MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

PROGRAM AND ABSTRACTS

5TH MUI-START SYMPOSIUM

CENTER OF CHEMISTRY & BIOMEDICINE, INNRAIN 80

PROGRAM

13:30 H Welcome Address of Prof. Christine Bandtlow (Vice Rector of Research and International Relations)

TIME	*Oral presentations*	Abstract
13:40 – 13:50	**Mag.rer.nat Ingo Bauer PhD (Division of Molecular Biology)** "AN4022–A novel HDAC complex component as basis for antifungal therapy"	O 01
13:50 – 14:00	**Mag. Dr. Phil. Luca Fava (Division of Developmental Immunology)** "The Caspase-2-PIDDosome restrains the proliferative capacity of cells failing cytokinesis"	O 02
14:00 – 14:10	**Dr.med.univ. Gabriele von Gleissenthall (Department of Biological Psychiatry)** "Tryptophan and kynurenine metabolism in alcohol dependent patients in acute and medium-term withdrawal"	O 03
14:10 – 14:20	**Dr.rer.nat. Johanna Gostner (Division of Medical Biochemistry)** "Formaldehyde metabolism – On the role of formaldehyde in inflammation"	O 04
14:20 – 14:30	**Mag.rer.nat. Martin Puhr PhD (Department of Urology)** "Assessment of altered miRNA expression profiles to improve prostate cancer treatment"	O 05
14:30 – 14:40	**Lourdes Rocamora Reverte PhD (Division of Developmental Immunology)** "T-cell derived glucocorticoids: a conversion process from an inactive precursor"	O 06

Poster Presentations ***14:40 h – 15:00 h***	**Abstract**
Carlo Bavassano PhD (Institute for Neuroscience) "Generation and characterization of a human neuronal model of spinocerebellar ataxia Type 6 *via* Induced pluripotent stem cell differentiation"	P 01
Mag. Biol. Stefan Coassin PhD (Division of Genetic Epidemiology) "Evaluation of the genetic variability in the LPA KIV-2 copy number variation by new sequencing technologies"	P 02
Dr.med.univ. Alexandra Gratl and Dr.med.univ. Maria Gummerer (Department of Vascular Surgery) "Neuroprotective potential of tetrahydrobiopterin in spinal cord ischemia using a rat model"	P 03
Dr.med.univ. Franka Messner (Department of Visceral, Transplant and Thoracic Surgery) "Mechanical stress as a trigger of skin rejection in composite tissue allotransplantation"	P 04
Serena Quarta PhD (Division of Physiology) "Adam: the involvement and mechanisms of ADAM 17 in neuropathic pain"	P 05
Dr.med.univ. Sebastain Reinstadler and Dr.med.univ. Hans-Josef Feistritzer (Department of Cardiology and Angiology) "Prognostic significance of copeptin after ST-elevation myocardial infarction: Insights from cardiac magnetic resonance imaging"	P 06
Mag. Ruslan Stanika PhD (Division of Physiology) "Role of the endogenous L-type calcium channel $Ca_V1.3$ in dendritic spine morphogenesis"	P 07
Coffee and Poster discussion ***15:00 h – 15:30 h***	

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.

Poster presentations

The size of posters should be A0 = 80 cm HORIZONTAL x 120 cm VERTICAL.

The Poster should include: title, background, aims/hypotheses, results and conclusion.

Posters can be previously mounted on the stands placed outside in the corridor. Please mount the posters on the corresponding stands according to the numbers indicated in the program.

The PIs will be first invited to give a short presentation (1-2 min) on the poster's topic using a PowerPoint slide as support.

The aim of this short presentation is to draw the attention of the participants to the posters and promote further discussion at the stands.

Please send your PowerPoint presentation or slide to the Servicecenter-Forschung (maria.perez@i-med.ac.at) no later than the **12th of December 2016**.

ABSTRACTS

Oral Presentation

O 01: AN4022 – A novel HDAC complex component as basis for antifungal therapy

Silke Gross[1], Leopold Kremser[2], Herbert Lindner[2], Stefan Graessle[1], Ingo Bauer[1]

[1]Division of Molecular Biology, Biocenter, Medical University of Innsbruck, Austria
[2]Division of Clinical Biochemistry, Biocenter, Medical University of Innsbruck, Austria

Background: An efficient adaptation of opportunistic pathogenic fungi to the host environment is crucial for a successful establishment of infection. Distinct histone modifying enzymes like histone deacetylases (HDACs) are important factors for a proper regulation of genes required for such adaptation processes.
RPD3-type HDACs of filamentous fungi exhibit a C-terminal extension that is not found in other eukaryotes and is indispensable for fungal growth and development. Since RPD3-type HDACs exert their function as protein complexes, such sequence peculiarities might represent important binding sites for novel complex partners that in turn might serve as promising targets for novel antifungal compounds.
Previously, we were able to identify an uncharacterized conserved fungal protein (AN4022) being part of *Aspergillus nidulans* RpdA complexes. Orthologs of AN4022 can exclusively be found in Eurotiomycetidae, including a number of important fungal pathogens such as *A. fumigatus*, *A. terreus*, *A. flavus*, *Penicillium marneffei*, *Coccidioides immitis*, or *Histoplasma capsulatum*, indicating unique function in this fungal taxon.
Methods/Results: To characterize the role of AN4022 in fungal growth and development, deletion mutants and complemented strains as well as strains expressing Venus- or TAP-tagged AN4022 were generated. Moreover, for a comparative analysis of effects caused by the disruption of an RPD3 complex partner common in all eukaryotes, SntB, was also deleted in *A. nidulans*. Mutant strains were subjected to phenotypic analysis under different growth and stress conditions. Preliminary results indicate reduced growth and sporulation and higher susceptibility to osmotic and heat stress of both mutants, though these effects are more pronounced in the *sntB* mutant. We further used TAP-tagged AN4022 for affinity purification and were able to identify an additional protein AN8823 as novel RpdA complex component together with AN4022 in *A. nidulans*. Currently ΔAN8823 and ΔAN4022/AN8823 double mutant strains are being generated.
Conclusion: We propose that increased susceptibility of AN4022 and *sntB* mutant strains against different stressors might be important during infection since functional stress response pathways are known to be essential for full virulence. Coming experiments will include further phenotypic analysis under oxidative stress in order to select proper growth conditions for subsequent transcriptome analysis. Further, strains expressing TAP-tagged RpdA complex partners will enable determination of size and *in vitro* activity of distinct HDAC complexes.

Oral Presentation

O 02: The Caspase-2-PIDDosome restrains the proliferative capacity of cells failing cytokinesis.

Luca L. Fava[1], Fabian Schuler[1], Valentina Sladky[1], Manuel D. Haschka[1], Claudia Soratroi[1], Lisa Eiterer[1], Egon Demetz[2], Günter Weiss[2], Stephan Geley[3], Erich A. Nigg[4], Andreas Villunger[1]

[1]Division of Developmental Immunology, Biocenter, Medical University of Innsbruck, Austria.
[2]Department of Internal Medicine VI, Medical University of Innsbruck, Austria.
[3]Division of Molecular Pathophysiology, Biocenter, Medical University of Innsbruck, Austria.
[4]Biozentrum, University of Basel, Switzerland.

Background: Caspase-2 has been implicated in several essential cellular processes, most notably in the DNA damage response (DDR) and the maintenance of genome integrity. Strikingly though, murine models devoid of Caspase-2 or of members of its postulated activation platform, the PIDDosome, failed to reveal the phenotypes expected by a defective DDR, highlighting the fact that the physiological function of Caspase-2 is poorly understood.
Methods: Here we searched for a genuine activator of Caspase-2 by using a series of pharmacological and genetic tools to interfere with the faithfulness of cell division. We employed biochemical and genetic methods to assess the contribution of PIDDosome subunits to the activation of Caspase-2. Finally, murine models were employed to ultimately assess the physiological relevance of the PIDDosome.
Results: Cells failing cytokinesis assembled the PIDDosome, activated Caspase-2, ultimately resulting in reduced proliferative capacity.
Conclusion: Taken together, we postulate that the PIDDosome functions to limit the proliferative capacity of cells that failed cytokinesis.

Oral Presentation

O 03: Tryptophan and kynurenine metabolism in alcohol dependent patients in acute and medium term withdrawal

Gabriele F. von Gleissenthall[1]

[1]Department of Biological Psychiatry, Medical University of Innsbruck, Austria

Background: Chronic alcohol dependence constitutes a major disease burden in modern society. Although current treatments show a considerable success, the relapse rate in chronic alcohol dependent patients remains high. In order to efficiently treat alcohol dependency, it is vital to understand the underlying complex mechanisms of recovery during alcohol withdrawal. Recent research including our own study has suggested that tryptophan and kynurenine metabolism is profoundly disturbed during alcohol withdrawal both by immune-associated and cortisol-related mechanisms. Kynurenine production has been shown to be intensified during the alcohol withdrawal and to correlate with some alcohol-associated neuropsychiatric symptoms such as affective symptoms and sleep disturbances. The catabolism of kynurenine during alcohol withdrawal and in particular the role of its catabolites, which are substantially neuroactive (whether toxic or neuroprotective) are still unclear.

This study aims to investigate the dynamics of tryptophan metabolism and kynurenine catabolism in alcohol dependent patients during acute and medium-term alcohol withdrawal.

Methods/Results: Thirty patients (23 male, 7 female) were enrolled, three subjects had to be excluded (substance relapse or deliberate dropouts). The mean age was 44 years, the mean alcohol consumption per day 149,56 g. The self-reporting alcohol consumption (Audit - Alcohol Use Disorders Identification Test) showed a mean score of 30 (score 0 - 40 points). Heavy alcohol consumption seems to involve moderate depressive symptoms. We observed moderate depressive symptoms at the beginning of the alcohol withdrawal (Beck depression inventory, BDI = 24). During the ongoing withdrawal of the substance these symptoms attenuated (BDI score 17 at day 14, 9 after 4 weeks). We found an Indolamin 2-3 Dioxygenase (IDO) activation over the whole period ($p<0,000$). Tryptophan first decreased, then returned to its initial level. Both kynurenine and kynurenic acid increased throughout the whole period. Quinolinic acid first increased, then dropped below initial levels.

Conclusion: We observe an increase in kynurenine in patients with depressive symptoms. However, a detailed analysis of this process is still ongoing work. Understanding the mechanisms of recovery from alcoholism may lead to an improvement of therapeutic and rehabilitation schemes for this disease.

Oral Presentation

O 04: Formaldehyde metabolism – on the role of formaldehyde in Inflammation

Johanna M. Gostner[1]
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Background*:* Formaldehyde (HCHO) is an important metabolic intermediate that is present in all kingdoms. In human blood, formaldehyde concentrations range from 10-100 µM. Formaldehyde shows high reactivity towards cellular molecules due to its electrophilicity. It is detoxified either by formation of hydroxymethylglutathione (HMG), which is oxidized by alcohol dehydrogenase 5 (ADH5) and subsequently hydrolysed to give formate, or it can be directly oxidized to formate via mitochondrial aldehyde dehydrogenase (ALDH2). Formate then enters the one-carbon pool and is consumed in different biochemical pathways. Recent data shows that cancer cells can release considerable amounts of formaldehyde and this mechanism is hypothesized to contribute to tissue penetration by metastatic cells. Additionally, formaldehyde is a strong reducing agent and may be able to modulate immune responses by generating a reductive milieu. Since now, the role of endogenous formaldehyde formation under inflammatory conditions has not been investigated in detail.
Methods*:* Cell culture, HPLC and LC-MS analytics, western blotting, qPCR.
Results*:* Previously we could show that formaldehyde addition was able to suppress the activity of the central immunoregulatory enzyme indoleamine 2,3-dioxygenase-1 in mitogen-stimulated peripheral mononuclear cells to a larger extent than in unstimulated cells and this effect started already at sublethal concentrations. Stimulation of PBMC led to a shift of formaldehyde catabolic routes indicated by an upregulation of ADH5 and downstream thiolase ACAT1, while ALDH2 was down-regulated, pointing towards a differential regulation of formaldehyde metabolism under inflammatory status. Similarly, we observed an upregulation of ADH5 in A549 lung carcinoma cells when treated with either formaldehyde or formaldehyde-generating compounds aspartame and methanol. In addition, we established an LC-MS method to determine the amount of free formaldehyde in the cells and cell supernatants after derivatization with dinitrophenylhydrazine and will investigate whether the amount of formaldehyde itself will be affected under the different treatment conditions.
Conclusion*:* Both inflammatory conditions and the presence of formaldehyde-generating agents induce changes in formaldehyde metabolism. It remains to be estimated whether overall concentrations of formaldehyde increase or if there is a shift in the catabolic route only. Despite its importance as a carbon source for several biochemical pathways after its oxidation to formate, we postulate a signaling function of formaldehyde by exerting biphasic effects: The reductive capacity of formaldehyde may favor a reductive milieu and thus suppress Th1-type related immunobiochemical pathways on the one hand, while on the other hand its electrophilic properties may contribute to the activation of stress signaling pathways.

Oral Presentation

O 05: Assessment of altered miRNA expression profiles to improve prostate cancer treatment

Martin Puhr[1], Andrea Eigentler[1], Irma Sottsas[1], Georg Schäfer[2], Eberhard Steiner[1], Helmut Klocker[1]

[1]Department of Urology, Medical University of Innsbruck, Austria
[2]Department of Pathology, Medical University of Innsbruck, Austria

Background: In recent years much effort has been made to identify and study novel biomarkers to improve cancer treatment. In this context, micro-RNAs (miRNAs) and their potential use as diagnostic and/or prognostic biomarkers have become focus of investigation for many malignancies. Within this pilot project we therefore want to clarify, whether deregulated expression profiles of specific miRNAs can be used to discriminate indolent from aggressive prostate tumors, with the aim to establish a basis for the development of a prognostic miRNA biomarker panel to improve future prostate cancer therapies.

Methods: 40 prostate cancer patients who underwent radical prostatectomy were selected and matched for histopathological parameters like age, Gleason-score, and tumor stage. Frozen prostate tissues were macro-dissected in benign and malignant samples for each patient. Total RNAs including miRNAs were isolated with Direct-zol™ RNA MiniPrep kit. The quality of isolated RNA from all tissue samples was determined by assessment of the RNA integrity number (RIN) with the Agilent 2100 bioanalyzer system. Altered miRNA expression profiles were assessed on miRCURY LNA™ microRNA arrays (7th generation) including 3100 capture probes for all known human miRNAs and confirmed by qRT-PCR.

Results: The microRNA array data sets revealed more than 40 significantly regulated miRNAs in prostate cancer tissue. The expression of the most promising differentially regulated miRNAs was confirmed by qRT-PCR analysis. For this purpose specific miRNA primer and probe sets were spotted on 384 well costume made miRNA qRT-PCR-array plates. This validation process identified 31 miRNAs which are significantly deregulated in a direct comparison between benign and cancer samples concerning absolute expression and fold change. Furthermore, these identified miRNAs can be used to discriminate low from high Gleason score (GSC) tumors and to distinguish different prostate tumor stages.

Conclusion: The applicant has identified a specific tissue miRNA signature for prostate cancer. In a next step the expression of selected miRNAs will be evaluated in prostate cancer patient serum to assess a potential diagnostic value.

Oral Presentation

O 06: T-cell derived glucocorticoids: a conversion process from an inactive precursor

Lourdes Rocamora-Reverte[1], Andreas Villunger[1], Jan G. Wiegers[1]
[1]Division of Developmental Immunology, Biocenter, Medical University of Innsbruck, Austria

Background: Glucocorticoids (GC) are steroid hormones which take part in a feedback mechanism in the immune system shutting down inflammatory responses. There is increasing evidence that GC are key players in T cell selection in the thymus and are therefore important in shaping the peripheral T cell repertoire. GC are not only produced by the adrenal glands but also locally in the thymus. Whether thymic epithelial cells (TEC) or thymocytes are the main source of GC is a matter of debate. In order to elucidate which cells are capable to synthesize *de novo* GC, our research is focused on the study of the enzymes involved in GC synthesis in TEC and T cells at different developmental stages. Our work is centered on the study of the two main *de novo* GC synthesizing enzymes, CYP11A1 and CYP11B1, as well as paying special interest to the GC-activating enzyme 11bHSD1 which converts inactive 11-dehydrocorticosterone (11-DHC) into active corticosterone.
Methods/Results: Analysis of the expression of the GC pathway enzymes by qPCR shows expression of the first of the enzymes CYP11A1 and the activating enzyme 11bHSD1 at different T cell subsets in thymus and spleen, as well as in TEC. In contrast to previous studies, we did not find any detectable expression level of the final enzyme in the GC synthetic pathway, i.e. CYP11B1, neither in T cells nor in TEC. In order to address the effect of GC on T-cell development and selection, we performed fetal thymic organ culture incubated with different GC concentrations and found that low concentrations of GC were able to increase the number of CD4+CD8+ thymocytes whereas higher concentrations induced apoptosis. This effect was not observed in immature CD4-CD8- cells which were more resistant to high GC concentrations. In addition, using the OP9-DL1 *in vitro* system, we assessed the development of early immature thymocytes to mature T cells and confirmed that upon GC treatment CD4+CD8+ thymocytes did not progress in their maturation.
We further analyzed the capability of T cells to produce active GC *in vitro* and we found a clear conversion of the inactive precursor 11-DHC into corticosterone which resulted in T cell death that was specifically mediated by the glucocorticoid receptor (GR).
Conclusion: These results suggest that GC may modulate T cell development affecting thymocytes in a different way depending on their maturation status. The absence of CYP11B1 expression suggests that if GC are needed for T cell selection and development they may not be synthesized using the *de novo* synthesis pathway but rather generated by the conversion of inactive 11-DHC into active corticosterone by the action of the enzyme 11bHSD1 which is expressed throughout T cell development.

Poster Presentation

P 01: Generation and characterization of a human neuronal model of spinocerebellar ataxia Type 6 *via* induced pluripotent stem cell differentiation

Carlo Bavassano[1], Andreas Eigentler[1], Ruslan Stanika[2], Sylvia Bösch[3], Gerald Obermair[2], Roxana Nat[1], Georg Dechant[1]

[1]Institute for Neuroscience, Medical University Innsbruck, Austria
[2]Department of Physiology, Medical University Innsbruck, Austria
[3]Department of Neurology, Medical University Innsbruck, Austria

Background: Spinocerebellar Ataxia Type 6 (SCA6) is an autosomal dominant neurodegenerative disease characterized by a late onset, slow progression and pure cerebellar ataxia. SCA6 is an allelic disorder associated with the CACNA1A gene, coding for the alpha 1 A (α1A) subunit of P/Q type voltage-gated calcium channel $Ca_V2.1$, which, in the brain, is particularly highly expressed in the cerebellum. SCA6 mutation consists of a short expansion of a poly-glutamine stretch located in the cytoplasmic C-terminal domain of $Ca_V2.1$ channel protein.

Extensive studies, both using heterologous expression systems and transgenic animal models, have highlighted the complexity of the pathogenic molecular mechanism of SCA6. Currently, the cause of the disease remains elusive, and no therapy is known for SCA6.

We hypothesize that the analysis of patient-derived neurons expressing SCA6-$Ca_V2.1$ channels in their endogenous human neuronal microenvironment will help to shed light on the molecular cause/s of the disease. To this end, the aim of our study is to characterize the biophysical, cellular, and molecular properties of SCA6 patient-derived neurons differentiated from Induced Pluripotent Stem Cells (iPSC).

Methods/Results Control and SCA6 patient-derived iPSC lines were generated using Yamanaka´s reprogramming factors and neuronal differentiation was achieved following a milestone-based protocol. The resulting control and SCA6 patient-derived neurons expressed mature neuronal markers, and patch clamp recordings revealed their capability of firing action potentials and eliciting voltage-dependent calcium currents. CACNA1A gene was expressed at mRNA and protein level both in control and SCA6 neurons, as indicated by quantitative real-time PCR and immunocytochemistry staining.

The characterization of this human model for SCA6 will include the analysis of $Ca_V2.1$ currents and differential gene expression, both in control and SCA6 neurons.

In summary, we have generated functional SCA6 patient-derived neurons expressing the disease relevant protein *via* differentiation of iPSCs. This model will help to understand the effect of SCA6 mutation in human neurons.

Poster Presentation

P 02: Evaluation of the genetic variability in the LPA KIV-2 copy number variation by new sequencing technologies

Stefan Coassin[1]

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

Poster Presentation

P 03: Neuroprotective potential of tetrahydrobiopterin in spinal cord ischemia using a rat model

Alexandra Gratl[1], Maria Gummerer[1], Elisabeth Khuen[1], Manuel Maglione[2], Patrizia Moser[3], Gustav Fraedrich[1]

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[3]Department of Pathology, Medical University of Innsbruck, Austria

Background: Neurological complications such as paraplegia as a result of spinal cord ischemia (SCI) during thoracic or thoracoabdominal aortic aneurysm repair is a serious complication. Tetrahydrobiopterin (BH4) is one of five essential cofactors of the NOS and is crucial in the production of NO. It has shown to efficiently abrogate ischemia reperfusion in transplant surgery. We evaluate the effect of BH4 compared to Vitamin C and Saline Injection.
Methods: Male CD-rats were treated with either 50 mg/kg tetrahydrobiopterin, 20 mg/kg, vitamin C or saline injection with the sham group (n = 12 for each group). SCI will be induced by inserting a 2 French Fogarty catheter to the left femoral artery. Next the descending aorta will be occluded by inflating the balloon right after the left subclavian artery. Occlusion will be maintained for 10 minutes and 30 seconds. Neurological function was assessed using the Basso-Beattie-Bresnahan (BBB) motor rating scale until 7 days after surgery (day 1,2,3,5 and 7). Different tissue (spinal cord, cerebrum, aorta, pancreas, kidney, heart, liver, bloodserum and spleen) will be harvested for histopathological examinations and an evaluation of tetrahydrobiopterin level. A TUNEL assay will be performed using an in situ cell death detection kit, on paraffin-embedded sections. To validate immunohistochemical results and to further identify changes in protein levels and isometric changes in the NO pathway, we will perform Western Blot analyses.
First results: We operated the sham-group of 12 CD-rats. 3 of them got a ptosis on the operated side. The BBB score in the sham group was 21. The tissue level of tetrahydrobioperin was tested in 2 groups of each 5 animals. In both 50 mg/kg 15 minutes and in the other group 5 minutes before sacrificing. The final results are outstanding. We took of each CD-rat different tissue specimen: spinal cord, cerebrum, aorta, pancreas, kidney, heart, liver, bloodserum and spleen and gave it to the histopathological department for Histopathological and immunohistochemical evaluation.
Conclusion: At this point of the study we are not able to make a conclusion because examinations and results are still missing.

Poster Presentation

P 04: Mechanical stress as a trigger of skin rejection in composite tissue allotransplantation

Franka Messner[1]

[1]Department of Visceral-, Transplant- and Thoracic Surgery, Medical University of Innsbruck, Austria

Background: In the past 15 years reconstructive transplantation (RT) has become a rapidly advancing field with more than 100 hand/forearm transplantations and 20 face transplantations realized in transplant centers all over the world. Despite excellent functional and aesthetic outcomes repetitive acute rejections were seen in most patients.
It has been observed in a cohort of patients that some form of mechanical stress also might trigger skin rejection, which primarily manifests at the palm of the hand and includes dryness, scaling and thickness of the palmar skin. Besides differences in macroscopic features also histologic findings differ from classical rejection. This novel type of rejection is called “atypical” skin 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) or Brown Norway (donor) rats. Tacrolimus and ALS were used as immunosuppressive agents. Immunosuppression was tapered on postoperative day 10 (POD) to a minimal maintenance dose (0,2mg/kg KG), preventing primary alloimune response. On POD 20 mechanical irritation, using a specifically designed mechanical stimulation device applying irritation to the transplanted planta pedis, was performed twice daily for 10 minutes using 5 Newton pressure. Tissue biopsies (skin, muscle) were taken on POD 30 for histology and RTQ-PCR analysis.
First results: All seven proposed study groups (1=naive, 2=naive+stimulation, 3=syngenic transplantation, 4=syngenic transplantation+stimulation, 5=allotransplantation, 6=allotransplantation/rejection, 7=allotransplantation+stimulation; n=5) have been successfully transplanted and/or stimulated. Macroscopic features included a progressive dryness of the irritated planta pedis with hyperkeratosis, erythema and edema. The effect of the mechanical irritaion was in contrast to the pilot study less pronounced. Tissue biopsies (skin and muscle of planta pedis and thigh respectively) have been taken and histologic and gene expression analysis are currently performed.
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. We could successfully design a mechanical irritation device which was able to induce macroscopic features of atypical rejection in the setting of orthotopic hind limb allotransplantation in the rat. Histology and RTQ-PCR are currently in process.

Poster Presentation

P 05: ADAM: the involvement and mechanisms of ADAM 17 in neuropathic pain

Serena Quarta[1], Natalia Schiefermeier[1], Theodora Kalpachidou[1], Theresa Martha[1], Kathrin Braun[1], Stefan Rose-John[2], Michaela Kress[1]

[1]Division of Physiology, Medical University of Innsbruck, Austria
[2]Department of Biochemistry, Christian-Albrechts-University Kiel, Germany

Background: Tumor necrosis factor-α converting enzyme TACE/ADAM17 is a member of the ADAM (A Disintegrin And Metalloproteinase) protease family. ADAM17 regulates inflammatory and regenerative events in healthy and pathological conditions.
Some of the ADAM17 targets such as TNFα, TNFR, IL-6R and some chemokines were shown to be involved in the development of pain and hyperalgesia in inflammatory and neuropathic pain models. However, the role of ADAM17 in the generation and maintenance of pathological pain is largely unknown.
Methods: Adam17ex/ex mice, characterized by barely detectable ADAM17 mRNA expression, were used for the experiments. A battery of in vivo sensory behavioral tests was used to study the pain sensitivity in adult naïve mice and in a model of neuropathic pain (spared nerve injury, SNI). Adam17 levels were quantified by qRT-PCR. Visualization of the skin innervation and dorsal root ganglion (DRG) morphology was obtained through high resolution microscopy. Immunostainings on spinal cord slices were performed to visualize and evaluate the percentage of activated microglia in the dorsal horn from wild-type (wt) and Adam17ex/ex mice after SNI. Westernblot assay was used in order to quantify the levels of different ADAM17 targets.
First results: Our preliminary data shows that reduced ADAM17 expression correlated with reduced sensitivity to mechanical stimuli. No differences were detected in skin innervation and thickness of the stratum corneum in Adam17ex/ex mice. In DRG a significant reduction in the number of IB4-positive neurons was observed in Adam17ex/ex compared to wt mice. Nociceptors were sensitized to mechanical stimuli after SNI, however, Adam17ex/ex mice were protected from the severe effect of the peripheral injury. In a pilot study, Adam17ex/ex hypomorphic mice had reduced microglia activation in the dorsal horn compared to controls already 3 days after lesion. These results were in lane with the reduced levels of phosphorylation of p38 protein in Adam17ex/ex spinal cord, which are normally higher in activated microglia.
Conclusions: Our data support the hypothesis that ADAM17 could be implicated in regulating nociceptor excitability and contribute to pain associated with tissue injury maybe modulating microglia activation in spinal cord. Further experiments will be required to understand the molecular partners implicated in these processes.

Poster Presentation

P 06: Prognostic significance of copeptin after ST-elevation myocardial infarction: Insights from cardiac magnetic resonance imaging

Sebastian Reinstadler[1], **Hans-Josef Feistritzer**[1]

[1]Department of Cardiology and Angiology, Medical University of Innsbruck, Austria

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 associated with cardiac magnetic resonance (CMR) markers of adverse outcome (myocardial function, infarct size, microvascular obstruction). Accordingly, copeptin might improve risk stratification of STEMI patients. However, copeptin rapidly decreases within the first days after infarction and therefore the most appropriate time point for the measurement of copeptin to risk stratify these patients' remains to be determined. We aim to compare the use of multiple versus single time point copeptin measurements for the prediction of systolic dysfunction and myocardial damage 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, 90 patients (90%) have been enrolled. These patients underwent a standardized contrast-enhanced CMR imaging protocol for the measurement of myocardial function and infract characteristics (infarct size, infarct transmurality, myocardium at risk intramyocardial hemorrhage and microvascular obstruction).

Outlook: This study will provide for the first time data about the association 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.

Poster Presentation

P 07: Role of the endogenous L-type calcium channel CaV1.3 in dendritic spine morphogenesis

Ruslan Stanika[1], Jörg Striessnig[2], Bernhard E. Flucher[1], Gerad J. Obermair[1]

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Background: L-type Ca^{2+} channels (LTCC) are important for the activity-dependent regulation of neuronal development, synaptic plasticity and gene transcription. A deficiency of Ca_V1 channels or their increased activity leads to aberrant brain function and neurological disease. $Ca_V1.3$ channels critically contribute to the excitability, pacemaking and dendritic spine formation in GABA-ergic neurons in the substantia nigra and therefore have been implicated in the etiology of Parkinson's disease (PD). 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. Only the long splice variant contains a cytoplasmic PDZ ligand (ITTL) that binds the synaptic scaffolding proteins densin-180 and shank.

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.

First results: Hippocampal neurons lacking $Ca_V1.3$ calcium channel show normal development of dendritic tree with formation of spines by 21 days in culture. Reconstitution of $Ca_V1.3_L$ splice variant resulted in slight decrease of spine size with increasing of spine density along dendrite. In contrast, $Ca_V1.3_{\Delta ITTL}$ mutant (which similarly to short splice variants lacks PDZ interaction domain) significantly increase spine size simultaneously changing its morphology-from "thin", "stubby" and "mushroom" spines to filopodia-like structures or "branched" spines. These changes are accompanied by relative redistribution of surface AMPA receptors expression between shaft of dendrite and dendritic spines.

Conclusion: Taken together, our data revealed an important role of the $Ca_V1.3$ C-terminus in regulating the morphology of dendritic spines and thus postsynaptic stability.

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