors potentially promoted the increased migration of c-kit⁺-cardiac stem cells into the infarct area and subsequent vasculogenesis and myocardial regeneration. Modulation of the HO-1/CO axis may thus provide a new tool for the repair of cardiac injury and prevention of post MI cardiac failure. The novel findings on cardiac stem cells open a completely new possibility avenue for to improve the treatment of chronic heart failure in the future.

Publications

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- Lakkisto P, Csonka C, Fodor G, Bencsik P, Voipio-Pulkki L-M, Ferdinandy P, Pulkki K. The heme oxygenase inducer hemin protects against cardiac dysfunction and ventricular fibrillation in ischaemic/reperfused rat hearts: role of connexin 43. *Scand J Clin Lab Invest* 2009; 69: 209-218
- Louhelainen M, Vahtola E, Forsten H, Merasto S, Kytö V, Finckenberg P, Leskinen H, Kaheinen P, Tikkanen I, Levijoki J, Mervaala E. Oral levosimendan prevents post-infarct heart failure and cardiac remodeling in Diabetic Goto-Kakizaki rats. *J Hypertens* 2009; 27: 2094-2107
- Tikkanen I, Lakkisto P, Segersvärd H, Siren J-M, Forsten H, Palojoki E, Laine M. Hjärtats stamceller och regeneration vid hjärtskada. *Finska Läkaresällskapets Handlingar* 2009; 169: 2, 30-35
- Vaajanen A, Lakkisto P, Virtanen I, Kankuri E, Oksala O, Vapaatalo H, Tikkanen I. Angiotensin receptors in the eyes of arterial hypertensive rats. *Acta Ophthalmol*; Epub 2009 Sep 16



► **Members of the Unit:**

Heikki Koistinen, M.D., D.Msc., Docent, head
 Paulina Skrobuk, M.Sc., Ph.D. student
 Johannes Kärkkäinen, M.B., graduate student
 Stephanie von Kraemer, student of genetics,
 Master's student
 Cynthia Moed, student of genetics, Master's
 student
 Maria Semenova, M.Sc., researcher

Funded by the Minerva Foundation, the
 Academy of Finland, Sigrid Jusélius
 Foundation, Helsinki Uusimaa Hospital
 District and Novo Nordisk Foundation.

UNIT OF METABOLISM

Insulin stimulates glucose transport activity in skeletal muscle ~4-fold, and a large part of this stimulation is due to a net translocation of GLUT4 from an intracellular compartment to the cell surface. In a collaborative study with investigators from Sweden and the UK, we have examined the extent to which insulin or the AMPK activator AICAR can lead to stimulation of the exocytosis limb of the GLUT4 translocation pathway. We have tagged the endogenous GLUT4 using a biotinylated photoaffinity label to study the kinetics of exocytosis of the tagged protein in rat and human skeletal muscle in response to insulin or AICAR. In rat epitrochlearis muscle, insulin exposure leads to a 6-fold stimulation of the GLUT4 exocytosis rate. In human vastus lateralis muscle, insulin stimulates GLUT4 translocation by a similar 6-fold increase in the exocytosis rate constant. AICAR treatment does not markedly increase the exocytosis rate constant in either rat or human muscle. Thus, insulin-stimulation of the GLUT4 exocytosis rate constant is sufficient to account for most of the observed increase in glucose transport activity in rat and human muscle (1).

The upstream transcription factor 1 (USF1) gene is associated with familial combined hyperlipidemia, the most common genetic dyslipidemia in humans. In collaboration with professor Leena Palotie and professor Marja-Riitta Taskinen, we have explored the molecular mechanism through which the strongest-associating intronic single-nucleotide polymorphism variant in USF1 is involved in the development of dyslipidemia. We have studied the effects of the risk variant on gene expression in human adipose tissue and muscle. Global transcript profiles of 47 fat biopsies ascertained for carriership of the risk allele were tested for differential expression of known USF1 target genes as well as for broader effects on the transcript profile. Allelic imbalance of USF1 in fat was assessed using a quantitative sequencing approach. The possible allele-specific effect of insulin on the expression of USF1 was studied in 118 mus-



cle biopsies before and after a euglycemic hyperinsulinemic clamp. The risk allele of single-nucleotide polymorphism rs2073658 abolishes the inductive effect of insulin on the expression of USF1 in muscle and fat. The expression of numerous target genes is in turn altered in adipose tissue. Thus, in risk allele carriers, a defective response of USF1 to insulin results in the suboptimal response of relevant target genes. This may contribute to the enhanced risk of developing dyslipidemia and coronary heart disease (2).

In 2008, the head of the Metabolic group was granted a 3-year funding from the Academy of Finland to promote his Clinical Researcher's Career. This funding enables him to devote 50% of his annual working time to research 2009-2011. Consequently, the research activity of the group has increased during 2009, and the group has expanded. Among the new investigators, students of genetics Stephanie von Kraemer and Cynthia Moed started research aiming at Master's theses. Medical student Johannes Kärkkäinen started Ph.D. studies in Autumn 2009, and M.Sc. Maria Semenova joined the team in the beginning of December 2009.

Publications

- Karlsson HKR, Chibalin AV, Koistinen HA, Yang J, Koumanov F, Wallberg-Henriksson H, Zierath JR, Holman GD. Kinetics of GLUT4 trafficking in rat and human skeletal muscle. *Diabetes* 2009; 58: 847-854
- Naukkarinen J, Nilsson E, Koistinen HA, Söderlund S, Lyssenko V, Vaag A, Poulsen P, Groop L, Taskinen MR, Peltonen L. Functional variant disrupts insulin induction of USF1: mechanism for USF1-associated dyslipidemias. *Circulation: Cardiovascular Genetics* 2009; 2: 522-9
- Skrobuk P, von Kraemer S, Kärkkäinen J, Koistinen H. Reglering av glukosmetabolism i skelettmuskel hos människan. *Finska Läkaresällskapets Handlingar* 2009; 169 (2): 56-62



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Anna Kotronen, medical student, D.Msc.

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Ksenia Sevastianova, M.D., postgraduate
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Piia Simonen, M.D., D.Msc.

Julia Perttilä, Ph.D.

Leena Juurinen, M.D., postgraduate student

Anja Cornér, M.D., postgraduate student

Katja Sohlo, laboratory technician

Mia Urjansson, laboratory technician

Kaisa Makkonen, laboratory technician

UNIT OF ENDOCRINOLOGY

The group has continued to study the diagnosis, pathogenesis and treatment of fat accumulation in the liver due to non-alcoholic causes (non-alcoholic fatty liver disease, NAFLD). Fat accumulation in the liver is necessary to observe insulin resistance or the metabolic syndrome in humans.

Causes of NAFLD

In a study using monozygotic and dizygotic twins, we found that approximately 60% of the variation in serum alanine aminotransferase (S-ALT), a marker of liver fat content, is genetically determined. Consistent with a contribution of genetic factors to NAFLD, we showed that genetic variation in PNPLA3 (adiponutrin), which is a lipase hydrolyzing hepatic triglycerides, is associated with a 60% increase in liver fat in humans with the rare GG as compared to the common CC genotype, independent of age, sex and BMI. Studies are currently underway both in vivo and in vitro to understand the clinical and biochemical significance of this gene. The in vitro studies are performed by Julia Perttilä in collaboration with docent Vesa Olkkonen at Minerva.

Diagnosis of NAFLD

We have developed a method to accurately predict NAFLD and liver fat content based on routinely available clinical and laboratory data and tested whether knowledge of the genetic variant (rs738409) in the PNPLA3 gene increases the accuracy of the prediction. The presence of metabolic syndrome and type 2 diabetes, fasting serum (fS) insulin, fS-aspartate aminotransferase (AST), and the AST/ALT ratio were independent predictors of NAFLD. The score had an area under the receiver operating characteristic curve of 0.87 in the estimation and 0.86 in the validation group. This study was accompanied by an editorial in Gastroenterology and the use of the equation has very recently been replicated in two different groups.

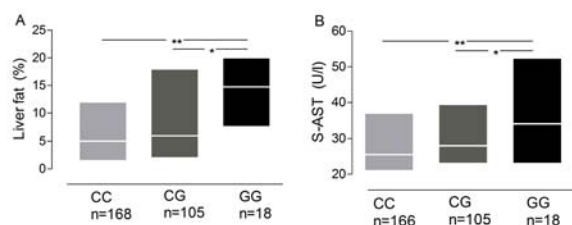


Fig. Medians and interquartile ranges of liver fat content measured by 1H-MRS ($p=0.011$) (panel A), and serum AST ($p=0.002$) (panel B) in different PNPLA3 (encoding adiponutrin) rs738409 genotypes. * $p<0.05$, ** $p<0.01$ for least-square difference post hoc test. GG is the rare and CC the common PNPLA3 genotype.

Pathogenesis of NAFLD

We have documented that lipolysis is increased independently of obesity in NAFLD, while hepatic lipid oxidation is unchanged. Analysis of liver samples using lipidomics/metabolomics approaches in collaboration with Matej Oresic revealed that hepatic stearyl-CoA desaturase (SCD)-1 activity and diacylglycerol but not ceramide concentrations are increased in the non-alcoholic human fatty liver. We also found that the proportion of saturated fatty acids is increased. The increase in SCD-1 activity and saturated fatty acids supports the idea that, in addition to lipolysis, de novo lipogenesis contributes to increased liver fat content in humans.

Lipodystrophy

Jussi Sutinen has continued studies addressing the metabolic effects of different antiviral drugs in patients with highly active antiretroviral therapy (HAART)-induced lipodystrophy.

Therapeutic aspects

We have completed the following intervention studies: i) a 12-month study on the effects of rimonabant (cannabinoid 1-receptor blocker) vs. placebo on the amount of liver fat in obesity ii) a 4-year study on antihyperglycemic and lipid effects of GLP-1 (Glucagon like peptide-1) agonist exenatide in patients with type 2 diabetes.

Publications

- Sievers M, Walker U A, Sevastianova K, Setzer B, Wagsater D, Eriksson P, Yki-Järvinen H, Sutinen J. Gene expression and immunohistochemistry in adipose tissue of HIV type-1 infected patients with nucleoside analogue reverse – transcriptase inhibitor – associated lipodystrophy. *Journal of Infectious Diseases* 2009; 200: 252-262
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- Makkonen J, Pietiläinen KH, Rissanen A, Kaprio J, Yki-Järvinen H. Genetic factors contribute to variation in serum alanine aminotransferase activity independent of obesity and alcohol: A study in monozygotic and dizygotic twins. *J Hepatol* 2009; 50: 1035-1042

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Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, Lundbom N, Rissanen A, Ridderstråle M, Groop L, Orho-Melander M, Yki-Järvinen H Prediction of Non-Alcoholic Fatty Liver Disease and Liver Fat Using Metabolic and Genetic Factors. *Gastroenterology* 2009; 137: 865-872

Kotronen A, Bergholm R, Yki-Järvinen H. Fettlever vid metabola syndromet och typ 2-diabetes *Finska Läkaresällskapets Handlingar* 2009; 169 (2): 42-49

Reviews

Kotronen A, Yki-Järvinen H. Intrahepatic fat accumulation: a major determinant of human metabolism. *International Diabetes Monitor* 2009; 21: 16-23



► Members of the Unit:

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

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

Emad Afrasiabi, M.D., D.Msc.
Christoffer Löf, M.Sc.
Paramod Sukumaran, M.Sc.
Kati Kemppainen, M.Sc.
Veronica Kalhori, M.Sc.
Ilari Pulli, B.Sc.

The Unit was part of the Centre of Excellence in Cell Stress (Åbo Akademi University) during 2006-2009. The Centre obtained a renewal of the Centre of Excellence status for 2010-2014.

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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 was investigated. In these studies, sphingomyelin derivatives (ceramide, sphingosine and sphingosine 1-phosphate [S1P]) proved to be very effective modulators of calcium signals. The investigations during 2009 aimed at understanding the physiological significance of the putative sphingosine-S1P "rheostat".

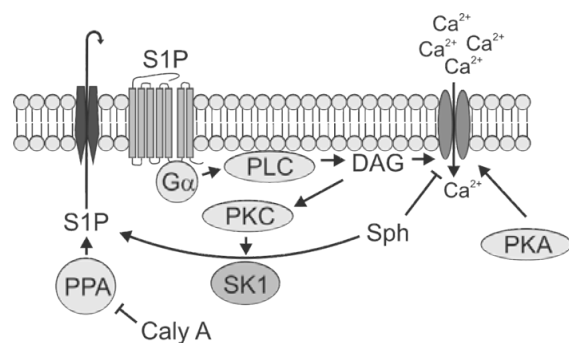


Figure 1. Schematic representation of the autocrine S1P-evoked calcium signalling in thyroid cells. DAG, diacylglycerol; Sph, sphingosine; PKA, protein kinase A; PKC, protein kinase C; SK1, sphingosine kinase 1; PPA; protein phosphatase; Caly A; Calyculin A (a phosphatase inhibitor); PLC, phospholipase C. The picture was reproduced from Gratshev et al., *Endocrinology* 150:5125-5134 (2009), with permission of the Endocrine Society.

Currently, the Unit is elucidating the mechanisms by which S1P and sphingosine kinase, i.e. the kinase phosphorylating sphingosine to S1P, regulate calcium entry. Our results revealed that activation or overexpression of sphingosine kinase resulted in a pronounced entry of calcium into thyroid cells. Furthermore, this effect was the result of intracellular S1P being transported out of the cells, by at least the ATP-binding cassette multidrug resistant protein C1 (ABCC1). S1P then potently activated S1P receptors on the plasma membrane, resulting in enhanced calcium entry. The result thus revealed a novel mechanism by which S1P can activate calcium entry, i.e. by an autocrine effect. This is probably of significant physiological importance. Preliminary results from our laboratory suggest that S1P activates a member of the transient receptor potential family of calcium channels.

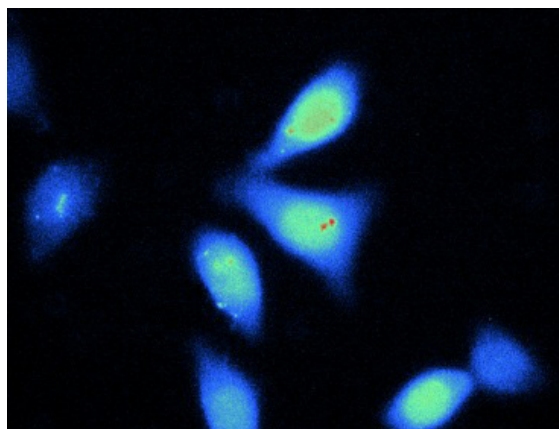


Figure 2. Intracellular calcium signals in cells stimulated with 1 μ M S1P. The brighter the colour, the higher are the levels of intracellular free calcium concentrations in the cells.

Investigations on the importance of S1P in regulating thyroid cancer cell migration have been particularly successful. The 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, recent studies clearly show that S1P may stimulate the migration of thyroid cancer cells by an autocrine mechanism. In these studies, the importance of the sphingosine-S1P "rheostat" is again highlighted. Over-expression of sphingosine kinase dramatically increased the migratory potential of follicular thyroid cancer cells. In these cells, the S1P produced by sphingosine kinase is transported out from the cells by ABCC1 (and probably some other mechanisms as well) and, by an autocrine mechanism, enhances cell migration. Considering that sphingosine kinase may function as an oncogene, these observations may be of significant clinical relevance. Furthermore, in thyroid cancer cells, vascular endothelial growth factor (VEGF) receptor 2 is an important part of the signaling complex activated by S1P. This is emphasized by recent results, which show that blocking VEGF receptor 2 potentially attenuates the migratory response evoked by S1P, and that S1P phosphorylates VEGF receptor 2.

An exciting series of investigations aim at understanding the mechanisms of ceramide action on potassium channels. Our previous investigations in the Unit have shown that ceramide evokes internalization and ubiquitin-mediated degradation of the HERG potassium channel. Understanding the mechanisms by which ceramide (i.e. as a result of cellular stress) regulates HERG channel function is of great importance, given the central role of HERG in the repolarization of e.g. cardiac action potentials. An important issue is to investigate the mechanisms by which ceramide internalizes HERG and which ubiquitin ligase participates in this process. The project is quite complicated, and more work is required. Another recent observation is that HERG has an important role in regulating migration and proliferation of cancer cells. We have now shown that in MDA-MB-435S cells, HERG is expressed and regulates both proliferation and migration. Blocking HERG potentially inhibits both proliferation and migration. Studies are planned to further investigate this phenomenon.

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UNIT OF TARGETED GENE-EXPRESSION ANALYSIS

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In addition the following researchers working at the Department of Clinical Chemistry, University of Helsinki:

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Collaborators

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Laboratoriot, HYKS Instituutti and Expression

Analytics Oy.

The aim of the Unit 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, at diagnosis the disease has spread outside the bowel but not into regional lymph nodes. 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 of is to identify gene expression patterns that would reflect aggressive behavior associated with an increased risk of disease recurrence. This would allow for 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.



Gene expression analysis from frozen versus FFPE samples using Genome-Controlled RT-PCR. The upper row shows expression profiles of snap frozen samples from three colon cancer specimens that were stored at -80°C for 5 years. The lower row shows expression profiles of FFPE samples from the same three specimens that were stored at room temperature during the same time period.

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 (estrogen receptor, ER), trastuzumab (HER2 or erbB2 tyrosine kinase), anthracyclins (TOP2A -enzyme), lapatinib (erbB1 and erbB2 tyrosine kinases) and bevasizumab (VEGF growth factor, VEGFR2 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. The 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.

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Stenman J. Riktad analys av genuttryck med genomkontrollerad RT-PCR. Finska Läkaresällskapets Handlingar 2009; 169: 63-67

ADDITIONAL RESEARCH



Frej Fyhrquist, M.D., D.M.Sc., professor emeritus, together with his team consisting of Outi Saijonmaa, Ph.D., docent, Anders Eriksson, DVM, Emilie Kvist, student, and Anna Nyman, student, has focused on the regulation of renin-angiotensin system components, notably ACE2 regulation in endothelial cells. Telomeres in cardiovascular disease has become our new field of interest.

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Fyhrquist F, Saijonmaa O. Reply to A.Dupont et al. (On the importance of Angiotensin IV). *J Intern Med* 2009; 265: 404-405

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Fyhrquist F, Saijonmaa O. Renin-angiotensinsystemet – en uppdatering (Renin-angiotensin system – an update. Review in Swedish) *Finska Läkaresällskapets Handlingar* 2009; 169: 23-29



Ralph Gräsbeck, M.D., D.M.Sc., Dhc, professor emeritus, a founder of the Minerva Institute, led the small Unit of Biochemistry. The Unit also consisted of a junior scientist, Mirkka Sarparanta, M.Sc. and Ph.D. student, and Prof. Benoît Dugué, Ph.D., who periodically visited the Unit. In 2009, Mrs. Sarparanta left the group to take up a new position. Consequently, and considering his age (79), Gräsbeck decided to abandon his radiochemical project, which in practice means the cessation of the Unit and an end to his experimental research, which started around 1952. However, he continues to study and give advice concerning rare forms of cobalamin deficiency, especially the Imerslund-Gräsbeck syndrome (IGS) and a congenital error in the gastric intrinsic factor gene. This has led to invitations to write about the subject in *Current Pediatric Reviews* and *Klinisk kemi i Norden*. The first case of a mutated intrinsic factor gene in Scandinavia was found and a manuscript describing the case was submitted. Another manuscript about the toxicity of cyanocobalamin was accepted by the *Journal of Child Neurology*. In addition, Gräsbeck has continued to write historical articles, including descriptions of the founding of the Minerva Institute and reviews on the antique books of the *Finska Läkaresällskapet* (Finnish Medical Society).

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