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Tuulia Huhtala et al.

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

Winter School 2011 Abstracts

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

UNIVERSITY OF
EASTERN FINLAND

A.I.VIRTANEN
INSTITUTE

TUULIA HUHTALA, ANNE JÄÄSKELÄINEN, LAURI LEHTO, TEEMU NATUNEN, JANNE RUOTSALAINEN, 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 5th Annual Post-Graduate Symposium of the Doctoral Program in Molecular Medicine to be held in Katinkulta, Vuokatti, on March 15 – 16, 2011.

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DOCTORAL PROGRAM IN MOLECULAR MEDICINE

WINTER SCHOOL 2011

MARCH 15 – 16, KATINKULTA VUOKATTI

PROGRAM

Tuesday, March 15

7:00	Departure from Bioteknia 1 (Neulaniementie 2)
9:45 – 10:15	Coffee, Poster set up
10:15 – 10:25	Opening of the Symposium ***Seppo Ylä-Herttuala***
10:25 – 11:45	**ORAL SESSION I** *Chairs: Merja Jaronen and Sara Paulo*
10:25 – 10:45	Brain disorders: from mechanisms to treatments ***Gundars Goldsteins***
10:45 – 11:00	IVIG alleviates cognitive deficits after global cerebral ischemia in C57/ Bl6 mice and provides time and dose dependent neuroprotection ***Riikka Heikkinen***
11:00 – 11:15	Minocycline inhibits formation of insoluble TAU aggregates in vitro ***Taisia Rolova***
11:15 – 11:30	Contribution of monocytic cells derived from hematopoietic stem cells of bone marrow in the clearance of Aβ of Alzheimer's disease ***Ekaterina Savchenko***
11:30 – 11:45	Stem cell derived human neural progenitors restore gait impairments in a mouse model of cerebral stroke ***Kaisa Savolainen***
11:45 – 12:45	**LUNCH & EXHIBITION**
12:45 – 14:10	**ORAL SESSION II** *Chairs: Emilia Kansanen and Heini Belt*
12:45 – 13:00	A naturally occurring dominant-negative splice variant regulates the responsiveness of Galphaq-regulated RhoGTPase activators ***Lili Li***
13:00 – 13:15	Androgen- and glucocorticoid-induced activation of FKBP51 locus through long-range interactions ***Ville Paakinaho***
13:15 – 13:30	Effects of SUMO (small ubiquitin-related modifier) modification on androgen receptor ***Päivi Sutinen***
13:30 – 13:45	The effect of SIRT1 on antioxidant response element mediated gene regulation ***Hanna Leinonen***
13:45 – 14:00	The absence of macrophage NRF2 promotes atherogenesis in early but not in late stages of atherosclerosis ***Anna-Kaisa Ruotsalainen***
14:00 – 14:10	Sigma Life Sciences – Product update ***Marko Santala***
14:10 – 15:10	**COFFEE, POSTER SESSION & EXHIBITION**

15:10 – 16:45 ORAL SESSION III

Chairs: Sofya Ziyatdinova and Lakshman Puli

15:10 – 15: 30 DPMM PhD student feedback summary ***Riikka Pellinen***

15:30 – 15:45 Urokinase-type plasminogen activator receptor modulates epileptogenesis in mouse model of temporal lobe epilepsy ***Xavier Ekolle Ndode-Ekane***

15:45 – 16:00 Role of amyloidogenic APP processing in epileptogenesis ***Diana Miszczuk***

16:00 – 16:15 Loss of GABAergic neurons in the rat dentate gyrus during epileptogenesis depends on the type of epileptogenic insult ***Noora Huusko***

16:15 – 16:30 Migraine mediator CGRP up-regulates pacemaker channels in meningeal nerves and in isolated trigeminal neurons: a novel mechanism of headache? ***Azat Abdullin***

16:30 – 16:45 The role of serine 275 in shaping of the binding pocket of ATP-gated P2X3 receptor ***Natalia Petrenko***

16:45 – 20:00 CHECK IN, OUTDOOR ACTIVITIES

20:00 DINNER

DOCTORAL PROGRAM IN MOLECULAR MEDICINE

WINTER SCHOOL 2011

MARCH 15 – 16, KATINKULTA VUOKATTI

PROGRAM

Wednesday, March 16

7:00 – 8:20	Breakfast
8:25 – 9:45	**ORAL SESSION IV** *Chairs: Jussi Paananen and Sanna-Kaisa Häkkinen*
8:25 – 8:45	In the light of nutrigenetics: you are what you eat? ***Tiina Lappalainen***
8:45 – 9:00	Microfibrillar associated protein 5 (MFAP5) is linked with markers of obesity-related extracellular matrix remodeling and inflammation ***Maija Vaittinen***
9:00 – 9:15	TCF7L2 splicing is regulated by weight loss and differs between fat depots in humans ***Dorota Kaminska***
9:15 – 9:30	Association of indices of liver and adipocyte insulin resistance with 19 confirmed susceptibility loci for type 2 diabetes in 6,733 non-diabetic Finnish men ***Jagadish Vangipurapu***
9:30 – 9:45	Prediction of microRNA targets in C. Elegans using a self organizing map ***Liisa Heikkinen***
9:45 – 10:30	**BREAK, POSTER SESSION AND EXHIBITION**
10:30 – 11:45	**ORAL SESSION V** *Chairs: Janne Ruotsalainen and Joanna Huttunen*
10:30 – 10:45	Calcification detection in vivo and ex vivo in injured rat brain using SWIFT ***Lauri Lehto***
10:45 – 11:00	Simultaneous fMRI and local field potential measurements of pentylenetetrazol induced epileptic seizures in traumatic brain injury rats ***Antti Airaksinen***
11:00 – 11:15	Rotating frame spin lattice relaxation time mapping and cine MRI of myocardial infarcted mice in vivo at 9.4 T ***Haja-Sherief N. Musthafa***
11:15 – 11:30	Interferon-beta sensitivity of tumour cells correlates with response to oncolytic VA7 virotherapy in mouse glioma models ***Miika Martikainen***
11:30 – 11:40	Fisher Scientific – Efficient sample homogenisation with Precellys 24 ***Isto Jänönen***
11:40 – 12:40	**LUNCH AND EXHIBITION**

12:40 – 14:15 **ORAL SESSION VI**
Chairs: Timo Sarajärvi and Suvi Jauhiainen

12:40 – 13:00 Why are photoreceptors of different insect species using a different set of Kv-channels? ***Matti Weckström***

13:00 – 13:15 Effects of human intravenous immunoglobulin on amyloid pathology and neuroinflammation in a mouse model of Alzheimer's disease ***Lakshman Puli***

13:15 – 13:30 Genetic analysis of genes involved in amyloid-beta degradation and clearance in AD ***Teemu Natunen***

13:30 – 13:45 Chronic ibuprofen treatment does not affect the secondary pathology in the thalamus or improve behavioural outcome in MCAO rats ***Anu Lipsanen***

13:45 – 14:00 The effects of local VEGF gene transfer on regional lymph nodes ***Galina Dragneva***

14:00 – 14:15 Effect of spermidine/ spermine-N^1-acetyltransferase (SSAT) on hematopoiesis ***Sini Pirnes-Karhu***

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

WINTER SCHOOL 2011

MARCH 15 – 16, KATINKULTA VUOKATTI

PROGRAM

POSTERS

1. Evaluation of pharmacological responses by quantitative T2 fMRI ***Joanna Huttunen***
2. Implementation of migraine aura model for fMRI studies ***Artem Shatillo***
3. New insights into susceptibility microstructure using phase spin-locking magnetic resonance imaging ***Joonas Autio***
4. Anti-migraine medicine Naproxen inhibits ATP gated channels and Calcium oscillations in trigeminal neurons ***Taneli Hautaniemi***
5. Generation of patient specific induced pluripotent stem cells ***Sara Paulo***
6. A non-selective Calcium channel blocker, Bepridil, decreases soluble aβ and Calcium levels in the thalamus after MCAO in rats. ***Timo Sarajärvi***
7. Differential roles of MA3PKs in MAP Kinase related Neurodegenerative Diseases: Development of novel MAP3Ks inhibitory compounds ***Maykel López-Rodríguez***
8. Roles and functions of the NOS1AP adaptor protein ***Xiaonan Liu***
9. Nitro-oleic acid regulates endothelin receptor B in human endothelial cells in a Nrf2 dependent manner ***Emilia Kansanen***
10. VEGF-D stimulates VEGF-A, stanniocalcin-1 and neuropilin-2 and has potent angiogenic effects ***Suvi Jauhiainen***
11. Identification of novel glioma stem cell specific biomarkers and targeting molecules ***Heini Belt***
12. VA7 infects human glioma derived slice cultures and primary cell cultures and has only limited replication in healthy macaque brain tissue ***Janne Ruotsalainen***
13. The effect of ketogenic diet on epileptiform EEG abnormalities in a mouse model of Alzheimer's disease ***Sofya Ziyatdinova***
14. Protein disulphide isomerase regulates SOD1 activity and controls cytochrome C-catalyzed peroxidation in amyotrophic lateral sclerosis models ***Merja Jaronen***
15. Granulocyte colony stimulating factor reduces inflammation in a mouse model of ALS ***Eveliina Pollari***
16. NRF2 knockout mice exhibit impaired motor function recovery and increased TNF-alpha production after contusion spinal cord injury ***Yuriy Pomeshchik***

Oral Session I

Chairs:

– Merja Jaronen & Sara Paulo –

IVIG ALLEVIATES COGNITIVE DEFICITS AFTER GLOBAL CEREBRAL ISCHEMIA IN C57/BL6 MICE AND PROVIDES TIME- AND DOSE-DEPENDENT NEUROPROTECTION

R. Heikkinen [1], T. Malm [1], H. Koivisto [1], M. Takalo [1], A. Muona [2], H. Tanila [1], M. Koistinaho[2], J. Koistinaho [1]

[1] Department of Neurobiology, A I V Institute for Molecular Sciences, University of Eastern Finland, [2] Medeia Therapeutics Ltd, Kuopio Finland.

Intravenous immunoglobulin (IVIg) has traditionally been used to treat autoimmune diseases. Recent studies suggest that IVIg is neuroprotective also against acute ischemic attack. The aim of this study was to test the neuroprotective potential of IVIg in a mouse model of global ischemia (GI), including the most effective therapeutic dose and the therapeutic time window for IVIg treatment. Mice were treated with a single dose of 0.1, 0.5 or 1.0 g/ kg IVIg or diluent i.v., 1, 3 or 6 hours after GI. A neurological test battery for motor functions, anxiety, and spatial learning and memory was performed during post-operative weeks 3 and 4. Histological outcome was analyzed with NeuN-staining. Impairment in spatial learning and reduction in survival of the CA1 hippocampal neurons were preveted by a 1.0 g/ kg dose of IVIg given 1 or 3 hours after ischemia. Treatment with IVIG did not protect from motor deficits but decreased post-ischemic anxiety when administered at 1h time point. A single dose of 1g/ kg IVIg given 1 or 3 hours after GI proved to be the most efficient in restoring spatial learning, which correlated well with neuroprotection seen in CA1. Differential effects seen in motor and navigation tasks confirm IVIg as valuable treatment for conditions with hippocampal damage.

MINOCYCLINE INHIBITS FORMATION OF INSOLUBLE TAU AGGREGATES IN VITRO

Taisia Rõlova [1,2], Yuji Yoshiike [2], Miyuki Murayama [2], Jari Koistinaho [1], Akihiko Takashima [2]

[1] Department of Neurobiology, University of Eastern Finland, Finland, [2] Lab for Alzheimer's Disease, RIKEN Brain Science Institute, Japan

Abnormal aggregation of microtubule-associated protein tau contributes to neuronal dysfunction and death that occur in neurodegenerative tauopathies, including Alzheimer's disease (AD). Previous studies have demonstrated that antibiotic minocycline can improve cognitive function in transgenic mouse models of AD and suggested that this drug might have a direct effect on tau pathology. The aim of the present work was to confirm the inhibitory effect of minocycline on tau aggregation. To avoid any confounding factors, we first studied heparin-induced assembly of recombinant wild-type human tau in the presence or absence of the drug. Thioflavin T assay showed that minocycline dose-dependently inhibited formation of beta-sheet-structured tau aggregates. The finding was further confirmed by sucrose gradient fractionation of the samples and protein content analysis of each fraction. We also demonstrated that minocycline inhibited tau aggregation in SHSY-5Y and Neuro2a cell lines overexpressing P301L mutated human tau. Furthermore, in Neuro2a cell line, minocycline reduced tau phosphorylation at Ser202 / Ser205 residues, possibly by upregulating AKT and inhibiting GSK-3-beta activity. In conclusion, minocycline may be a promising drug candidate for the treatment of tauopathies.

CONTRIBUTION OF MONOCYTIC CELLS DERIVED FROM HEMATOPOIETIC STEM CELLS OF BONE MARROW IN THE CLEARANCE OF AB OF ALZHEIMER'S DISEASE

Ekaterina Savchenko [1], Johanna Magga [1], Tarja Malm [1], Piia Valonen [1], Anu Muona [2], Milla Koistinaho [2] and Jari Koistinaho [1]

[1] Department of Neurobiology, University of Eastern Finland, Kuopio, Finland; [2] Medeia Therapeutics Ltd, Kuopio, Finland

Alzheimer Disease (AD) is a progressive neurodegenerative disorder characterized by the presence of β-amyloid (Aβ) deposits and neurofibrillary tangles in the brain. During AD pathology microglia become activated and participate to some extent in the clearance of Aβ. Moreover, it has been shown that during the progression of AD pathology monocytes with morphological features of endogenous microglia are able to migrate through the blood brain barrier (BBB) and participate in the reduction of Aβ. In this study, we investigated the ability of bone marrow (BM) hematopoietic stem cell (HSC) derived monocytic cells to phagocytose Aβ. HSC were isolated from BM and differentiated into monocytic cells. To study the phagocytic activity of these cells ex vivo assay was performed. The reduction of Aβ was analyzed by ELISA and immunocytochemistry. We also transplanted BM HSC derived monocytic cells into the hippocampus of 2-year-old APdE9 transgenic mice. Four days after the transplantation the phagocytic activity and phenotype of transplanted cells was determined with immunostaining and confocal microscopy. The degradation of Aβ was observed both ex vivo and in vivo. These findings suggest the role BM HSC monocytic cells as phagocytic cells which participate in clearance of Aβ.

STEM CELL DERIVED HUMAN NEURAL PROGENITORS RESTORE GAIT IMPAIRMENTS IN A MOUSE MODEL OF CEREBRAL STROKE

Kaisa Savolainen [1], Katja Puttonen [1], Marika Ruponen [1], Tarja Malm [1], Jonna Koponen [2], Seppo Ylä-Herttuala [2], Outi Hovatta [1,3], Jari Koistinaho [1], Anu Muona [1,4]

[1] Department of Neurobiology and [2] Biotechnology and Molecular Medicine, University of Eastern Finland, Kuopio, [3] Karolinska Institute, Sweden, [4] Medeia Therapeutics Ltd, Kuopio, Finland

Cerebral stroke is the third leading cause of death worldwide and the current treatment is restricted to tPA-therapy within 3-4 hour time window and rehabilitation. Human embryonic pluripotent stem cells (hESC) are able to divide indefinitely and differentiate into any kind of cells. Previous studies have shown that transplanted ESC-derived neural progenitor cells (NPCs) survive and enhance functional recovery in animals with cerebral stroke. Modulation of inflammation is suggested to be mostly behind the beneficial outcome. In this study, we transplanted 200 000 hESC-derived GFP-labeled NPCs into the striatum of 5-month-old Balb/ c mice after middle cerebral artery occlusion. Already 2 wks after transplantation, CatWalk™ revealed a very significant functional recovery in treated vs. untreated mice in several gait parameters i.e. print area ($p=0.000006$) and diagonal paw support ($p=0.003$), reaching the level of sham-operated mice lasting up to 12 wks, the end of follow-up time. Previous studies confirmed the survival and neuronal characteristics of the transplanted cells along with mild gliosis and accumulation of monocytic cells at the transplantation site. The results indicate that transplantation of hESC can promote a dramatic long-term motor recovery in mouse models of ischemic stroke.

Oral Session II

Chairs:

– Emilia Kansanen & Heini Belt –

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

Lili Li, Tim Church, Soila Tossavainen, Veronika Redai, Michael Courtney
Molecular Signalling Laboratory, Department of Neurobiology, A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland

RhoGTPases have a powerful influence on cell behavior and fate via regulation of transcription and cytoskeleton. Small GTPases such as members of Rho family are activated by exchange of bound GDP for GTP, which is mediated by guanine-nucleotide exchange factors (GEFs). Extracellular signals activate RhoGTPases via G protein coupled receptors. Gα12/ 13-regulated GEFs are well studied, whereas not much is known about Gαq-regulated neuronally-enriched 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. This variant is expressed in different cell types. This variant is not merely inactive as a Rho activator, but also acts as a dominant negative inhibitor of PKT-GEFs. Deletion analysis reveals the region sufficient for inhibition, which we reveal possesses an unexpected yet strong homophilic interaction which may be responsible for the inhibitory action. We propose a model whereby activation of PKT-GEFs renders them competent to interact in trans with the inhibitory region of the variant. 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.

ANDROGEN- AND GLUCOCORTICOID-INDUCED ACTIVATION OF FKBP51 LOCUS THROUGH LONG-RANGE INTERACTIONS

Ville Paakinaho, Harri Makkonen, Tiina Jääskeläinen and Jorma J. Palvimo
Institute of Biomedicine/Medical Biochemistry, University of Eastern Finland, Kuopio, P.O. Box 1627, FI-70211 Kuopio, Finland

FKBP51 (an immunophilin) is a sensitive biomarker of corticosteroid and androgen responsiveness in lung and prostate. We have elucidated the molecular mechanisms underlying the induction of FKBP51 by the glucocorticoid receptor (GR) in A549 lung cancer cells and by the androgen receptor (AR) in VCaP prostate cancer cells. Both cell lines show a robust accumulation of FKBP51 mRNA in response to exposure of their cognate steroid. ChIP scans and enhancer activity analyses indicate that activation of the FKBP51 locus by glucocorticoids and androgens is triggered by the loading of GR/ AR to enhancers at ~34 kb 5' and ~87 kb 3' from the TSS, both acting the major enhancers for AR but only the latter one for GR. Steroid exposure also resulted in significant recruitment of RNA polymerase II and SWI/ SNF chromatin remodeling complexes within the locus. Furthermore ChIP scans show that the FKBP51 region encompassing the steroid-regulated enhancers is similarly bordered by CTCF- and cohesin-binding (RAD21) sites in both cell lines. Taken together, these results indicate that GR/ AR are similarly capable of activating transcription and evoking changes in chromatin structure through distant-acting enhancers by means of chromatin loops that are probably stabilized by CTCF and cohesin complexes.

EFFECTS OF SUMO (SMALL UBIQUITIN-RELATED MODIFIER) MODIFICATION ON ANDROGEN RECEPTOR

Päivi Sutinen, Jorma Palvimo
Institute of Biomedicine, University of Eastern Finland, Kuopio

SUMOylation is post-translational modification in which small ubiquitin-like modifiers (SUMO-1, -2, -3) are covalently attached to specific lysine residues of target proteins. One of the known SUMO targets is androgen receptor (AR). The AR is a ligand-regulated transcription factor which has an important role in the development and progression of prostate cancer. The AR is known to be SUMOylated at two sites (K386 and K520). In this work, we constructed HEK293 cell lines that stably express wild-type (wt) or SUMOylation-deficient AR (K386 and K520 converted to arginines). By using these cell lines, we compared the stabilities of the wt and the mutant AR, their abilities to regulate gene expression and bind to chromatin. Interestingly, the half-life of the mutant AR was shorter than that of the wt AR, suggesting that the SUMO modifications protect the receptor from degradation. qRT-PCR analyses confirmed that certain AR target gene mRNAs, such as S100P mRNA and SPOCK1 mRNA, were more strongly expressed in the mutant AR cells. ChIP analyses further indicated that the AR SUMOylation sites influence the kinetics of the receptor binding to chromatin; the mutant AR bound faster and stronger to androgen response element-containing chromatin sites of the latter two AR target genes. Further studies addressing the role of the SUMOylation sites in the receptor chromatin interactions on a genome-wide scale as well as their effect on the mobility of the AR are currently under way.

THE EFFECT OF SIRT1 ON ANTIOXIDANT RESPONSE ELEMENT MEDIATED GENE REGULATION

Hanna Leinonen, Emilia Kansanen, Seppo Ylä-Herttuala, Anna-Liisa Levonen
Department of Biotechnology and Molecular Medicine

Oxidative stress is involved in the pathogenesis of age-related diseases such as cardiovascular diseases, cancer and neurodegenerative disorders. Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor regulating the expression of several oxidative stress inducible genes via binding to the Antioxidant Response Element (ARE). Sirt1 is a class III histone deacetylase known to deacetylate many stress-related transcription factors leading either to their activation or inactivation. Both Nrf2 and Sirt1 are activated in oxidative stress, and by a polyphenol resveratrol. We therefore hypothesized that Sirt1 would impact on Nrf2-ARE signalling. To test this hypothesis, Sirt1 inhibitor, nicotinamide (NAM), and Sirt1 overexpression were used to assess ARE activity with reporter constructs containing the luciferace gene under the control of Nrf2 target gene promoter. NAM inhibited both the basal and inducible ARE activity, whereas Sirt1 overexpression increased the ARE activation by Nrf2 inducing agents. In addition, NAM attenuated Nrf2 target gene expression induced by ARE activators. Sirt1 also interacted with Nrf2 in both basal as well as inducible conditions, assessed by immunoprecipitation and western blotting. We conclude that Sirt1 positively regulates Nrf2 activity.

THE ABSENCE OF MACROPHAGE NRF2 PROMOTES ATHEROGENESIS IN EARLY BUT NOT IN LATE STAGES OF ATHEROSCLEROSIS

Anna-Kaisa Ruotsalainen [1], Matias Inkala [1], Jari Lappalainen [1], Mervi Partanen [1], Suvi Heinonen [1], Janne Heikkilä [2], Seppo Ylä-Herttuala [1], Anna-Liisa Levonen [1]

[1] Department of Biotechnology and Molecular Medicine, University of Eastern Finland, [2] Department of Oncology, Kuopio University Hospital

Nrf2 (NF-E2-related factor 2) is a positive regulator of many antioxidant and detoxifying enzymes. Our previous studies indicate that Nrf2 has vasculoprotective functions. However, it has been suggested that Nrf2 promotes atherosclerosis by enhancing foam cell formation through upregulation of CD36 in macrophages. Therefore, we assessed the effect of macrophage-specific loss of Nrf2 on atherogenesis. $Nrf2^{-/-}$ or wild type (WT) bone marrow was transplanted to $LDLr^{-/-}$/ $ApoB^{100/100}$ -mice followed by high-fat diet (HFD) for 6 or 12 weeks. The cross-sectional lesion area of the aortic root and the lesion coverage of the aorta was assessed. In addition, peritoneal macrophages from WT and $Nrf2^{-/-}$ mice were isolated for the assessment of CD36 expression after treatment with oxLDL or acLDL. CD36 induction by oxLDL and acLDL was markedly reduced in $Nrf2^{-/-}$ peritoneal macrophages suggesting a role of Nrf2 in mediating the effect. The lesion coverage of the aortas was 4.9 ± 0.6 % and 2.2 ± 0.5 % ($p < 0.01$) after 6 weeks in $Nrf2^{-/-}$ and WT groups but no difference at 12 weeks. There was no significant difference between the groups in cross-sectional lesion area after 6 or 12 weeks of HFD. We concluded that absence of macrophage Nrf2 promotes atherogenesis in early but not in advanced stages of atherosclerosis.

Poster Session

EVALUATION OF PHARMACOLOGICAL RESPONSES BY QUANTITATIVE T2 FMRI

Joanna Huttunen [1], Antti Airaksinen [1], Kimmo Lehtimäki [2], Artem Shatillo [1], Juha Yrjänheikki [2], Olli Gröhn [1]

[1] Department of Neurobiology, University of Eastern Finland, [2] Cerebricon Ltd / Charles River Labs, Discovery and Imaging Services, Kuopio

Pharmacological magnetic resonance imaging (phMRI) is a novel application of functional MRI where the activation in the brain is induced by a pharmacological agent and measured e.g. with blood oxygenation level dependent (BOLD) contrast. The possible fluctuations (e.g. room temperature, hardware drifts) in the BOLD time series that are in the time scale of the pharmacological activation may not be filtered easily but could be eliminated with the T2 maps, since the drifts are presumed to be roughly similar in two sequential datasets with different echo times (32 ms and 50 ms). 11 male Spraque-Dawley rats were anesthetized with urethane (1.25 g/ kg, i.p.), ventilated and paralyzed with pancuronium bromide (0.5 mg/ kg/ h, i.v.). A bolus of nicotine (n=5, 0.25 mg/ kg, s.c.) or apomorfine (n=6, 0.25 mg/ kg, s.c.) was administered after 500 baseline images (250 T2 maps) and the functional scan was continued for 1000 images (500 T2 maps) using 7.0 T magnet. Nicotine caused large positive cortical activation while smaller positive apomorfine responses were mainly detected bilaterally in the lateral entorhinal cortices. The T2 map method in pharmacological studies could be beneficial in studying new pharmacological agents with small or unknown responses in the brain.

IMPLEMENTATION OF MIGRAINE AURA MODEL FOR FMRI STUDIES.

Artem Shatillo, Rashid Giniatullin, Olli Gröhn

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

The main underlying event in aura phase of migraine is cortical spreading depression (CSD) which is a slow wave of neuronal and glial depolarization that spreads across the cortex with a speed of 2-7 mm/ min. The aim of this work was to implement a robust protocol for induction of CSD in the 9.4T magnet for continuous BOLD fMRI data acquisition with simultaneous local field potentials (LFP) recording in rats. Animal preparations, consisting of femoral artery and vein canulation, cranial window opening and insertion of LFP electrode to ipsilateral frontal cortex was conducted under isoflurane anesthesia. Urethane anesthesia 1.25 g/ kg and muscle relaxation with ventilation (pancuronium bromide, 0.5 mg/ kg/ h i.v.) was used for data collection. We induced CSD after 100 baseline BOLD images by applying 1M KCl solution (10µl) to intact meninges for 13 Wistar rats. The following imaging time was 1h (900 images). During that period, 1-5 CSD waves were observed on LFP and BOLD recordings. Based on BOLD data we calculated CSD properties: mean propagation speed of 5.3 ± 1.4 mm/ min and duration of 129 ± 25 s. Developed protocol allowed us to elicit CSD with very characteristic properties in all KCl treated animals, which makes this model usable for further migraine fMRI studies.

NEW INSIGHTS INTO SUSCEPTIBILITY MICROSTRUCTURE USING PHASE SPIN-LOCKING MAGNETIC RESONANCE IMAGING

Joonas Autio, Lauri Lehto, Olli Gröhn
A.I. Virtanen Institute, Department of Neurobiology, University of Eastern Finland

Magnetic resonance imaging (MRI) can provide noninvasive measurements of magnetic susceptibility differences in biological tissues and phase MRI has been used for clinical and research applications. Currently used phase MRI techniques are sensitive on field inhomogeneities at all spatial length scales. The aim of this study was to investigate whether a novel phase spin-lock MRI can distinguish between large and small field inhomogeneities. Para- and diamagnetic phantoms were measured using SL MRI. The results show that the SL MRI is sensitive to small field inhomogeneities, whereas the larger field inhomogeneities do not contribute to SL MRI contrast. The presented method is expected to be valuable for evaluating microscopic tissue magnetic field inhomogeneities.

ANTI-MIGRAINE MEDICINE NAPROXEN INHIBITS ATP GATED CHANNELS AND CALCIUM OSCILLATIONS IN TRIGEMINAL NEURONS

T. Hautaniemi, N. Petrenko and R. Giniatullin
Department of Neurobiology, University of Eastern Finland

In migraine pain, sensitized trigeminal ganglion (TG) neurons innervating meninges are hyperexcitable, thus producing and relaying nociceptive signals. In the current project we tested whether the popular anti-migraine medicine naproxen could change excitability of cultured TG neurons in fast manner via direct interaction with pain transducing receptors. To this end we used calcium imaging and patch-clamp recordings of rat trigeminal neurons activated by the ATP analogue and the TRPV1 agonist capsaicin. We found that naproxen immediately blocked intracellular calcium transients activated by the ATP analogue and spared those elicited by capsaicin indicating a selective sensitivity of ATP-driven mechanisms. Using patch-clamp recordings we directly tested the action of naproxen on P2X3 mediated membrane currents. Naproxen produced the use-dependent blocking action on ATP receptors which are known to be sensitized in migraine-like states (Fabbretti et al., 2006). The use-dependent mechanism of action presumes that the blocking action of this agent will be primarily expressed in most active tissues. Our data present a novel fast mechanism for the anti-migraine action of naproxen which can act in synergy with the COX inhibition.

GENERATION OF PATIENT SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Sara Paulo [1], Marika Ruponen [1,3], Katja Puttonen [1], Juha Rinne [4], Seppo Heinonen [5], Outi Hovatta [2], Jari Koistinaho [1]

[1] Dept. of Neurobiology and [2] Dept. of Biotechnology & Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, UEF, [3] School of Pharmacy, UEF, [4] University of Turku, [5] Department of Obstetrics and Gynaecology Kuopio University Hospital

Induced pluripotent stem cells (iPSCs) are stem cells derived from somatic cells that resemble embryonic stem cells (ESCs). Two iPSC lines from healthy patients have been already established and their characterization is in underway. Fibroblasts cultured from skin samples were used to transduce with a polycistronic lentiviral vector carrying Oct4, Sox2, Klf4 and c-Myc. The transduced cells are grown on feeder cells and the cultures are observed to see that iPSCs form colonies with similar appearance to ESC-colonies. After 10 passages the cells are considered to be bona fide iPSCs. We will perform karyotyping and also DNA methylation assay to check the methylation status of promoters of Oct4 and Nanog. To confirm the iPSC's ability to form cells of all embryonic germ layers, we will conduct embryoid body and teratoma formation assays. The pluripotency of the cells is also confirmed with RT-PCR and immunocytochemical staining of pluripotency markers. Our aim is to produce patient specific iPSCs that carry either a mutation P264L or a deletion PS-1Delta E9 in the presenilin-1 gene, both of which cause an inherited form of Alzheimer's disease (AD). After the characterizations we will differentiate the iPSCs into neural progenitor cells and fully matured neurons to study mechanisms of AD.

A NON-SELECTIVE CALCIUM CHANNEL BLOCKER, BEPRIDIL, DECREASES SOLUBLE Aβ AND CALCIUM LEVELS IN THE THALAMUS AFTER MCAO IN RATS

T. Sarajärvi [1], A. Lipsanen [1], A. Haapasalo [1], P. Mäkinen [1], S. Peräniemi [2], H. Soininen [1,3], J. Jolkkonen [1], M. Hiltunen [1]

[1] Inst. of Clinical Medicine – Neurology and [2] Dept. of Biosciences, UEF and [3] Dept. of Neurology, Kuopio University Hospital, Kuopio

Alzheimer's disease and cerebral ischemia share similar features in terms of altered amyloid precursor protein processing and β-amyloid (Aβ) accumulation. We previously observed that Aβ and calcium deposition are robustly increased and that β-secretase levels and activity are altered in ipsilateral thalamus after transient middle cerebral artery occlusion (MCAO) in rats. Here we investigated whether chronic bepridil treatment affects thalamic Aβ and calcium levels and functional recovery after MCAO in rats. Male Wistar rats were subjected to sham-operation or transient MCAO. Bepridil (50 mg/ kg/ day, p.o.) or vehicle treatment was started two days after MCAO. Cylinder and tapered/ ledged beam walking tests were used as behavioural outcome measures. After the follow-up, animals were sacrificed for analysis of Aβ40, Aβ42, and calcium levels in contra- and ipsilateral thalami. Bepridil treatment improved forelimb use in MCAO rats in the cylinder test, which coincided with decreased calcium and soluble Aβ40 and Aβ42 levels in the ipsilateral thalamus compared to vehicle-treated rats. Bepridil did not affect astrogliosis or TNFα expression. Our findings suggest that bepridil decreases soluble Aβ and calcium levels in the thalamus and concomitantly improves sensorimotor recovery in MCAO rats.

DIFFERENTIAL ROLES OF MA3PKS IN MAP KINASE RELATED NEURODEGENERATIVE DISEASES: DEVELOPMENT OF NOVEL MAP3KS INHIBITORY COMPOUNDS

Maykel López-Rodríguez, Michael Courtney
Molecular Signalling Lab. Dept. of Neurobiology. A.I. Virtanen Institute. University of Eastern Finland. Kuopio

No effective treatment is available for Alzheimer's Disease, Parkinson's Disease and Cerebral Ischemia. It is therefore crucial to continue studying the pathological mechanisms to develop new therapies. MAPK pathways are strongly implicated in the pathogenesis of these diseases and nhibition of downstream MAPKs JNK and p38 has been found to be neuroprotective. However, the activation signals converge from a larger group of MAP3Ks into a smaller group of MAPKs. Thus, we predict that inhibition of MAP3Ks will result in a more specific inhibitory effect. Within this project we aim to determine the differential roles of MAP3Ks in several models of neuronal stress responses. Based on structure-function studies we are developing a set of peptide-based inhibitors that can be evaluated in vivo. A plasmid-based RNA interference (RNAi) library targeting all MAP3Ks has been created and is currently being validated in cell lines. The effects on neuronal models are evaluated using high-throughput imaging assays. We also started developing a set of constructs producing peptides based on MAP3Ks regulatory domains. These are being selected based on the capacity to inhibit MAP3Ks judged to be relevant to disease models based on our results from the RNAi library.

ROLES AND FUNCTIONS OF THE NOS1AP ADAPTOR PROTEIN

Xiaonan Liu [1], Tuomo Laitinen [2], Zoher Hoosenally [3], Antti Poso [2], Michael Courtney [1]
[1] Department of Neurobiology, University of Eastern Finland, [2] Department of Pharmaceutical Chemistry, University of Eastern Finland [3] Faculty of Health and Medical Sciences, University of Surrey, UK

Neuronal nitric oxide synthase adaptor protein (NOS1AP) is associated with diseases and conditions including sudden cardiac death (SCD) and schizophrenia, however their association with NOS1AP is without mechanistic explanation. Neuronal nitric oxide synthase (NOS1) is also an important factor in disease, and NOS1AP is reported to regulate NOS1. The aim of this project is to obtain further insights into the mechanism of action of NOS1AP. The N-terminus of NOS1AP contains a PTB domain, while the C-terminus includes a PDZ domain-binding motif. We used C-terminal deleted forms of recombinant, purified NOS1AP containing the PTB domain and confirmed the C-terminal region of RasD1 could act as a ligand. Meanwhile, we generated a model of NOS1AP-PTB using molecular docking methods to select a panel of candidate ligands for evaluation in future binding experiments. The most promising ligands can then be used to address biological questions and be considered for use in disease models. In addition we used deletion and point mutant forms of nNOS in binding assays with N-terminally deleted forms of NOS1AP to map the regions within the NOS1AP that are responsible for interaction with nNOS disease relevance will be subsequently evaluated primarily in cell culture models of neuronal and cardiac systems.

NITRO-OLEIC ACID REGULATES ENDOTHELIN RECEPTOR B IN HUMAN ENDOTHELIAL CELLS IN A NRF2 DEPENDENT MANNER

Emilia Kansanen, Anna-Kaisa Ruotsalainen, Heidi Laitinen, Seppo Ylä-Herttuala, Anna-Liisa Levonen

Department of Biotechnology and Molecular Medicine, A.I.Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland

Nitro-fatty acids (NO_2-FAs) are signaling mediators formed in vivo via nitric oxide and nitrite dependent reactions. NO_2-FAs modulate signaling cascades via covalent post-translational modifications of nucleophilic amino acids in regulatory proteins, thus altering downstream signaling events, such as Keap1-Nrf2-antioxidant response element (ARE) regulated gene expression. We have recently explored the effects of nitro-oleic acid (OA-NO_2) on endothelial cell transcriptome and found that one of the genes induced by OA-NO_2 and very tightly regulated by Nrf2 was endothelin receptor B (ET_B), which mediates the vasodilatory effects of endothelin-1 (ET-1). In the present study, control and Nrf2-siRNA transfected human aortic and vein endothelial cells and mouse endothelial cells isolated from either $Nrf2^{-/-}$ or wt mice were treated with vehicle or OA-NO_2 and the levels of endothelial receptor type A and B were determined by quantitative PCR. The result revealed that the upregulation of ET_B is tightly regulated by Nrf2. Furthermore, in silico analysis of ET_B gene discovered four putative ARE sites in the ET_B gene. These data suggests that Nrf2 regulates the transcription of ET_B gene, potentially affecting the signaling events related to blood pressure regulation and vascular remodeling.

VEGF-D STIMULATES VEGF-A, STANNIOCALCIN-1 AND NEUROPILIN-2 AND HAS POTENT ANGIOGENIC EFFECTS

Suvi Jauhiainen, Sanna-Kaisa Häkkinen, Pyry Toivanen, Suvi Heinonen, Henna-Kaisa Jyrkkänen, Emilia Kansanen, Hanna Leinonen, Anna-Liisa Levonen, Seppo Ylä-Herttuala

Department of Biotechnology and Molecular Medicine, University of Eastern Finland

Mature form of human vascular endothelial growth factor-D (VEGF-$D^{\Delta N\Delta C}$) is an efficient angiogenic factor under normoxic conditions but its full mechanism of action and target genes have remained unclear. We studied the effects of VEGF-$D^{\Delta N\Delta C}$ in human umbilical vein endothelial cells (HUVECs) and TIME cells using gene array, signalling, cell culture and *in vivo* gene transfer techniques. Concomitant with the angiogenic and proliferative responses VEGF-$D^{\Delta N\Delta C}$ enhanced the phosphorylation of VEGFR-2, Akt and eNOS in HUVECs. Gene arrays, qRT-PCR and western blot revealed increases in VEGF-A, stanniocalcin-1 (STC1) and neuropilin (NRP)-2 expression by VEGF-$D^{\Delta N\Delta C}$-stimulation, whereas induction with VEGF-A_{165}, a well known activator of endothelial cells, altered the expression of STC1 and NRP1, another co-receptor for VEGFs. The effects of VEGF-$D^{\Delta N\Delta C}$ were only seen at high-serum conditions, whereas for VEGF-A_{165} the strongest response was observed at low-serum conditions. The VEGF-$D^{\Delta N\Delta C}$-induced upregulation of STC1 and NRP2 was evident also *in vivo* in mouse hind limb skeletal muscle treated with VEGF-$D^{\Delta N\Delta C}$ by adenoviral gene delivery. The importance of NRP2 in VEGF-$D^{\Delta N\Delta C}$ signaling was further studied with NRP2 siRNA and NRP antagonist which were able to block VEGF-$D^{\Delta N\Delta C}$-induced survival of HUVECs.

IDENTIFICATION OF NOVEL GLIOMA STEM CELL SPECIFIC BIOMARKERS AND TARGETING MOLECULES

Heini Belt [1], Outi Rautsi [1], Kirsi Vuorinen [1], Juha E. Jääskeläinen [2] and Pirjo Laakkonen [1, 3]

[1] AIVI, University of Eastern Finland, [2] Dept. of Neurosurgery, Kuopio University Hospital, [3] Research Programs Unit, University of Helsinki

The goal of this study is to create novel tools to study and treat malignant gliomas. Despite of aggressive conventional treatments and rapid development of novel cancer therapies, prognosis of patients diagnosed with malignant glioma remains poor, and more specific therapies are needed. Gliomas, as most cancers, contain a subpopulation of cells that share properties with normal stem cells and have an ability to initiate and maintain the tumor. The lack of reliable glioma stem cell (GSC) specific markers, however, hambers the investigation of these cells. This study aims at identifying novel GSC specific markers and targeting molecules. We will isolate GSC from human glioma specimens with the aid of FACS and/ or magnetic beads, expand them *in vitro* and inject intracranially into mice. To identify novel GSC targeting molecules, we will screen a phage displayed peptide library (CX_7C peptide library on the T7Select415-1-phage) *ex vivo* and *in vivo* to identify peptide(s) that recognize(s) specific structures on the surface of GSC. These peptides will be used to identify their receptor molecules = GSC biomarkers, and as delivery vehicles to target GSC specific therapies to brain tumors.

VA7 INFECTS HUMAN GLIOMA DERIVED SLICE CULTURES AND PRIMARY CELL CULTURES AND HAS ONLY LIMITED REPLICATION IN HEALTHY MACAQUE BRAIN TISSUE

Janne Ruotsalainen [1], Miika Martikainen [1], Kirsi Vuorinen [1], Markus Vähä-Koskela[2], Juha Jääskeläinen [3] John Bell[2], Pirjo Laakkonen [1], Ari Hinkkanen [1]

[1] A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland, [2] Ottawa Health Research Institute, Ottawa, Canada, [3] Neurosurgery, Neurocenter, Kuopio University Hospital, Kuopio, Finland

Semliki Forest virus (SFV) is a non-pathogenic alphavirus, which replicates efficiently in a number of different cancer cell lines. In our recent study intravenously administered SFV VA7 completely eliminated subcutaneous and intracranial U87 human glioma xenografts in nude mice. Here we demonstrate the susceptibility of a human malignant glioma biopsy derived tissue slice culture and a primary cell explant culture to VA7 mediated infection and oncolysis, and limited replication in healthy Macaque brain tissue culture. Live tissue slice cultures and primary cell explants were established from a human malignant glioma biopsy samples and a healthy macaque brain and they were infected with oncolytic SFV VA7 alphavirus vector. VSV Δ51-EGFP and wtVSV-EGFP viruses were used as controls in some of these infections. The cells and spontaneously forming tumour spheres were infected with VA7 virus with or without human IFNβ pretreatment. VA7 infected human malignant glioma tissue slice cultures and killed biopsy derived malignant glioma cell explants. The replication was either restricted or delayed in the glioma cell explant cultures after human IFNβ pretreatment. VA7 had only limited and focal replication profile in healthy macaque brain tissue slice culture.

THE EFFECT OF KETOGENIC DIET ON EPILEPTIFORM EEG ABNORMALITIES IN A MOUSE MODEL OF ALZHEIMER'S DISEASE.

Sofya Ziyatdinova [1], Misha Zilberter [2], Yuri Zilberter [2], Tibor Harkany [2], Asla Pitkänen [1], Heikki Tanila [1].

[1] Department of Neurobiology, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland. [2] Karolinska Institutet, Stockholm, Sweden.

Alzheimer's disease (AD) is one of the priority mental health disorders, affecting around 24 million people worldwide. AD patients have about 8-times higher risk to develop unprovoked seizures than age-matched control population. Mice with amyloid precursor protein mutation (APP) and presenilin-1 (PS1) mutation have amyloid plaques formation and cognitive impairment. We recently showed that about 65% of APP/ PS1 transgenic mice have at least one unprovoked seizure by the age of 5 month. Reduced glucose utilization in AD brain could lead to neuronal damage. We aim to compensate for impaired glucose metabolism by complementing glucose with alternative energy substrates. We administered pyruvate and beta-hydroxybutyrate enriched diet to female 12-13 wk old APP/ PS1 mice (n=9) during 5 wk, control group (n=8) received regular mouse show. Video-EEG monitoring was done 24/ 7 during 1 wk before the diet started, during the last 2 wk of the diet and during 2 wk thereafter. 3 of 17 mice included in the study had seizures at baseline, 8/ 9 mice in diet group and 6/ 8 in control had 1-245 EDs per week (24±59). Number of EDs was reduced during and after the ketogenic diet ($p<0.05$). Analysis of epileptiform spikes is ongoing to verify the effect of the diet on suppression of epileptiform activity.

PROTEIN DISULPHIDE ISOMERASE REGULATES SOD1 ACTIVITY AND CONTROLS CYTOCHROME C-CATALYZED PEROXIDATION IN AMYOTROPHIC LATERAL SCLEROSIS MODELS

Merja Jaronen [1], Toni Ahtoniemi [2], Jari Koistinaho [1] and Gundars Goldsteins [1]

[1] Dept of Neurobiology, A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland. [2] Medeia Therapeutics Ltd, Kuopio, Finland.

Increased mitochondrial production of ROS is a hallmark in several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Some forms of ALS are associated with mutations in superoxide dismutase -1 (SOD1) gene. SOD1 is present in an inactive form in the mitochondrial intermembrane space (IMS) where its activity is controlled by the redox state of an intramolecular disulphide bond which is executed by protein disulfide isomerase (PDI). We have shown that mutant SOD1 is up regulated in the IMS and SOD1 activity in the IMS increases mitochondrial ROS production by enhancing hydroperoxide production, resulting in augmented cytochrome c-catalyzed peroxidation. PDI expression peaks in the spinal cord of ALS animal models at presymptomatic stage. We investigated whether PDI can exercise redox control of SOD1 activity leading to increased hydroperoxide production and cytochrome c-catalysed peroxidation. We show that PDI catalyzes reactivation of SOD1 after inactivation by disulphide bond reduction. This resulted in increased hydroperoxide production and cytochrome c-catalysed peroxidation which were inhibited by bacitracin. Inhibition of PDI suppressed also paraquat-induced hydroperoxide production. 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

GRANULOCYTE COLONY STIMULATING FACTOR REDUCES INFLAMMATION IN A MOUSE MODEL OF ALS

E. Pollari [1], K. Kanninen [1], R. Giniatullina [1], T. Ahtoniemi [2], T. Malm [1], R. Giniatullin [1], J. Koistinaho [1], J Magga [1]

[1] Department of Neurobiology, A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland; [2] Medeia Therapeutics Ltd, Kuopio, Finland.

Amyotrophic lateral sclerosis (ALS) is a lethal motoneuron disease without cure. We propose that granylocyte colony stimulating factor (G-CSF), a neuroprotective agent, is a promising drug candidate for treatment of ALS. Our aim was to assess the G-CSF mediated protection in the central nervous system (CNS) and in inflammatory status in mouse model of ALS. Transgenic mice modelling ALS received G-CSF treatment and at different stages of the disease an array of inflammatory markers in the CNS were analyzed and the survival was monitored. Neuroprotective effect of G-CSF was confirmed in primary spinal cord (SC) cultures. G-CSF treatment prolonged the survival of the transgenic mice (from 173±6.7 to 185±6.7 days $p<0.01$) and reduced production of proinflammatory TNF-alpha in microglia and monocytes. In addition, G-CSF treatment reduced gliosis in the ventral horn of lumbar SC and diminished ROS levels in the CNS. In primary SC cultures G-CSF significantly protected against glutamate toxicity. Our data reveal new anti-inflammatory properties of G-CSF in a mouse model of ALS. We were able to demonstrate that G-CSF prolongs survival of transgenic mice and protects neurons in culture. These results validate beneficial properties of G-CSF as a neuroprotective factor for the treatment of ALS.

NRF2 KNOCKOUT MICE EXHIBIT IMPAIRED MOTOR FUNCTION RECOVERY AND INCREASED TNF-ALPHA PRODUCTION AFTER CONTUSION SPINAL CORD INJURY

Yuriy Pomeshchik [1], Tarja Malm [1], Katja Kanninen [1], Anna-Liisa Levonen [2], Jari Koistinaho [1]

[1] Department of Neurobiology, University of Eastern Finland; [2] Department of Biotechnology and Molecular Medicine, University of Eastern Finland

Spinal cord injury (SCI) pathology is a sequence of primary injury followed by secondary injury playing a pivotal role in the outcome of patients. Free radical damage is an important mechanism contributing to secondary damage in SCI. NF-E2 related factor 2 (NRF2) is an important transcription factor responsible for upregulating antioxidant-responsive element (ARE)-mediated gene expression. Previously we have shown activation of Nrf2-ARE pathway after SCI, but the effect of Nrf2-deficiency on SCI outcome is still unknown. In the present study a model of contusion SCI was produced by using the Infinite Horizon Impactor after T_{10} laminectomy using Nrf-/ - mice and their C57BL/ 6J wild-type (wt) controls. The mRNA level of TNF-alpha was determined using qRT-PCR at 7 days after SCI. The motor function was tested using the open-field BMS once a week until the end of the study by two observers. Nrf2-/ - mice had significantly lower BMS score as compared with wt-injured controls starting already 7 days after surgery. At that time-point the mRNA level of TNF-alpha was significantly upregulated in Nrf2-/ - as compared with wt-injured injured mice. Our results show that the Nrf2 plays important role on SCI pathogenesis limiting TNF-alpha production and improving motor function recovery after contusion SCI.

Oral Session III

Chairs:

– Sofya Ziyatdinova & Lakshman Puli –

UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR MODULATES EPILEPTOGENESIS IN MOUSE MODEL OF TEMPORAL LOBE EPILEPSY

Xavier EKOLLE N and Asla Pitkänen
Department of neuroscience, University of Eastern Finland

Urokinase-type plasminogen activator (uPA) and its receptor (uPAR) are induced by brain insults in animals and humans but their functional role in the post-injury aftermath is poorly understood. Here we tested a hypothesis that uPAR contributes to tissue remodeling after brain injury and consequent epileptogenesis. Epileptogenesis was induced by intrahippocampal injection of kainic acid (KA) in adult wild type (Wt, n=7) and uPAR knockout (uPAR-/ -, n=8) mice. After KA injection, mice were video-EEG monitored for 30 d (24/ 7). EEG recordings were analyzed for severity of status epilepticus (SE) and occurrence of spontaneous seizures. The severity of SE during the first 48 h after KA administration was lower in uPAR-/ - mice compared to Wt ($p<0.01$). Also, during the 30-d follow-up, daily seizure frequency was lower in uPAR-/ - mice as compared to Wt (0.7 ± 0.2 vs. 3.0 ± 0.9, $p<0.01$). Histological analysis revealed more severe degeneration in the ipsilateral CA1 pyramidal cells in Wt than uPAR-/ - mice ($p<0.05$). Also, degenration of ipsilateral hilar cells was more severe in Wt than uPAR-/ - mice (1036 ± 152 vs. 2336 ± 346 remaining, $p<0.01$). These data suggest that uPAR predisposes neurons to post-injury degeneration augmenting epileptogenesis after status epilepticus.

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ROLE OF AMYLOIDOGENIC APP PROCESSING IN EPILEPTOGENESIS.

Diana Miszczuk [1,2], Heikki Tanila [1], Katarzyna Lukasiuk [2], Asla Pitkänen [1]
[1] Department of Neurobiology, University of Eastern Finland, [2] The Nencki Institute of Experimental Biology, Polish Academy of Science

Traumatic brain injury (TBI) is a major cause of epilepsy. Mechanisms of epileptogenesis are poorly understood but epidemiological and pathological studies demonstrate the association between TBI and development of Alzheimer diseases. To address the question if there is causality between amyloidogenic APP processing and the epileptogenesis after TBI we induced TBI using controlled cortical impact model in wild type (n=8) and APP/ PS1 over-expressing mice (n=9). Sham operated wild type controls (n=7) and sham injured APP/ PS1 (n=7) were generated. Motor recovery was assessed with Neuroscore 2, 7, 14 days post-TBI. APP/ PS1 injured mice showed significant motor deficits compared to APP/ PS1 sham mice 2 days ($p<0.01$) and 7 days ($p<0.05$) after TBI. Morris water-maze was used to evaluate spatial memory 2 weeks post TBI. There was no significant difference observed between groups. This preliminary data suggest that APP/ PS1 genetic background does not have strong influence on behavioral changes after TBI. The future aims are to analyze results of fear conditioning and 2-wk continuous vEEG monitoring. Brain tissue will be used to evaluate level of mRNAs coding subunits of ion channels. We assume that in APP/ PS1 mice TBI results in more robust APP accumulation and facilitated epileptogenesis.

LOSS OF GABAERGIC NEURONS IN THE RAT DENTATE GYRUS DURING EPILEPTOGENESIS DEPENDS ON THE TYPE OF EPILEPTOGENIC INSULT

Noora Huusko, Christine Römer, Asla Pitkänen

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

Brain insults can trigger epileptogenic process which after a latency phase leads to the epilepsy. The changes in GABAergic network in post-injury hippocampus can play a major role in epileptogenesis. To address a question, whether the severity of damage to GABAergic neurons in the rat dentate gyrus depends on the type of epileptogenic insult, we compared the neuronal numbers at 6 months after status epilepticus (SE) and traumatic brain injury (TBI) in adult male rats. Both SE and TBI rats were video-EEG monitored to detect spontaneous seizures. TBI rats were also tested in seizure threshold test. Brains were processed for immunohistochemical analysis of GABAergic neurons (NPY, PARV, CR, CCK, SOM). After TBI, there was a loss of all different subtypes of GABAergic neurons ($p<0.05$) in the ipsilateral and CR-, PARV- and CCK-immunoreactive (ir) neurons ($p<0.05$) in the contralateral dentate gyrus. These rats had no spontaneous seizures but seizure threshold was lowered. Rats with spontaneous seizures after SE (50 %) had loss of NPY- and CR-ir neurons ($p<0.05$) in the ipsilateral and only NPY-ir neurons ($p<0.05$) in the contralateral dentate gyrus. Our results suggest that epileptogenic process is not similar after TBI as compared to SE.

MIGRAINE MEDIATOR CGRP UP-REGULATES PACEMAKER CHANNELS IN MENINGEAL NERVES AND IN ISOLATED TRIGEMINAL NEURONS: A NOVEL MECHANISM OF HEADACHE?

Azat Abdullin, Nikolay Naumenko, Andrei Skorinkin, Genet Assefa, Pasi Tavi and Rashid Giniatullin

Department of Neurobiology, University of Eastern Finland

It is generally accepted that the neuropeptide calcitonin gene related peptide (CGRP) plays a central role in migraine pathology by sensitizing trigeminal neurons innervating meninges. But the mechanism of this sensitization is little understood. Since CGRP receptor induced signaling involves cAMP in the current project we tested whether this migraine mediator could increase activity of cAMP-dependent pacemaker Ih channels in trigeminal sensory neurons. RT-PCR indicated expression of mRNA for several subtypes of Ih channels in rat trigeminal neurons. Voltage clamp recordings revealed functional Ih channels in isolated trigeminal neurons which were up-regulated after CGRP application. Using a novel hemiskull preparation we found that CGRP increased the frequency of spikes in trigeminal nerves in meninges from 0.84 ± 0.26 to 4.17 ± 0.62 Hz ($n=10$, $P=0.0008$). The specific blocker of Ih channels ZD-7288 reduced the Ih currents in CGRP treated cells and spiking activity in dura mater providing additional evidence on involvement of these channels in sensitization in migraine-like states. Taken together our data suggested a novel mechanism for migraine pain based on enhanced activity of pacemaker channels.

THE ROLE OF SERINE 275 IN SHAPING OF THE BINDING POCKET OF ATP-GATED P2X3 RECEPTOR

Nataliia Petrenko [1], Kamil Khafizov [2] and Rashid Giniatullin [1]

[1] Dept. Neurobiology, University of Eastern Finland; [2] Computational Structural Biology group, Max Planck Institute of Biophysics, Frankfurt am Main.

ATP-activated P2X3 receptors are expressed in nociceptive sensory neurons and play important role in transmission of pain signals. One important characteristic of these receptors is fast and persistent desensitization (inactivation induced by the agonist ATP). Persistence of desensitization should depend on the rate of agonist dissociation from the binding site. However, the structure of ATP binding site is not well known. In the current work, by exploring the structural model of P2X receptor, we identified a candidate residue S275 in the ectodomain, which probably contributes to the closure of the agonist-binding pocket. Indeed, experimental testing of the S275A mutant using patch clamp technique not only revealed a key role of the S275 in agonist binding but also indicated its crucial role in receptor desensitization. Thus, some full agonists of P2X3 receptor, like beta-, gamma-meATP became only partial agonists of the S275A mutant. Moreover, unlike the WT, the S275A mutant showed reduced onset of desensitization, it was almost insensitive to nanomolar ATP and desensitization was less persistent. Thus, our data indicate that S275 is a key contributor to the closure of agonist-binding pocket in P2X3 receptor and its presence determines high rate and persistence of desensitization.

Oral Session IV

Chairs:

– Jussi Paananen & Sanna-Kaisa Häkkinen –

MICROFIBRILLAR ASSOCIATED PROTEIN 5 (MFAP5) IS LINKED WITH MARKERS OF OBESITY-RELATED EXTRACELLULAR MATRIX REMODELLING AND INFLAMMATION

Maija Vaittinen [1], Marjukka Kolehmainen [1], Ursula Schwab [1,2], Matti Uusitupa [1,3], Leena Pulkkinen [1]

[1] Institute of Public Health and Clinical Nutrition, University of Eastern Finland; [2] Institute of Clinical Medicine, Internal Medicine, Kuopio University Hospital, Finland; [3] Research Unit, Kuopio University Hospital, Finland

We have earlier shown that the expression of MFAP5 is down-regulated in adipose tissue along with weight reduction in persons with metabolic syndrome (The Genobin study). Our aim was to examine if the change of MFAP5 mRNA expression due to weight reduction is correlated with measures of glucose metabolism and obesity. Altogether 46 obese subjects with impaired glucose tolerance and features of metabolic syndrome were randomized to weight reduction (n=28) or control group (n=18). Circulating glucose and insulin concentrations and subcutaneous AT biopsies were performed before and after the intervention. The mRNA expression was studied by QPCR. The mRNA expression of MFAP5 correlated with BMI at baseline, and there was a correlation between the change in MFAP5 mRNA expression and the change of body fat mass. MFAP5 mRNA expression correlated also with fasting serum or plasma concentrations of adiponectin, IL1β, insulin, leptin and IL1Ra at baseline. In addition, there were correlations between MFAP5 mRNA expression and the expressions of PPARγ, ADAM12 and CCND2 both at baseline and after the intervention. This study demonstrates that MFAP5 is highly expressed in AT and is down-regulated by weight reduction along with improved insulin sensitivity.

TCF7L2 SPLICING IS REGULATED BY WEIGHT LOSS AND DIFFERS BETWEEN FAT DEPOTS IN HUMANS.

Dorota Kaminska [1, 2], Tiina Kuulasmaa [2], Tiina Sistonen [2], Sari Grönlund [3], Pirjo Käkelä [3] Jussi Pihlajamäki [1, 2]

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

Alternative splicing leads to transcript diversity. A gene encoding transcription factor 7-like 2 (TCF7L2) is the gene most strongly linked to type 2 diabetes (T2DM). TCF7L2 has several isoforms but their role in T2DM and obesity is not clear. We investigated whether alternative splicing of TCF7L2 gene is influenced by substantial weight loss or between fat depots. In order to determine this relationship we implemented PCR-capillary electrophoresis method allowing for efficient screening of alternatively spliced exons. Subcutaneous and visceral adipose tissue samples taken during and 1 year after gastric bypass surgery were analyzed from 55 individuals. Additionally, we examined 83 liver samples taken during the surgery. We found alternative splicing in exon 4 and several 3' exons of TCF7L2 gene in response to weight loss, between fat depots and between adipose tissue and liver (p-values 0.04-1.7E-07). Interestingly, we discovered correlation between fasting glucose and expression of isoform lacking exons 14 and 15 of TCF7L2 in adipose tissue ($r_{spearman}$=0.34, p=0.02). Finally, adipose tissue expression of this isoform was higher in 24 obese subjects with T2DM compared to 27 subjects with normal glucose tolerance (p=0.001). Our results show that alternative splicing of TCF7L2 gene is tissue specific, is regulated by weight loss and associates with T2DM.

ASSOCIATION OF INDICES OF LIVER AND ADIPOCYTE INSULIN RESISTANCE WITH 19 CONFIRMED SUSCEPTIBILITY LOCI FOR TYPE 2 DIABETES IN 6,733 NON-DIABETIC FINNISH MEN.

Jagadish Vangipurapu [1], Alena Stančáková [1], Jussi Pihlajamäki [1], Teemu Kuulasmaa [1], Tiina Kuulasmaa [1], Jussi Paananen [1], Johanna Kuusisto [1], Ele Ferrannini [2], Markku Laakso [1].

[1] Department of Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland, [2] Department of Internal Medicine, University of Pisa School of Medicine, Pisa, Italy

Of the confirmed type 2 diabetes (T2D) susceptibility loci only a few are known to affect insulin sensitivity. We examined the association of indices of hepatic and adipocyte insulin resistance (IR) with 19 confirmed T2D risk loci in a large population-based study. Non-diabetic participants (n=8,460, age 57.3±7.0 years, BMI 26.8±3.8 kg/ m^2) from a population-based cohort underwent an OGTT. Of them, 6,733 non-diabetic men were genotyped for single nucleotide polymorphisms (SNPs) in/ near the 19 confirmed T2D risk loci. We investigated hepatic IR with a new index of liver IR and used an adipocyte IR index that was previously described. T2D risk SNPs in/ near *KCNJ11* and *HHEX* were significantly (p<0.0013), and those in/ near *CDKN2B*, *NOTCH2* and *MTNR1B* were nominally (p<0.05), associated with decreased liver IR index. The Pro12 allele of *PPARG2* was significantly associated with a high adipocyte IR index and nominally associated with high liver IR. The Pro12 allele of *PPARG2* seems to impair insulin's antilipolytic effect, leading to high NEFA release in the fasting state and IR. In addition, the T2D risk alleles of *KCNJ11* and *HHEX* were associated with increased hepatic insulin sensitivity.

PREDICTION OF MICRORNA TARGETS IN C. ELEGANS USING A SELF-ORGANIZING MAP

Liisa Heikkinen [1, 2], Mikko Kolehmainen [3], Garry Wong [1, 2]

[1] Department of Neurobiology; A.I. Virtanen Institute for Molecular Sciences, [2] Department of Biosciences, [3] Department of Environmental Science, University of Eastern Finland, Kuopio, Finland.

MicroRNAs are small non coding RNAs that regulate transcriptional processes via binding to the target gene mRNA. In animals, this binding is imperfect, which makes the computational prediction of animal microRNA targets a challenging task. The accuracy of microRNA target prediction can be improved with the use of machine learning methods. Previous work has described methods using supervised learning, but they suffer from the lack of adequate training examples, a common problem in microRNA target identification, which often leads to deficient generalization ability. In this work, we introduce mirSOM, a microRNA target prediction tool based on clustering of short 3' untranslated region (UTR) substrings with self-organizing map (SOM). Because our method uses unsupervised learning and a large set of verified C. elegans 3' UTRs, we did not need to resort to training using a known set of targets. Our method outperforms six other methods in predicting the experimentally verified C. elegans true and false microRNA targets.

Oral Session V

Chairs:

– Joanna Huttunen & Janne Ruotsalainen –

CALCIFICATION DETECTION IN VIVO AND EX VIVO IN INJURED RAT BRAIN USING SWIFT

Lauri Lehto [1], Alejandra Sierra [1], Curtis Corum [2], Djaudat Idiyatullin [2], Michael Garwood [2] and Olli Gröhn [1]

[1] Department of Neurobiology, University of Eastern Finland, [2] Center for Magnetic Resonance Research, University of Minnesota.

Phase imaging using conventional magnetic resonance imaging (MRI) techniques require post-processing of the images to remove large spatial frequency fluctuations. Interestingly, a novel MRI technique SWIFT also shows contrast in phase images without any need for post-processing. To demonstrate the use of SWIFT in phase imaging, calcifications induced by brain injuries were imaged. Two rat animal models causing brain injury and calcifications in chronic phase were used: systemic pilocarpine injection inducing status epilepticus (n=8) and lateral fluid percussion traumatic brain injury (TBI, n=5). Epileptic animals were imaged ex vivo and TBI animals in vivo and ex vivo six and five months post-injury, respectively. The volumes of the calcifications were estimated from the SWIFT ex vivo magnitude images and correlated with histology. A semiquantitative explanation for phase formation in SWIFT was developed. Five pilocarpine and 5 TBI animals showed calcification formation. Calcifications were detected in SWIFT phase images using their dipole field effect. Pooling all the calcifications (n=43) in all of the animals, the correlation of the calcification size between histology and MRI was very good (r=0.84). Thus, SWIFT was shown to be highly useful for brain calcification detection.

SIMULTANEOUS FMRI AND LOCAL FIELD POTENTIAL MEASUREMENTS OF PENTYLENETETRAZOL INDUCED EPILEPTIC SEIZURES IN TRAUMATIC BRAIN INJURY RATS

Airaksinen Antti, Huttunen Joanna, Shatillo Artem, Pitkänen Asla, Gröhn Olli.

Department of Neurobiology, UEF

Traumatic brain injury (TBI) is a major cause of death and disability worldwide, and can lead to significant and long-lasting disabilities, such as cognitive and motor impairments and post-traumatic epilepsy. In this study, we aimed to characterize the origin of the seizures caused by pentylenetetrazol (PTZ) administration in sham operated and TBI brain using functional magnetic resonance imaging (fMRI). Simultaneous local field potential (LFP) and blood oxygenation level dependent (BOLD) fMRI experiments were performed for 14 TBI and 6 sham operated rats 2 months after TBI. Animals were sedated and paralyzed with mixture of medetomidine (0.1 mg/kg/h) and pancuronium bromide (2 mg/kg/h). For LFP recordings, tungsten wire electrode was implanted in the frontal cortex. Our preliminary results showed that, the PTZ caused bilateral cortical activation in BOLD signal which was also detected as 6-8 Hz spiking in the LFP signal in all rats. Results are likely to increase our understanding of the origin of seizures after TBI. This is an ongoing study which may provide an approach to study functional brain activity in abnormal brain, recovery processes after TBI and the progression of the seizures.

ROTATING FRAME SPIN LATTICE RELAXATION TIME MAPPING AND CINE MRI OF MYOCARDIAL INFARCTED MICE IN-VIVO AT 9.4T

Haja-Sherief N. Musthafa [1], L. Petrov [2], G. Dragneva [1], S. Ylä-Herttuala [1], O. Gröhn [1], T. Liimatainen [1]

[1] A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland ; [2]Ark Therapeutics Ltd., Kuopio, Finland

Myocardial infarction is one of the leading causes of death in the western countries despite improved diagnosis and treatments. Locating the irreversible injury areas in myocardium is one of the main approaches for diagnosis of infraction. Spin-lattice relaxation time in the rotating frame of reference (T1rho) was found as a promising surrogate marker for cerebral ischemia, and glioma gene therapy response in experimental models. In this study, T1rho was measured before and after occlusion of left anterior descending coronary artery (LAD) in mice at 9.4 T MRI scanner. T1rho and cine imaging was repeated 1, 3, 7 and 20 days after LAD surgery. For T1rho spin lock radio frequency pulse with five durations between 0 and 54 ms was used. The average T1rho was calculated on the infarcted and loaded myocardium based on cine images. The T1rho was found to increase by 32 % between 1st and 7th days after LAD occlusion. In our knowledge, this is the first time when T1rho has been measured from mouse heart. The increase in T1rho after infarction reflects most likely physicochemical changes during ventricular remodelling. The research is continued by resolving the reasons for increased T1rho by histological analyzes, and application of T1rho for imaging of gene therapy after LAD occlusion.

INTERFERON-BETA SENSITIVITY OF TUMOUR CELLS CORRELATES WITH RESPONSE TO ONCOLYTIC VA7 VIROTHERAPY IN MOUSE GLIOMA MODELS

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

[1] A. I. Virtanen Institute for Molecular Medicine, University of Eastern Finland; [2] Department of Biochemistry and Pharmacy, Åbo Akademi; [3] OHRI, Canada

In our previous studies, intravenously administered attenuated Semliki Forest virus VA7 effectively infects and eradicates orthotopic U87 gliomas in nude mice. However, in treatment of syngeneic GL261 gliomas in immunocompetent mouse model, VA7 (also in combination with rapamycin or cyclophosphamide) fails to infect the tumour cells and shows no positive effect on survival. As virally induced cytokines are powerful inducers of antiviral response, possibly explaining the poor infectivity in vivo, we tested our cell lines for their interferon-beta (IFN-beta) sensitivity in vitro. For this, cultured U87 and GL261 cells were infected with VA7 before and after treating the cells with IFN-beta. Similar studies were done to GL261 cells explanted from tumor-bearing VA7-treated and untreated C57BL/ 6 mice. IFN-beta blocks VA7 infectivity in GL261 cells in vitro. As compared to GL261 cell line, U87 cell line shows lower sensitivity to IFN-beta. Our findings suggest that tumour cell infectivity in vivo and good response to systemically administered oncolytic VA7-treatment correlates to low sensitivity to vector-induced interferons in the targeted cells. Strategies to safely overcome vector immunity are needed.

Oral Session VI

Chairs:
– Timo Sarajärvi & Suvi Jauhiainen –

WHY ARE PHOTORECEPTORS OF DIFFERENT INSECT SPECIES USING A DIFFERENT SET OF KV-CHANNELS?

Matti Weckström and Mikko Vähäsöyrinki
Department of Physics and Biocenter Oulu, University of Oulu, Oulu, Finland

Voltage-dependent potassium channels (Kv-channels) are membrane proteins that have essential functions in action potentials, but in the last decades it has become clear that they are important also in fine-tuning other, graded potential signals in insect photoreceptors and interneurons. After about two decades of experimental and computational research it is possible to draw together the different lines of investigation that have arisen out of those works, with the aim of developing novel, mechanistic insights on the function and importance of the Kv-channels in neural signalling in the insect compound eyes of several species with different ways-of-life. This will have general relevance in understanding the function of the nervous system, including that of humans.

EFFECTS OF HUMAN INTRAVENOUS IMMUNOGLOBULIN ON AMYLOID PATHOLOGY AND NEUROINFLAMMATION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

Lakshman Puli, Yuriy Pomeshchik, Tarja Malm, Jari Koistinaho, Heikki Tanila.
A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio.

Human intravenous immunoglobulin (hIVIG) preparation has been hypothesized to confer beneficial effects in Alzheimer's disease (AD). Here, we set out to determine the effects of hIVIG treatment on amyloid pathology and inflammation in vivo in APP/PS1 mouse model of AD. We treated 4-month-old female APP/PS1 mice with weekly intraperitoneal injections of hIVIG (Gammagard, Baxter A/G) or saline for 8 months. After the treatment the mice brains were rapidly retrieved and processed for routine histology, immunostainings and biochemical evaluations. hIVIG treatment did not cause any changes in amyloid plaque burden in hippocampus. Also, serum Aβ40 levels did not change, indicating the absence of peripheral sink mechanism. Most immunohistochemical markers for inflammatory changes including GFAP, CD68 and Iba-1a did not change. However, we noticed a change in hippocampal CD45 immunoreactivity in hIVIG treated group. We found no evidence for a peripheral sink or microglia mediated clearance of amyloid load after hIVIG treatment. However, hIVIG suppressed subset of microglial cells positive for CD45 antigen. These immunomodulatory effects of hIVIG may account for its beneficial effect in AD patients.

GENETIC ANALYSIS OF GENES INVOLVED IN AMYLOID-BETA DEGRADATION AND CLEARANCE IN AD

*Teemu Natunen, Seppo Helisalmi, Saila Vepsäläinen, Timo Sarajärvi, Leila Antikainen, Petra Mäkinen, Sanna-Kaisa Herukka, Anne Maria Koivisto, Annakaisa Haapasalo, Hilkka Soininen, and Mikko Hiltunen**

Institute of Clinical Medicine – Neurology, University of Eastern Finland and Department of Neurology, Kuopio University Hospital, P.O.Box 1627, FIN-70211 Kuopio, Finland

Accumulation of amyloid β-peptide (Aβ) in the brain of Alzheimer's disease (AD) patients has been postulated to reflect defects in Aβ degradation or clearance. Here, we have selected 12 genes involved in Aβ degradation or clearance and elucidated their genetic role in AD among Finnish case-control cohort consisting of ~1300 AD patients and controls in total. Association analysis of the liver X receptor α (NR1H3) gene SNPs showed a protective effect for C allele carriers of rs7120118 (p=0.014; OR=0.70, 95% CI 0.53-0.93). Consistent with this, the phospho-tau levels were significantly decreased in the cerebrospinal fluid (CSF) of AD patients carrying the C allele. Moreover, a significant decrease in the age of onset was observed in AD patients carrying the A allele of rs723744 and the C allele of rs3794884 in transthyretin (TTR) gene. The phospho-tau levels in CSF were again increased among AD patients carrying the G allele of rs1080093 in TTR gene. These results suggest that genetic alterations in NR1H3 and TTR may play a role in AD pathogenesis.

CHRONIC IBUPROFEN TREATMENT DOES NOT AFFECT THE SECONDARY PATHOLOGY IN THE THALAMUS OR IMPROVE BEHAVIOURAL OUTCOME IN MCAO RATS

Anu Lipsanen, Mikko Hiltunen and Jukka Jolkkonen

Department of Clinical Medicine – Neurology, University of Eastern Finland, Kuopio, Finland

Anti-inflammatory drug ibuprofen decreases the β-amyloid (Aβ) deposition and associated inflammation in transgenic Alzheimer disease mice. Based on this, we studied whether ibuprofen could modulate the secondary pathology described in the thalamus of middle cerebral artery occlusion (MCAO) rats. Our hypothesis was that ibuprofen could decrease inflammatory reaction and Aβ load in the thalamus of MCAO rats, which reflects in behavioral outcome. Forty Wistar rats were subjected to sham-operation or transient occlusion of the right MCA (120 min). Ibuprofen (40 mg/ kg/ day, per os)/ vehicle was administrated for 27 days beginning the treatment on post-operative day 2. Sensorimotor impairment was assessed using the limb-placing, tapered/ ledged beam-walking and cylinder tests during the follow-up. The rats were perfused for histology on postoperative day 29. Histological data showed that ibuprofen did not affect Aβ or calcium load in the thalamus of MCAO rats. In addition, behavioral tests did not show significant difference between vehicle- and ibuprofen-treated MCAO rats. The present data do not support the idea that ibuprofen reduces the secondary Aβ/ calcium pathology in the thalamus or associated sensorimotor impairment following cerebral ischemia.

THE EFFECTS OF LOCAL VEGF GENE TRANSFER ON REGIONAL LYMPH NODES

Galina Dragneva, Ivana Kholová, Laura Alasaarela, Felisitas Yongabi, Seppo Ylä-Herttuala.
Dept. of Biotechnology and Molecular Medicine, University of Eastern Finland

In this study we aim to outline the histopathological and molecular biological effects of different VEGFs on regional lymph nodes after local gene transfer into mouse skeletal muscles. Intramuscular injections of AdhVEGF-A, AdhVEGF-D (proteolytically processed form), AdhVEGF-C or AdLacZ were performed on ApoB48-LDLR$^{-/-}$ mouse skeletal muscles. We carried out detailed morphological analysis to determine any tissue changes in the regional lymph nodes by means of hematoxylin-eosin, special stains and immunohistochemistry. The transgene expression was assessed by qRT-PCR. The transgene protein levels were determined by ELISA. Histopathological results in regional lymph nodes demonstrated sinus dilation, activated lymphatic follicles and paracortical hyperplasia as well as generation of angiogenesis and lymphangiogenesis. The highest level of angiogenesis was recorded after AdhVEGF-D gene transfer, while the highest level of lymphangiogenesis was detected after AdhVEGF-C gene transfer. All the transgenes were detected both on mRNA and protein level on D7. The evaluation of the transgene expression in different non-target organs such as lymphoid tissue may prove important in assessing the safety pattern of any gene transfer vector.

EFFECT OF SPERMIDINE/SPERMINE-N^1-ACETYLTRANSFERASE (SSAT) ON HEMATOPOIESIS

Sini Pirnes-Karhu [1], Sisko Juutinen [1], Petri Mäkinen [1], Sohvi Hörkkö [2], Esa Jantunen [3], Pentti Mäntymaa [4], Leena Alhonen [1], Anne Uimari [1]
[1] Department of Biotechnology and Molecular Medicine, University of Eastern Finland, [2] Department of Microbiology, University of Oulu, [3] Institute of Clinical Medicine, Internal Medicine, Kuopio University Hospital, [4] Eastern Finland Laboratory Centre

Polyamines are small cationic molecules essential for many cellular processes, including cell growth and differentiation. The mice over-expressing spermidine/ spermine-N^1-acetyltransferase (SSAT), a catabolic regulator of polyamines, have several phenotypic and metabolic changes when compared with their non-transgenic littermates. The hematopoietic features of these mice were examined by histology, blood count and FACS analyses, bone marrow transplantation and by treating mice with inhibitors of NF-κB and DNA/ histone modifying enzymes. The SSAT over-expressing mice exhibited enlarged secondary lymphoid tissues and elevated amount of leukocytes in blood and bone marrow. In addition, the relative proportions of leukocyte subpopulations were altered in blood, spleen and bone marrow. The changes in leukocyte populations seemed to originate from SSAT over-expression in the hematopoietic stem and/ or progenitor cells, as demonstrated by the bone marrow transplantation. Also human leukemia samples showed altered polyamine metabolism. This study will give new information about the role of polyamines and their metabolism in cell proliferation and differentiation processes.

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Tuulia Huhtala et al.

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

Winter School 2011 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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