MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

6TH MUI-START SYMPOSIUM

PROGRAM AND ABSTRACTS

MZA, Anichstraße 35

PROGRAM

13:00 H Welcome Address of Prof. Christine Bandtlow (Vice Rector of Research and International Relations)

TIME	*Oral presentations*	Abstract
13:10 – 13:20	**Priv.-Doz. Dr.rer.nat Laura Zamarian PhD (Department of Neurology)** "Medical judgements can improve after cognitive training – A study with healthy people and patients with multiple sclerosis"	O 01
13:20 – 13:30	**Assoz. Prof. Priv.-Doz. Dr.med. Astrid Grams (Department of Neuroradiology)** "Intracranial aneurysm as a hypertensive disease"	O 02
13:30 – 13:40	**Assoz. Prof. Priv.-Doz. Dr.med.univ. Isabel Heidegger PhD (Department of Urology)** "Robo 4 - the double-edged sword in prostate cancer: impact on cancer cell aggressiveness and tumor vasculature"	O 03
13:40 – 13:50	**Mag. Biol. Stefan Coassin PhD (Division of Genetic Epidemiology)** "Investigation of genetic variability in the LPA KIV-2 repeat region: looking into a white sport of the genome using new sequencing technologies"	O 04
13:50 – 14:00	**Dr.med.univ. Alexandra Gratl (Department of Vascular Surgery)** "Neuroprotective potential of tetrahydrobiopterin in spinal cord ischemia using a rat model"	O 05
14:00 – 14:10	**Dr.med.univ. Franka Messner (Department of Visceral, Transplant and Thoracic Surgery)** "Mechanical stress as a trigger of skin rejection in composite tissue allotransplantation"	O 06

TIME	*Oral presentations*	Abstract
14:10 – 14:20	**Dr.med.univ. Sebastian Reinstadler (Internal Medicine III: Cardiology and Angiology)** "Prognostic significance of copeptin after ST-elevation myocardial infarction: Insights from cardiac magnetic resonance imaging"	O 07
14:20 – 14:30	**Mag. Ruslan Stanika PhD (Division of Physiology)** "Role of the endogenous L-type calcium channel $Ca_V1.3$ in dendritic spine morphogenesis"	O 08
14:30 – 14:40	**Dr. med. univ. Romana Gerner (Internal Medicine I: Gastroenterology, Hepatology and Endocrinology)** "Lethal graft−versus−host disease is promoted by NAMPT and can be ameliorated by its inhibition"	O 09
14:40 – 14:50	**Dr. rer. nat. Markus Keller (Division of Human genetics)** "Nutrition dependent reconfiguration of the mitochondrial cardiolipidome in ageing mice"	O 10
Coffee and discussion		

Oral presentations

Oral presentations should last no more than 7 min to have 3 min for discussion. Please make clear during your presentation which aims your project had and to which extend you managed to achieve them.

Please send your PowerPoint presentation or slide to the Servicecenter-Forschung: (maria.perez@i-med.ac.at) no later than the **12th of December 2017**.

ABSTRACTS

O 01: Medical judgements can improve after cognitive training – A study with healthy people and patients with multiple sclerosis

Laura Zamarian[1]

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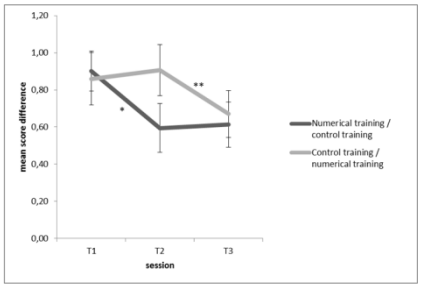
Background: Risk understanding is essential for participating in health care and making informed, advantageous decisions. Typically, people's preferences and decisions are influenced by the way information is presented, known as *framing effect*[1]. For example, people demonstrate a more favourable attitude towards positively-framed medications than towards negatively-framed medications[2]. Previous investigations have shown that patients with multiple sclerosis have some difficulties in advantageous decision making[3,4]. Recently, we have also found that patients with relapsing-remitting multiple sclerosis (RRMS) are more prone to make irrational decisions than healthy controls, although both groups do not differ in the quantity of information they sample before making a decision[5]. Aim of this study was to evaluate whether a targeted cognitive training would improve medical judgements and performance on a framing task in both healthy people and patients with RRMS.

Method: 37 patients with RRMS and 73 healthy controls participated in the study. In a controlled cross-over design, half of the participants underwent a week of numerical training followed by a week of control training (text comprehension); the other half had first the control training and then the numerical training. Before any intervention started (T1), participants performed a comprehensive neuropsychological background assessment and a framing task where they evaluated the success of fictive medications on a 7-point scale. Medications were described either in positive terms or in negative terms. Parallel forms of the framing task were used after each training week (T2, T3).

Results: Both, patients and controls, showed a significant effect of training type. Indeed, the framing effect decreased after a week of numerical training, while performance did not change following a week of control training. Regardless of the training type, patients were influenced more strongly than controls by the frame of information. We also found that the framing effect of the whole sample correlated with both demographical variables and cognitive scores (logical reasoning, working memory, mental calculation, ratio processing), with a stronger framing effect being associated with older age, lower education, and lower cognitive functioning. However, only logical reasoning emerged as significant predictor of variance in the framing task.

Figure 1. Framing task.



* P<.05, ** P<.01.

Conclusion: Our results show that (1) training alters the way medical information is evaluated; (2) patients with RRMS as well as healthy controls can profit from cognitive training; and (3) the type of training has specific effects. In light of these findings, we suggest that a targeted cognitive training may enhance the evaluation of new information and reduce framing effects. This in turn should favor informed and advantageous decision making in the health context.

References: [1]Tversky A, Kahneman D. Science 1981;211:453-8. [2]Moxey A, et al. J Gen Intern Med 2003;18:948–59. [3]Simioni S, et al. PLoS One. 2012;7(12):e50718. doi: 10.1371/journal.pone.0050718. [4]Farez MF, et al. BMJ Open. 2014; 4(7):e004918. [5]Zamarian L, et al. Reflection impulsivity in patients with multiple sclerosis. In preparation..

O 02: Intracranial aneurysm as a hypertensive disease

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Background: One main risk factor for the development of intracranial aneurysms and their rupture may be arterial hypertension, from which arterial calcification displays an epiphenomenon. In addition there is a known correlation between arterial hypertension and the presence of adrenal tumors, but a correlation between ruptured intracranial aneurysms and adrenal tumors has never been investigated.

Methods: In total 23 patients with aneurysmal SAH were included, who agreed in a magnetic resonance imaging (MRI) examination of the upper abdomen, which was performed between six and 12 months after the SAH. The MRI scans were screened for adrenal masses, and calcifying macroangiopathy of the intracranial vessels was quantified on computed tomography (CT) scans, which have been performed in the acute stage after SAH. In addition 46 age and gender matched controls from a historical patient group, who did not suffer from aneurysmal SAH and who did receive a cerebral CT or a MRI of the upper abdomen, were included.

Results: In the present population two of the SAH patients and none of the controls displayed an adrenal tumor. Patients with ruptured intracranial aneurysms displayed a significantly higher amount of supraaortal arterial calcification than the controls (p=0.03).

Conclusion: A positive connection between ruptured intracranial aneurysms and arterial calcification could be confirmed. A positive correlation between ruptured intracranial aneurysms and adrenal masses is difficult to prove with the present data, as only two patients displayed adrenal masses. The inclusion rate of patients so far was lower than expected, mostly due to the clinical condition or a missing consent

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O 03: Robo 4 - the double-edged sword in prostate cancer: impact on cancer cell aggressiveness and tumor vasculature

Andreas Pircher[1], Andrea Eigentler[2], Renate Pichler[2], Martin Puhr[2], Eberhard Steiner[2]. Georg Schäfer[3], Eberhard Gunsilius[1], Helmut Klocker[2], Isabel Heidegger[2]

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Background: The magic roundabout receptor 4 (Robo 4) is a tumor endothelial marker expressed in various tumor entities. However, the role of Robo 4 in prostate cancer (PCa), the second common cause of cancer death among men in European countries, has not been described yet. Thus, the present study investigates for the first time the impact of Robo 4 on PCa aggressiveness both *in vitro* as well as in the clinical setting.

Material and Methods: Robo 4 was overexpressed (plasmid) or downregulated (siRNA) in metastatic PCa cell lines (PC3, DU145, LNCaP). Cell proliferation was assessed using 3H Thymidine incorporation assay, cell viability was measured by WST assay, respectively. Moreover, we analyzed 95 patients diagnosed with PCa who underwent a radical prostatectomy (RPE) at our department and generated a tissue-micro-array (TMA) from RPE specimens for the analysis of Robo4 and its ligand Slit 2 using immunohistochemistry.

Results: In contrast to the as yet described role of Robo 4 as tumor endothelial cell marker we observed that Robo 4 is expressed in PC3 PCa tumor cells. Overexpression of Robo 4 in PC3 as well as in Robo 4 negative DU145 and LNCaP PCa cells was associated with a significant decrease in cell-proliferation and cell-viability. Furthermore, analyses of benign and malign tissue samples of 95 PCa patients, who underwent RPE, revealed significant elevated Robo 4 and Slit 2 (Robo 4 ligand) protein expression in cancerous compared to benign tissue specimens. Moreover, increased Robo 4 expression was associated with higher Gleason score and pT stage. We also observed in a small patient collective (n=39) a hypothesis generating (but not significant) trend towards a reduced Robo 4 expression in patients with a biochemical recurrence after RPE.

Summary: We described a dual effect of Robo 4 in PCa as it is associated with more aggressive cancers in the localized disease setting as well as a trend towards a protective effect of Robo 4 regarding tumor recurrence after RPE.

O 04: Investigation of genetic variability in the LPA KIV-2 repeat region: looking into a white sport of the genome using new sequencing technologies

Stefan Coassin[1]

[1]Division of Genetic Epidemiology, Medical University of Innsbruck, Austria

Background*:* Lp(a) concentrations represent the most important genetic cardiovascular risk factor in the general population. They vary by >1000-fold and are nearly exclusively controlled by one single gene locus (*LPA*). Up to 70% of the *LPA* coding sequence is located in a 5.5 kb large, hypervariable copy number variation (1 to >40 copies *per allele*) named "KIV-2", which determines 40-70% of Lp(a) levels. At least three subtypes of KIV-2 with different haplotypes (A, B, C) exist, but no detailed variation and haplotype data in the KIV-2 is available. The region is not covered by 1000 Genomes, ExaC or GnomAD data. Low molecular weight isoforms (LMW; ≤13 KIV-2) are associated with increased Lp(a), but even within the same isoform the Lp(a) levels can still vary by >200-fold for unknown reasons. We hypothesized that the poorly investigated KIV-2 region may contain novel functional variants and used 2nd and 3rd generation (Nanopore) sequencing to investigate this region.

Methods: The KIV-2 region was ultra-deep sequenced on a MiSeq System and KIV-2 variants detected as low level variants using specifically developed bioinformatics. Findings were validated in publically available 1000-Genomes raw data. The variant 4925G>A was followed up by castPCR in the complete KORA-F4 population (n=2,892) and effects estimated by isoform-adjusted linear regression. Minigene assays and sequencing in human mRNA and pre-mRNA (n=4 liver biopsies) were used to investigate splicing defects. Using the variation data of the MiSeq approach, we explored the generation of single molecule haplotypes by both standard Nanopore sequencing (DNA and cDNA) and by Intramolecular-ligated Nanopore Consensus Sequencing (INC-Seq).

Results: In contrast to previous studies using Sanger sequencing, we observed >400 variants in the KIV-2 region. The frequent (22% carriers in KORA-F4) splice site variant 4925G>A was associated with a Lp(a) reduction of 30.6 mg/dL in LMW carriers (i.e. -70%; p=3.0e-36) and carrier status explained 19.3% of the Lp(a) variance additionally to the KIV-2 number. Minigene assays indicated a reduction of splicing efficiency by this variant and a reduced protein amount originated in-vivo from the mutated allele. While common Nanopore sequencing was too error prone for direct generation of molecular haplotypes, INC-Seq was promising and indeed able to clearly detect full length (5.5 kb) KIV-2B haplotypes present at 10% level in a background of KIV-2A haplotypes. Read lengths >>50 kb were routinely observed. Despite low specificity (11.8% ± 14.7%; mean±SD), variant detection sensitivity was similar to MiSeq data (98.8% ± 1.2%; mean±SD). We also observed the occurrence of chimeric molecules which must be taken into account when analyzing future Nanopore data.

Conclusion: We were able to provide the first comprehensive collection of variation in the KIV-2 in a large cohort. A highly frequent variant in the KIV-2 was associated with strongly reduced Lp(a) concentrations and accounts for several peculiarities of the Lp(a) trait. MUI-START allowed establishing Nanopore Sequencing at the MUI and successfully determining single molecule haplotypes. This opens new opportunities to characterize variation in the KIV-2 region and is potentially generalizable to any other CNV region. Finally, fruitful collaborations with the University Hospital for Visceral Surgery and the Divisions for Cell Biology and RNomics and Genomics were established and are currently deepened in follow-up projects.

O 05: Neuroprotective potential of tetrahydrobiopterin in spinal cord ischemia using a rat model

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Background: Spinal cord ischemia (SCI) is one of the major complications in thoracoabdominal aortic aneurysm repair and occurs with an incidence of 5 – 12%. There are several clinical strategies, such as reperfusion of segmental arteries or using motor evoked potentials to monitor spinal cord function during surgery, to prevent this devastating complication. Numerous substances have been tested in experimental settings to provide evidence about their neuroprotective potential but none of them has been tested in clinical settings so far. Tetrahydrobiopterin (BH4) is one of five essential cofactors of nitric oxide synthase (NOS) and a decrease of intracellular BH4 is known to induce an uncoupling of NOS. This mechanism leads to an increased production of hydrogen peroxide and superoxide anion inducing the formation of the strong oxidative agent peroxynitrite resulting in endothelial function. Regarding current literature, data from cardiovascular and transplant research proved a protective function of BH4 in ischemia reperfusion injury and the aim of this study was to investigate the neuroprotective potential of BH4 in SCI using a rat model.

Methods: SCI has been induced in male CD rats by insertion of a Fogarty catheter via the left femoral artery to occlude the thoracic descending aorta. Animals have been grouped into a sham group (n=12, no treatment), a treatment group (n=11, i.m. administration of 50mg/kg BH4 15 minutes before SCI) and a control group (n=12, i.m. administration of saline 15 minutes before SCI). Blood samples have been taken just before inducing SCI and 5 minutes after reperfusion and animals have been monitored by cannulation of the tail artery (distal arterial pressure – DAP) and the left carotid artery (proximal arterial pressure – PAP). Neurofunctional testing was performed immediately after awakening and 1, 2, 3, 5 and 7 days after SCI using the Basso-Beattie-Breshnahan locomotor rating scale (range 0 – 21; 0 - no hind limb movement, 21 - normal hind limb movement). Additional histopathological evaluation (counting of normal neurons), TUNEL-assays (determination of apoptotic neurons) as well as investigation of oxidative stress (using nitrotyrosine antibody for immunohistochemistry and western blotting) are currently performed and results are expected within the next months.

Results: Regarding hemodynamic parameters (DAP, PAP before and during aortic occlusion) as well as blood parameters (pO2, pCO2, glucose, lactate, haemoglobin and pH value before SCI and 5 minutes after reperfusion) there were no significant differences between the treatment group and the control group. BBB score directly postoperative showed significantly increased scores within the treatment group compared to the control group (BBB score 18.4 treatment group vs. 15.3 control group; p=0.014). 8 out of 12 animals (66,7%) within the control group and 7 out of 11 animals (63,6%) within the treatment group survived until day 7, reason of death was mainly segmental mesenteric ischemia. BBB score at day 7 showed increased scores within the treatment group compared to the control group without being statistical significant (BBB score 21.0 treatment group vs. 18.3 control group; p=0.205). Results from histopahtology, TUNEL-assays, immunohistochemistry as well as western blot are not available yet.

Conclusion: Regarding these first results, a neuroprotective potential of BH4 has been observed after inducing SCI in a rat model using neurofunctional testing so far. After establishing the surgical

procedure, the model itself showed relatively high perioperative mortality with a high rate of deaths due to segmental mesenteric ischemia. Considering results from neurofunctional testing, we are excited to get results from outstanding evaluations as, despite being performed blinded, neurofunctional testing remains observer-related and thus subjective. Using further histological, immunohisochemical methods as well as western blotting, objective determinants will hopefully confirm these first results.

O 06: Mechanical stress as a trigger of skin rejection in composite tissue allotransplantation

Franka Messner[1], Theresa Hautz[1] , Anna Fischer[1] ,Elias Rungaldier[1], Bettina Zegler[1], Bernhard Zegler[1], Dietmar Öfner[1], Stefan Schneeberger[1]

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Background: In four hand transplanted patients (Austria, Belgium, Italy and the United States) a novel type of skin rejection referred to as „atypical“ rejection has been observed. All patients experienced previous to the rejection some form of intense mechanical or thermal stress (intense physical hand therapy, intense manual work, hot water). Besides differences in macroscopic features also histologic findings differed from classical rejection. The aim of the study is to investigate skin irritation and its effect on skin rejection after limb transplantation in a rat model.

Methods: Syngenic and allogenic orthotopic hind limb transplantations have been performed using male Lewis (recipent/donor) and Brown Norway (donor) rats (n=7). Immunosuppressive therapy consisted of anti-lymphocyte serum (ALS, 0,5ml) and tacrolimus (Prograf®) which was individually tapered (final dose of 0,1-0,2mg/kg KG) to prevent primary alloimmune response. Then mechanical irritation was applied to the planta pedis of the transplanted hind limb graft using a specifically designed mechanical stimulation device. Stimulation was performed four times daily for 10 minutes using 5 Newton pressure. Tissue biopsies (skin, muscle) were taken after 10 days of stimulation for further analysis.

Results: All seven stimulated allotransplanted animals showed macroscopic signs of rejection including a progressive dryness of the irritated planta pedis with hyperkeratosis, erythema and edema. Histology of the stimulated skin of the planta pedis showed a moderate to severe inflammatory infiltrate with epidermal involvement, dyskeratosis and keratinolysis (median rejection grade II°; range I-IV°), the muscle of the planta pedis displayed mild to moderate signs of inflammation with a median rejection grade I° (range 0-II°). The planta pedis of stimulated syngenic transplanted animals and naive controls did only show mild to moderate histopathologic alterations including perivascular infiltration and rare epidermal involvement (grade 0-II°). Histopathologic alterations were pronounced at the area of stimulation and markedly declined in more proximal tissue biopsies.

Conclusion: Atypical rejection is a rare immunological process which has first been described in four hand transplanted patients after been exposed to some form of mechanical or thermical irritation. By applying standardized mechanical irritation, we were able to show that rejection can be triggered by an external stimulus in the setting of vascularized tissue allotransplantation. The induced allograft rejection is mainly restricted to the challenged skin area and underlying muscle and exceeds the alterations induced by mechanical irritation alone.

O 07: Prognostic significance of copeptin after ST-elevation myocardial infarction: Insights from cardiac magnetic resonance imaging

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Background: We have previously shown that single time point copeptin concentrations measured between day 1 and day 3 after ST-elevation myocardial infarction (STEMI) are related with cardiac magnetic resonance (CMR) markers of adverse outcome (myocardial function, infarct size, microvascular obstruction). As such, copeptin could enhance risk prediction for survivors of acute STEMI. Nevertheless, copeptin rapidly decreases early after infarction and thus the most adequate time point for the assessment of copeptin for risk stratification remains to be evaluated. Our aim is to investigate the value of multiple versus single time point copeptin determinations for the prediction of systolic dysfunction and infarct severity as visualized by CMR at baseline, 4 months, and 12 months after primary percutaneous coronary intervention (p-PCI) in patients with acute STEMI.

Methods: Serial blood samples for determination of plasma copeptin (admission, 6 h, 12 h, 24 h, 48 h, 4 months, and 12 months after p-PCI) will be obtained from 100 consecutive STEMI patients treated with p-PCI. So far, 100 patients have been enrolled. All patients underwent a baseline CMR investigation, but follow-up CMR is not completed yet in all patients. Median baseline infarct size is 16 % of left ventricle [8-27%] and median ejection fraction is 55 % [49-61%]. Microvascular obstruction was present at baseline in 44 patients (50%).

Outlook: Baseline results for myocardial function and infarct severity are comparable with other contemporary CMR studies. After finalization, this study will provide for the first time data about the relationship between serially measured copeptin concentrations and CMR markers of adverse outcome in patients after acute STEMI. The results will further elucidate the role of copeptin in enhancing the early classification of STEMI patients into different risk groups.

O 08: Role of the endogenous L-type calcium channel CaV1.3 in dendritic spine morphogenesis

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Background: L-type Ca^{2+} channels (LTCC) regulate the activity-dependent neuronal development, synaptic plasticity, and gene transcription and their altered activity has been associated with aberrant brain function and neurological disease. Alternative splicing of the $Ca_V1.3$ α_1 subunit gives rise to a long ($Ca_V1.3_L$) and two short ($Ca_V1.3_{42A}$, $Ca_V1.3_{43S}$) C-terminal variants, which differ with respect to the voltage-dependence of activation and Ca^{2+}-dependent inactivation and may thus differentially contribute to the neuronal loss in PD. Recently we demonstrated that the LTCC $Ca_V1.3$ modulates postsynaptic dendritic spine stability, thereby providing a potential mechanism linking channel function to pathological alterations. In order to provide insights into the underlying mechanisms, here we studied dendritic spine modulation by $Ca_V1.3$ channels in hippocampal cultures from $Ca_V1.3$ knockout mice and analyzed the consequences on postsynaptic AMPA receptor distribution, CaMKII autophosphorylation, and synaptic activity (mEPSC).

Methods: Low-density cultures of hippocampal neurons were prepared from 1-2 do $Ca_V1.3^{-/-}$ mice. Dissected hippocampi were dissociated by trypsin treatment and trituration. Neurons were plated on poly-L-lysine-coated glass coverslips in 60 mm culture dishes at a density of ~3500 cells/cm2. After plating, cells were allowed to attach for 3-4 h before transferring the coverslips neuron-side down into a 60 mm culture dish with a glial feeder layer. Reconstitution of $Ca_V1.3$ KO cultures of hippocampal neurons with $Ca_V1.3_L$ splice variants or $Ca_V1.3_{\Delta ITTL}$ mutant was performed by expression of corresponding variants of Cav1.3 α1 subunit DNA and soluble eGFP fluorescent protein for visualization of cell morphology For staining of surface AMPA transfected neurons were incubated with antibody recognizing extracellular domain of GluR2 for 20 min at 37°C. Coverslips were rinsed in HBSS and fixed with 4% paraformaldehyde for 10 min. After fixation, neurons were washed with PBS for 30 min, blocked with 5% goat serum for 30 min, and labeled with anti-rat Alexa Fluor 594 (1:4000, 1h). Analysis of AMPA expression and spine morphology was done using MetaMorph software.

Results: Hippocampal neurons lacking $Ca_V1.3$ calcium channel show normal development of dendritic tree with formation of spines by 21 days in culture. Reconstituting $Ca_V1.3$ knockout neurons with full-length $Ca_V1.3_L$ resulted in a slightly increased spine density and a larger fraction of mature mushroom-like spine. Deletion of the C-terminal PDZ-binding sequence ($Ca_V1.3_{\Delta ITTL}$) significantly increased spine size and induced the formation of more filopodia-like spines. Dendritic spine stabilization by $Ca_V1.3_L$ was associated with increased spine and decreased dendritic AMPA-Rs. In contrast, spine elongation induced by $Ca_V1.3_{\Delta ITTL}$ was paralleled by decreased spine and increased dendritic AMPA-Rs. Overall AMPA-R cluster intensity was significantly larger in neurons expressing $Ca_V1.3_{\Delta ITTL}$ compared to $Ca_V1.3_L$ and control ($Ca_V1.3^{-/-}$). Reconstitution of $Ca_V1.3^{-/-}$ neurons with $Ca_V1.3_L$ or $Ca_V1.3_{\Delta ITTL}$ both reduced mEPSC amplitudes, but did not altered CaMKII autophosphorylation at T286.

Conclusion: $Ca_V1.3$ channels regulate dendritic spine morphogenesis depending on the presence of C-terminal PDZ-domain binding sequence. Spine elongation induced by $Ca_V1.3_{\Delta ITTL}$ was accompanied by a redistribution of surface AMPA receptors from dendritic spines to shafts. Taken together, $Ca_V1.3$ channels regulate dendritic spine and postsynaptic AMPA-receptor abundance.

O 09: Lethal graft−versus−host disease is promoted by NAMPT and can be ameliorated by its inhibition

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Background: Graft−versus−host disease (GVHD) remains the major complication after allogeneic hematopoietic stem cell transplantation (HSCT) with high rates of treatment failure and mortality upon gastrointestinal (GI) involvement. GVHD is a consequence of complex interactions of allo−reactive donor T cells with host antigen−presenting cells (APC) resulting in an immunological attack against host cells and tissue. Upon immune activation or proliferation, immune cells and cancer cells undergo a profound metabolic reprogramming towards increased glucose uptake for aerobic glycolysis and production of biomass. The molecule nicotinamide adenine dinucleotide (NAD) is essential in these energy and signal transduction pathways and is constantly replenished by the rate−limiting enzyme Nicotinamide phosphoribosyl−transferase (NAMPT) in the NAD *salvage pathway*. Lymphocytes are particularly dependent on a proper NAD homeostasis and supply. Along these lines, targeting the energy metabolism in acute GVHD as a highly inflammatory condition might constitute a promising treatment strategy.

Methods The therapeutic efficacy of the NAMPT inhibitor FK866 was investigated in various fully−mismatched mouse models for GVHD and in a graft−versus−leukemia (GVL) model. *In vivo* experiments were completed by extensive *in vitro* approaches, investigating FK866's anti-proliferative, anti-inflammatory and anti-neoplastic activity in human mixed lymphocyte cultures (MLC), colonic organ cultures (COC) and on the B-cell lymphoma cell line A20.

Results Here we show, that NAMPT is highly elevated in serum of patients during acute GI- GVHD correlating with disease severity. Therapeutic application of FK866 during fully established disease effectively mitigated clinical and histological severity outcomes probably by suppressing activated donor T cells and host APC. FK866 further displayed potent anti−lymphoma activity, which was also beneficial in a GVL model. Notably, NAMPT blockade effectively suppressed the proliferative response of human lymphocytes in MLC and suppressed cytokine release in COC from patients with severe GI-GVHD.

Conclusions Our findings reveal a critical role for NAMPT in acute GVHD, that fuels activation and proliferation of lymphocytes and APC. The increased NAD demand of immune− and cancer cells implies an enhanced susceptibility towards NAD−depletion and suggests, that FK866−mediated NAMPT inhibition may provide a novel strategy to reduce inflammatory responses in GVHD and further eradicate residual malignant cells.

O 10: Nutrition dependent reconfiguration of the mitochondrial cardiolipidome in ageing mice

Markus Keller[1]

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Background: Mitochondria are organelles with important metabolic functions, such as the citric acid cycle and oxidative phosphorylation. Of central importance for correct mitochondrial functioning are their unique phospholipid bilayers, assembled in an outer and inner membrane structure and establishing the basis for respiratory proton gradient formation and a multitude of linked processes such as ATP synthesis and mitochondrial protein import. One factor in which mitochondria fundamentally differ from other cellular compartments or membranes is their lipid composition that contains approximately 20% of cardiolipins, a mitochondria specific phospholipid species. Cardiolipins are unique lipids with a glycerol bridged diphosphatidylglycerol backbone, substituted with up to four acyl chains. They are required for stabilisation of respiratory protein complexes, for cristae formation, to buffer pH fluctuations, and for apoptotic signalling. The biosynthesis of cardiolipins is however unspecific in respect to the acyl side chains of substrates and thus a post-biosynthetic side-chain remodeling process is required to generate a mature and functional cardiolipin profile.

Methods*:* Recently we have developed a novel HPLC – tandem mass spectrometric methodology which is capable to identify and quantify up to 140 different cardiolipin species in cells and tissues. Instead of targeting on only a few selected subspecies this method allows analyzing a complete cardiolipidome, including monolyso-, oxidized, and peroxidised species. Furthermore by applying mathematical modeling techniques we are able to extract detailed structural information about total and intramolecular fatty acyl side chain distributions, their length and degree of saturation form fragment spectra which are generated in parallel.

Results*:* Applying this methodology in a broad set of murine tissues, we could systematically characterize the structural variability of molecular cardiolipin species and quantified their respective acyl side chain and double bond distribution patterns. Interestingly, the obtained cardiolipin compositions indicated substantial differences in the structural variability of this lipid class. Thus, these results indicate that different types of mitochondria - as characterized by their individual cardiolipin profile - do exist within the same organism in a tissue specific manner. Additional integrative analysis using tissue-specific RNA-seq datasets showed that transcriptional regulations are unlikely a prominent factor in the regulation of cardiolipin variability.

Conclusion*:* Hence our results raise further questions about the regulatory/biochemical origin of the observed cardiolipin variability and their functional consequences. In order to tackle these research questions, we now focus on studying the concurrent action of enzyme and transporter specificities, physico-chemical effects, and fatty acyl pool availability for defining the strong tissue specificities of mitochondrial cardiolipin compositions

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