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TUULIA HUHTALA ET AL.

The Fourth Annual Post-Graduate Symposium of the Graduate School of Molecular Medicine

Winter School 2010 Abstracts

PUBLICATIONS OF THE UNIVERSITY OF EASTERN FINLAND
Reports and Studies in Health Sciences

UNIVERSITY OF
EASTERN FINLAND

A.I.VIRTANEN
INSTITUTE

TUULIA HUHTALA, SUSANNA BOMAN, TEEMU LAITINEN, HENRI LUMIVUORI, MIIA RYTINKI, TIMO SARAJÄRVI, RIIKKA PELLINEN

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1

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ABSTRACT

The Graduate School of Molecular Medicine combines systematic doctoral education of the highest quality to the best research expertise in molecular medicine at the A. I. Virtanen Institute for Molecular Sciences and Department of Medicine in the Faculty of Health Sciences in the University of Eastern Finland. The doctoral students of the school work as active researchers in the participating research groups whose scientific activities belong to six research programs: Cardiovascular Diseases, Type 2 Diabetes and Cardiovascular Diseases, Type 2 Diabetes and Obesity, Neurodegenerative Diseases, Stem Cell Research, and Inflammatory States.

This book compiles the abstracts of the 4th Annual Post-Graduate Symposium of the Graduate School of Molecular Medicine to be held in Tahkovuori, Nilsiä, on March 16 – 17, 2010.

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Contents

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GRADUATE SCHOOL OF MOLECULAR MEDICINE

WINTER SCHOOL 2010

MARCH 16 – 17, TAHKOVUORI NILSIÄ

PROGRAM

Tuesday, March 16

7:30 –	Departure from Bioteknia 1 (Neulaniementie 2)
8:45 – 9:15	Coffee (TahkoSpa) Poster set up
9:15 – 9:20	Opening of the Symposium, ***Seppo Ylä-Herttuala*** (TahkoSpa, Multifunction Arena)
9:20 – 10:55	**ORAL SESSION I** *Chairs: Lili Li and Antti Kurronen*
9:20 – 9:40	Current view of the pathogenesis of Alzheimer's disease. ***Heikki Tanila***
9:40 – 9:55	Alzheimer's disease-associated ubiquilin-1 regulates presenilin-1 accumulation and aggresome formation ***Jayashree Viswanathan***
9:55 – 10:10	Genetic study of 22 potential Alzheimer's disease risk genes selected on the basis of the AlzGene database meta-analyses ***Timo Sarajärvi***
10:10 – 10:25	Alzheimer's disease-associated protein ubiqulin-1 regulates BACE1 translation through decreased eIF-2-alpha phosphorylation in ER-stress ***Teemu Natunen***
10:25 – 10:40	Sodium channel blocking antiepileptic drugs suppress spontaneous epileptiform discharges in APdE9 mouse model of Alzheimer's disease ***Sofya Ziyatdinova***
10:40 – 10:55	Loss of hippocampal GABAergic interneurons during epileptogenesis: comparison of SE and TBI as epileptogenic etiology ***Noora Huusko***
10.55 – 11.45	**LUNCH & EXHIBITION (TahkoSpa)**
11:45 – 12:50	**ORAL SESSION II** *Chairs: Miika Martikainen and Hanna Leskinen*
11:45 – 12:05	Tumor metastasis and angiogenesis as well as novel therapy options ***Pirjo Laakkonen***
12:05 – 12:20	MDGI in cancer progression and metastasis ***Katja Häkkinen***
12:20 – 12:35	15-LO-1 induced apoptosis inhibits tumour growth and extends survival in rat glioma model ***Agnieszka Pacholska***
12:35 – 12:50	Susceptibility to alphavirus-mediated oncolysis of GL261 and CT-2A syngeneic mouse gliomas in vitro and in vivo ***Janne Ruotsalainen***
12:50 – 13:00	**BREAK**

13:00 – 14:05 **ORAL SESSION III**
Chairs: Suvi Jauhiainen and Anastasia Shakirzyanova

13:00 – 13:15 Vascular endothelial growth factor (receptor) expression during adipocyte differentiation in vitro ***Marike Dijkstra***
13:15 – 13:30 The role of VEGF-A / PLC-gamma pathway in cardiac myocytes ***Genet Assefa***
13:30 – 13:50 A short story about migraine aura and migraine pain ***Rashid Giniatullin***
13:50 – 14:05 Oxidative stress in migraine pain transduction ***Olga Kyrylenko***

14:05 – 15:05 **COFFEE, POSTER SESSION & EXHIBIOTION**

15:05 – 16:25 **ORAL SESSION IV**
Chairs: Teemu Laitinen and Dorota Kaminska

15:05 – 15:20 Transgenic rat models ***Sigma-Aldrich***
15:20 – 15:40 MRI – a non-invasive tool for biomedical research ***Olli Gröhn***
15:40 – 15:55 Implementation of pharmacological MRI ***Joanna Huttunen***
15:55 – 16:10 Simultaneous BOLD fMRI and local field potential measurements of epileptic seizures in awake and medetomidine sedated rats ***Antti Airaksinen***
16:10 – 16:25 MRI detection of short T2 component in brain by SWIFT ***Lauri Lehto***

16:25 – 20:30 Check in
Outdoor activities

20:30 – **DINNER (SokosHotel Tahkovuori)**

GRADUATE SCHOOL OF MOLECULAR MEDICINE

WINTER SCHOOL 2010

MARCH 16 – 17, TAHKOVUORI NILSIÄ

PROGRAM

Wednesday, March 17

7:00 – 8:30	Breakfast (SokosHotel Tahkovuori)
8:30 – 9:50	**ORAL SESSION V** *Chairs: Kati Pulkkinen and Jagadish Vangipurapu*
8:30 – 8:50	Vascular and metabolic effects of Nrf2 ***Anna-Liisa Levonen***
8:50 – 9:05	Nitro-fatty acids activate Nrf2 by Keap1 cysteine 151-independent mechanism ***Emilia Kansanen***
9:05 – 9:20	Antioxidant response element – regulated vectors for cancer gene therapy ***Hanna Leinonen***
9:20 – 9:35	SUR1-E1506K knock-out mouse: a disease model for impaired insulin secretion and type 2 diabetes ***Maija Tusa***
9:35 – 9:50	Characterization of aP2-SSAT mouse line ***Taina Roiha***
9:50 – 10:00	**BREAK**
10:00 – 11:20	**ORAL SESSION VI** *Chairs: Sini Pirnes-Karhu and Mitja Kurki*
10:00 – 10:15	SIRT1 regulates TCF7L2 in pancreatic beta-cells and adipocytes ***Shalem Raju Modi***
10:15 – 10:30	Association of 16 SNPs increasing fasting glucose level with indices of insulin release, proinsulin conversion, and insulin sensitivity in 6,696 non-diabetic Finnish men ***Alena Stanèáková***
10:30 – 10:45	Biodistribution of IGF-1 in transgenic CLN mice – a novel candidate for the treatment of INCL ***Tuulia Huhtala***
10:45 – 11:00	Biofunctionalization of mesoporous silicon nanoparticles for targeted peptide delivery ***Jussi Rytkönen***
11:00 – 11:20	Programming organogenesis: the kidney as a model system ***Seppo Vainio***
11:20 – 12:20	**LUNCH & EXHIBITION (TahkoSpa)**
12:20 – 13:20	**ORAL SESSION VII** *Chairs: Sara Paulo and Yuriy Pomeshchik*
12:15 – 12:35	Multifaceted nature of inflammation in neurodegenerative diseases ***Tarja Malm***
12:35 – 12:50	Role of NF-kappa-B p50 gene on behavior and neuropathology of APP/ PS1 transgenic mouse model of Alzheimer's disease ***Lakshman Puli***

12:50 – 13:05	Neuroinflammation – stem cell derived neural progenitors and inflammatory response in a mouse model of cerebral stroke ***Kaisa Savolainen***
13:05 – 13:20	Novel beneficial properties of granulocyte colony stimulating factor treatment in mouse model of amyotrophic lateral sclerosis ***Eveliina Pollari***
13:20 – 13:30	**BREAK**
13:30 – 14:15	**ORAL SESSION VIII** *Chairs: Maykel Lopez Rodriguez and Juha-Pekka Niskanen*
13:30 – 13:45	Protein disulphide isomerase regulates SOD1 activity and controls cytochrome C-catalyzed peroxidation – implications for mitochondrial ROS production in ALS models ***Merja Jaronen***
13:45 – 14:00	Characterization of the phagocytic capacity of bone marrow derived monocytic cells towards A-beta ***Ekaterina Savchenko***
14:00 – 14:15	Organotypic slice culture in spinal cord injury ***Susanna Boman***
14:15 – 14:45	**COFFEE**
14:45 – 15:05	Awards committee Best presentations Director of the GSMM, Best doctoral thesis 2009 Closing of the symposium
15:05 –	Departure

GRADUATE SCHOOL OF MOLECULAR MEDICINE

WINTER SCHOOL 2010

MARCH 16 – 17, TAHKOVUORI NILSIÄ

PROGRAM

Posters:

1. Creation of rtTA-shVEGF-A mice ***Riina Rissanen***
2. Mutant-SOD1 toxicity in BV2 microglial cells ***Sara Paulo***
3. fMRI based detection of brain regions involved in activation of trigeminal nociceptive system in migraine-like states ***Artem Shatillo***
4. Independent enrichment analysis of gene sets ***Mitja Kurki***
5. A naturally occurring dominant-negative splice variant regulates the responsiveness of G-alphaq-regulated rhoGTPase activators ***Lili Li***
6. Effects of CD36 inhibition in the outcome of cerebral ischemia ***Hiramani Dhungana***
7. Alternative splicing is regulated by weight loss and differs between fat depots in humans ***Tiina Kuulasmaa***
8. Immunological characterization of spermidine/ spermine-N1-acetyltransferase (SSAT) transgenic mice ***Sini Pirnes-Karhu***
9. Identification of genes involved in short term and long term hyperexcitable brain states ***Natalia Sherina***
10. Targeting MAPKKK as molecular therapeutic candidates for MAPK-related pathological conditions ***Maykel Lopez Rodriguez***
11. VEGF-B167 over expression cardiac hypertrophy and exercise ***Anniina Muhonen***
12. Evaluation of functional deficit and recovery in the rat somatosensory cortex after moderate traumatic brain injury ***Juha-Pekka Niskanen***
13. Impact of A-beta conformers on hippocampal and cortical cell activity ***Dorota Kaminska***
14. ***Kimmo Nikkanen*** (BCO)

Oral Session I

Chairs:

– Lili Li & Antti Kurronen –

ALZHEIMER'S DISEASE-ASSOCIATED UBIQUILIN-1 REGULATES PRESENILIN-1 ACCUMULATION AND AGGRESOME FORMATION

Jayashree Viswanathan, Annakaisa Haapasalo, Claudia Böttcher, Riitta Miettinen, Kaisa Kurkinen, Christa Maynard, Alice Lu, Lars Bertram, Hilkka Soininen, Rudolph Tanzi, Nico Dantuma and Mikko Hiltunen

Institute of Clinical Medicine - Neurology, University of Eastern Finland, Kuopio.

Ubiquilin-1 is a ubiquitin-like protein genetically and functionally associated to Alzheimer's disease (AD). It mediates proteasomal degradation of proteins including AD-associated presenilin-1 (PS1) and causes PS1 accumulation. Here we characterized the effects of different ubiquilin-1 transcript variants (TV), full-length TV1 and the proteasome-interaction domain lacking TV3, on PS1 accumulation and ubiquitin-proteasome system (UPS). TV1 or TV3 and PS1 cDNAs were transiently transfected to human embryonic kidney cells overexpressing APP (HEK293-AP-APP). The effects were assessed by Western blotting, fluorescence and electron microscopy (EM) and ELISA. Co-expression of especially TV3 with PS1 induced the accumulation and stabilization of PS1, effects not caused by a general impairment of UPS. Accumulated PS1 co-localized with TV1 and TV3 in juxtanuclear aggresomes in both HEK293-AP-APP and cortical cells. EM confirmed the presence of TV1 and TV3 in aggresomes and autophagosomes. PS1 accumulation and aggresome formation coincided with decreased production of A-beta, particularly in TV3-expressing cells. Our results suggest that specific ubiquilin-1 TVs regulate PS1 accumulation and targeting into the aggresome-autophagosome pathway reducing the available PS1 for gamma-secretase complex hence reducing A-beta production.

GENETIC STUDY OF 22 POTENTIAL ALZHEIMER'S DISEASE RISK GENES SELECTED ON THE BASIS OF THE ALZGENE DATABASE META-ANALYSES

Timo Sarajärvi, Seppo Helisalmi, Leila Antikainen, Petra Mäkinen, Anne Maria Koivisto, Annakaisa Haapasalo, Hilkka Soininen, Mikko Hiltunen

Institute of Clinical Medicine – Neurology, University of Eastern Finland and Department of Neurology, Kuopio University Hospital, Kuopio, Finland.

Alzheimer's disease (AD) is a genetically complex disorder encompassing several individual susceptibility genes with low risk effects. In order to assess the risk gene profile among Finnish AD patients, we selected 22 most promising candidate genes on the basis of the meta-analyses retrieved from the AlzGene database. Single nucleotide polymorphisms (SNP) from 22 genes were genotyped from 647 Finnish AD patients and 687 cognitively healthy controls using Sequenom platform. Subsequently, logistic regression and Kaplan-Meier analyses were performed to assess single SNP effects on the AD risk and the age of onset. SNP data were also correlated to cerebrospinal fluid (CSF) A-beta-42, total tau and phosphorylated tau levels in AD patients. A statistically significant genotype and allele association with AD was observed with rs1800529 in the TNF-alpha gene ($p<0.05$). APOE, gender and age-adjusted logistic regression analysis revealed a protective effect for A allele carriers of rs1800529 ($p=0.02$: OR=0.69, 95% CI 0.49-0.95). Also, AD patients carrying the minor allele of rs600879 in SORCS1 showed a statistically significant increase in CSF A-beta-42 levels ($p<0.05$), whereas rs1143634 allele in IL1B associated with increased phosphorylated tau levels in the CSF ($p<0.05$). These findings suggest that rs1800529 in the TNF-alpha gene encompasses a protective effect among Finnish AD patients.

ALZHEIMER'S DISEASE-ASSOCIATED PROTEIN UBIQUILIN-1 REGULATES BACE1 TRANSLATION THROUGH DECREASED EIF2-ALPHA PHOSPHORYLATION IN ER-STRESS

Teemu Natunen, Kaisa Kurkinen, Jayashree Viswanathan, Petra Mäkinen, Hilkka Soininen, Annakaisa Haapasalo, Mikko Hiltunen

Institute of Clinical Medicine – Neurology, University of Eastern Finland and Department of Neurology, Kuopio University Hospital, Kuopio, Finland.

Endoplasmic reticulum (ER) stress plays an important role in the pathogenesis of Alzheimer's disease (AD). We have previously shown that over-expression of the AD-associated protein, ubiquilin-1 alleviates pro-apoptotic C/ EBP homologous protein (CHOP) induction during ER-stress and increases cell viability. Interestingly, phosphorylation of Ser51 site in the translation initiation factor eIF2-alpha has been shown to correlate with beta-secretase (BACE1) levels in different stress conditions. Here we have assessed whether ubiquilin-1 regulates BACE1 translation through eIF2-alpha phoshorylation in ER-stress. To address changes in BACE1 translation, BACE1 cDNA constructs with or without endogenous 5'-UTR were transfected into human neuroglioma H4 cells stably over-expressing ubiquilin-1. ER-stress was induced using tunicamycin and protein lysates were analyzed by Western blotting. Over-expression of ubiquilin-1 decreased Ser51 phosphorylation of eIF2-alpha on average by 40% after 5-hour tunicamycin treatment when compared to naïve cells. Decreased eIF2-alpha phoshorylation status in turn correlated with reduced BACE1 levels in ubiquilin-1 over-expressing H4 cells. These findings suggest that ubiquilin-1 regulates BACE1 translation through eIF2-alpha phosphorylation in ER-stress.

SODIUM CHANNEL BLOCKING ANTIEPILEPTIC DRUGS SUPPRESS SPONTANEOUS EPILEPTIFORM DISCHARGES IN APDE9 MOUSE MODEL OF ALZHEIMER'S DISEASE

Sofya Ziyatdinova[1], Kestutis Gurevicius[1], Tamuna Bolkvadze[1], Heikki Tanila[1,2], Asla Pitkänen[1,2]

[1] Department of Neurobiology, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, [2] Department of Neurology, Kuopio University Hospital, Kuopio, Finland.

Alzheimer's disease (AD) patients have a higher risk of developing epileptic seizures as compare with general population. Spontaneous seizures and frequent epileptiform discharges (EDs) are reported in mouse models of AD with increased amyloid beta peptide production. Sodium channels deficiency in GABAergic interneurons has been proposed to contribute to hyperexcitability. This gave rise to hypothesis that drugs blocking sodium channels might exacerbate spontaneous seizures or EDs. We aimed to study the effect of common used sodium channel blocking antiepileptic drugs (AEDs) on spontaneous seizures or EDs in APdE9 mice. 12-13 wk old APdE9 mice were successively treated with vehicle, followed by CBZ (10 mg/ kg, t.i.d.), DPH (10 mg/ kg, t.i.d.), or VPA (260 mg/ kg, b.i.d.) for 3 d. After wash-out and new vehicle treatment, higher doses of CBZ (40 mg/ kg, t.i.d.), DPH (40 mg/ kg, t.i.d.), or VPA (400 mg/ kg, b.i.d.) were administered for 5 d. During the experiment, mice were under continuous (24/ 7) video-EEG monitoring. Each treatment reduced the number of spontaneous electrographic EDs. VPA was the most effective by reducing the ED frequency below 50% of that at baseline in 75% of mice. No exacerbation of spontaneous seizures or EDs was seen even at AED doses close to TD50.

LOSS OF HIPPOCAMPAL GABAERGIC INTERNEURONS DURING EPILEPTOGENESIS: COMPARISON OF SE AND TBI AS EPILEPTOGENIC ETIOLOGY

Noora Huusko, Christine Römer, Asla Pitkänen

Epilepsy Research Laboratory, Department of Neurobiology, A.I. Virtanen Institute for Molecular Science, University of Eastern Finland, Kuopio.

Brain insults can trigger epileptogenic process which after a latency phase leads to the development of spontaneous seizures and epilepsy. In adult brain, the changes in GABAergic network in post-injury hippocampus can play a major role in epileptogenesis. Two models of temporal lobe epilepsy were used, one induced by traumatic brain injury (TBI) and another with status epilepticus (SE). After 7 months both SE and TBI animals were video-EEG monitored to detect spontaneous seizures. TBI rats were also tested in pentylenetetrazol seizure threshold test. Brains were processed for immunohistochemical analysis of GABAergic neurons (NPY, PARV, CR, CCK, SOM). After TBI, there was a decrease in the number of all different types of GABAergic neurons in the hilus ($p<0.05$). These rats had no spontaneous seizures during video-EEG monitoring but seizure threshold was lowered. Only NPY and PARV-ir neurons were reduced after SE ($p<0.05$). Interestingly, these rats had spontaneous seizures. These observations suggest that reduced perisomatic inhibition in the dentate gyrus can contribute to seizure susceptibility. However, even a dramatic loss in hilar inhibitory neurons is not necessarily associated with occurrence of spontaneous seizures.

Oral Session II

Chairs:

– Miika Martikainen & Hanna Leskinen –

MDGI IN CANCER PROGRESSION AND METASTASIS

Katja Häkkinen[1], Johanna Lammi[2], Kirsi Vuorinen[1], Outi Rautsi[1], Pirjo Laakkonen[1,2]
[1] A. I. V. Institute, Department of Biotechnology & Molecular Medicine, University of Eastern Finland, Kuopio, [2] Molecular Cancer Biology Research Program & Institute of Biomedicine, University of Helsinki.

We have recently identified a novel peptide that homes and binds specifically to malignant brain tumors. Mammary derived growth inhibitor (MDGI) was identified as the receptor molecule for our peptide. MDGI belongs to the family of fatty acid –binding proteins and it is the only FABP that affects cell proliferation. FABPs reside normally in the cytoplasm. However, in tumors MDGI is found outside of the cells and associated with the tumor vasculature where it is accessible to circulatory ligands. The role of MDGI in tumor genesis is controversial. In order to study the functional role of MDGI in tumor progression and metastatic spread we created stable MDA-MB-231 human breast carcinoma cell lines expressing MDGI. The parental MDA-MB-231 cells express MDGI at very low level. In the current study we compared the growth and metastatic spread of MDGI-MDA and the parental MDA-MB-231 subcutaneous tumors in Balb/ c/ Sca-nu/ nu mice. Preliminary data from the growth curves of the xenograft tumors show that tumors with high MDGI expression grow slower than the parental tumors. The analysis of the reasons for the growth difference and metastatic potential of these tumors is ongoing. Our results will provide new information about the role of MDGI in cancer progression and metastasis.

15-LO-1 INDUCED APOPTOSIS INHIBITS TUMOUR GROWTH AND EXTENDS SURVIVAL IN RAT GLIOMA MODEL

Agnieszka Pacholska, Helena Viita, Farizan Ahmad, Essi Uusitalo, Anna Hyvärinen, Thomas Wirth and Seppo Ylä-Herttuala
Department of Biotechnology and Molecular Medicine, A. I. Virtanen Institute for Molecular Sciences.

Gliomas are among the most vascularised tumours in humans, what makes them a very promising target for anti-angiogenic therapies. It is known that 15-LO-1 may possess strong anti-angiogenic properties. We have proved that it as well induces apoptosis by induction of lipid peroxidation in vivo. Therefore, we concluded that 15-LO-1 gene therapy could be used as a promising cancer treatment acting through two independent mechanisms with pro-apoptotic therapeutic effects additionally being complemented by inhibition of tumour angiogenesis. BDIX male rats were injected with 10^4 BT4C glioma cells into the right corpus callosum. The rats were divided into two cohorts: group I, which received adenoviral vector carrying 15-LO-1 transgene and group II, which was a control group. Tumour growth was verified 14 days after cell injection by magnetic resonance imaging (MRI). On the consecutive day animals received 3 injections of Adh15-LO-1, followed by two additional ones applied the next day. We have shown that 15-LO-1 delayed tumour growth and extended survival by 18 % in rat malignant glioma model. Those results support the pro-apoptotic role of 15-LO-1 and prove that it could be a potential new treatment gene for the therapy of malignant glioma.

SUSCEPTIBILITY TO ALPHAVIRUS-MEDIATED ONCOLYSIS OF GL261 AND CT-2A SYNGENEIC MOUSE GLIOMAS IN VITRO AND IN VIVO

Janne Ruotsalainen[1], Miika Martikainen[1], Tytti Aaltonen[2], Jari Heikkilä[2], Markus Vähä-Koskela[3], Ari Hinkkanen[1]

[1] A. I. Virtanen Institute for Molecular Medicine, University of Eastern Finland, Kuopio, [2] Department of Biochemistry, Åbo Akademi, Turku, Finland, [3] Ottawa Health Research Institute, Canada.

Semliki Forest virus derived vector VA7 effectively infects and kills a number of human and murine tumor cells in vitro and in vivo when implanted in immune compromised mice. Cell cultures of syngeneic glioma (GL261) and astrocytoma (CT2A) were established and VA7-EGFP infectivity and innate immune responses were analyzed with fluorescence microscopy and Western blotting respectively. For in vivo studies GL261 cells were injected intracranially into the caudate putamen of immunocompetent C57BL/6 mice and after the tumors had developed, 10>6 pfu of VA7 vector was injected via tail vein. Both cell lines were infected by VA7 in culture and 70% and 95% lysis was reached after 72 hrs p.i. for CT-2A and GL261 cells, respectively. Infection with VA7 of GL261 cell line resulted in expression of antiviral oligoadenylate synthase (OAS) active (40 kDa) form, whereas PKR and eIF-2a levels remained unchanged. In the pilot in vivo studies, none of the treated mice survived even when immunosuppsessed with CPA. We conclude that innate immune responses including interferon-related factors expressed in tumor cells upon infection with VA7 pose hurdles to vector replication and oncolytic efficacy. Alternative approaches to evade the immunoresponse are needed.

Oral Session III

Chairs:

– Suvi Jauhiainen & Anastasia Shakirzyanova –

VASCULAR ENDOTHELIAL GROWTH FACTOR (RECEPTOR) EXPRESSION DURING ADIPOCYTE DIFFERENTIATION IN VITRO

Marike Dijkstra, Eija Pirinen, Seppo Ylä-Herttuala
Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio.

Adipogenesis is tightly associated with angiogenesis. VEGF-A accounts for most of the angiogenic activity of adipose tissue. VEGF-A is an angiogenic factor expressed in and secreted by adipocytes in vivo and in vitro. The expression pattern of the VEGF receptors and the other VEGF family members have not been investigated fully and their role in adipocyte differentiation in vivo and in vitro is still unclear. Therefore, the aim of this study was investigate the expression pattern of VEGF(R)s in differentiated adipocytes in vitro. 3T3-L1 mouse fibroblasts were differentiated into adipocytes following standard protocol. Messenger RNA levels of VEGF-A, VEGF-B, VEGF Receptor-1(VEGFR1), VEGFR2 and neuropilin-1 (nrp1) and nrp2 were analyzed at different time points using Taqman RT-PCR. Adipocyte differentiation was confirmed by Oil Red O staining and increased mRNA expression of PPAR-gamma and CEBP-alpha. During adipocyte differentiation, expression of VEGF-A, VEGF-B, and nrp1 were increased while VEGFR1 and nrp2 mRNA levels decreased. No VEGFR2 mRNA was detected. These results suggest that the main role for VEGF-A in adipose tissue is activation of endothelial cells as VEGFR2 is exclusively expressed in endothelial cells in adipose tissue. In addition, the switch in expression levels of the VEGF-B binding receptors VEGFR1 and nrp1 in adipocytes implies different signaling pathways induced by VEGF-B in lipid loaded compared to non-differentiated adipocytes.

THE ROLE OF VEGF-A / PLC-GAMMA PATHWAY IN CARDIAC MYOCYTES

Genet S. Assefa, Marike H. Dijkstra, Hanna Leskinen and Pasi Tavi
Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio.

Vascular endothelial growth factor A (VEGF-A) is a potent angiogenic factor. In endothelial cells the signaling of VEGF-A is mediated by two tyrosine kinase receptors, VEGFR-1 & -2. VEGF-A activates phospholipase C-gamma (PLC-gamma) leading to formation of inositol 1, 4, 5 -trisphosphate (IP3) and diacylglycerol (DAG). The IP3 increases cytosolic Ca2+, whereas DAG activates protein kinase C (PKC) pathway. PLC-gamma/ PKC-pathway is known regulator of cardiomyocyte contractility. Aim of the study is to characterize the effects of VEGF-A on PKC and Ca signaling in cardiac myocytes. Neonatal mouse or rat cardiomyocytes were incubated with VEGF-A164 in concentrations of 0, 5, 25 or 100 ng/ ml for 48 h. RNA was extracted for quantitative RT-PCR and cytosolic and membrane fraction of proteins was extracted for Western blotting. VEGF-A164 dose-dependently increased expression of VEGFR-1 & -2 in cardiac myocytes (n=4/ group). VEGF-A164 (25 ng/ ml) induced VEGFR-1 expression to 2.1-fold ($p<0.05$) and VEGFR-2 expression to 5.2-fold ($p<0.01$). Translocation of PKC-e and PKC-d from cytosolic fraction to membrane fraction increased to 3.0-fold ($p<0.01$) and 2.2-fold ($p=0.05$) in VEGF-A164 (25 ng/ ml) treated cells (n=3 / group), whereas no change in PKC-a translocation was seen. In conclusion, our data show that VEGF-A164 up regulates the expression of VEGF receptors and activates PKC signaling in cardiac myocytes suggesting a potential role for VEGFs in the regulation of cardiomyocyte contractility.

OXIDATIVE STRESS IN MIGRAINE PAIN TRANSDUCTION

O. Kyrylenko, A. Shakirzyanova, R. Giniatullina,, N. Naumenko, G. Bart, D. Fayuk, K. Kanninen, J. Koistinaho, R. Giniatullin

Department of Neurobiology, A.I.Virtanen Institute, University of Eastern Finland.

Migraine is a type of chronic pain associated with oxidative stress but whether pain transduction in migraine is affected by reactive oxygen species (ROS) is unknown. In this study, using patch clamp and Ca2+ imaging technique, together with various biochemical approaches (NAD and FOX1 assays), we tested the role of ROS on expression and function of pain transducing ATP-gated P2X3 and capsaicin-activated TRPV1 receptors of rat trigeminal neurons. Application of hydrogen peroxide for 5 h to cultured trigeminal neurons increased expression and activity of TRPV1 receptor, whereas expression and function of P2X3 receptor was not affected. We also observed a dramatic decrease of NAD+ in peroxide-treated cells. Interestingly, in Nrf2 knock-out mice, with compromised antioxidant defense, we found decreased expression and activity of TRPV1 receptor along with increased expression of P2X3 receptor, with no changes in NAD+ level. These data indicate high plasticity of pain transducers associated with different profile of ROS and the level of intracellular NAD.

Oral Session IV

Chairs:

– Teemu Laitinen & Dorota Kaminska –

IMPLEMENTATION OF PHARMACOLOGICAL MRI

Joanna Huttunen[1], Antti Airaksinen[1], Juha-Pekka Niskanen[1], Kimmo Lehtimäki[2], Olli Gröhn[1]

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Pharmacological magnetic resonance imaging (phMRI) is a novel MRI method where the pharmacological agent causes local activation or deactivation in the brain and the sequential changes in the cerebral blood flow, volume and oxygenation can be measured with BOLD contrast. The aim of this work was to implement a robust approach to pharmacological MRI. Nicotine (n=4, 0.25-0.5 mg/ kg, i.v.) caused large cortical activation in freely breathing animals under urethane anesthesia. Donepezil (n=9, 3 mg/ kg, s.c.), which is an acetyl cholinesterase inhibitor, induced also muscle twitching and hence a huge motion artifact to the MR images. Ventilation of the animals and a dosage of muscle relaxant (pancuronium bromide, 0.5 mg/ kg/ h, i.v.) abolished all motion artifacts but had interaction with donepezil. Therefore the effects of apomorfine (n=3, 0.25 mg/ kg, s.c.), which is a dopamine receptor agonist, was also investigated. The selection of anesthetics, muscle relaxants and pharmacological agents is important and should be carefully considered to avoid any undesirable interaction effects. Despite that, pharmacological MRI offers unique, non-invasive view to study pharmacokinetics, receptor activity and drug actions in normal and pathological brain.

SIMULTANEOUS BOLD FMRI AND LOCAL FIELD POTENTIAL MEASUREMENTS OF EPILEPTIC SEIZURES IN AWAKE AND MEDETOMIDINE SEDATED RATS

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In the present study, we focused on the effects of medetomidine (domitor) sedation on simultaneous local field potential (LFP)/ fMRI variables. First, we determined the baseline cerebral blood flow (CBF) in awake and anesthetized (domitor and isoflurane) rats. Second, simultaneous LFP/ fMRI measurements were performed to compare BOLD signal changes, caused by kainic acid (KA) induced epileptic seizures in awake and domitor sedated rats. CBF was studied using CASL technique. fMRI data were acquired using EPI sequence. After KA injection (i.p., 10 mg/ kg), image acquisition was continued for 1.5 hours for each rat. The highest CBF values were observed in isoflurane anesthetized rats, the lowest ones were detected in domitor sedated animals. After KA injection in awake and domitor sedated rats, the LFP data showed epileptic seizures in the hippocampus. During this period, there were strong bilateral BOLD signal changes in the hippocampus. In all rats, positive signal increases in EPI time courses were observed coincide with the observed seizures in LFP signal. We conclude that domitor sedation is well suited for studies of the normal and pathologic rat brain, but a basal CBF level that is lower than that of awake rats should be taken into account when interpreting the fMRI results.

MRI DETECTION OF SHORT T2 COMPONENT IN BRAIN BY SWIFT

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Tissue components such as lipids in cell membranes are likely to have very short transverse relaxation times, T2, making their detection practically impossible with conventional MRI methods. SWIFT is an imaging technique capable of detecting them, but since virtually the whole spectrum of signals with different T2's is detected, short T2 components are overpowered by the long T2 components. In this study, two simple long T2 suppression methods combined with SWIFT were investigated. Additionally, an alternative to suppression is introduced. First method was based on the idea that short T2 components do not follow long pulses since their relaxation rate exceeds the excitation rate of the pulse. The preparation consisted of an adiabatic inversion followed by a delay. Two different pulse lengths were compared. The second method was based on a subtraction of a normal and a short T2 suppressed image. An ex vivo mouse brain was used as a sample. All methods highlighted the white matter tracts. The subtraction method had a considerably higher SNR than the long pulse method. Using the first method, suppression was higher with a shorter pulse. Results give promise that short T2 components can be highlighted in SWIFT with different long T2 suppression methods.

Poster Session

CREATION OF RTTA-SHVEGF-A MICE

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It is known that the inhibition of VEGF-A induces for example hypertension, proteinuria and renal endothelial dysfunction. In humans VEGF-A antibody Avastin, that is used in anticancer therapy, also causes severe thromboembolic complications in up to 5 % of treated patients. Therefore, we wanted to study how much VEGF-A levels can be down-regulated without severe side effects. For these purposes, lentiviral vector with doxycyclin inducible shRNA silencing VEGF-A via RNAi was created. Functionality of the vector was tested in our laboratory and the in vitro results show efficient and doxycyclin dose dependent manner of VEGF-A silencing. Also doxycyclin induced positive feedback loop was observed. By using this vector we have further created a mouse strain via lentiviral transgenesis. Copynumber analysis done with southern blotting revealed that most of the founder mice had only few transgene copies in their genome. The mice have been bred further successfully. In the future we will characterize the mouse strain and the in vivo functionality of our viral construct in more detail. We will study the effects of VEGF-A down-regulation in different tissues by ultrasound and immunohistological methods.

MUTANT-SOD1 TOXICITY IN BV2 MICROGLIAL CELLS

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Mutations in Cu,Zn superoxide dismutase (SOD1) causes familial amyotrophic lateral sclerosis (ALS). ALS is a non-cell-autonomous disease, where mutant SOD1 expression in microglia has been suggested to contribute to degeneration of motor neurons. Here, we studied whether expression of various mutant SOD1s in microglial BV2 cell line is toxic and alters nitric oxide (NO) production in these cells. Some antioxidative compounds were tested regarding their ability to rescue the cells. BV2 microglial cells were transfected with SOD1 dimers (G37R-G37R, G37R-WT, G85R-G85R, G85R-WT and WT-WT). After 24 h transfection western blot was used to assess the transgene expression. Cell survival after transfection was determined with a resazurin assay and NO-release was measured using Griess reagent. Catalase, ebselen, minocycline and apocynin were applied for the duration of transfection. We found that G37R-G37R-SOD1 was the most toxic to BV2 cells, resulting in a loss of about 40 % viable cells compared to non-transfected cells and loss of 24 % compared to WT-WT-transfected cells after 24 h transfection. Minocycline reduced G37R-G37R-SOD1 toxicity and had a tendency to suppress NO-release. Apocynin suppresed NO-production but did not inhibit the toxicity. We conclude that G37R-G37R-SOD1-toxicity to BV2 cells is not attributable to increased NO-production and that minocycline protects microglial cells from this toxicity.

FMRI BASED DETECTION OF BRAIN REGIONS INVOLVED IN ACTIVATION OF TRIGEMINAL NOCICEPTIVE SYSTEM IN MIGRAINE-LIKE STATES

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Migraine is the most common neurological disease but, paradoxically, the mechanism of persisting pain in migraine remains unknown. We plan to study brain processing of sensory inputs induced by activation of trigeminal nociceptive system, utilizing its unique property to express tetrodotoxin (TTX) insensitive sodium channels. Stimulating sensory neurons innervating whiskers pad of Wistar rats in the presence of TTX and register using fMRI change of central presentation of peripheral nociception. After urethane anesthesia stimulatory and recording electrodes will be implemented into the whiskers pads and brain respectively. Rats will obtain a sc injection of TTX in the area between stimulatory electrodes and then transferred to 4.7 T or 9.4 T Varian horizontal MRI system. Area for fMRI will be identified electrophysiologically as active regions responding to electrical stimulation of whickers. We will use multislice spin-echo sequence (TR 2.5s, TE 60ms, 256×256, FOV 5 cm) for anatomical images and spin echo EPI sequence (TR 2s, TE 60ms, 64x64, FOV 2.5x2.5cm2, slice thickness 1.5 mm, 5 slices) for fMRI. We aim to increase understanding of the pathophysiological mechanism of the migraine pain and develop novel animal model which can provide a platform for pre-clinical drug testing.

INDEPENDENT ENRICHMENT ANALYSIS OF GENE SETS

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Standard enrichment analysis of differentially expressed (DE) genes obtained from gene expression studies finds over-represented annotations such as functions or pathways. However, DE genes often include multiple gene subsets associated to distinct annotations that may be overlooked by standard EA. We present a novel method and software tool TAFFEL that eases the analysis of DE genes by clustering the genes to groups that have similar biological functions or are co-regulated by common transcription factors (TFs). As major improvement to previous methods TAFFEL performs Independent Enrichment Analysis (IEA), which finds functionally homogeneous gene clusters that enrich TFs and gene clusters homogeneous in regulatory TFs that enrich functional annotations. This is used as evaluation to find relevant clusters for the user among several and to discover the control between regulatory proteins and functions in studied conditions. We show that TAFFEL can find expected and novel results by testing it with gene expression data obtained from human hepatocyte treated with forskolin. Secondly, we present analysis of DE from comparison of ruptured and unruptured saccular cerebral artery aneurysm walls. The results show usefulness of our method in analysis of such uncharacterized conditions.

A NATURALLY OCCURRING DOMINANT-NEGATIVE SPLICE VARIANT REGULATES THE RESPONSIVENESS OF G-ALPHAQ-REGULATED RHOGTPASE ACTIVATORS

Lili Li, Tim Church, Soila Tossavainen, Suvi Parviainen, Veronika Redai, Michael Courtney

RhoGTPases have a powerful influence on cell behavior and fate via regulation of transcription and the cytoskeleton. Extracellular signals activate RhoGTPases via G protein coupled receptors. This is mediated via well studied G-alpha-12/ 13-regulated GTPase activators and poorly characterized G-alphaq-regulated activators of the neuronally-enriched p63-Kalirin-Trio family (PKT-GEFs) implicated in schizophrenia and other diseases. We have identified and used molecular biological and biochemical methods to characterize a novel PKT-GEF splice variant, x12. This variant is expressed in several different cell types. x12 is not merely inactive as a Rho activator, lacking critical Dbl-homology residues, but it also acts as a dominant negative inhibitor of PKT-GEFs. Deletion analysis reveals the region sufficient for inhibition, which we find possesses an unexpected yet strong homophilic interaction which may be responsible for the inhibitory action of this variant. We propose a model whereby activation of PKT-GEFs renders them competent to interact in trans with the inhibitory region of x12. The variant may selectively target the activated PKT-GEF population, thereby sharpening the response curve to activation signals to one with a more all-or-none character.

EFFECTS OF CD36 INHIBITION IN THE OUTCOME OF CEREBRAL ISCHEMIA

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Cerebral ischemia is the leading cause of disability and death worldwide and requires long term hospitalization. It is caused by cessation of blood flow to the brain resulting in insufficient delivery of oxygen and nutrients to neuronal tissues. Ischemic damage initiates inflammatory process as a defence mechanism in an attempt to restore tissue homeostasis. However, in acute phase of stroke, inflammation contributes to the development of delayed brain damage. Therefore, it has been suggested that anti-inflammatory treatment may be beneficial for stroke patients. Fatty acid translocase, CD36, is a class B scavanger receptor that induces inflammatory response by activating NF-kappa-B. Since CD36 has been shown to be specifically involved in inflammatory reactions in ischemic brain, pharmacological inhibition of CD36 has been suggested a potential treatment for stroke. In this study we inhibited CD36 by using sulfosuccinimidyl oleate sodium (SSO) to elucidate the role of CD36 in cerebral ischemia. Balbc mice underwent permanent middle cerebral artery occlusion and received SSO at the doses of 20mg/ kg intraperitoneally 1 hour after induction of ischemia. Ischemic cell death was determined by 2,3,5-triphenyltetrazolium chloride (TTC) staining after 48h time point. Preliminary data from TTC staining suggest a protective role of SSO by reducing acute edema associated with ischemia. Further studies will be carried out to confirm the effect of SSO and CD36 inhibition in ischemic damage.

ALTERNATIVE SPLICING IS REGULATED BY WEIGHT LOSS AND DIFFERS BETWEEN FAT DEPOTS IN HUMANS

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Alternative splicing leads to several mRNA isoforms, and often to multiple protein variants. Our overall aim is to study the contribution of alternative splicing in type 2 diabetes (T2DM) and obesity. We therefore utilized Affymetrix Human Exon 1.0 ST arrays analyzed with Partek Genomics Suite and Bioconductor's Exonmap to examine the effect of weight loss on splicing of T2DM candidate genes and novel genes, using adipose tissue samples taken from 11 subjects prior to and following gastric bypass surgery. Additionally, PCR-capillary electrophoresis method was developed for efficient screening of alternative spliced exons in candidate genes. In response to weight loss we observed alternative splicing in exon 4 and 3' exons of TCF7L2, in exon 9 of GIPR and in exon 11 of INSR (all $p<0.0001$) Interestingly, we also observed altered splicing patterns between subcutaneous and visceral fat, including TCF7L2 in both exon 4 and 3' exons, and in LPIN1 exon 7. Additional alternative spliced genes in response to weight loss and between subcutaneous and visceral fat depots have been identified, and results will be presented. We conclude that alternative splicing is regulated by both environmental effects (e.g. weight loss) and anatomical location of adipose depot. Together, our data suggest that non-coding SNPs in known T2DM genes may contribute to disease risk by modifying alternative splicing of candidate genes.

IMMUNOLOGICAL CHARACTERIZATION OF SPERMIDINE/SPERMINE-N1-ACETYLTRANSFERASE (SSAT) TRANSGENIC MICE

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Polyamines are small cationic molecules essential for many cellular processes, including cell growth and differentiation. The mice over-expressing spermidine/ spermine-N1-acetyltransferase (SSAT), a catabolic regulator of polyamines, have several phenotypic and metabolic changes when compared with their non-transgenic littermates. The immunological features of these mice were investigated for instance by histology, blood count, leukocyte FACS analyses, immunoglobulin measurements and haematopoietic stem cell transplantation. The SSAT over-expressing mice exhibited enlarged secondary lymphoid tissues, elevated amount of leukocytes in blood and bone marrow and the relative proportions of T, B, CD4+, CD8+ and Treg cells were altered in blood and spleen. In addition, the relative proportion of IgG was altered in blood. The changes in leukocyte populations seemed to originate from SSAT over-expression in the haematopoietic stem and/ or progenitor cells, as demonstrated by the bone marrow transplantation. The characterization of the disturbed haematopoiesis in the SSAT over-expressing mice will give new information about the role of polyamines and their metabolism in cell proliferation and differentiation processes.

IDENTIFICATION OF GENES INVOLVED IN SHORT TERM AND LONG TERM HYPEREXCITABLE BRAIN STATES

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The transcriptional response to cortical spreading depression (CSD) is crucial for understanding mechanisms responsible for migraine with aura. CSD is potentially able to induce multiple plastic changes including gene transcription. A certain number of genes are up-regulated in response to CSD (for example, COX-2, TNF-a, IL-beta;, iNOS). Nonetheless, a comprehensive evaluation of multiple genes activated in animal models of migraine is not yet available. The aim of this study is to uncover transcriptional responses following induction of migraine-like states in rats. These states will be induced in rats by i.p. injection of nitroglycerin, i.p. injection of LPS, after CSD, and by combination of these factors. mRNA from cortex, trigeminal ganglia and meninges will be used to identify a large cohort of genes activated in migraine states on Microarray chips. The polymerase chain reaction in real time will be used to validate the expression profile of differentially expressed genes involved in various cellular events with stress on those which are involved in pain transduction. This project should provide a comprehensive view on multiple genes activated in the cortex, trigeminal ganglia and provide further explanation of the pathophysiological mechanisms of migraine-like states.

TARGETING MAPKKK AS MOLECULAR THERAPEUTIC CANDIDATES FOR MAPK-RELATED PATHOLOGICAL CONDITIONS

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Mitogen Activated Protein Kinases (MAPK) signalling pathways play key roles in both normal and aberrant physiology. Despite the extensive findings about the roles of MAPK in pathological conditions, it is poorly understood how- under which conditions a specific MAPKKK (upstream MAPK activator) leads to the activation of a MAPK. Within this project we will study further mechanisms of activation of MAPKKK and the specific individual or family contribution to the activation of effectors in cellular conditions where the MAPK axis is implicated. We have developed MEKK-MAPKKK-derived constructs and we have found that two MEKK1 constructs reduced the level of phosphorylated activated JNK in COS-7 cells. We are developing a panel of miRNA plasmids for most of MAPKKK and for its downstream targets MAPKK. These tools will be used in models of neurodegeneration, excitotoxic neuronal death, development, cancer and migration. As results of our project we anticipate a better understanding on the activation of MAPKKK and its individual-family role in specific cellular conditions. As several MAPK- based therapeutic strategies have failed because of lack of specificity, the above outcomes will allow us to develop more specific inhibitory compounds as molecular therapeutics candidates.

VEGF-B167 OVEREXPRESSION, CARDIAC HYPERTROPHY AND EXERCISE

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Coronary artery disease remains the leading cause of death in the Western World. VEGF-B_{167}, the member of vascular endothelial growth factor (VEGF) family, has shown comprehensive effects in myocardial metabolism. The aim was to study how much exercise and cardiac VEGF-B_{167} overexpression could slow down or even prevent already physiologically hypertrophic heart from drifting to the pathological condition. We compared wildtype and transgenic mice in their correspondence to physiological, pathological and both of the previously mentioned hypertrophy types. Physiological hypertrophy was induced with voluntary treadmill exercise (4 or 6 weeks) and pathological hypertrophy with two weeks of angiotensin II treatment. The angiogenic and functional effects were analyzed by ultrasound and histological analyses. RT-PCR was used to analyze mRNA levels of hypertrophy markers and VEGFs. The hypertrophy markers responded as expected while VEGF-A levels remained unchanged. We saw that heart function decreased in transgenic mice with pathological hypertrophy compared to wildtype mice. However, the wildtype mice had more fibrosis. The role of VEGF-B_{167} will be clarified in the future by studying VEGF-B KO animals.

EVALUATION OF FUNCTIONAL DEFICIT AND RECOVERY IN THE RAT SOMATOSENSORY CORTEX AFTER MODERATE TRAUMATIC BRAIN INJURY USING FMRI

Juha-Pekka Niskanen[1,2], Antti Airaksinen[1], Jari Nissinen[1], Asla Pitkänen[1], Olli Gröhn[1]

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Traumatic brain injury (TBI) is a major cause of death and disability worldwide. Impact to the head causes immediate damage and launches complex cascade of secondary injury and recovery processes. The aim of this study was to evaluate the lost of function and potential recovery in the periphery of the lesion in primary somatosensory cortex after moderate TBI using functional magnetic resonance imaging (fMRI). Sprague-Dawley rats with moderate TBI (n=10) and sham operated controls (n=6) were imaged with a 4.7 T MRI scanner during electrical stimulation of the forepaws before TBI and 1, 2 and 8 weeks after TBI. A separate study using simultaneous local field potential and fMRI was conducted to investigate the neuronal coupling of the fMRI response. Ipsilateral somatasensory cortex appeared normal in structural T2-wt MRI. However, response to forepaw stimulation was lost after TBI in trauma animals but was maintained in the contralateral side and in controls. Partial recovery in some of the animals was detected 8 weeks after induction of TBI. Furthermore, simultaneous electrophysiological recordings and fMRI confirmed that the observed hemodynamical changes were of neuronal origin. The results suggest that fMRI could serve as non-invasive tool to evaluate functional recovery after TBI.

IMPACT OF A-BETA CONFORMERS ON HIPPOCAMPAL AND CORTICAL CELL ACTIVITY

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by an accumulation of amyloid plaques, neurofibrillary tangles and synaptic failure that leads to progressive memory loss. A growing body of evidence suggest that the longer 42 aa residue form of the amyloid beta-protein (A-beta), plays a crucial role in AD pathogenesis. A-beta can exist as a monomer, a variety of oligomeric forms and in fibrillar polymers. Recent findings suggest that soluble oligomeric or protofibrillar species may be responsible for impairment of neuronal function. We investigate the impact of defined A-beta species on cell and synaptic activity in both primary hippocampal and cortical cultures and the mechanisms responsible for this modulation. Signalling under basal conditions and in response to synaptic activity are examined by measurement of cytoplasmic free calcium, and activation of small G protein and protein kinase pathways. These are monitored by combinations of fluorescence imaging for single cells recordings and immunoblotting for whole cell population responses. Both oligomeric and protofibrillar A-beta conformers were prepared. We observe selective increase in cytoplasmic free calcium and calcium spiking in response to protofibrillar but not oligomeric A-beta.

Oral Session V

Chairs:

– Kati Pulkkinen & Jagadish Vangipurapu –

NITRO-FATTY ACIDS ACITVATE NRF2 BY KEAP1 CYSTEINE 151-INDEPENDENT MECHANISM

Emilia Kansanen[1], Gustavo Bonacci[2], Henna-Kaisa Jyrkkänen[1], Francisco J. Schopfer[2], Steven R. Woodcock[2], Seppo Ylä-Herttuala[1], Bruce Freeman[2], Anna-Liisa Levonen[1]

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Nitro-fatty acids (nitro-FAs) are electrophilic signaling mediators formed via NO-dependent reactions. Transcriptional profiling of human endothelial cells responses to nitro-OA revealed that nitro-OA activates two distinct pathways, one being activation of the transcription factor Nrf2 and the other involving HSF-mediated transcription. We now report the molecular mechanisms by which nitro-FAs activate Nrf2 signaling, focusing on the hypothesis that post-translational modification of Keap1 cysteines occurs by Michael addition of nitro-FAs to Keap1, and that this event is a significant component of Nrf2 activation and cardiovascular signaling by nitro-FAs. We identified the most nitro-OA-reactive Keap1 cysteines by mass spectrometry and found that nitro-OA adducts C38, C226, C257, C273, C288 and C489. In addition, we show that nitro-OA binds to Keap1 at nanomolar concentrations in a cellular milieu. Notably, the mutation of the highly-reactive C151 of Keap1 did not affect nitro-OA binding. We also utilized reporter gene expression analysis to reveal the functional role of Keap1 C151 and found that, in contrast to "classical" Nrf2-reactive electrophiles, nitro-OA activated Nrf2 independent of Keap1 C151. Keap1 is a critical molecular target for nitro-FAs in Nrf2 activation. Moreover, Keap1 C151-independent activation of Nrf2 contributes to the protective effects of nitro-FAs in the vasculature.

ANTIOXIDANT RESPONSE ELEMENT – REGULATED VECTORS FOR CANCER GENE THERAPY

Hanna Leinonen, Seppo Ylä-Herttuala, Anna-Liisa Levonen

Department of Biotechnology and Molecular Medicine, University of Eastern Finland.

Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor regulating the expression of detoxifying enzymes and other cytoprotective genes via binding to the antioxidant response element (ARE). Several drug-resistant cancer cell lines have high constitutive Nrf2 activity due to mutations in Nrf2 or its inhibitor Keap1. Moreover, certain anticancer drugs activate Nrf2. The aim of this study was therefore to examine whether these attributes could be exploited for cancer gene therapy. To this end, we chose to study two cancer cell lines, non-small lung cancer A549 cells with constitutively high Nrf2 activity, and human breast carcinoma MCF-7 cells, in which ARE can be activated by chemotherapeutic drugs. Using lentiviral ARE-luciferase reporter constructs we found that in A549 cells, constitutive ARE activity is high and cannot be further increased by Nrf2 inducing agents, whereas MCF-7 cells have a low basal activity inducible up to 4.7 fold by Nrf2 inducing agents such as diethylmaleate and an anticancer drug carmustine. We next examined whether high ARE activity in A549 cells is sufficient to drive thymidine kinase (TK) gene for TK-ganciclovir (GCV) suicide gene therapy approach. A549 cells transduced with lentiviral ARE-TK construct had up to 70% reduced viability assessed by MTT assay upon treatment with increasing concentrations of GCV. We conclude that ARE-regulated vectors are promising novel tools for cancer gene therapy especially in cells with high constitutive Nrf2 activity.

SUR1-E1506K KNOCK-OUT MOUSE: A DISEASE MODEL FOR IMPAIRED INSULIN SECRETION AND TYPE 2 DIABETES

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The objective was to identify the mechanism causing decreased insulin secretion and type 2 diabetes in the patients with a point mutation E1506K in the sulfonylurea receptor 1 (SUR1) gene. Although hyperinsulinemic in infancy, the mutation carriers paradoxically develop insulin deficiency in adulthood. To address how the mutation predisposes to diabetes, we acquired a transgenic knock-in mouse carrying the Sur1-E1506K mutation. We studied the glucose metabolism, islet morphology, SUR1 protein levels, and performed gene mRNA expression arrays on liver, islets and beta-cells. Insulin secretion and intracellular calcium responses were measured in vitro. The knock-in mice had abnormal glucose metabolic phenotype, very similar to the adult human SUR1-E1506K carriers. Morphologically the Sur1-E1506K mice had normal islets; Unlike previously hypothesized, there was no apoptosis. Thus, the insulin secretion deficiency was caused by a functional defect. In vitro studies revealed abnormal islet calcium and insulin secretion responses to secretagogues. The gene expression pattern of the beta cells was intricately altered. The next challenge is to pinpoint the detailed molecular mechanism behind the hypoinsulinemic phenotype of the Sur1-E1506K mice.

CHARACTERIZATION OF AP2-SSAT MOUSE LINE

Taina Roiha[1], Eija Pirinen[1], Anne Uimari[1], Susanna Vuohelainen[1], Sini Pirnes-Karhu[1], Markku Laakso[2], Leena Alhonen[1]

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Previous studies with transgenic mice having whole-body overexpression of the key enzyme in polyamine catabolism, spermidine/ spermine N1-acetyltransferase (SSAT), have shown that enhanced polyamine metabolism regulates glucose and energy metabolism. The aim of this study is to specify the consequences of enhanced polyamine catabolism to glucose and energy metabolism in white adipose tissue (WAT). Therefore we have generated a transgenic mouse line (aP2-SSAT mice) overexpressing spermidine/ spermine-N1-acetyltransferase under an adipose tissue specific promoter. Our results showed that, in comparison with wild-type mice, aP2-SSAT mice had significantly reduced WAT mass and the white adipocytes had increased amount of mitochondria. In addition, their oxygen consumption was significantly higher revealing that their energy expenditure is enhanced. These findings were explained by increased expression of the key regulator of mitochondrial biogenesis and energy metabolism, peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC-1 a), in WAT of aP2-SSAT mice. Based on glucose and insulin tolerance tests, no changes in glucose metabolism were observed. When challenged with high-fat diet (HFD), aP2-SSAT mice were resistant to HFD-induced body weight gain. The results of this study suggest that enhanced polyamine catabolism is an important regulator of energy metabolism in WAT. Therefore, this study may help to develop novel therapeutic applications for the treatment of obesity and type 2 diabetes.

Oral Session VI

Chairs:
– Sini Pirnes-Karhu & Mitja Kurki –

SIRT1 REGULATES TCF7L2 IN PANCREATIC BETA-CELLS AND ADIPOCYTES

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Type 2 diabetes is characterized by insulin resistance and impaired insulin secretion. Among 20 susceptibility loci, variants of the transcription factor 7 -like 2 gene (TCF7L2) have been found to have the strongest association with the risk of type 2 diabetes. TCF7L2 plays a key role in insulin secretion and Wnt signaling. We tested whether insulin secretion requires energy and therefore SIRT1, a histone deacetylase and a master regulator of energy balance could be involved in the regulation of TCF7L2 function. We investigated the interaction of TCF7L2 and SIRT1 in pancreatic beta-cells and adipocytes. Treatment with resveratrol enhanced both TCF7L2 and SIRT1 protein levels by 1.6 –fold in MIN6 beta-cells. In contrast, resveratrol did not alter protein levels of AMPK, another key regulator of cellular energy status and metabolism. Resveratrol, a known SIRT1 activator, significantly stimulated insulin secretion in beta-cells in the absence of glucose. In adipocytes (3T3L1 cells) resveratrol induced a significant down-regulation of TCF7L2 by 5 -fold in contrast to our findings in beta-cells. Resveratrol increased SIRT1 and phospho-AMPK protein expression in 3T3L1 cells. Our results suggest that there is a tissue specific regulation of TCF7L2 by SIRT1 activation. In beta-cells TCF7L2 and SIRT1 interaction may involve a cross- talk between GLP-1 mediated Wnt signaling and insulin signaling. The mechanisms why and how SIRT1 activation downregulates TCF7L2 in adipocytes remains to be elucidated.

ASSOCIATION OF 16 SNPS INCREASING FASTING GLUCOSE LEVEL WITH INDICES OF INSULIN RELEASE, PROINSULIN CONVERSION AND INSULIN SENSITIVITY IN 6 696 NON-DIABETIC FINNISH MEN

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We investigated the effects of 16 loci influencing fasting glucose (FG) level on insulin secretion, conversion of proinsulin to insulin, and insulin sensitivity, in order to find out the mechanisms whereby they affect FG levels. A total of 6,696 non-diabetic men (age 57±7 years, BMI 26.8±3.8 kg/ m2) from a large population-based cohort were examined. Oral glucose tolerance test (OGTT) and genotyping of SNPs in/ near TCF7L2, SLC30A8, MTNR1B, ADRA2A, FAM148A, CRY2, ADCY5, SLC2A2, FADS1, DGKB, PROX1, GCK, G6PC2, GLIS3, GCKR, and MADD were performed.Six SNPs were significantly (TCF7L2, SLC30A8, MTNR1B) ($P<0.001$) or nominally (CRY2, FADS1, GCK) ($P<0.05$) associated with early-phase insulin release (InsAUC0-30/ GluAUC0-30). Seven SNPs were significantly (MADD, SLC30A8, TCF7L2, MTNR1B) or nominally (ADCY5, GCKR, G6PC2) associated with indices of proinsulin conversion. Only GCKR was nominally associated with insulin sensitivity (Matsuda ISI). Most of the 16 loci influencing FG levels were associated with early-phase insulin release and/ or indices of proinsulin-to-insulin conversion, whereas only one locus was associated with insulin sensitivity. These results give evidence that impaired insulin secretion and proinsulin processing are important regulators of FG levels.

BIODISTRIBUTION OF IGF-1 IN TRANSGENIC CLN MICE – A NOVEL CANDIDATE FOR THE TREATMENT OF INCL

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Infantile Neuronal Ceroid Lipofuscinoses (INCL) is a recessive autosomal genetic trait. The disorder is progressive, degenerative, fatal and extremely rare worldwide but relatively common in Finland. The development of children starts to regress after 8 – 18 months and the average lifespan is 9 – 11 years. Treatment is limited, focused mainly to relieve the symptoms. It has been shown that low concentration of insulin-like growth factor (IGF) 1 in cerebrospinal fluid is linked with pathogenesis in autism at an early age. In previous study with Mnd/ mnd animal model it has been shown that only one week treatment with IGF-1 partially restored interneuronal number and reduced hypertrophy in subhippocampal subregions. In this study the accumulation of I-125 labeled IGF-1 and IGF-1-binding protein complex (IGF-BP-3) to the brains was studied in transgenic CLN mouse model with SPECT. BP-3 was used as a control. Our results showed that the accumulation of IGF-1 to the brains was 43 % and IGF-BP-3 14 % higher than control 20 min after an i.v. injection. After 2 h the accumulation of IGF-BP-3 was 39 % higher than control. These results show that IGF-1 accumulates to the brains and it may offer medical remedy to extremely problematic disease. These results are beneficial base for further studies concerning more accurate research of kinetics and dose intermediates to IGF-1 and IGF-BP-3 before treatment studies.

BIOFUNCTIONALIZATION OF MESOPOROUS SILICON NANOPARTICLES FOR TARGETED PEPTIDE DELIVERY

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We have studied dispersivity, opsonisation, peptide hormone (IGF-1) release and targeting properties of thermally hydrocarbonized porous silicon nanoparticles derivatized with undecylic acid (UnTHCPSi). They are negatively charged, relative hydrophobic particles, which are hard to suspense in physiological buffers due to agglomeration. We have modulated the dispersivity of the nanoparticles with different size (5, 10 and 20 kDa) of polyethylene glycols (PEG) using either covalently conjugated C-PEG or adsorbed oleic acid-PEG (O-PEG). When immersed in human plasma, the size of the O-PEG-particles increased less than the size of C-PEG or the original particles suggesting the lowest opsonisation degree. After the loading with the peptide, O-PEG removed weakly adsorbed IGF-1 from the surface and plugged the rest of the pores of the nanoparticles. When incubated in human plasma majority of the peptide hormone is released from the pores within the first hour. Our particles provide good platform for the development of targeted nanoparticles with low opsonisation and modulated release of hydrophilic peptide hormones.

Oral Session VII

Chairs:

– Sara Paulo & Yuriy Pomeshchik –

ROLE OF NF-KAPPA-B P50 GENE ON BEHAVIOUR AND NEUROPATHOLOGY OF APP/PS1 TRANSGENIC MOUSE MODEL OF AD

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Transcription factor nuclear factor kappa-B (NFkB) plays a prominent role in immune and inflammatory responses. Alzheimer's disease (AD) is a chronic inflammatory disease associated with glial cell activation. NFkB immunoreactivity has been found around the beta-amyloid (A-beta) deposits in AD brains. To investigate further the role of NFkB in AD pathology progression, we have crossed APP/ PS1 transgenic mice with NFkB knockout mice and established a mouse line carrying mutated APP and PS1 transgenes on NFkB p50 null background to see the impact of p50 gene knock-out on behavioral alternations and A-beta related neuropathology in APP/ PS1 mice. The mice were taken through a battery of behavioral tests for neophobia, spontaneous activity, motor coordination, anxiety and spatial memory at the age of 12 months before collecting their brains for biochemical and histological evaluations. APP/ PS1 x p50-knock-out mice exhibited a robust behavioral phenotype. They had better motor coordination and less anxiety when compared to their littermate controls. APP/ PS1 transgenic mice were impaired in spatial memory and possession of p50- knock-out did not rescue the memory deficits. Histopathology revealed no differences with respect to A-beta load or associated microglial activation. To conclude, NFkB p50 null background does not affect A-beta plaque pathology or spatial memory deficits in APP/ PS1 mice. Whether there are changes in the local inflammatory milieu surrounding the A-beta plaques remains to be investigated.

NEUROINFLAMMATION - STEM CELL DERIVED NEURAL PROGENITORS AND INFLAMMATORY RESPONSE IN A MOUSE MODEL OF CEREBRAL STROKE

Savolainen Kaisa[1], Puttonen Katja[1], Marika Ruponen[1], Tarja Malm[1], Jonna Koponen[2], Seppo Ylä-Herttuala[2], Jari Koistinaho[1], Outi Hovatta [1,3], Anu Muona [1,4]
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Cerebral stroke is the third leading cause of death worldwide and the current treatment is restricted to the tPA-therapy in 3 hour window and rehabilitation afterwards. Human embryonic pluripotent stem cells (hESC) are able to divide indefinitely and differentiate into any kind of cells. Studies have shown that transplanted ESC-derived neural progenitor cells (NPCs) survive and enhance functional recovery in animals with cerebral stroke. It has been suggested that modulation of inflammation is mostly behind the beneficial outcome. We transplanted 200 000 hESC-derived, either ultra small superparamagnetic iron-oxide (USPIO) or green fluorescent protein (GFP)-labeled NPCs into the striatum of middle cerebral artery occluded Balb/ c mice. MRI showed that USPIO-labeled NPCs migrated towards the ischemic site in stroke animals. 12 wks after transplantation, tape test revealed a slight recovery ($p=0.059$) in treated vs. untreated animals. In our previous studies immunohistochemistry has shown that the transplanted cells have been positive for neuronal markers and surrounding cells have been mostly positive for astrocyte and panhematopoietic markers indicating that they are endogenous. Next we will analyze the inflammation associated factors more thoroughly.

NOVEL BENEFICIAL PROPERTIES OF GRANULOCYTE COLONY STIMULATING FACTOR TREATMENT IN MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a lethal, incurable and progressive neurodegenerative disease with distinctive features including loss of motor neurons, denervation of target muscles, muscle atrophy and paralysis. Our data suggest that granulocyte colony stimulating factor (GCSF), a neuroprotective agent, has beneficial effects on transgenic G93A-SOD1 mice, a disease model of ALS. We assessed GCSF mediated protection to CNS, peripheral hematopoietic system and neuromuscular junction activity. In this study setup G93A-SOD1 mice and wild-type mice received weekly GCSF injections (0,3 mg/ kg) starting at pre-symptomatic stage. Treatment prolonged survival of male transgenic mice. Effect of GCSF on motoneuron survival in spinal cord will be assessed at end-stage of disease from histological samples. Properties of neuromuscular junction were measured from diaphragm at symptomatic stage. In comparison to age-matched wild-type mice, G93A-SOD1 mice displayed decline in several characteristics of neuromuscular transmission and in transgenic mice GCSF treatment significantly improved all these functions. These results further validate beneficial properties of GCSF as a neuroprotective factor and suggest this neurotrophin for the treatment of ALS.

Oral Session VIII

Chairs:

– Maykel Lopez Rodriguez & Juha-Pekka Niskanen –

PDI REGULATES SOD1 ACTIVITY AND CONTROLS CYTOCHROME C-CATALYZED PEROXIDATION – IMPLICATIONS FOR MITOCHONDRIAL ROS PRODUCTION IN ALS MODELS

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We have demonstrated that in G93A-SOD1 rats the mutant superoxide dismutase 1 (SOD1) is up regulated in the intermembrane space (IMS) and possess increased ability to bind the inner membrane of isolated mitoplasts. In parallel, protein disulfide isomerase (PDI) expression peaks in the spinal cord of G93A-SOD1 in rat and mouse models at presymptomatic stage of the disease. These findings allow us to hypothesize that increased PDI expression may lead to the aberrant control of SOD1 activity in the mitochondrial IMS and may cause increased hydroperoxide production in this compartment, eventually resulting in neuronal vulnerability. Our aim was to investigate whether PDI can exercise redox control of SOD1 activity leading to increased hydroperoxide production and cytochrome c-catalysed peroxidation in vitro and HEK-293 cell culture. Our results show that PDI catalyzes reactivation of SOD1 after inactivation by disulphide bond reduction. This reactivation resulted in increased hydroperoxide production and cytochrome c-catalysed peroxidation. Both reactivation and increased peroxidation were inhibited by bacitracin, a PDI inhibitor. Inhibition of PDI by bacitracin suppressed also paraquat-induced hydroperoxide production in HEK-293 cells. These results elucidate the possible role of PDI in controlling SOD1 activity within the IMS and its impact on mitochondrial ROS production in ALS models.

CHARACTERIZATION OF THE PHAGOCYTIC CAPACITY OF BONE MARROW DERIVED MONOCYTIC CELLS TOWARDS A-BETA

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Alzheimer's disease (AD) is the most common form of dementia characterized by age-dependent and progressive accumulation of beta-amyloid (AB) plaques. An imbalance between production and clearance of toxic AB peptides causes abnormal accumulation and eventually results in cell death. Monocytic cells derived from bone marrow (BM) have been shown actively participate in the reduction of AB burden but the reasons for insufficient clearance are still unknown. The aim was to investigate the phagocytic capacity of BM-derived monocytic cells towards AB depending on the different way of their isolation. In direct way, CD11b monocytes were isolated from mouse BM by positive and negative selection whereas indirect way included proliferation and differentiation of monocytic cells from BM hematopoietic stem cells. The phagocytic capacity of monocytic cells was assessed in ex vivo assays in which the cells were plated on hippocampal brain sections prepared from aged APP/ PS1 mice. Our results show that monocytic cells obtained both directly from BM and differentiated from BM stem cells were able to degrade AB. Moreover, CD11b monocytes isolated by negative selection degraded AB more efficiently than CD11b monocytes isolated by positive selection. Prolonged proliferation of stem cells did not reduce cells phagocytic capacity but led to generation of monocytic cells with increased phagocytic ability towards AB.

ORGANOTYPIC SLICE CULTURE IN SPINAL CORD INJURY

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Spinal cord injury (SCI) is a devastating event leading to serious long lasting consequences. Due to limited recovery and medical treatment after SCI, cell replacement therapy has become a promising therapeutic research area. Although primary and secondary injury mechanisms have been studied in many in vivo models, the in vitro organotypic slice cultures offer an opportunity to investigate both intra- and extracellular pathophysiological mechanisms in live cytoarchitecture after injury. The purpose of this study was to set up an in vitro model of SCI and study the migration and differentiation of human embryonic derived neuronal progenitor cells (NPC) injected into slices after in vitro trauma. Lentivirally transduced NPCs expressing green fluorescent protein (GFP) were injected into the intact and injured slices and their migration and maturation was determined microscopically. Preliminary findings suggest that NPCs cultivated more 5 days in neural induction medium without fibroblast growth factor differentiated into neuronal cells. Neuronal projections were seen in one week after injection to slices and persisted at least up to five weeks. NPCs migrated inside the slices. Our findings indicate that NPCs survive and have capacity to migrate and possibly maturate into neuronal cells on organotypic slice cultures. This model proved to be a potential method for research of cell replacement after SCI.

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Participants

Tuulia Huhtala et al.

The Fourth Annual Post-Graduate Symposium of the Graduate School of Molecular Medicine

Winter School 2010 Abstracts

The Graduate School of Molecular Medicine combines systematic doctoral education of the highest quality to the best research expertise in molecular medicine at the A. I. Virtanen Institute for Molecular Sciences and Department of Medicine in the Faculty of Health Sciences in the University of Eastern Finland.

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