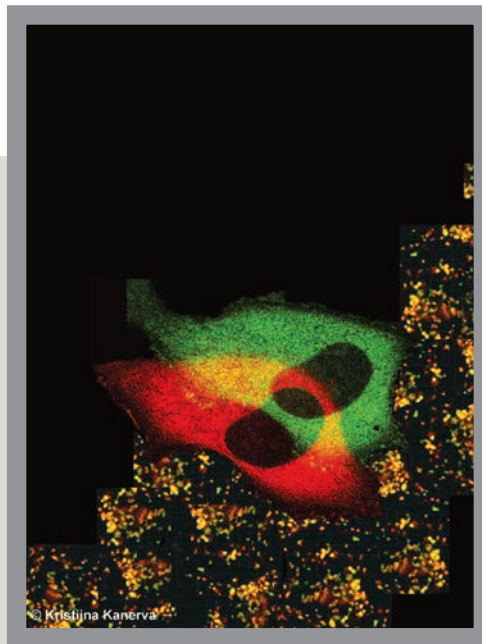




MINERVA FOUNDATION
&
MINERVA FOUNDATION INSTITUTE
FOR MEDICAL RESEARCH



Scientific Report 2013



Front page image: The first and last images from a confocal time-lapse series of migrating cells overexpressing focal adhesion marker zyxin-cherry (presented in pseudo-color green/red). On the background, lysosome related organelles harbouring LDL-derived Bodipy-cholesterol (green) and late endosomal marker CD63-cherry (red) (courtesy of Kristiina Kanerva/Membrane biology).

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SUMMARY OF RESEARCH AND ACTIVITIES DURING THE YEAR 2013

Minerva Foundation Institute for Medical Research is a privately owned research institute located at the Biomedicum Helsinki Research Center at Meilahti campus. The Institute, the history of which dates back to 1959, creates a fruitful combination of basic biomedical research and clinical investigation relevant for common disease and provides infrastructure for top quality research in this area. The objectives of study range from the fundamental molecular mechanisms of disease addressed in cultured cells and genetically manipulated animal models to investigation of human patients. The aim is to generate a new knowledge base as well as innovations for the development of future diagnostic approaches, preventive measures and treatments for common diseases such as diabetes, cardiovascular diseases and neurodegenerative disorders. The work carried out in the eight research groups of the Institute during the year 2013 is outlined in this report.

The funds of Minerva Foundation are directed at maintaining and further developing a research infrastructure that serves, in the most effective way, the substantive activities of the groups, and encourages the scientists to maximise efforts to acquire external research funds. During 2013, 50% of the Institute budget was covered by funds from the Minerva Foundation. Despite the current challenging financial situation in science, Minerva scientists succeeded in raising a substantial amount of external funds, covering the remaining 50% of the budget.

To enhance the research impact and maximise the gain from the investment in the Institute, it is essential to continually encourage integration both within the Institute and with the other organizations located at the campus. One important area of development towards this goal is generation of common technology cores in collaboration with neighboring institutions. In 2013, Minerva scientists organized the purchase of an advanced cell metabolism analyzer, which was acquired as a joint effort of seven different organizations and is now employed in use as a core facility. In addition to building external collaboration, the internal integration and spirit of the Institute were promoted by weekly internal meetings and an institute-wide social event, a boat cruise to the historical town of Porvoo organized on June 18.

To inform other research organizations and the public of the work carried out at Minerva, a symposium in which four Minerva senior scientists presented their research, was arranged on September 16. Moreover, the Institute organized four special seminars held by invited guest lecturers during 2013. The international connections of the Institute were enhanced by invitation of a visiting scholar, Prof. Andrew Brown, University of New South Wales, Sydney, Australia, who worked at the Institute during June 2013.

The work at the Institute in 2013 was efficient and productive. Minerva scientists published a total of 35 articles in international peer reviewed journals, with a mean impact factor of 5.60 (median 4.78). In addition, four Ph.D. theses were finalized and defended during the year.

EVENTS AT MINERVA 2013

Seminars and symposia

TIMO YLIKOMI: *A cultured cell model for human adipose tissue.* February 1st, Biomedicum Helsinki 1

ANDREW BROWN: *Cholesterol in the balance.* June 11th, Biomedicum Helsinki 1

MINERVA SYMPOSIUM
September 16th, Biomedicum Helsinki 1

MINGFENG XIA: *The principle and application of ultrasound quantitative method for liver fat content.* September 27th, Biomedicum Helsinki 1

MATTI LANG: *Molecular mechanism for control of intracellular bilirubin levels, A protective system against oxidative stress.* October 8th, Biomedicum Helsinki 1

Dissertations

You Zhou: *Oxysterol binding proteins homologues: Novel functions in intracellular transport and adipogenesis.* January 25, 2013. (Lipid signalling and homeostasis)

Raili Koivuniemi: *Regulation of neural progenitor cell proliferation and fate by proteolytic pathways and inflammatory signals in the brain.* September 6, 2013. (Neuroscience)

Wei Wang: *Studies on the transcriptional and enzymatic control of steroid metabolism: Regulation by lysosomal acid lipase, 24-dehydrocholesterol reductase, and amyloid precursor protein.* November 28, 2013. (Membrane biology)

Anders Eriksson-Palojärvi: *Neurohumoral response and pathophysiologic changes during progression of mitral regurgitation in the Cavalier King Charles spaniel.* November 29, 2013. (Telomere research)

ADMINISTRATION

THE MINERVA FOUNDATION

The main purpose of the Foundation is to promote research in medicine and biosciences by maintaining the Minerva Foundation Institute for Medical Research. This scientific review covers the period from January 1, 2013, to December 31, 2013. During this period, the board of trustees included the following persons:

Professor Jim Schröder, chair
Professor Caj Haglund, vice chair
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Professor Per-Henrik Groop
Professor Carola Grönhagen-Riska
Professor Carl-Gustaf Nilsson
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M.Sc. Ann-Christine Sundell, secretary
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The administration was handled by following groups:

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Carola Grönhagen-Riska
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Caj Haglund

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Per-Henrik Groop, chair
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THE MINERVA FOUNDATION INSTITUTE FOR MEDICAL RESEARCH

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Professor Dan Lindholm
Docent Jakob Stenman
Docent Ilkka Tikkanen
Professor Kid Törnquist
Professor Hannele Yki-Järvinen
Carita Estlander-Kortman
Cia Olsson, secretary



Group Members

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 Hanna Forsten, V.M.D.
 Jere Paavola, M.D.
 Heli Segersvärd, M.D.
 Juuso Siren, M.D.
 Karri Kalervo, M.B.
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 Katariina Immonen, B.Sc., laboratory technician
 Riikka Kosonen, M.Sc., laboratory technician
 Sanni Perttunen, B.Sc., laboratory technician, (started 1.12.2013)

Collaborators

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Funded by

The Aarne Koskelo Foundation
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 The Finnish Foundation for Cardiovascular Research
 The Finnish Medical Foundation / the Finnish Medical Society Duodecim
 The Finska Läkaresällskapet
 The Ida Montin Foundation
 The Liv och Hälsa Foundation
 The Maud Kuistila Memorial Foundation
 The Minerva Foundation
 The Orion-Farmos Research Foundation
 The Oskar Öflund Foundation
 The Paavo Nurmi Foundation
 Research Funding of Helsinki-Uusimaa Hospital District (EVO)

CARDIOVASCULAR RESEARCH

Main research activities

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

We have shown previously that heme oxygenase-1 (HO-1) and carbon monoxide (CO) have potential roles in post-myocardial infarction (MI) cardiac repair. Our novel findings demonstrated that treatment with a specific CO-donor, CORM-3, improved both structural and functional cardiac recovery after myocardial injury. Induction of HO-1-CO pathway may prove to be a useful tool to protect against pathological myocardial changes and development of heart failure after MI, thereby facilitating cardiac recovery. In 2013, the research of HO-1-CO pathway continued. In collaboration with Hannele Laivuori, we studied the association of HO-1 gene (*HMOX1*) polymorphisms with an increased risk of pre-eclampsia. Our group also participates in the COST Action BM1005, European Network on Gaseotransmitters. In 2013 Päivi Lakkisto was nominated as the Management Committee Member of BM1005.

We studied the cardiac manifestations of polycystic kidney disease (PKD), in collaboration with Professor Ehrlich, in a zebrafish model lacking the polycystin-2 protein. These fish demonstrated impaired intracellular calcium handling and

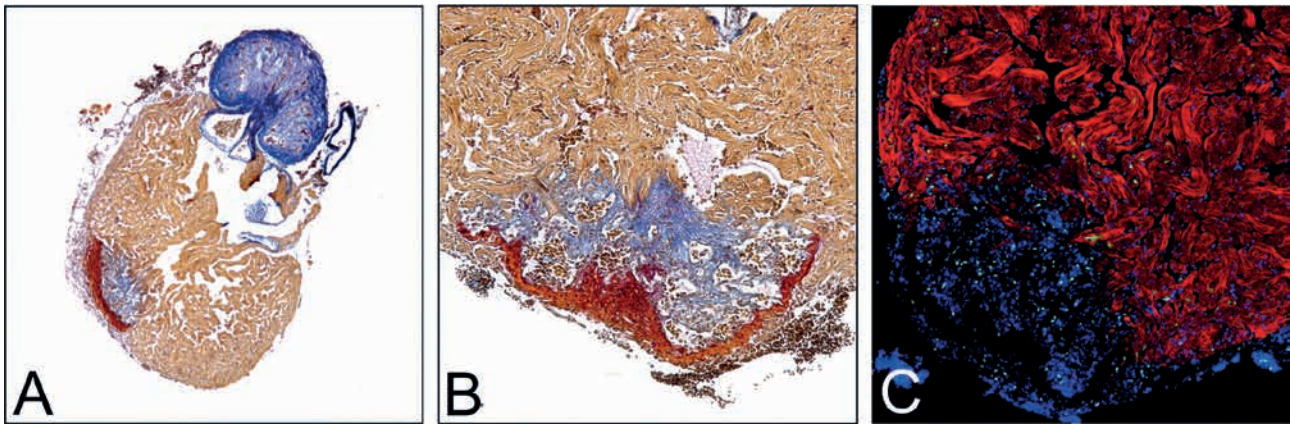


Figure. (A) and (B) AFOG stained zebrafish hearts 14 days after cryoinjury. (C) BrdU (green) and cardiac myosin (red) staining of zebrafish hearts 14 days after cryoinjury. Nuclei are stained with DAPI (blue).

developed heart failure and arrhythmias. Interestingly, a high prevalence of idiopathic dilated cardiomyopathy was found in human PKD patients harboring mutations in polycystin-2.

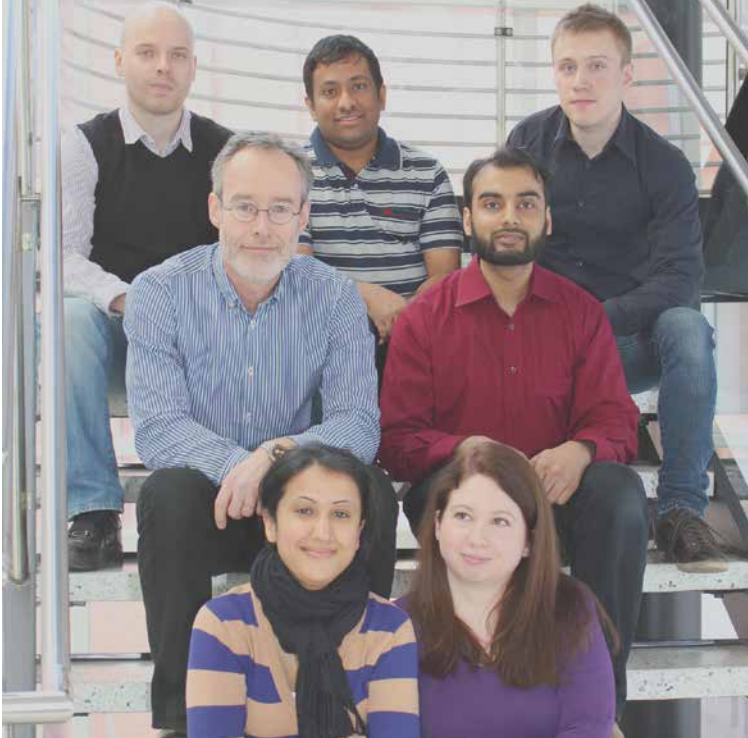
Using the cardiac cryoinfarction model in adult zebrafish, we carried out pilot experiments on the effect of micro-RNAs on cardiac regeneration. We validated protocols for efficient delivery of micro-RNAs and synthetic anti-micro-RNAs, or Antagomirs, to the adult zebrafish. This method enables the study of molecules and pathways related to cardiac regeneration in a fast-growing, affordable model organism with an amazing regenerative capacity. Additionally, we developed a drug-induced cardiac hypertrophy/failure model in embryonic zebrafish. With easy delivery of drugs and methodology for gene silencing, our model provides a robust platform for studying the development of cardiac hypertrophy and failure, as well as factors affecting recovery of cardiac function from heart failure.

We have been working in several collaborative clinical studies that will continue in 2014. For example, in collaboration with Veli-Pekka Harjola and Yvan Devaux, we have been searching for potential miRNAs associated with the outcome of cardiogenic shock patients (CardShock project). The effects of drug treatment on the progression of disease in aortic stenosis patients have been investigated in collaboration with Professor Markku Kupari (ROCK-AS study). In collaboration with Professor Ville Pettilä, potential new biomarkers of acute kidney injury (FINNAKI study) have been studied.

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

Publications

- Paavola J**, Schliffke S, Rossetti S, Kuo IY, Yuan S, Sun Z, Harris PC, Torres VE, Ehrlich BE. Polycystin-2 mutations lead to impaired calcium cycling in the heart and predispose to dilated cardiomyopathy. *J Mol Cell Cardiol* 2013; 58:199–208
- Segersvärd H, Lakkisto P, Forsten H, Immonen K, Kosonen R**, Palojoiki E, Kankuri E, Harjola A, **Laine M, Tikkanen I**. J Effects of angiotensin II blockade on cardiomyocyte regeneration after myocardial infarction in rats. *Renin Angiotensin Aldosterone Syst*. Epub 2013 Apr 29
- Tikkanen I**, Lappalainen K. Renal denervation in the treatment of hypertension. *KATSAUS. Duodecim*. 2013; 129(8):825–32



Group members

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

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

Collaborators

at the Åbo Akademi University, Turku, Finland:

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Dan Lindholm, Professor, University of Helsinki and Minerva Foundation Institute for Medical Research, Finland
Tomas Blom, Docent, University of Helsinki, Finland

Funded by

The Liv och Hälsa Foundation
The Minerva Foundation
The Sigrid Juselius Foundation
Åbo Akademi University

CELLULAR PHYSIOLOGY

Main research activities

The research group focuses on investigating calcium and lipid signaling mainly in thyroid cancer cells. The importance of the canonical transient receptor potential (TRPC) -family of ion channels, as well as the actions of the sphingomyelin metabolite sphingosine 1-phosphate (S1P), are topics of great interest. In addition, the interactions between TRPC-channels and S1P are actively investigated.

We have previously shown that S1P induces migration of follicular ML-1 thyroid cancer cells. Hypoxia-induced factor-1 (HIF-1) is an oxygen-sensitive transcription factor, which adapts cells to hypoxic conditions through increased survival, motility and angiogenesis. Due to these properties and its increased expression in response to intratumoral hypoxia, HIF-1 is considered a significant regulator of tumor biology. In our recent work, we found that S1P treatment increased expression of the regulatory HIF-1 α subunit, as well as HIF-1 activity and expression of HIF-1 target genes, in normoxic ML-1 cells. Importantly, inhibition or knock-down of HIF-1 α attenuated S1P-induced migration of ML-1 cells. S1P-induced HIF-1 α expression was mediated by S1P receptor 3 (S1P₃), Gi proteins and their downstream effectors MEK, PI3K, mTOR and PKC β I. Half-life measurements using cycloheximide-induced protein synthesis inhibition indicated that S1P treatment stabilized the HIF-1 α protein. On the other hand, S1P treatment activated translational regulators eIF-4E and p70S6K, which are known to control HIF-1 α synthesis. Thus, based on our studies, S1P seems to be a regulator of HIF-1 α , and HIF-1 α participates in the regulation of thyroid cancer cells.

We have previously shown that the TRPC2 ion channel is an important regulator of thyrotropin receptor expression, calcium homeostasis, and the regulation of calcium-activated chloride channels

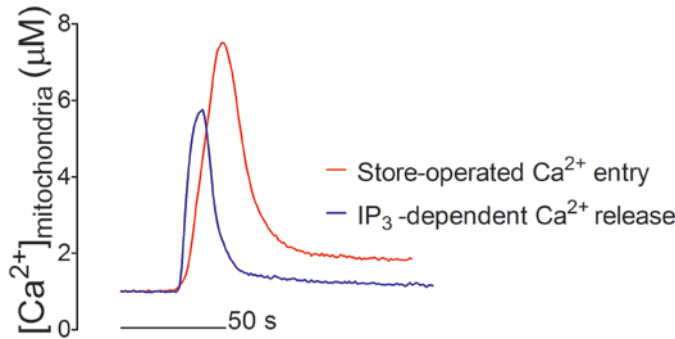


Figure. Measurements of mitochondrial calcium concentrations using mitochondria-targeted aequorin. The cells were stimulated with 10 μM histamine (blue trace; Inositol triphosphate (IP_3) dependent Ca^{2+} release) in a calcium-free buffer, and mitochondrial uptake of Ca^{2+} released from the endoplasmic reticulum is measured. The red trace (Store-operated Ca^{2+} entry) shows mitochondrial Ca^{2+} uptake after readdition of Ca^{2+} to cells where the endoplasmic reticulum has been depleted of Ca^{2+} . Readdition of Ca^{2+} to these cells evokes a massive Ca^{2+} influx, and part of this Ca^{2+} is taken up by the mitochondria. Please note the difference in the uptake kinetics and the transient nature of the responses.

(Anoctamins) in rat thyroid cells. We have now also studied the significance of the TRPC2 channel in the regulation of rat thyroid FRTL-5 cell proliferation, migration, adhesion and invasion, using stable TRPC2 (shTRPC2) knock-down cells. As a result of the loss of TRPC2 gene expression in these cells, proliferation was decreased due to a prolonged G1/S cell cycle phase. Furthermore, the p53 tumor suppressor and p27 and p21 cyclin-dependent kinase inhibitors were upregulated. Cell invasion, adhesion and migration were also attenuated in shTRPC2 cells, probably due to decreased activity of both the Rac small GTPase and calpain protease and decreased secretion and activity of matrix metalloproteinase 2. The attenuated proliferation, migration, invasion and ATP-evoked calcium entry was mimicked by overexpressing a non-conducting, truncated TRPC2 in wild type cells, and was reversed by overexpression of TRPC2-GFP in shTRPC2 cells. Together with our previous data these studies show that the TRPC2 channel is an important regulator of rat thyroid cell function.

Current, ongoing efforts are aimed at understanding the importance of TRPC channels in human thyroid cancer cells and the interactions of

these channels with S1P signaling in regulating the expression of matrix-metalloproteinases and HIF-1 α . Furthermore, mechanisms regulating S1P-evoked migration are also investigated.

Publications

- Hyrskyluoto A**, Pulli I, **Törnqvist K**, **Huu Ho T**, **Korhonen L**, **Lindholm D**. Sigma-1 receptor agonist PRE084 is protective against mutant huntingtin-induced cell degeneration: involvement of calpastatin and the NF- κB pathway. *Cell Death Dis* 2013; 23;4:e646
- Kalhari V**, Kempainen K, Asghar MY, **Bergelin N**, Jaakkola P, **Törnqvist K**. Sphingosine-1-phosphate as a regulator of hypoxia-induced factor-1 α in thyroid follicular carcinoma cells. *PLOS One* 2013; 8(6):e66189
- Sukumaran P, Löf C, Pulli I, Kempainen K, **Viitanen T**, **Törnqvist K**. Significance of the transient receptor potential canonical 2 (TRPC2) channel in the regulation of rat thyroid FRTL-5 cell proliferation, migration, adhesion and invasion. *Mol Cell Endocrinol* 2013; 374(1–2):10–21
- Sukumaran P, Lönnfors M, Långvik O, Pulli I, **Törnqvist K**, Slotte JP. Complexation of c6-ceramide with cholesteryl phosphocholine - a potent solvent-free ceramide delivery formulation for cells in culture. *PLoS One* 2013; 8(4):e61290
- Törnqvist K**. Sphingosine 1-phosphate and cancer: Lessons from thyroid cancer cells. *Biomeolecules* 2013, 3:303–315



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 Marju Orho-Melander, Professor, Malmö, Sweden
 Ronald Kahn, Professor, Boston, MA, USA
 Barbara Kahn, Professor, Boston, MA, USA
 Bart Staels, Professor, Lille, France
 Xin Gao, Professor, Shanghai, China

Funded by

The Academy of Finland
 European Medical Information Framework (EMIF)
 The Liv och Hälsa Foundation
 The Minerva Foundation
 The Novo Nordisk Foundation
 Research Funding of Helsinki-Uusimaa Hospital District (EVO)
 The Sigrid Jusélius Foundation

ENDOCRINOLOGY

Main research activities

During 2013, we have continued our efforts to understand the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and develop a diagnostic marker for this condition. Regarding pathogenesis, the rs738409 C>G single-nucleotide polymorphism in the PNPLA3 gene leads to a missense mutation (I148M) which increases liver fat but does not cause insulin resistance. We hypothesized that patients with non-alcoholic fatty liver disease (NAFLD) due to the PNPLA3 variant ('PNPLA3 NAFLD' = PNPLA3-148MM) do not have adipose tissue (AT) inflammation, in contrast with those with NAFLD due to obesity ('obese NAFLD'). Biopsy specimens of AT were taken, and PNPLA3 genotype and liver fat content (proton magnetic resonance spectroscopy (1)H-MRS)) were determined in volunteers, who were divided previously into obese and non-obese groups based on either median body mass index (BMI) or PNPLA3 genotype. All groups were similar with respect to age and sex. Our results showed that liver fat was similarly increased in obese NAFLD and PNPLA3 NAFLD, and fasting serum insulin was higher in the obese than the non-obese group but similar in PNPLA3-148MM and PNPLA3-148II. In obese but not PNPLA3 NAFLD, markers of adipose tissue inflammation were significantly elevated. These data show that PNPLA3 NAFLD is characterized by an increase in liver fat but not insulin resistance or AT inflammation, while obese NAFLD has all three of these features. We also showed that adipocyte size, a well-established determinant of whole body insulin sensitivity, is associated with an increase in liver fat content independent of obesity, fat distribution and PNPLA3 genotype.

Regarding diagnosis of NAFLD, we have developed a biomarker, based on molecular lipids, that can help in diagnosing NAFLD. Specifically, concentrations of molecular lipids by ultra-performance

liquid chromatography-mass spectrometry (UPLC-MS) were measured in blood samples of 679 well-characterized individuals in whom liver-fat content was measured using proton magnetic resonance spectroscopy ((1)H-MRS) or liver biopsy. The participants were divided into biomarker-discovery (n = 287) and validation (n = 392) groups to build and validate the diagnostic models, respectively. Individuals with NAFLD had increased triacylglycerols with low carbon number and double-bond content while lysophosphatidylcholines and ether phospholipids were diminished in those with NAFLD. Furthermore, a serum-lipid signature comprising three molecular lipids ('lipid triplet') was developed to estimate the percentage of liver fat. This signature test had a sensitivity of 69.1% and specificity of 73.8% when applied for diagnosis of NAFLD in the validation series. The usefulness of the lipid triplet was demonstrated in a weight-loss intervention study. A concluding sentence is needed here. What is the final summary/future direction of using the lipids as a biomarker?

Publications 2013

- Lallukka S, Sevastianova K, Perttilä J, Hakkarainen A, Orho-Melander M, Lundbom N, Oikkonen VM, Yki-Järvinen H.** Adipose tissue is inflamed in NAFLD due to obesity but not in NAFLD due to genetic variation in PNPLA3. *Diabetologia* 2013; 56(4):886–92
- Petäjä EM, Sevastianova K, Hakkarainen A, Orho-Melander M, Lundbom N, Yki-Järvinen H.** Adipocyte size is associated with NAFLD independent of obesity, fat distribution, and PNPLA3 genotype. *Obesity* (Silver Spring) 2013; 21(6):1174–9
- Varewijck AJ, **Yki-Järvinen H**, Schmidt R, Tennagels N, Janssen JA. Concentrations of Insulin Glargine and Its Metabolites During Long-Term Insulin Therapy in Type 2 Diabetic Patients and Comparison of Effects of Insulin Glargine, Its Metabolites, IGF-I, and Human Insulin on Insulin and IGF-I Receptor Signaling. *Diabetes* 2013; 62(7):2539–44
- Yki-Järvinen H, Kotronen A.** Is there evidence to support use of premixed or prandial insulin regimens in insulin-naïve or previously insulin-treated type 2 diabetic patients? *Diabetes Care* 2013; 36 Suppl 2: S205–11
- Yki-Järvinen H, Rosenstock J, Durán-García S, Pinnett S, Bhattacharya S, Thiemann S, Patel S, Woerle HJ.** Effects of adding linagliptin to basal insulin regimen for inadequately controlled type 2 diabetes: a ≥52-week randomized, double-blind study. *Diabetes Care* 2013; 36(12):3875–81



Group members

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 Raghavendra Mysore, M.Sc.
 Jarkko Soronen, M.Sc.
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 Liisa Arala, laboratory technician

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 Timo Ylikomi, Professor, FICAM and University of Tampere, Finland

Funded by

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

LIPID SIGNALING AND HOMEOSTASIS

Main research activities

A major aim of the group is to elucidate mechanisms that cells employ to sense their internal lipid status and to relay this information to control a number of vital cellular processes, including organelle lipid compositions, signal transduction, and vesicle transport. Oxysterols are oxidized derivatives of cholesterol that act as potent regulators of cellular lipid metabolism, signaling and differentiation. In this context, the cytoplasmic oxysterol-binding proteins, ORPs (Figure), are of major interest. The project aims to unravel the function of these proteins as lipid sensors and transporters with major impacts on a variety of downstream processes and their roles in diseases involving skewed lipid signaling.

In 2013 we finalized and published a study on the first ORP knock-out mouse model, that of Osbpl8, demonstrating specific lipid and lipoprotein metabolism phenotypes (Béaslas et al. 2013). A major activity was directed at understanding the spatial and temporal regulation of the interaction of ORPs with their endoplasmic reticulum (ER) anchors, the VAMP-associated proteins (VAPs). The results implied that ORP sterol liganding modifies the interaction with VAPs, and several ORP-VAP complexes localize at membrane contact sites, which play central roles in the inter-organelle transport of lipids, control of Ca^{2+} fluxes, and other signaling processes. Of notable importance, a complex of human ORP2 with VAPA at endoplasmic reticulum-lipid droplet junctions was demonstrated to specifically impact the cellular metabolism of triacylglycerols (TAG; manuscript in preparation). Collaborative efforts with the group of D. Yan (Guangzhou, China) provided evidence for an interaction of ORP8 with the spindle and kinetochore protein Astrin and a functional role of ORP8 in sterol regulation of the cell cycle. Moreover, the mode of action of ORP4 in control of endoplasmic reticulum Ca^{2+} release

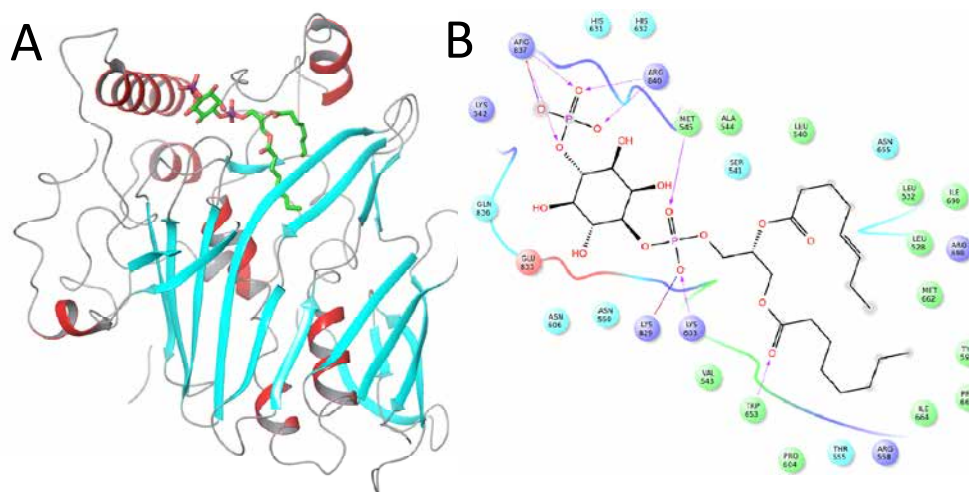


Figure. A homology model of human ORP3. (A) The overall structure. Beta-strands are displayed in blue, alpha-helices in red, and a bound phospholipid (PI4P) in green. (B) Ligand interaction diagram of ORP3 with PI4P. (Courtesy of Dr. Gerd Wohlfahrt)

and leukemia cell viability was elucidated together with prof. Yan (work submitted).

A collaborative project on the molecular mechanisms underlying non-alcoholic fatty liver disease (NAFLD) and distortions of insulin signaling is carried out with the group of Endocrinology led by Prof. Hannele Yki-Järvinen. NAFLD affects more than 20% of the population in Western countries and predisposes to insulin resistance/metabolic syndrome as well as steatohepatitis and liver cirrhosis. During 2013, we produced evidence that the PNPLA3 protein facilitates the remodeling of cellular TAGs, an activity defective in the I148M variant (manuscript submitted). In addition, we focused major effort on the role of microRNAs in NAFLD as well as the insulin regulation and function of Angiopoietin-like proteins 3, 4 and 8, important new controllers of lipid metabolism and insulin sensitivity (in preparation).

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- Béaslas O**, Metso J, **Nissilä E**, Laurila PP, Kaiharju E, Krishna Chaithanya Batchu, Kaipainen L, Mikko I. Mäyränpää, Yan D, Gylling H, Jauhiainen M, **Oikkonen VM**. Osbp18 Deficiency in Mouse Causes an Elevation of High-Density Lipoproteins and Gender-Specific Alterations of Lipid Metabolism. *PLoS ONE* 2013; 8(3):e58856
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- Ikonen E**, **Oikkonen VM**. Nobelin palkinto solunsisäisen kalvoliikenteen tutkijoille. *PALKINNOT. Duodecim* 2013; 129:75–76
- Korhonen JT, **Oikkonen VM**, Laheesmaa R, Puolakkainen M. ABC-cassette transporter 1 (ABCA1) expression in epithelial cells in Chlamydia pneumoniae infection. *Microb Pathog* 2013; 61–62:57–61

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Oikkonen VM. OSBP-related proteins: Liganding by glycerophospholipids opens new insight into their function. *Molecules* 2013; 18(11):13666–13679

Oikkonen VM, Li S. Oxysterol-binding proteins: Sterol and phosphoinositide sensors coordinating transport, signaling and metabolism. *Prog Lipid Res* 2013; 52(4):529–538

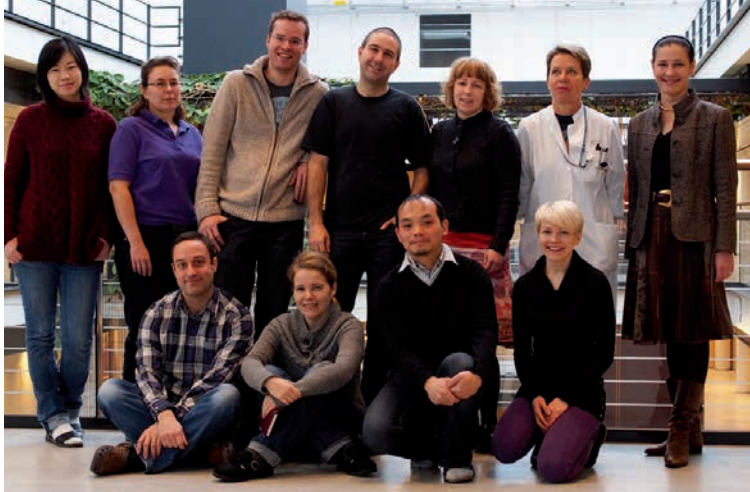
Qin SY, Liu J, Jiang HX, Hu BL, **Zhou Y**, **Oikkonen VM**. Association between baseline lipoprotein (a) levels and restenosis after coronary stenting: Meta-analysis of 9 cohort studies. *Atherosclerosis* 2013; 227(2):360–6

Weber-Boyvat M, Zhong W, Yan D, **Oikkonen VM**. Oxysterol-binding proteins: Functions in cell regulation beyond lipid metabolism. *Biochem Pharmacol* 2013; 86(1):89–95

Thesis completed in the group in 2013

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

You Zhou: Oxysterol binding proteins homologues: Novel functions in intracellular transport and adipogenesis. January 25, 2013.



Group members

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 Tomas Blom, Ph.D., Academy Research Fellow
 Riku Fagerlund, Ph.D.
 Maarit Hölttä-Vuori, Ph.D.
 Kristiina Kanerva, Ph.D.
 Shiqian Li, Ph.D.
 Maarit Neuvonen, Ph.D.
 Simon Pfisterer, Ph.D.
 Sanjeev Ranjan, Ph.D.
 Boris Vassilev, M.Sc.
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 Veijo Salo, medical student
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 Pekka Lappalainen, Professor, Institute of Biotechnology, University of Helsinki, Finland
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Funded by

The Academy of Finland: Academy Professorship, Photonics and Modern Imaging Techniques Programme
 Sydäntutkimussäätiö
 The Minerva Foundation
 The Sigrid Jusélius Foundation
 Biocentrum Helsinki, Biocenter Finland
 University of Helsinki, Research excellence and infrastructure funding

MEMBRANE BIOLOGY

Main research activities

Our group focuses on understanding how cholesterol functions in the cellular context, both under physiological conditions and in human diseases.

Cholesterol is an essential component of cell membranes. It is enriched in the plasma membrane, where it dictates important biophysical properties of the bilayer and participates in lipid-protein interactions. Although physiologically highly important, these aspects often receive less attention than the adverse effects of cholesterol. In addition to cardiovascular diseases, disturbances in cholesterol metabolism are implicated e.g. in several neurodegenerative diseases.

We have recently investigated mechanisms by which altered cellular lipid handling may contribute to neurodegenerative diseases. First, we have proposed that impaired lipid trafficking and defective differentiation of myelinating cells contribute to the pathogenesis of demyelinating neuropathy Charcot-Marie-Tooth disease 4D (Pietiäinen et al., 2013). We have also found that hereditary spastic paraplegia caused by mutations in seipin, in a disease known as seipinopathy, may result from lipid imbalance. We generated a zebrafish model for seipinopathy, showed that the seipinopathy is accompanied by reduced triglyceride content in the brain and demonstrated that the motor defect can be alleviated by lipid supplementation (Hölttä-Vuori et al., 2013). Finally, we have discovered a new physiological link between amyloid precursor protein (APP; mutated in familial Alzheimer's disease) and cholesterol metabolism (Wang et al., 2013). This new link may help to explain some of the clinically observed connections between cholesterol and Alzheimer's disease.

In addition to our studies on neurodegenerative diseases, during 2013, we also examined the cell biological mechanisms and consequences of cholesterol transport. It is well appreciated that

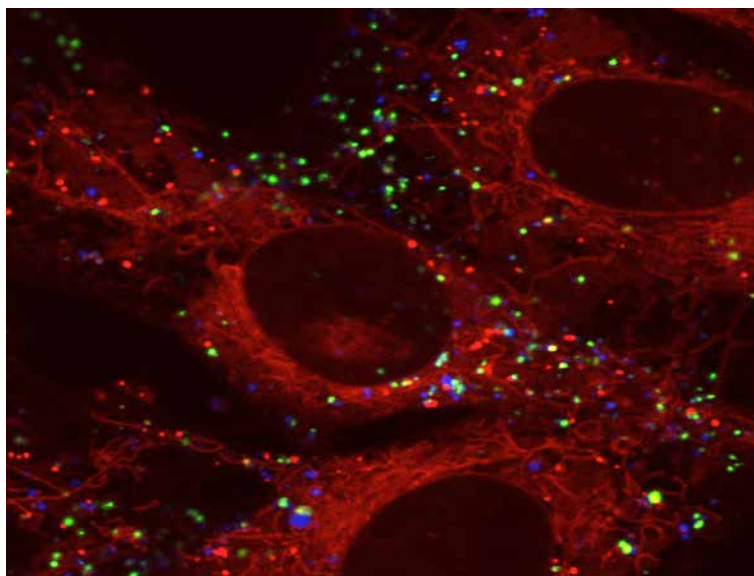


Figure. Triple labeling of living fibroblasts reveals LDL-cholesterol uptake (green; BODIPY-cholesteryl linoleate labeled LDL, as in Kanerva et al., 2013), lysosomes (blue), lipid droplets (red, bright dots) and endoplasmic reticulum (red, tubules) (courtesy of Kristiina Kanerva and Simon Pfisterer).

Publications 2013

most cells acquire cholesterol via low-density lipoprotein (LDL) uptake and distribute it from endosomes to other organelles, particularly the plasma membrane. However, how this delivery happens has been obscure. We have recently identified a molecular pathway by which LDL-cholesterol is transported from late endosomes to the plasma membrane (Kanerva et al., 2013). This route consists of late endosome-derived CD63- and cholesterol-containing vesicles that move to the cell leading edge and become anchored to the cortical actin, in a Rab8a- and myosin5b-dependent manner. Importantly, we also show that this route of cholesterol delivery ends in cell attachment sites, focal adhesions, and is physiologically important for cell migration.

Hölttä-Vuori M, Salo VT, Ohsaki Y, Suster ML, **Ikonen E**. Alleviation of seipinopathy-related ER stress by triglyceride storage. *Hum Mol Genet* 2013; 22(6):1157–66

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Siljamäki E, Rintanen N, Kirsi M, Upla P, **Wang W**, Karjalainen M, **Ikonen E**, Marjomäki V. Cholesterol dependence of collagen and echovirus 1 trafficking along the novel $\alpha 2 \beta 1$ integrin internalization pathway. *PLoS One* 2013; 8(2):e55465

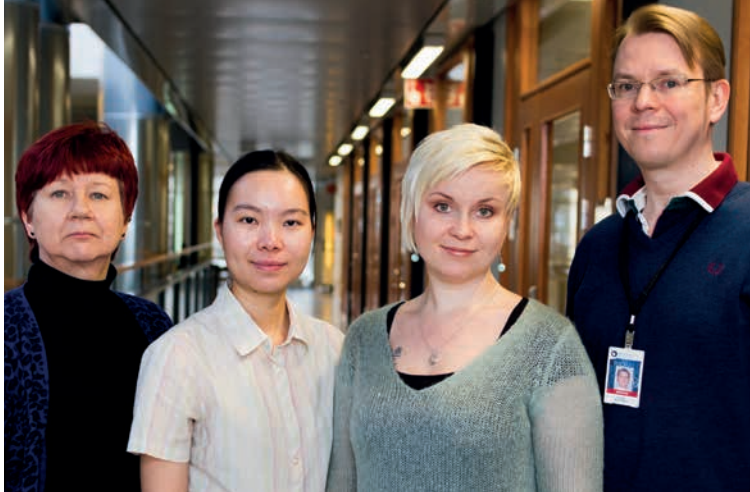
Zerenturk EJ, Sharpe LJ, **Ikonen E**, Brown AJ. Desmosterol and DHCR24: Unexpected new directions for a terminal step in cholesterol synthesis. *Prog Lipid Res* 2013; 52(4):666–680

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Thesis completed in the group in 2013

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

Wei Wang: Studies on the transcriptional and enzymatic control of steroid metabolism: regulation by acid lipase, 24-dehydrocholesterol reductase and amyloid precursor protein. November 28, 2013.



Group members

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 Selina Mäkinen, M. Sc.
 Riitta Päivärinta, laboratory technician

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 Hannele Laivuori, Docent, Haartman Institute, University of Helsinki, Finland
 Nina Lundbom, Professor, Department of Radiology, Helsinki University Central Hospital, Finland

FUSION (Finnish-United States Investigation of NIDDM Genetics) consortium
 (fusion.sph.umich.edu/generated/people.php):

Michael Boehnke, Professor, University of Michigan, Ann Arbor, USA
 Richard Bergman, Professor, Cedars-Sinai Diabetes and Obesity Research Institute, Los Angeles, USA
 Francis Collins, MD, PhD, director of National Institutes of Health, USA
 Markku Laakso, Professor, University of Eastern Finland, Finland
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Funded by

The Academy of Finland
 Jalmari and Rauha Ahokas Foundation
 Finnish Diabetes Research Foundation
 The Finska Läkaresällskapet
 The Liv och Hälsa Foundation
 The Minerva Foundation
 The Novo Nordisk Foundation
 Research Funding of Helsinki-Uusimaa Hospital District (EVO)
 The Sigrid Jusélius Foundation

METABOLISM

Main research activities

In 2013, the research activity of the group continued with the primary focus on the interaction between fatty acid and glucose metabolism in skeletal muscle, using isolated human skeletal muscle strips and primary human muscle cells. Moreover, sample collection for "MAMA – The Molecular Mechanisms of Insulin Resistance in Gestational Diabetes (PI docent Heikki Koistinen)" continued actively.

In March 2013 we completed the clinical part in FUSION (Finnish United States Investigation on NIDDM Genetics) Tissue Biopsy study (PI prof. Jaakko Tuomilehto) where we collected muscle, adipose and skin samples from 331 clinically characterized people across all glucose tolerance stages (normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), untreated type 2 diabetes (T2D)). This cohort is one of the largest existing collections of human tissue samples relevant for metabolic diseases. We are currently analysing muscle and adipose transcriptomes by RNA sequencing, and genome-wide genotype and DNA methylation data using the Illumina Omni 2.5M-Quad SNP chip and Illumina HumanMethylation-450 Beadchip, respectively, for these samples. In order to generate induced pluripotent stem cell cultures, we have also established fibroblast cell lines from these subjects. Our biological sample collection allows measurements of genotype-specific gene and protein expression, and will provide in depth information critical to our functional genomic efforts to identify genetic variants predisposing to the development of T2D. First data freeze from muscle RNA sequencing was presented as posters at the American Society of Human Genetics and Novo Nordisk Genomics in Metabolism meetings in the Fall 2013. Docent Heikki Koistinen became one of the FUSION Principal Investigators in 2013.



Figure 1. Showtime: the set-up in the laboratory just before the experiment.

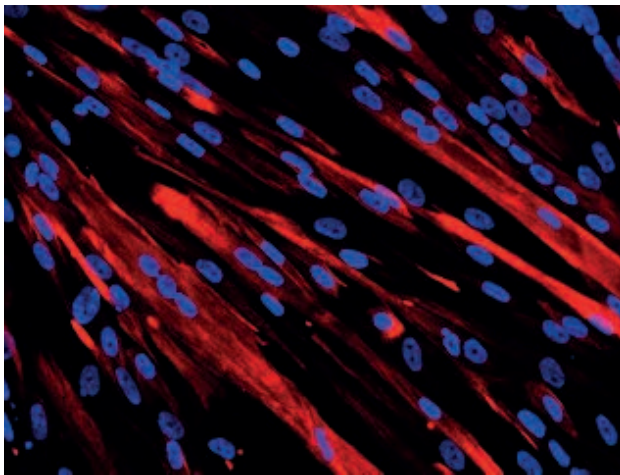


Figure 2. Primary human skeletal muscle cells have been differentiated into multi-nucleated myotubes.

Publications

Mäkinen S, Skrobuk P, Nguyen YH, Koistinen H. Insuliiniresistenssin mekanismit. KATSAUS.
Duodecim 2013; 129:2115–22



Group members

Dan Lindholm, M.D., Dr.Med.Sci., Professor, Head
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 Céline Bruelle, Ph.D.
 Olga Maloletkina, Ph.D.
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 Timofey Tselykh, Ph.D.
 Hai Thi Do, M.Sc.
 Julia Döhla, M.Sc., HBGP
 Alise Hyrskyluoto, M.Sc., FGSN
 Jenny Kivinen, M.Sc.
 Raili Koivuniemi, M.Sc., FGSN, Ph.D. fall 2013
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 Trin Laksperre, B.Sc., TRANSMED student

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 Ove Eriksson, Docent, Institute of Biomedicine, University of Helsinki, Finland
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The group is a member of the Nordic Autophagy Network (<http://nordicautophagy.org/>) and of the European network to integrate research on intracellular proteolysis pathways in health and disease (PROTEOSTASIS). The group PI is a member of the Brain & Mind Doctoral Programme and the Helsinki Biomedical Graduate Programme (HBGP).

Funded by

The Academy of Finland
 The Finska Läkaresällskapet
 The Liv och Hälsa Foundation
 The Magnus Ehrnrooth Foundation
 The Minerva Foundation
 The Sigrid Jusélius Foundation

NEUROSCIENCE

Main research activities

We study basic mechanisms underlying neurodegenerative diseases and cell damage in the brain. Our specific areas of focus are protein ubiquitination and the role of mitochondrial and endoplasmic reticulum (ER) stress pathways as well as autophagy in the pathogenesis and models of brain diseases. Furthermore, we examine neurotrophic factors, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) and their tyrosine kinase receptors, such as Trk and p75, in regulation of cell-specific events. We employ various proteomic and gene expression methods, cell culture and animal models of disease as well as genetically modified mice. Although the majority of the group is housed in the Institute of Biomedicine, Faculty of Medicine, the group is actively engaged in research at Minerva.

A. Signalling Mechanisms and Organelle Dysfunctions in Neurodegenerative Diseases

We have previously shown that cytoprotective autophagy plays a role in cell viability in Huntington's disease (HD) and that autophagy can be regulated by the upstream mechanistic target of rapamycin/GADD34 pathway in a cellular model Huntington's disease (Hyrskyluoto et al 2012). Mitochondria are in close apposition to the ER in contacts called Mitochondria-associated membranes (MAMs) which contain a subset of proteins involved in calcium regulation. We recently showed that an agonist for the Sigma-1 receptor (Sig-1R) present in MAMs increases cell survival and counteracts the deleterious effects caused by N-terminal mutant huntingtin proteins in neuronal cells (Hyrskyluoto et al, 2013). The results showed that the Sig-1R agonist had beneficial effects in models of HD, and suggested that Sig-1R may be a promising target for future drug development in HD.

The transcriptional coactivator protein peroxisome proliferator activated receptor-gamma coactivator 1-alpha (PGC-1) is a master regulator of mitochondrial and oxidative stress in cells. Using transgenic mice with overexpression of PGC1 in brain neurons, we have shown that dopaminergic neurons in the substantia nigra are resistant to the neurotoxin MPTP in an animal model of Parkinson's disease (Mudo et al 2012). To identify the proteins and cell pathways involved in the resistance, we profiled gene expression of different PGC-1 mice brain regions. The results revealed a novel pattern of gene expression in PGC-1 mice compared with control mice. The potential roles of these gene products in neuroprotection and metabolic changes induced by PGC-1 are currently under investigation.

Furthermore, we have also examined the effects of different neurotrophic factors in PGC-1 regulation in human dopaminergic cells (Mäkelä et al, in press). Currently, we are using chemical screening methods to search for novel compounds that may influence the activity and/or levels of PGC-1 in the dopaminergic system. Specifically, we utilize the recently acquired Seahorse XF Analyzer which allows the real-time study of mitochondrial functions in living neurons

B. Protein ubiquitination pathways in cell physiology

Ubiquitination of target protein by specific E3-ubiquitin ligases has emerged as an important step in the regulation of various cell processes and events with a link to diseases. We previously cloned the E3 ligase, Mir/Myip, also called Idol (Inducible degrader of the low density lipoprotein receptor, LDLR) that reduces LDLR levels in cells. We recently observed that Myip/Idol also influences very low density lipoprotein receptor (VLDLR) levels in brain neurons via protein ubiquitination (Do et al., 2013). We also observed that while the neurotrophic factor BDNF increases VLDLR expression, whereas Reelin decreases it, suggesting an important interaction of these molecules in neurodevelopment and plasticity.

MIR-interacting saposin-like protein (Msap) is an antagonist of Myip/Idol, belonging to the Canopy (Cnpy) family of proteins. We recently showed that Cnpy2/Msap is involved in the regulation of LDLRs in human hepatocytes acting in conjunction with the metabolic regulator FGF21 (Do et al, 2012). We are currently investigating using proteomics interactions partners of Msap in the ER. We also use study the effects of increased Msap expression and its downregulation using different cell types.

Deubiquitinating Enzymes (DUBs) are important for maintaining ubiquitinated protein levels in the cell. Our research has focused on Usp-14, that is widely expressed in brain neurons and linked to nerve cell signaling as shown in an ataxia (ax^l) mouse models. Our data suggest that Usp-14 plays an unprecedented role

in the clearing of protein aggregates and influences cell responses to oxidative stress that we are now studying in more details.

C. Regulation of Neuronal Progenitor Cells (NPCs)

NPCs are highly dividing cells with a capacity for self-renewal and the potential to give rise to both neurons and glial cells. To determine how cell-surface proteins influence cell proliferation of NPCs, we focused on the transmembrane serine protease inhibitors, HAI-1 and HAI-2. We observed that bone morphogenic proteins (BMPs) important in neurodevelopment as well as HAI-1 influence progenitor cell proliferation and glial cell differentiation in an age-dependent manner (Koivuniemi et al. 2013). These results show how quiescent NPCs can be activated by the action of HAI and cell-associated proteases in the neuroepithelium.

Publications

Do HT, Bruelle C, Tselykh T, Jalonon P, Korhonen LT, Lindholm D. Reciprocal regulation of very low-density lipoprotein receptors (VLDLRs) in neurons by brain-derived neurotrophic factor (BDNF) and Reelin: Involvement of the E3 ligase Myip/Idol. *J Biol Chem* 2013; 288(41):29613–20

Hyryskyluoto A, Pulli I, Törnqvist K, Huu Ho T, Korhonen L, Lindholm D. Sigma-1 receptor agonist PRE084 is protective against mutant huntingtin-induced cell degeneration: involvement of calpastatin and the NF-κB pathway. *Cell Death Dis* 2013; May 23;4:e646

Koivuniemi R, Mäkelä J, Hokkanen ME, Bruelle C, Ho TH, Ola R, Korhonen L, Schröder J, Kataoka H, Lindholm D. Hepatocyte growth factor activator inhibitor-1 is induced by bone morphogenetic proteins and regulates proliferation and cell fate of neural progenitor cells. *PLoS One* 2013; 8(2):e56117

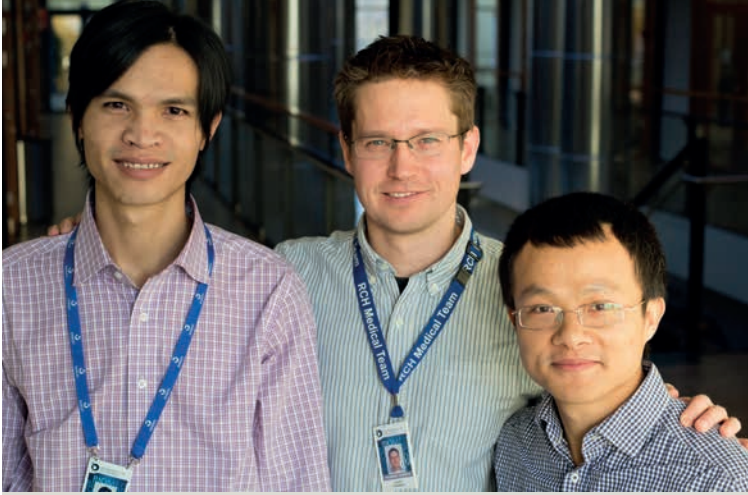
Theses completed in the group in 2013

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

Raili Koivuniemi: Regulation of neural progenitor cell proliferation and fate by proteolytic pathways and inflammatory signals in the brain. September 6, 2013.

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

Julia Döhla: Analysis of neuronal transcripts of PGC-1 alpha transgenic mice.



Group members

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 Ho Huu Tho, M.D.
 Kien Dang, M.D.
 Anne Ahmanheimo, bioanalyst

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 Marko Lempinen M.D., Dr.Med.Sci., Department of Transplantation and Liver Surgery Clinic
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Funded by

The Finska Läkaresällskapet
 The Liv och Hälsa Foundation
 Tekes (The Finnish Funding Agency for Technology and Innovation)
 The Minerva Foundation

TARGETED GENE-EXPRESSION ANALYSIS

Main research activities

The aim of the research group is to develop new gene expression-based assays for cancer diagnostics with the main focus on colon, prostate and breast cancer. We have extended our research interests to cover genetic and viral diseases for situations where there are specific technical difficulties related to amplification-based techniques.

During the past year we have worked intensively on a novel technique for ultra-sensitive detection of expressed KRAS mutations in patients with colorectal cancer. This technique could provide a means not only for disease monitoring following treatment, but also for early detection of malignant transformation in pre-malignant conditions such as Barrett's esophagus and Primary Sclerosing Cholangitis, where there are specific difficulties with currently available diagnostic tests. The results are promising and a manuscript describing the technique has been submitted for publication. In the coming year we are planning to apply this technique to clinical sample series in order to evaluate whether it can improve the sensitivity of KRAS testing.

In addition, we have continued development of a novel PCR amplification technology called Heat Pulse Extension PCR (HPE-PCR). Specifically, we have shown that difficulties in amplifying long GC rich and repetitive sequences can be overcome by using pulsatile temperature cycling during the extension step of each PCR cycle to destabilize secondary structures in the template. The utility of this technique has been demonstrated by amplifying repetitive expansions in Fragile X syndrome as well as in Type I Myotonic Dystrophy (Orpana et al 2012, Orpana et al 2013). Currently, we are focusing on improving the reaction efficiency further in order to enable amplification of longer repeat expansions that are present in Amyotrophic Lateral Sclerosis (ALS), Huntington's disease and Type 2 diabetes mellitus.

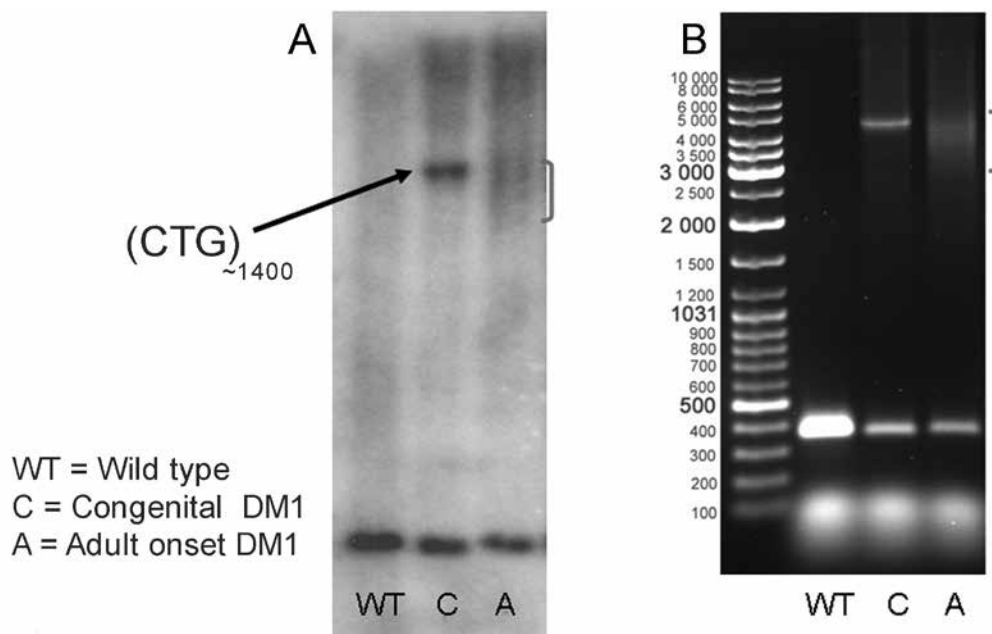


Figure. Concordance between Southern blot and HPE-PCR results. Southern blot (A) and corresponding HPE-PCR (B) analysis of DNA samples from a healthy individual (WT), a patient with congenital DM1 (C) and a patient with adult-onset DM1 (A).

Publications

- Kakkola L, Denisova OV, Tynell J, Viiliäinen J, Ysenbaert T, Matos RC, Nagaraj A, Ohman T, Kuivanen S, Paavilainen H, **Feng L**, Yadav B, Julkunen I, Vapalahti O, Hukkanen V, **Stenman J**, Aittokallio T, Verschuren EW, Ojala PM, Nyman T, Saelens X, Dzek K, Kainov DE. Anticancer compound ABT-263 accelerates apoptosis in virus-infected cells and imbalances cytokine production and lowers survival rates of infected mice. *Cell Death Dis* 2013; Jul 25;4:e742
- Orpana AK, **Ho TH**, Alagrund K, Ridanpää M, Aittomäki K, **Stenman J**. Novel heat pulse extension-PCR-based method for detection of large CTG-repeat expansions in myotonic dystrophy type 1. *J Mol Diagn* 2013; 15(1):110–5
- Koivuniemi R**, **Mäkelä J**, Hokkanen ME, **Bruelle C**, **Ho TH**, Ola R, **Korhonen L**, **Schröder J**, Kataoka H, **Lindholm D**. Hepatocyte growth factor activator inhibitor-1 is induced by bone morphogenetic proteins and regulates proliferation and cell fate of neural progenitor cells. *PLoS One* 2013; 8(2):e56117
- Hyrskyluoto A**, Pulli I, **Törnqvist K**, **Huu Ho T**, **Korhonen L**, **Lindholm D**. Sigma-1 receptor agonist PRE084 is protective against mutant huntingtin-induced cell degeneration: involvement of calpastatin and the NF-κB pathway. *Cell Death Dis* 2013; May 23;4:e646



TELOMERE RESEARCH

Group members

Frej Fyhrquist, M.D., Dr.Med.Sci., Dhc, Professor emeritus

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Anders Eriksson-Palojärvi, DVM

Anna Nyman, M.Sc.

Collaborators

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Antti Jula, Research Professor, National Institute for Health and Welfare, Turku, Finland

Funded by

The Finska Läkaresällskapet

The Liv och Hälsa Foundation

The Minerva Foundation

Main research activities

The team has studied leucocyte telomere length (LTL) in more than 4500 subjects to-date and examined LTL in comparison with various behaviors and medical and genetic profiles. For example, in the Helsinki Businessman Study, LTL was inversely related to smoking and body mass index during a long follow-up period, from 1964–1973 to 2002–2003, during which DNA for telomere measurement was isolated from blood leucocytes of 622 participants (collaboration with Professor T. Strandberg). In another study comprising the same subjects, high cholesterol in the beginning of the study combined with low cholesterol at the end was related to the shortest LTL and highest mortality. Moreover, we discovered associations of LTL with 1) alcohol consumption, 2) physical activity, and 3) insertion deletion (I/D) polymorphism of the angiotensin-I converting enzyme (ACE) gene.

A new study, focusing on telomeres and cardiovascular health in the young, was started in 2012 in collaboration with Profes-

sor A. Jula (Institute of Health and Welfare, Turku) as a substudy of the STRIP project (Sepelvaltimotaudin Riskitekijöiden Interventio Projekti) comprising >500 subjects each in the intervention and control groups with follow-up covering early childhood to maturity. This study has been ongoing during 2013, and results will be analyzed during 2014.

In the thesis by Anders Eriksson-Palojärvi the earlier evidence of the predictive value of N-terminal pro A-type natriuretic peptide (NT-proANP) in mitral regurgitation was confirmed and refined in a 5-year longitudinal natural dog model, corresponding to a 35-year follow-up in humans. Specifically, the study confirmed associations between 1) age and NT-proANP in dogs, 2) increased pulmonary transit time and degree of mitral regurgitation, and 3) increase in pulmonary blood volume and increased pulmonary transit time in dogs with serious mitral regurgitation. Although these associations have been reported in early work in both humans and animals, they had not previously been confirmed in natural mitral regurgitation in dogs.

Publications

Fyhrquist F, Saijonmaa O, Strandberg T. The roles of senescence and telomere shortening in cardiovascular disease. *Nat Rev Cardiol* 2013; 10(5):274-83

Strandberg TE, **Saijonmaa O, Fyhrquist F.** RE: "Association of leukocyte telomere length with breast cancer risk: nested case-control findings from the Shanghai women's health study". *Am J Epidemiol.* 2013; 178(4):662-3

Thesis completed in the group in 2013

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

Anders Eriksson-Palojärvi: Neurohumoral response and pathophysiologic changes during progression of mitral regurgitation in the Cavalier King Charles spaniel. November 29, 2013.

PUBLICATIONS 2013

Original articles

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Reviews

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