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Teemu Natunen et al.

The Sixth Annual Post-Graduate Symposium of the Doctoral Program in Molecular Medicine

Winter School 2012 Abstracts

Publications of the University of Eastern Finland
Reports and Studies in Health Sciences

UNIVERSITY OF
EASTERN FINLAND

A.I.VIRTANEN
INSTITUTE

TEEMU NATUNEN, HEINI BELT, NOORA HUUSKO, ANNE JÄÄSKELÄINEN, PÄIVI SUTINEN, RIIKKA PELLINEN

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ABSTRACT

The Doctoral Program in 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 6th Annual Post-Graduate Symposium of the Doctoral Program in Molecular Medicine to be held in Tahkovuori, Nilsiä, on March 13 – 14, 2012.

Universal Decimal Classification: 577
National Library of Medicine Classification: W 20.5, QU 4, QZ 52, WG 120, WL 140, WL 359, WL 385, WK 810, QY 58, WN 185
Medical Subject Headings: Biomedical Research; Congresses; Molecular Biology; Gene Therapy; Genetic Vectors; Gene Transfer Techniques; Glioma/therapy; Vascular Endothelial Growth Factor; Collagen; Diabetes Mellitus, Type 2; Insulin; Sirtuins; Cardiovascular Diseases; Epilepsy; Neurodegenerative Diseases/therapy; Alzheimer Disease; Amyotrophic Lateral Sclerosis; Disease Models, Animal; Magnetic Resonance Imaging; Microarray Analysis; Signal Transduction.

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DOCTORAL PROGRAM IN MOLECULAR MEDICINE

WINTER SCHOOL 2012

MARCH 13 – 14, TAHKOVUORI NILSIÄ

PROGRAM

Tuesday, March 13

8:00	Departure from Bioteknia 1 (Neulaniementie 2)
9:00 – 9:30	Coffee, Poster set up
9:30 – 9:45	Opening of the Symposium, DPMM Vice Chair ***Hilkka Soininen***
9:45 – 11:45	**ORAL SESSION I** *Chairs: Lakshman Puli and Timo Sarajärvi*
9:45 – 10:05	Alzheimer's disease – From genes to function and potential therapies ***Annakaisa Haapasalo***
10:05 – 10:20	Genetic and functional elucidation of GGA3 in Alzheimer's disease pathogenesis ***Teemu Natunen***
10:20 – 10:35	High fat diet increases four-repeat tau expression in the brain tissue of mice with combined T2DM and AD phenotype ***Mari Takalo***
10:35 – 10:50	SFRS10 as a regulator of MAPT alternative splicing in obesity ***Dorota Kaminska***
10:50 – 11:00	Break, exhibition
11:00 – 11:15	Can stimulation of brain recovery by enriched environment prevent epileptogenesis after TBI? ***Olena Shatillo***
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11:30 – 11:45	UEF Graduate School ***Anu Liikanen***
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12:30 – 16:00	**CHECK IN, OUTDOOR ACTIVITIES**
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17:35 – 17:50	Endothelial lipase is regulated by sterol regulatory element-binding proteins (SREBPs) in endothelial cells ***Marike H. Dijkstra***
17:50 – 18:00	Quantitative, multiplexed western blot detection with infrared fluorescence ***Isto Jänönen, Fisher Scientific***
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PROGRAM

Wednesday, March 14

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8:50 – 9:05	Gender-specific mechanism of synaptic impairment and its prevention by GCSF in a mouse model of amyotrophic lateral sclerosis ***Eveliina Pollari***
9:05 – 9:20	Interleukin-33 diminishes the lesion size in the mouse model of permanent ischemia ***Paula Korhonen***
9:20 – 9:35	Intra-arterial infusion of human bone marrow derived mesenchymal cells results in transient entrapment in the brain after cerebral ischemia in rats ***Bhimashankar Mitkari***
9:35 – 9:45	How to create a transgenic or knockout rat in less than 6 months ***Marko Sankala, Sigma-Aldrich***
9:45 – 10:30	**BREAK, POSTER SESSION AND EXHIBITION**
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10:30 – 10:45	Regulation of insulin secretion in sulfonylurea receptor 1 knock-in mouse ***Nagendra Yaluri***
10:45 – 11:00	Hippocampal volume, tensor-based morphometry and voxel-based morphometry in Frontotemporal dementia ***Miquel Muñoz***
11:00 – 11:15	Cortical spreading depression investigated using spin-lock functional magnetic resonance imaging ***Joonas Autio***
11:15 – 11:25	Targeted Resequencing Solutions from Illumina ***Reija Laitinen, Illumina***
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13:00 – 13:15 Interferon β sensitivity of tumor cells correlates with poor response to VA7 virotherapy in mouse glioma models ***Janne Ruotsalainen***

13:15 – 13:30 The effect of knockdown of XIAP in a switch from Type II to Type I TRAIL signaling in human follicular lymphoma cells ***Jemal Adem***

13:30 – 13:45 Characterization of hNPC derived from hES and hiPS cells injected into intact and injured spinal cord ***Iurii Kidin***

13:45 – 14:00 Preventing atherosclerosis with IK17-antibody in mice ***Antti Määttä***

14:00 – 14:15 Selection of tumor and tumor vasculature specific antibodies from phage display library and their genetic delivery *in vivo* ***Emmi Kokki***

14:15 – 14:45 **COFFEE**

14:45 – 15:00 Awards committee
Best presentations
Director of the DPMM, Best doctoral thesis
Closing of the symposium

15:00 – Departure

DOCTORAL PROGRAM IN MOLECULAR MEDICINE

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PROGRAM

POSTERS

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2. Inflammatory cells and pain transducers in rat meninges ***Juha Ropponen***
3. Molecular mechanisms of seladin-1 in pathways relevant for Alzheimer's disease pathogenesis ***Henna Martiskainen***
4. Development of a cell-permeable peptide that acts downstream of nNOS to protect neurons against excitotoxicity ***Lili Li***
5. Post-traumatic epileptogenesis in APP/PS1 mouse model with amyloidogenic APP processing ***Diana Miszczuk***
6. Loss of hippocampal parvalbumin immunoreactivity during epileptogenesis ***Noora Huusko***
7. T1rho MRI for evaluation of VEGF-B angiogenic therapy ***Haja-Sherief N. Mustafa***
8. Attenuation of lethal semliki forest virus neurovirulence in mice by neuronal microRNA targeting ***Miika Martikainen***
9. Expression of microfibrillar-associated protein 5 (MFAP5) is differently modified by inflammatory factors in adipocytes ***Maija Vaittinen***
10. Nuclear factor (erythroid-derived 2)-like 2 regulates neurogenesis in adult hippocampus after brain injury ***Ekaterina Savchenko***
11. Early detection of VEGF gene therapy response in ischemic hind limb mouse model by MRI rotating frame relaxation ***Hanne Hakkarainen***
12. Novel diffusion based MRI contrast based on double pulsed field gradients ***Tuukka Miettinen***
13. A Novel method to separate various phenotypes of microglia ***Lakshman Puli***
14. Characterization of novel PGC1alpha isoforms ***Heidi Laitinen***
15. Implementation of pharmacological MRI ***Jaakko Paasonen***
16. Effect of hypoxia on the physiology of developing cardiomyocytes ***Tomi Tuomainen***
17. Utilizing Galaxy framework for sequence data preprocessing in lentivirus vector integration site analysis ***Oskari Timonen***
18. Adeno-associated virus mediated gene transfer to mouse myocardium – transduction efficiency and effect on left ventricular function ***Line Lottonen***

Oral Session I

Chairs:
– Lakshman Puli & Timo Sarajärvi –

GENETIC AND FUNCTIONAL ELUCIDATION OF GGA3 IN ALZHEIMER'S DISEASE PATHOGENESIS

Teemu Natunen [1], Seppo Helisalmi [1], Juha-Pekka Pursiheimo [2], Timo Sarajärvi [1], Petra Mäkinen [1], Anne-Maria Koivisto [1], Irina Alafuzoff [3], Annakaisa Haapasalo [1], Hilkka Soininen [1] and Mikko Hiltunen [1]

[1] Institute of Clinical Medicine - Neurology, University of Eastern Finland and Department of Neurology, Kuopio University Hospital; [2] Turku Centre for Biotechnology, University of Turku, Finland; [3] Department of Immunology, Genetics and Pathology, Uppsala University, Sweden

Alzheimer's disease (AD) is a neurodegenerative disorder with genetically complex background. Aggregation of amyloid-β peptide (Aβ) and hyper-phosphorylated tau protein are key neuropathological hallmarks in AD brain. β-secretase (BACE1) protein is an initial and rate-limiting enzyme in the production of Aβ, while GGA3 (Golgi-localized γ-ear-containing ARF-binding protein) is a transport protein, which has been shown to affect the degradation of BACE1. Here, we assessed the genetic and functional role of *GGA3* in AD using clinic-based case-control and neuropathological AD sample sets. Six single nucleotide polymorphisms in *GGA3* gene were genotyped among 673 AD patients and 686 controls. *APOE*-, gender-, and age-adjusted logistic regression analyses revealed a decreased risk of AD by ~30% for A allele carriers of rs2242230 as compared to non-carriers. Comparison of mRNA and protein levels of GGA3 with respect to the severity of AD pathology or *GGA3* genotype (GG vs. GA) did not reveal significant alterations in the inferior temporal cortex samples of AD patients. Although the genetic study revealed a significant association with *GGA3* variation, further studies are needed to fully establish the genetic and functional role of GGA3 in AD.

HIGH FAT DIET INCREASES FOUR-REPEAT TAU EXPRESSION IN THE BRAIN TISSUE OF MICE WITH COMBINED T2DM AND AD PHENOTYPE

Mari Takalo [1], Annakaisa Haapasalo [1], Susanna Kemppainen [2], Petra Mäkinen [1], Hilkka Soininen [1], Markku Laakso [3], Heikki Tanila [2], Mikko Hiltunen [1]

[1]Institute of Clinical Medicine – Neurology, [2] A.I. Virtanen Institute, and [3] Institute of Clinical Medicine - Medicine, University of Eastern Finland

Based on the numerous epidemiological studies, type-2 diabetes mellitus (T2DM) increases the risk of Alzheimer's disease (AD). However, molecular mechanisms underlying this co-morbidity are not well understood. Intraneuronal deposits of microtubule-associated protein tau are a common feature of several neurodegenerative diseases, including AD. Moreover, aberrant splicing of tau exon 10 leads to the altered ratio of four-repeat (4R) vs. three-repeat (3R) isoforms in neurodegenerative diseases. To investigate the effects of both genetic and high fat-induced diabetic phenotype on tau pathology, we used a well-characterized AD mouse model with hyperglycemia induced by the over-expression of pancreatic insulin-like growth factor 2. High fat diet induced up to five-fold increase in 4R tau expression in the ventral cortex of female mice. Although the expression of 3R tau was also induced, the ratio of 4R vs. 3R isoforms was significantly increased on average two-fold. These changes were independent of the genotype. This study provides experimental *in vivo* evidence that high fat diet can alter tau exon 10 splicing, which in turn may ultimately modulate tau pathology. Finally, these results suggest that tau-related pathological mechanisms can be affected by active dietary choices.

SFRS10 AS A REGULATOR OF *MAPT* ALTERNATIVE SPLICING IN OBESITY

Dorota Kaminska [1,2], Sari Venesmaa [3], Pirjo Käkelä [3], Maija Vaittinen [2], Leena Pulkkinen [2], Helena Gylling [2], Markku Laakso [1] and Jussi Pihlajamäki [1,2]

Departments of [1] Medicine and [2] Clinical Nutrition, University of Eastern Finland; [3] Department of Surgery, Kuopio University Hospital

We have recently published that expression of splicing factor *SFRS10* is reduced in liver and muscle of obese humans and that *SFRS10* contributes to increased lipogenesis and lipid accumulation due to altered splicing of exon 6 of *LPIN1*. Moreover, *SFRS10* is known to regulate alternative splicing of human microtubule associated protein tau (*MAPT*) gene. In this study we investigate the role of *SFRS10* in alternative splicing of *MAPT* in adipocytes, determined by PCR capillary electrophoresis. Our hypothesis is that *MAPT* may alter microtubules function in adipocytes, as shown in neurons, and regulate adipocyte metabolism. We observed that *MAPT* exon 10 splicing correlates with the *SFRS10* mRNA expression in subcutaneous and visceral fat ($p< 0.05$). Lack of exon 10 is associated with type 2 diabetes in both fat depots ($p< 0.005$). Finally, we observed an association between inclusion of *MAPT* exon 10 and serum free fatty acid level in subcutaneous and visceral fat tissue of obese individuals ($p< 0.05$), suggesting a physiological role of *MAPT* in adipose tissue. Our future plans include testing the effect of *SFRS10* siRNA on alternative splicing of *MAPT,* Tau phosphorylation and cell metabolism in SGBS cell line.

CAN STIMULATION OF BRAIN RECOVERY BY ENRICHED ENVIRONMENT PREVENT EPILEPTOGENESIS AFTER TBI?

Olena Shatillo [1], Jukka Jolkkonen [2], Asla Pitkänen [1]

[1] Department of Neurobiology, University of Eastern Finland; [2] Institute of Clinical Medicine – Neurology

Traumatic brain injury (TBI) is a cause of 10-20% cases of symptomatic epilepsies. It was shown that enriched environment (EE) enhances motor and cognitive recovery after TBI in rats. The aim of the present work was to determine effect of EE on the development of lowered seizure threshold after TBI, and to correlate it with motor and cognitive recovery. To study posttraumatic epileptogenesis lateral fluid percussion TBI was performed in 2 groups of male Sprague-Dawley rats. One group of TBI rats (n=7) and corresponding sham-operated group (n=3) were housed in standard conditions. Second TBI group (n=8) and sham control (n=3) were placed in the EE. Behavior tests (neuroscore, beam-walking and Morris water maze) were done at 5 different time points (2d, 1wk, 2wk, 4wk, 8wk) to investigate posttraumatic recovery. Continuous 1 week video-EEG recording was performed 3 month after TBI. Then seizure threshold was tested with pentyleneterazol (25 mg/kg), followed by 24h video-EEG recording. Results show that EE was not beneficial for functional recovery. We found increase ($p\leq0,05$) in number of spontaneous epileptiform discharges in TBI animals with EE compared to standard conditions during 1 week video-EEG monitoring. It implies that EE rather facilitates epileptogenesis then prevents it.

SPONTANEOUS SEIZURES IN MICE CARRYING APP ARCTIC MUTATION

Sofya Ziyatdinova [1], Annica Rönnbäck [2], Caroline Graff [2], Bengt Winblad [2], Asla Pitkänen [1,3], and Heikki Tanila [1,3]

[1] A.I.Virtanen Institute for Molecular Sciences, University of Eastern Finland; [2] Karolinska Institutet; [3] Kuopio University Hospital

The Arctic mutation (E693G) of amyloid precursor protein (APParc) causes early onset autosomal dominant dementia. Mice with single Arctic APP mutation have cognitive dysfunction and amyloid β deposition, starting in the subiculum at 4 month of age. Arctic APP mutation increases formation of amyloid β protofibrils, without increasing amyloidogenic β- and γ-secretase cleavage of APP. We aim to verify suspected presence and characteristics of seizure activity in APParc mice. 21 homozygous APParc tg female mice and their 11 wt littermates were implanted with cortical electrodes. 3 video-EEG sessions (24/7, 2 wk) were performed at age of 15-20 wk, 23-24 wk and 32-35 wk. The behavioral phenotype of the seizure was assessed with Racine's scale. At the age of 15-20 wk seizures were observed in 6/21 (29%) APParc tg mice and in 2/11 (18%) wt mice. At age of 23-24 wk, seizures were observed in 6/20 (30%) APParc tg (2 mice with new-onset seizures) and in 2/11 (18%) wt (2 mice with new-onset seizures). Seizure frequency was 1 seizure per 2 wk, seizure duration in APParc tg mice was 18±10 sec in Session 1 and longer (33±17 sec, $p<0.05$) in Session 2. Our data confirm occurrence of spontaneous epileptic seizures in tg APParc mice that is possibly related with the presence of soluble Aβ protofibrils.

Poster Session

CONFORMATIONAL REGULATION OF $\alpha_1\beta_1$ INTEGRIN AND ITS EFFECTS ON CELL ADHESION AND SIGNALING

Henri Niskanen, Johanna Jokinen, Jarmo Käpylä and Jyrki Heino
Department of Biochemistry & Food Chemistry, University of Turku

$\alpha_1\beta_1$ integrin belongs to the collagen receptor family of integrins, which regulate cell adhesion, migration and proliferation and have significant roles in many diseases, for example in cancer, thrombosis and inflammation. Our studies focused on the activation of $\alpha_1\beta_1$ integrin and how structural changes regulate its function on cellular level. We introduced site specific mutations to α_1 integrin and produced CHO cell lines expressing α_1 integrin variants. Cell adhesion on type I and IV collagens was monitored with real time cell analyzer, confocal and live microscopy. We also investigated the effects of these mutations on integrin-mediated signaling pathways, for example ERK and Rho GTPases. Cell adhesion on type I and IV collagens was enhanced when ligand binding α_1I domain was activated with mutation E317A. Mutation E335A, which disrupts allosteric regulation between α and β subunits, prevented cell adhesion, as expected. Interestingly the double variant E317A/E335A expressing cells were able to attach on type IV collagen, but their spreading was partially prevented. α_1 integrin was also able activate ERK signaling even in presence of inactivating mutation E335A. Our results suggest that activation mechanisms of collagen receptor integrins might differ from other integrins.

INFLAMMATORY CELLS AND PAIN TRANSDUCERS IN RAT MENINGES

Juha Ropponen [1], Giedrius Kalesnykas [2], Dmitriy Fayuk [1] and Rashid Giniatullin [1]
[1] Dept Neurobiology, AIVI, University of Eastern Finland; [2] Dept Ophthalmology, University of Eastern Finland

Migraine is the most common neurological disease associated with aseptic neuroinflammation around blood vessels in meninges. It has been suggested that the severe migraine headache originates from meninges but the final proof of the peripheral basis and the mechanism of pain transduction remains unclear. In the current project we analyzed the distribution of mast cells, suspected players in migraine, using staining of rat dura mater with Toluidine Blue and performed live imaging of meningeal cells loaded with the calcium fluorescent dye, Fluo-3AM. We show here that mast cells are abundantly expressed in meninges and their number was increased after induction of migraine-like states with the NO donor. Intracellular Ca^{2+} imaging allowed us to visualize meningeal vessels and other residual meningeal cells in control and after application of the P2X and TRPV1 agonists, ATP and capsaicin, respectively. We obtained experimental evidence that application of ATP little changed the diameter of meningeal vessels but induced Ca^{2+} waves in dura mater cells while capsaicin induced strong vasoconstriction. Immunogold/P2X3 labeling of dura mater indicated that pain transducing P2X3 receptors were localized in meningeal nerves. Thus, the combination of various imaging technique allowed us to track the dynamics of meningeal cells and indicated expression of pain transducers in dura mater. Taken together these data support the hypothesis of peripheral origin of migraine pain.

MOLECULAR MECHANISMS OF SELADIN-1 IN PATHWAYS RELEVANT FOR ALZHEIMER'S DISEASE PATHOGENESIS

Henna Martiskainen, Timo Sarajärvi, Petra Mäkinen, Annakaisa Haapasalo, Hilkka Soininen, Mikko Hiltunen

Institute of Clinical Medicine – Neurology, University of Eastern Finland

Seladin-1/DHCR24 is neuroprotective protein, which is selectively down-regulated in the brain regions affected in Alzheimer's disease (AD). Seladin-1 confers resistance against Aβ- and oxidative stress-induced apoptosis and decreased levels of seladin-1 lead to the increased β-amyloidogenic processing of APP in the apoptotic conditions. Collectively, these data suggest that seladin-1 has an important role in the AD pathogenesis. Here, we investigated whether the over-expression of seladin-1 has beneficial effects on pathways relevant for AD, such as APP processing and Aβ-generation in human neuroblastoma SH-SY5Y cells over-expressing APP751 isoform (SH-SY5Y-APP751). SH-SY5Y-APP751 cells were transfected with seladin-1 plasmid and total protein lysates as well as cell culture medium samples were analyzed using Western blotting and Aβ-ELISA. Over-expression of seladin-1 significantly increased the levels of total APP on average 1.6-fold. Conversely, total APP normalized APP CTF levels were decreased, which again coincided with the decreased Aβ40 and Aβ42 levels in the cell culture medium. These results suggest that elevated seladin-1 expression in neuronal cells may exert beneficial effects on APP processing by reducing the Aβ generation.

DEVELOPMENT OF A CELL-PERMEABLE PEPTIDE THAT ACTS DOWNSTREAM OF nNOS TO PROTECT NEURONS AGAINST EXCITOTOXICITY

Lili Li [1], Xiaonan Liu [1], Olga Vergun [1], Vanessa Ginet [2], Anita Truttmann [3], Michael J. Courtney [1]

[1] Dept. of Neurobio., University of Eastern Finland; [2] DBCM, Univ. de Lausanne; [3] Dept. of Pediatrics and Pediatric Surgery, CHUV, Lausanne, Switzerland

The scaffold protein PSD95 assembles a ternary complex with the NMDA receptor subtype (NR) and neuronal nitric oxide synthase (nNOS). We found that rapid activation of p38MAPK by NR-gated calcium requires the catalytic activity of nNOS and early p38 activity is required for cell death. We have investigated the excitotoxicity-evoked activation mechanism and identified a region of nNOS, which when expressed, reduces excitotoxicity but without effect on either NO production or calcium elevation. This finding suggests that excitotoxic signaling downstream of NO may require a previously unidentified downstream binding partner of the region. We therefore designed a cell-permeable peptide (CPP) which binds to this region in order to compete with its endogenous interacting partners. The CPP inhibits excitotoxic activation of p38 in cortical cultures, consistent with an action downstream of NO. In an animal model of neonatal hypoxia-ischaemia, the CPP enters neuronal cells, reduces p38 activity and doubles surviving tissue, exhibiting as much reduction in lesion size as a p38 inhibitor. These results suggest not only the CPP is a novel neuroprotectant but also that excitotoxic activation of p38 downstream of NO requires nNOS-binding partners that are yet to be identified.

POST-TRAUMATIC EPILEPTOGENESIS IN APP/PS1 MOUSE MODEL WITH AMYLOIDOGENIC APP PROCESSING

Diana Miszczuk [1,2], Heikki Tanila [1], Katarzyna Lukasiuk [2], Asla Pitkänen [1]
[1] A. I. Virtanen Institute, University of Eastern Finland; [2] The Nencki Institute of Experimental Biology, Polish Academy of Science

Growing evidence suggests an association between Alzheimer disease (AD) and epilepsy. It has been showed that 65% of APP/PS1 mice display at least one seizure by the age of 5 months. To address the question whether increased amyloid load facilitates post-traumatic epileptogenesis we induced traumatic brain injury (TBI) in 13-15wk old APP/PS1 mice and Wt littermates. Long-term (24/7) 2-wk EEG recording was performed starting at 6 and 14wk post-TBI. AD injured showed motor deficits compared to AD controls at 2d ($p<0.01$), 7d ($p<0.01$) and 14d post-TBI ($p<0.05$). AD injured were more impaired than Wt injured at 14d post-TBI ($p<0.01$). Latency to find the platform in MWM was longer in AD injured than in Wt injured group ($p<0.05$). There was no difference in EEG results between groups. 1stEEG showed spontaneous seizures in AD injured and AD controls (43% of each group). Epileptiform discharges (EDs) were observed in 29% of AD controls. In 2ndEEG seizures and EDs continued in AD controls (25%) and occurred as well in Wt injured (14%). AD injured mice displayed more behavioral alterations than Wt injured and AD controls. Epileptiform activity started from 2ndEEG in Wt injured suggests that longer follow-up or increase injury severity may reveal whether TBI facilitates epileptogenesis in AD mice.

LOSS OF HIPPOCAMPAL PARVALBUMIN IMMUNOREACTIVITY DURING EPILEPTOGENESIS

Noora Huusko, Christine Römer, Asla Pitkänen
Epilepsy Research Laboratory, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Finland

Parvalbumin (PARV) labels about 20 % of GABAergic interneurons which are responsible for inhibition to principal cells. Impaired function of inhibition is presumed to contribute to seizure susceptibility. To address a question, whether the severity of damage to PARV positive neurons depends on the type of insult, we compared the number of cells at 1 and 6 months after *status epilepticus* (SE) and traumatic brain injury (TBI) in rats. Presence of seizures was investigated with v-EEG at 6 months post-insult and TBI rats also underwent test of seizure susceptibility. Decrease of PARV positive cells depends on etiology being greatest at 6 months post-TBI in ipsilateral dentate gyrus (35 % of cells remaining, $p<0.001$). Rats in this group had lowered seizure threshold but no seizures. Unexpectedly, the loss of PARV positive neurons (84 % of cells remaining, $p>0.05$) in rats with post-SE epilepsy is much milder than in the TBI group and not associated with seizure frequency. However, lower the number of PARV positive neurons in the contralateral granule cell layer, longer the duration of spontaneous seizures. In conclusion, reduced number of PARV positive cells can contribute to seizure susceptibility, but it is not necessarily associated with occurrence of seizures.

T1RHO MRI FOR EVALUATION OF VEGF-B ANGIOGENIC THERAPY

Haja-Sherief N. Musthafa, L Lottonen, S Ylä-Herttuala,O Gröhn, T Liimatainen
A.I. Virtanen Institute, University of Eastern Finland, Kuopio

The possibility of creating new blood vessels in ischemic heart has lead to many angiogenic preclinical trials involving delivery of vascular endothelial growth factor for treatment of myocardial ischemia. VEGF-B167 has been used in previous experiments to reduce the structural and functional disorder of cardiac muscle in dilated cardiomyopathy (DCM) and preserve contractility of heart muscles. In this research, we used MRI methods such as spin lattice relaxation time (T1rho), & spin-spin relaxation time (T2) to monitor gene therapy response in mouse models of DCM which are formed by a myocardial specific promoter Myosin heavy chain alpha transgenic strain. We used seven transgenic mice (MHCA-VEGFB) and five wild type mice which were imaged at 9.4 T. T1rho, T_2 weighted and cine images were acquired on 5^{th}, 7^{th} and 10^{th} months. A significant difference was found in T1rho between 5^{th} and 10th months in transgenic mice (*p=0.025). The mean left ventricle mass of the transgenic mice was found to be larger (124.3±12.9) than that of control mice (107.7±3.3) (*p=0.04) in 10^{th} month. No significant differences were found in stroke volume, ejection fraction, cardiac output, end systolic and end diastolic volumes. The research will be continued for more time periods and monitor the gene therapy response.

ATTENUATION OF LETHAL SEMLIKI FOREST VIRUS NEUROVIRULENCE IN MICE BY NEURONAL MICRORNA TARGETING

Miika Martikainen [1], Erkko Ylösmäki [2], Ari Hinkkanen [1], Kalle Saksela [2]
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MicroRNAs (miRNAs) are small non-coding RNA molecules that have important regulatory roles in gene expression by targeting mRNAs for cleavage or translational repression. The miRNA machinery has recently been successfully exploited to modify tropism of both RNA and DNA viruses to prevent viral replication in specific unwanted tissues. Semliki forest virus (SFV) is an enveloped positive-strand RNA virus of the family *Togaviridae*. SFV strains such as SFV4 and L10 are highly neurovirulent in mice of all ages causing fatal encephalitis while avirulent strain A7(74) is attenuated in the central nervous system (CNS) of adult mice. Replicative SFV vectors based on strain A7(74) have been shown as promising tools for oncolytic virotherapy with particular interest in therapy of brain tumours. To further increase the safety of SFV virotherapy, additional measures to restrict SFV replication in the CNS are needed. We have generated miRNA-targeted SFV vectors by inserting target elements for neuron-specific miRNAs into the SFV genome. Results indicate that SFV carrying target elements for miR124 has significantly attenuated replication potency in the CNS of Balb/c mice while retaining oncolytic properties *in vitro*.

EXPRESSION OF MICROFIBRILLAR-ASSOCIATED PROTEIN 5 (MFAP5) IS DIFFERENTLY MODIFIED BY INFLAMMATORY FACTORS IN ADIPOCYTES

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We have earlier shown that the expression of MFAP5 is down-regulated in adipose tissue (AT) along with weight reduction in the persons with metabolic syndrome (The Genobin study). Our aim was to study the role of MFAP5 in cultured human SGBS adipocytes to examine if inflammatory factors regulate the MFAP5 mRNA expression in preadipocytes and mature SGBS adipocytes. Immunohistochemistry of human AT and the gene expression pattern in SGBS cells showed that MFAP5 was highly expressed in AT and its expression was decreased during preadipocyte differentiation. Furthermore, down-regulation of the MFAP5 mRNA by siRNA resulted in a reduction of several genes related to extracellular matrix (ADAM12, NOTCH1) and inflammation (TGFβ1, IL1β, IL-6). Treatment of mature adipocytes with TGFβ1, TNF-α, IL1β or IL-6 decreased the mRNA expression of MFAP5, and treatment with IL1β or the highest (10 ng/ml) TNF-α concentration decreased the expression of MFAP5 in preadipocytes, whereas a treatment of preadipocytes with TGFβ1, IL-6 or the lowest (0.1 ng/ml) TNF-α concentration increased the MFAP5 mRNA expression. These results suggest that inflammatory factors which facilitate low-grade inflammation and promote insulin resistance in obesity modulate the expression of MFAP5 in adipocytes.

NUCLEAR FACTOR (ERYTHROID-DERIVED 2)-LIKE 2 REGULATES NEUROGENESIS IN ADULT HIPPOCAMPUS AFTER BRAIN INJURY

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The generation of new neurons within the dentate gyrus of the mature hippocampus is critical for spatial learning, object recognition and memory. Free radical damage is one of the main contributory factors of impaired neurogenesis in chronic neurodegenerative diseases. Transcription factor nuclear factor E2-related factor 2 (Nrf2) has been shown to be central for protection against oxidative stress by binding to the antioxidant response element (ARE) enhancer sequence of numerous protective genes. Using Nrf2 ($Nrf2^{-/-}$) deficient mice, we assessed the importance of Nrf2 in neurogenesis within the hippocampus after seizures or global ischemic injury. Seizure induction with kainic acid as well as global ischemia resulted in elevated doublecortin (DCX) expression, a marker of neurogenesis, and Ki-67 expression (marker of cell proliferation) both in *wild-type* (wt) and $Nrf2^{-/-}$ mice in comparison with vehicle-treated mice in dentate gyrus of hippocampus. Relative to wt mice, $Nrf2^{-/-}$ mice showed significantly fewer DCX^{+} and $Ki\text{-}67^{+}$ cells. This finding suggests that Nrf2 is important for proliferation of progenitor neural cells in the dentate gyrus in response to brain injury. We hypothesize that pharmacologigal or gene therapy promoting Nrf2 expression increases neurogenesis in the hippocampus and is beneficial after brain injury.

EARLY DETECTION OF VEGF GENE THERAPY RESPONSE IN ISCHEMIC HIND LIMB MOUSE MODEL BY MRI ROTATING FRAME RELAXATION

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Current knowledge of potential non-invasive MRI markers to detect therapy response in ischemic skeletal muscle is limited. However, the longitudinal relaxation time in the rotating frame ($T_{1\rho}$) has shown to be a potential quantitative MRI marker for tissue remodeling after myocardial infarction. Relaxation Along a Fictitious Field (RAFF) time constant T_{RAFF} has been found to correlate with cell density in rat glioma gene therapy model, implying that T_{RAFF} could be a sensitive marker of cell death in gene therapy model. The potential of rotating frame relaxation times to detect the gene therapy response in ischemic skeletal muscle will be studied in mouse gene therapy model of hind limb ischemia *in vivo*. The mice femoral artery of single hind limb will be ligated and adenovirus mediated vascular endothelial growth factor (VEGF-A and VEGF-D) will be injected. Lac-Z serves as control. The mice will be imaged using 7T MRI scanner before and 0, 1, 3, 7, 14 and 28 days after ligation. $T_{1\rho}$, T_{RAFF2} and T_{RAFF4}, T_1, T_2 and water diffusion will be measured. The results will be compared with histology derived severity of infarction and ultrasound perfusion. The most potent gene therapy MRI markers will be applied for monitoring a gene therapy response in a rabbit hind limb ischemia model.

NOVEL DIFFUSION BASED MRI CONTRAST BASED ON DOUBLE PULSED FIELD GRADIENTS

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Diffusion-based techniques are commonly used to generate contrast in Magnetic Resonance Imaging (MRI). Diffusion Tensor Imaging (DTI) is an effective non-invasive method to study coherently oriented white matter structures in brain. However, DTI is limited when tissue is more randomly oriented, like in gray matter. Novel double-pulsed-field-gradient (d-PFG) techniques may provide new MRI contrasts, when the tissue consists of smaller and randomly oriented structures. In this approach, two diffusion gradient pairs are applied offering many degrees of freedom, and the diffusion periods, mixing time and the angle between the gradient pairs can be varied. D-PFG has potential to be used in characterization of axon sizes and compartment size distribution in the grey matter. In the present, study this approach will be, for the first time, used to characterize pathological changes in rat brain after traumatic brain injury. Results will be verified using histology and electron microscopy of tissue samples.

A NOVEL METHOD TO SEPARATE VARIOUS PHENOTYPES OF MICROGLIA

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Microglia are dynamic and help maintaining homeostasis by actively monitoring their microenvironment. Microglia can be activated by a plethora of microbial and aseptic stimuli. One can visually appreciate various sub-populations of microglia; however, currently we lack analysis method to quantify such sub-populations of microglia within a tissue. Flow cytometry segregates microglia efficiently but we lose details of tissue microenvironments. Here, we describe a novel method of segregating microglia based on their morphology and expression of CD45/Iba-1 markers. Coronal brain sections from transgenic Alzheimer's mice were double labelled with fluorescent labels for CD45 and Iba-1a. Using confocal microscope, Z stack images of microglia were acquired and then, image J analysis software was used to derive integrated intensities as well as circularity data from 955 microglial cells in a random manner. Data when plotted on 3D graph, three different sub-populations of microglia were demarcated both visually and statistically. Investigating subpopulations of microglia in their microenvironments may reveal their functional identities in health and disease.

CHARACTERIZATION OF NOVEL PGC1ALPHA ISOFORMS

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Peroxisome proliferator-activated receptor gamma co-activator alpha (PGC1α) is a master regulator of energy metabolism, and an important factor in the maintenance of the cardio-vascular system. Recently four novel isoforms of PGC1α have been found that differ in structure and expression. These isoforms are suspected to have different effects in the cardio-vascular system. The aim of this research is to elucidate the function and expression of different PGC1α isoforms (PGC1α1-4) in cardio-vascular tissues. We study the basal and stimulated expression of PGC1α isoforms in cardiomyocytes and endothelial cells, the expression of PGC1α isoforms in cardiomyocytes from mice subjected to exercise or cardiac pressure overload evoked by transverse aortic constriction (TAC), the isoform specific target genes by isoform specific lentiviral overexpression in cardiomyocytes and endothelial cells, and the effect of isoform specific AAV9 overexpression in the TAC-mice hearts. In case differences in the expression and effects of the PGC1α isoforms in cardio-vascular tissues are found, the individual isoforms can potentially be utilized in therapeutic applications against cardio-vascular diseases.

IMPLEMENTATION OF PHARMACOLOGICAL MRI

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Pharmacological MRI (phMRI) is a modern imaging method providing indirect information of the actions of pharmaceuticals in tissue. The conventional functional MRI (fMRI) method for studying brain activation is the blood oxygenation level dependent (BOLD) fMRI, in which changes in blood oxygenation, flow and volume are used for localization of the activated areas. The general idea of phMRI has been discussed for years, but several issues still remain. These issues include weak BOLD fMRI signal changes, selection of suitable anesthetics, controlling of animal physiology, and deficient data analysis models. In this project, the main focus is to develop phMRI methodology in order to construct a robust measurement protocol and data analysis method for the use of phMRI in preclinical drug development as well as in characterization on experimental disease models. The goals are to 1) construct a robust phMRI data acquisition protocol, 2) incorporate pharmacokinetic model into phMRI data analysis, and 3) characterize brain function and its recovery after brain injury using phMRI.

EFFECT OF HYPOXIA ON THE PHYSIOLOGY OF DEVELOPING CARDIOMYOCYTES

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Oxygen is an important regulator of cellular differentiation. For example it has a well-known role in vascular formation. During early developmental phases tissues sustain remarkably lower oxygen levels than in adulthood. This is also true with embryonic heart whereas adult myocardium is highly energetic and requires a lot of oxygen to function properly. What is the role of oxygen tension in the development of cardiomyocyte function? To answer this question primary cultures of embryonic and neonatal mice cardiomyocytes are exposed to hypoxic conditions and the effects of low oxygen on their physiolological properties are analyzed. Size of the cells is assessed with Coulter counter. The amount of mitochondria will be measured by flow cytometric analysis of cells stained with a mitochondrial dye and mitochondrial function analyzed with Seahorse XF24 extracellular flux analyzer. Intracellular calcium signals are measured with line-scanning confocal microscopy of Fluo-4 loaded cardiomyocytes. Results of this study will gain our knowledge in the development of cardiomyocytes. Furthermore, findings can potentially elucidate molecular events which occur during hypoxic stress in adult heart tissue.

UTILIZING GALAXY FRAMEWORK FOR SEQUENCE DATA PREPROCESSING IN LENTIVIRUS VECTOR INTEGRATION SITE ANALYSIS

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Integration site analysis is an important part in risk assessment of gene therapy concerning retro- and lentivirus vectors. Distribution of integration sites in host genome gives valuable information on safety and effectiveness of used vectors. Easily reproducible way for determining integration sites from high-throughput sequence data may possess a challenge by itself. Sequences need to be filtered and cleaned up to the point where sequences that are left only contain host's genomic DNA. These sequences can then be cross-referenced to appropriate genome in order to determine integration sites of the vector. Galaxy platform provides an accessible and reproducible way for this kind of computationally multilayered processing and analysis of genomic data. Custom tools programmed for specific purpose may also be incorporated as a part of local instance of the galaxy platform. 454 sequence data from lentivirus transduced cells was preprocessed using Galaxy with an integrated custom tool for deleting the adapter sequences and everything after or before a specific sequence. Such tool was programmed using Perl and BioPerl modules which then could be incorporated as a part of Galaxy workflow along with other operations needed for the preprocessing step.

ADENO-ASSOCIATED VIRUS MEDIATED GENE TRANSFER TO MOUSE MYOCARDIUM –TRANSDUCTION EFFICIENCY AND EFFECT ON LEFT VENTRICULAR FUNCTION

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Severe coronary artery disease is a leading cause of death in developed countries and angiogenic gene therapy is studied as a promising new approach in treating myocardial ischemia in no-option patients. Here we tested the myocardial transduction efficiency of adeno-associated virus mediated gene transfer and its effects on left ventricular function and myocardial morphology. Adeno-associated virus serotype 2 (AAV-2) mediated LacZ gene transfer was done under ultrasound guidance to the anterior wall of left ventricle in mice. Left ventricular function and ECG were followed in several time points up to three months after the gene transfer. Myocardial morphology, inflammation, scar tissue formation and transduction efficiency of the virus were analyzed from histological samples in three different end points after the gene transfer. Transduction efficiency of AAV-2 in mouse myocardium was good. Ejection fraction started to decrease a month after the injection probably due to moderate inflammation and scar tissue formation in heart. We conclude that in terms of transduction efficiency, AAV-2 seems to be a good vector candidate for myocardial gene transfer but its harmful side effects need to be further studied.

Oral Session II

Chairs:
– Emilia Kansanen & Nicholas Downes –

LENTIVIRAL PROTEIN TRANSDUCTION WITH GENOME-MODIFYING INTEGRASE-I-PPOI FUSION PROTEIN

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In addition to gene transfer, lentiviral vectors can also be harnessed for protein transduction purposes. We have previously presented a cis-packaging method for foreign protein incorporation into lentiviral vectors while retaining the gene transfer activity. Here we demonstrate the principle of cis-packaging application in genome editing using I-PpoI meganuclease fused to lentiviral integrase. Third generation self-inactivating lentiviral vectors were produced in 293T cells with standard cotransfection of four plasmids, one of which contained the I-PpoI cDNA fused C-terminal to integrase in the gag-pol sequence. Correct packaging of the fusion protein and its ability to cut target sequence were verified. The fusion protein was able to enter the nucleus and cause double-strand breaks. We also found that cancer cell lines recover ineffectively from the meganuclease-induced double-strand breaks, whereas non-cancerous cells suffer no loss of viability. Remarkable cytotoxicity of IN-I-PpoI LVV for cancer cells lines *in vitro* led to the testing of the fusion protein *in vivo*, where the effect was preserved and growth of subcutaneous tumours in nude mice was restricted with an efficiency similar to well-studied thymidine kinase transgene combined with ganciclovir injections.

ENDOTHELIAL LIPASE IS REGULATED BY STEROL REGULATORY ELEMENT-BINDING PROTEINS (SREBPs) IN ENDOTHELIAL CELLS

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Endothelial lipase (EL) is a member of the lipoprotein lipase family which is mainly synthesized by endothelial cells (ECs). The main substrate for EL is high-density lipoprotein (HDL) and inverse correlation between HDL-cholesterol (HDL-C) levels and EL has been well established. As sterol regulatory element-binding proteins (SREBPs) are well known regulators of genes involved in lipid metabolism we hypothesized that SREBPs regulate EL in ECs and that EL expression can be modified by the SREBP activator VEGF-A. Western blot and quantitative PCR results showed SREBP2 up regulation and activation by starvation increased EL levels in HUVECs. Inhibition of the SREBP pathway by several inhibitors or siRNA confirmed the role of SREBPs in EL regulation. VEGF-A treatment of HUVECs inhibited EL expression via SREBPs *in vitro*, suggesting a possible mechanism for increased circulating HDL-C levels in atherosclerotic LDL-R/ApoB double knockout mice after adenoviral VEGF-A treatment *in vivo*. These data indicate that SREBPs are novel regulators of EL expression in ECs. Moreover, VEGF-A treatment induced a beneficial lipid profile *in vivo* by increasing systemic HDL-C levels possibly mediated by inhibition of EL expression via SREBPs.

NRF2 DEFICIENCY IN LDL-RECEPTOR DEFICIENT MICE EXPRESSING ONLY APOB^{100} LEADS TO SEVERE ATHEROSCLEROSIS AND OCCLUSIVE CORONARY ARTERY DISEASE

Anna-Kaisa Ruotsalainen, Mari Merentie, Mervi Partanen, Line Lottonen, Seppo Ylä-Herttuala, Anna-Liisa Levonen

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Oxidative stress is an imbalance of reactive oxygen species (ROS) and has an important role in the progression of atherosclerosis. Transcription factor NF-E2-related factor 2 (Nrf2) is a positive regulator of many antioxidant and detoxifying enzymes and thereby affording cytoprotection also in the cardiovascular system. Contradictory to its antioxidant and anti-inflammatory effects, the loss of Nrf2 in Apo E-deficient mice alleviates atherosclerosis likely via combined systemic and local effects. For this reason, we sought to determine the role of Nrf2 in atherosclerosis in a mouse model that more closely mimics human hypercholesterolemia, the LDL-receptor deficient mice expressing apoB^{100}-only (LDLR$^{-/-}$ApoB^{100}). Remarkably, our preliminary data demonstrated that Nrf2 deficiency increased the lesion area in aortic root and occlusive coronary artery disease in LDLR$^{-/-}$ApoB^{100} mice. In addition, Nrf2$^{-/-}$LDLR$^{-/-}$ApoB^{100} mice developed spontaneous myocardial infarction between the age of 5-11 months on a chow diet, with respective worsening of left ventricular function. We conclude that Nrf2 deficiency possibly leads to severe atherosclerosis, occlusive coronary artery disease and subsequent myocardial infarction in LDLR$^{-/-}$ApoB^{100} mice. This phenotype provides a new model for coronary artery disease and preclinical testing of novel therapies.

EFFECT OF SOLUBLE VASCULAR ENDOTHELIAL GROWTH FACTOR 3 (sVEGFR3) ON ATHEROGENESIS IN LDLR$^{-/-}$/APOB$^{100/100}$ MOUSE MODEL

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Lymphatic vessels collect and transport extravasated fluid from tissues to blood stream and participate in fat metabolism by taking up chylomicrons from intestinal microvilli. They are also present in outer layers of large arteries and therefore might play a role in arterial pathologies, such as atherosclerosis. In this study, mice expressing soluble VEGFR3, a molecule inhibiting normal lymphatic vessel development and function, was cross-bred with atherosclerotic LDLR$^{-/-}$/ApoB$^{100/100}$ mice. Both female and male mice were selected from age groups 3-4 months, 7-8 months and 11-12 months and LDLR$^{-/-}$/ApoB$^{100/100}$ mice served as controls. Mice were fed with western-type high-fat diet (42% of calories from fat and 0.15% from cholesterol) up to 3 months and blood samples were collected every two weeks. Plasma triglyceride and cholesterol levels were measured to analyze the lipid metabolism of the mice. Atherosclerosis was quantified from Sudan IV staining of *en face* aortas and hematoxylin-eosin staining of aortic roots. Effects of the impaired lymphatic vessel function on the development of atherosclerosis will be described.

Oral Session III

Chairs:
– Ekaterina Savchenko & Yuriy Pomeshchik –

GENDER-SPECIFIC MECHANISM OF SYNAPTIC IMPAIRMENT AND ITS PREVENTION BY GCSF IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of motoneurons which progresses differentially in males and females for unknown reason. Our aim was to measure electrophysiologically pre- and post-synaptic properties of neuromuscular junction in diaphragm of an ALS mouse model at early symptomatic stage and determine whether sex or anti-inflammatory treatment with granulocyte colony stimulating factor (G-CSF) affects the properties of synaptic transmission or level of reactive oxygen species (ROS) in spinal cord. Miniature and evoked endplate potentials (MEPPs and EPPs) were recorded in the diaphragm muscle using intracellular microelectrode technique. Electrophysiological testing revealed that in ALS mice the postsynaptic function was mainly preserved whereas the presynaptic properties were greatly affected by the disease. In male mice the G-CSF treatment improved the affected presynaptic properties. In the spinal cords the level of ROS was increased in males but not in females and was reduced by G-CSF treatment. This is the first detailed electrophysiological analysis of impaired synaptic function in a mouse model of ALS providing a sensitive biomarker for ALS research. Our results support previous findings of sexual dimorphism in ALS progression and drug response.

INTERLEUKIN-33 DIMINISHES THE LESION SIZE IN THE MOUSE MODEL OF PERMANENT ISCHEMIA

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Cerebral stroke is one of the leading causes of death and long-term disability in the world. Central and peripheral inflammatory responses contribute to the outcome after ischemia; Th1-shifted responses contribute to neuronal death whereas Th2-type responses are generally beneficial. IL-33 is recently found cytokine that shifts immune balance towards Th2-phenotype via anti-inflammatory cytokines IL-4, IL-5 and IL-13. IL-33 has been shown to be protective in atherosclerosis but there are no studies of IL-33 in brain diseases. The aim of this study was to test whether IL-33 is protective in a mouse model of ischemic stroke. Balb/c mice were treated with IL-33 a week before permanent middle cerebral artery occlusion, with a dose of 1 μg given twice a week i.p. Blood samples were taken from the saphenous vein before and after ischemia. The mice were imaged by MRI and sacrificed 3 days post ischemia. Cytokine levels in plasma and spleen were determined by flow cytometry. Pretreatment with IL-33 decreased the ischemic damage significantly ($p<0.05$) and increased the expression of several cytokines, including IL-4 in plasma and spleen. It is concluded that IL-33 regulates the extent of infarction after ischemic stroke and may represent a novel therapy for neurodegenerative diseases.

INTRA-ARTERIAL INFUSION OF HUMAN BONE MARROW DERIVED MESENCHYMAL CELLS RESULTS IN TRANSIENT ENTRAPMENT IN THE BRAIN AFTER CEREBRAL ISCHEMIA IN RATS

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Cell therapies from various sources have been under intense research. Efficient homing of the cells in the injured brain without complications is necessary to facilitate therapeutic potential of cell therapy. Intra-arterial infusion of cells bypasses the filtering organs and directs the cells to the target area. Here we studied biodistribution of human bone marrow derived mesenchymal cells (BMMSC) after infusion through external carotid artery in rats subjected to transient middle cerebral artery occlusion (MCAO). Transient MCAO was induced in male Wistar rats followed by intra-arterial injection of BMMSCs. Cells were cultured without animal-derived reagents and treated with a proteolytic enzyme to transiently modify certain cell adhesion proteins. Biodistribution of ^{111}In-oxine labeled cells were then studied by SPECT imaging immediately after injection and 24 h thereafter. Intra-arterial infusion through the external carotid artery was safe and efficient administration route resulting in a massive but transient entrapment of cells in the brain. Eventually most of the cells relocated into the internal organs. Thus, more work is needed to understand the functional role and mechanisms involved in long-term cell engraftment in ischemic brain.

Oral Session IV

Chairs:
– Maija Vaittinen & Shalem Raju Modi –

REGULATION OF INSULIN SECRETION IN SULFONYLUREA RECEPTOR 1 KNOCK-IN MOUSE

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We have previously described a new subtype of autosomal dominant diabetes in a Finnish family attributable to a dominant heterozygous mutation E1506 in the sulphonylurea receptor 1 gene (SUR1). Interestingly, this mutation causes congential hyperinsulinism in infancy, loss of insulin secretory capacity in early adulthood, and diabetes in middle age. We 'knocked-in' this SUR1/E1506K human mutation in mice aiming to investigate the molecular mechanisms of this mutation on the regulation of insulin secretion. We also performed experiments using the MIN6 cell lines. We observed that the pancreatic islets of the SUR1/E1506K mice do not respond normally to glucose and incretin (GLP-1) stimulation. The 1st phase of insulin secretion was decreased due to lower protein expression and activity of EPAC-2. We demonstrated that the hyperinsulinemia during infancy is likely caused by a cross-talk between two different insulin pathways regulating insulin secretion, namely the ATP dependent potassium channels hyperactivated by the mutation, and the cholinergic pathway, since the islets were hypersensitive to acetylcholine. Both pathways (but mainly the cholinergic pathway) lead to an increase in calcium influx into the beta-cells. High intra-cellular calcium levels and a subsequent activation of other regulatory pathways lead to a decrease in TRPM5 expression, and an increase in osteopontin gene expression, which further impair insulin secretion at later stages of diabetes.

HIPPOCAMPAL VOLUME, TENSOR-BASED MORPHOMETRY AND VOXEL-BASED MORPHOMETRY IN FRONTOTEMPORAL DEMENTIA

MA Muñoz [1], P Hartikainen [1,2], J Koikkalainen [3], R Wolz [4], V Julkunen [1,2], E Niskanen [5], S-K Herukka [1,2], M Kivipelto [1,6], R Vanninen [5], D Rueckert [4], J Lötjönen [3], and H Soininen [1,2]

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Introduction & objective: MRI is an important clinical tool for diagnosing dementia diseases. However there is a need of finding a more accurate and standardized method. We compare Frontotemporal Dementia (FTD) with Alzheimer's Disease (AD) and Mild Cognitive Impairment (MCI) stages using automatic MRI analysis methods: Hippocampal Volumetry (HV), Tensor-based Morphometry (TBM) and Voxel-based Morphometry (VBM), in specific regions of interest in order to find the highest accuracy. Methods: Thirty-seven FTD patients, 46 AD patients, 26 control subjects (C), 16 patients with progressive MCI (P-MCI) and 48 patients with stable-MCI (S-MCI) were included in the analyses. We calculated the Correct Classification Accuracy (CCR), sensitivity (SS) and specificity (SP) between the study groups. Results: We found significant results differentiating FTD from C with HV (hippocampus left side) (CCR=0.83; SS=0.83; SP=0.82), with TBM (hippocampus and amygdala) (CCR=0.83; SS=0.96 SP=0.72), and with VBM (all the regions studied, especially in hippocampus and amygdala) (CCR=0.86; SS=0.96; SP=0.78). VBM achieved the highest accuracy in differentiating FTD and AD (CCR=0.74; SS=0.78; SP=0.70). Conclusion: VBM resulted in highest accuracy in differentiating between FTD and AD.

CORTICAL SPREADING DEPRESSION INVESTIGATED USING SPIN-LOCK FUNCTIONAL MAGNETIC RESONANCE IMAGING

Joonas Autio, Artem Shatillo, Olli Gröhn

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Functional magnetic resonance imaging (fMRI) is based on the blood oxygen level-dependent (BOLD) contrast. However, because MRI signal is not only sensitive to BOLD contrast but also to tissue homeostasis, we hypothesized that intense metabolic events may cause endogenous tissue relaxation changes detectable with fMRI. Cortical spreading depression (CSD) was used as a suitable pathophysiological paradigm to evoke strong but transient homeostatic disturbances. The underlying fMRI signal changes during CSD were investigated using cerebral blood volume (CBV)-weighted fMRI and spin-lock RF-technique that attenuate blood and the extra-vascular BOLD signals, respectively, with an aim to detect changes in endogenous tissue signals. Our results show substantial differences between CBV-weighted and SL-weighted signals indicating that fMRI signal is modulated by endogenous tissue contrast. Our preliminary results suggest a new concept: BOLD-contrast is an inadequate model to fully describe fMRI signals during strong pathophysiological paradigms. The mechanisms of the endogenous tissue contrast remain elusive.

Oral Session V

Chairs:
– Heidi Laitinen & Tomi Tuomainen –

INTERFERON β SENSITIVITY OF TUMOR CELLS CORRELATES WITH POOR RESPONSE TO VA7 VIROTHERAPY IN MOUSE GLIOMA MODELS

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In our recent study replicative alphaviral vector VA7 was very effective against orthotopic human U87-glioma *in vivo* model. We tested here VA7 efficacy in immunocompetent orthotopic GL261 and CT-2A glioma models of C57BL/6 mouse. Despite general *in vitro* susceptibility, GL261 infection was highly restricted in confluent cell cultures and mouse IFNβ pretreatment prevented VA7 replication in both cell lines. When mice bearing orthotopic GL261 or CT-2A tumors were administered neurotropic VA7, either intravenously or intracranially, the vector was unable to infect the tumor and no survival benefit was achieved. Pre-treatments with CPA and rapamycin had no effect on tumor infection or survival. Intracranial GL261 tumors were refractory also in athymic C57BL/6 mice, which have serious defects in their adaptive immunity. Implanted VA7 preinfected GL261 cells also developed tumors excluding participation of physical barriers and indicating robust host interferon action. Mouse and human IFNβ seem not to be species cross-reactive, which might limit the translational relevance of xenograft models in oncolytic virotherapy. In our pilot experiments, valproic acid demonstrated efficacy in combination with VA7 in GL261 model doubling the survival.

THE EFFECT OF KNOCKDOWN OF XIAP IN A SWITCH FROM TYPE II TO TYPE I TRAIL SIGNALING IN HUMAN FOLLICULAR LYMPHOMA CELLS

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Follicular lymphoma (FL) is a common type of non-Hodgkin Lymphoma (NHL). It is not curable and the median survival of FL patients is eight to ten years. The anti-apoptotic Bcl-2 family proteins protect FL cells from apoptosis (a programmed cell death) by keeping mitochondrial integrity from a variety of apoptotic stimuli. Based on Bcl-X_L overexpression studies in human FL cell lines, we have shown that TRAIL induces apoptosis through type I apoptotic signaling (mitochondrial independent) or type II apoptotic signaling (mitochondrial dependent) pathways. We have also found that in Bcl-X_L overexpressing type II cells, TRAIL induced partial processing of caspase-3 whereas, the presence of a proteasome inhibitor MG-132 led to the full activation of caspase-3 and switched type II signaling to type I signaling. The switch of type II to type I apoptotic signaling helps to overcome the resistance of FL and other lymphomas against cancer therapy. We propose that X-linked inhibitory of apoptosis protein (XIAP), a potent suppressor of apoptosis prevents full activation of caspase-3. At present, we examine the effect of regulated knockdown of XIAP using lentiviral vector, pSLIK (Single Lentivector for inducible knockdown) to investigate the role of XIAP in the switch of the signaling pathway.

CHARACTERIZATION OF hNPC DERIVED FROM hES AND hiPS CELLS INJECTED INTO INTACT AND INJURED SPINAL CORD

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Transplantation of human neural progenitor cells (hNPCs) is thought to represent a promising novel approach in treating the spinal cord injury (SCI). However, human embryonic stem (hES) cells as a source of hNPCs has quantitative, immunological and ethical restrictions. These limitations can be overcome by the application of human induced pluripotent stem (hiPS) cells. To investigate the survivability, migration and differentiation potential of hES- and hiPS-derived hNPCs, the cells were labeled with lenti-GFP and injected into the intact or injured (only hES) spinal cord of C57BL/6J mice after T10 laminectomy and/or contusion SCI. Seven days after transplantation, the mice were sacrificed and spinal cords were dissected for immunohistochemical analysis. Transplanted cells survived and migrated along the intact and injured spinal cords. The transplanted cells did not express pluripotent marker Nanog, asrocyte marker GFAP, microglial marker Iba1 or neuronal marker Tuj1. Instead, GFP^+ cells were positive for DCX and Ki-67, markers of neurogenesis and cell proliferation. Our results show that grafted hNPCs derived from hiPS and hES cells are actively dividing and proliferating cells which survive at least up to 7 days in intact and injured SC. Whether transplantation of hiPS-derived hNPC into the injured spinal cord is beneficial, remains to be investigated.

PREVENTING ATHEROSCLEROSIS WITH IK17-ANTIBODY IN MICE

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Atherosclerosis and its clinical symptoms are one of the most common causes of mortality in Western World. The disease is caused by accumulation on modified cholesterols and inflammation in arterial walls, causing eventually complete blockage of an artery. IK17 is a single chain antibody(ScFv) against oxLDL. Short term expression of IK17 has been shown to reduce atherosclerotic plaques. Goal of our study was to investigate the effects of long term expression of IK17 in mouse models. We created transgenic(Tg) mice expressing IK17 under apoE-promoter and crossbred them to $LDLR^{-/-}$ mice. Secondly, to study the macrophage specific effects of IK17-expression irradiated $LDLR^{-/-}$ mice were also transplanted with bone marrow of IK17-Tg-mice. The mice were kept on high fat diet for 12 weeks. The cross-sectional area of the aortic root and the lesion coverage of the aorta were assessed. We produced a novel IK17-ScFv-Tg-mouse model to study the therapeutic effects of long term anti-oxLDL antibody production in the treatment of atherosclerosis. Throughout the study, we detected IK17 from the serum of Tg-mice in high levels. Preliminary results from bone marrow transplantation studies showed that macrophage-specific expression of IK17 is enough to reduce lesions size in heart leaflets.

SELECTION OF TUMOR AND TUMOR VASCULATURE SPECIFIC ANTIBODIES FROM PHAGE DISPLAY LIBRARY AND THEIR GENETIC DELIVERY *IN VIVO*

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Monoclonal antibodies hold great promises for cancer treatment but there are still some remarkable limitations (i.e. high cost, non-stable plasma concentration and side effects) in current antibody therapies. Genetic delivery of therapeutic antibodies can solve many of these problems. The aim of this study is to find new tumor and tumor vasculature specific antibodies and achieve stable and long-term gene expression *in vivo* using a single administration of a lentiviral vector expressing a therapeutic antibody. For that we are constructing a human scFv phage display library using blood samples of cancer patients. The constructed library will be screened *in vivo* using three different animal models with highly vascularised tumors. Variable regions of heavy and light chains of selected antibodies will be cloned into lentiviral vector and administered to tumor bearing animals. We will study the ability of antibody expressing lentiviral vector to suppress tumor growth aiming at high, long-lasting and stable gene expression. Study results are expected to be beneficial in cancer treatment, diagnostics and drug targeting to tumors or tumor vasculature.

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The Sixth Annual Post-Graduate Symposium of the Doctoral Program in Molecular Medicine

Winter School 2012 Abstracts

The Doctoral Program in 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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